USMANU DANFODIYO UNIVERSITY, SOKOTO

(POSTGRADUATE SCHOOL)

EFFECT OF TILLAGE, CROP ROTATION AND NITROGEN FERTILIZATION ON SOIL MICROBIAL DIVERSITY IN NIGERIAN SAVANNA ALFISOLS

A Dissertation

Submitted to the

Postgraduate school,

USMANU DANFODIYO UNIVERSITY, SOKOTO, NIGERIA

In Partial Fulfillment of the Requirements
for the Award of the Degree of
MASTER OF SCIENCE (SOIL SCIENCE)

BY

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JUNE, 2020

DEDICATION

This dissertation is dedicated to my parents, Mr. and Mrs. Daniel Danjuma, and siblings for their prayers, support and encouragement throughout the course of the programme, and to the resource poor farmers in Nigeria who cultivate the soil and produce crops.

CERTIFICATION

This dissertation by DANIEL, Jeremiah Danjuma (16210613002) has met the requirement for the award of the degree of Master of Science (Soil Science) of the Usmanu Danfodiyo University, Sokoto and is approved for its contribution to knowledge.

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ACKNOWLEDGEMENTS

My sincere gratitude goes to my supervisory team Professor Ado Adamu Yusuf, Professor Abubakar Umar Dikko and Professor Ahmadu Ali Farouq for painstakingly taking their time to guide, advice, support, scrutinize and criticize the research throughout its lifespan. Your fountain of knowledge gave this work its credibility. I am most indebted to Professor Ado Adamu Yusuf for the motivation, mentorship and encouragement in the course of this research. I really appreciate your effort, Sir.

I am also grateful to Professor Musa Audu for his invaluable advice and concern he has shown to me; not forgetting Dr. Garba Aliyu Abubakar, Mal. A. S. Lukman, Mal. M. Haliru, Mal. Yusif N. Sanusi, Mal. M. Sawa and the entire staff of the Department of Soil Science and Agricultural Engineering, Usmanu Danfodiyo University, Sokoto. My appreciation also goes to Mal. Abdulmalik Shehu of the Central Research Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto; Dr. Ibrahim Abubakar Aliyu of the Department of Soil Science, Faculty of Agriculture, Ahmadu Bello University, Zaria for his assistance in my data analysis; not forgetting Mal. Ibrahim Ilu and Mal. Abubakar Sani of the same Department for their support in the field work and soil analysis.

This work would not have been a success without the prayers, support and encouragement of my family members; siblings and parents. I am forever indebted to you. I also owe a great deal of gratitude to several people; among who are Dr. Enjugu Manasseh Achukwu, thank you for your encouragement and support, you are a friend indeed; Mr. Sabbat P Lama, Mr. Dudu E Danjuma, Mr. Jonathan Jacob, Mr. James D Tanko, Mr. Danjuma Bedi, Miss Chinyere Elizabeth Malizu; and others too numerous to mention, Thank you so much.

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ABSTRACT

The study assessed tillage practices, crop rotation and N-fertilizer on soil microbial diversity in Nigerian *Alfisol*s. The aim of the research was to evaluate the influence of long-term tillage practices, cereal/legumes rotation and N-fertilizer rates on soil microbial diversity and its effect on soil quality. The trial was a split split-plot experiment laid in a randomized complete block design replicated three times; with tillage as main plot, crop rotation as sub-plot and N-fertilizer rates as sub sub-plot. Soil properties were analyzed using standard procedures while serial dilution and molecular techniques (16S rRNA genes for bacteria) were used to study soil microbial dynamics. Results revealed soil texture as sandy loam, soil pH, exchangeable Ca and Mg, CEC and available P were moderate, bacterial and fungal populations were high. Phylogenetic diversity depicted *Bacillus* as the dominant genera studied*;* others are *Rhizobiale*, *Rhodobacter* and *Sinorhizobium* observed in cowpea/maize rotation. The influence of cropping systems on both bacterial population and clustering of microbial sequences were significance (P<0.05); tillage and N-fertilizer rates (T*NR) and crop rotation and N-fertilizer rates (CR*NR) on bacteria and fungi loads were significance (P<0.05). Shannon Index for bacteria negatively correlated with available P. (P<0.01) and silt but positively correlated with sand (P<0.01).

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of the Study

Tillage practices impact physical, chemical, and biological properties of soil and have immense effects on the productivity and sustainability of soil. Conventional tillage systems may greatly affect long-term soil productivity due to erosion and loss of organic matter in soils. Sustainable soil management can be practiced through conservation tillage (including no-tillage), high crop residue return, and crop rotation (Hobbs, Sayre. and Gupta, 2008). Conservation tillage refers to tillage practice that leaves more than 30% of the soil surface covered with crop residue; and it is one of the most effective, efficient and least costly methods of reducing soil erosion, fluctuation in temperature (Cullurn, McGregor, Mutchler and Johnson, 1998) as well as conserving labour, fuel and soil moisture (Allen and Musick, 1997). Studies conducted under several climatic conditions, soil types, and crop rotation systems indicated that soils under reduced tillage have significantly higher soil organic matter contents compared with conventionally tilled soils (Alvarez, 2005).

Conventional tillage has been practiced for years around the globe (Fowler and Rockstrom, 2001) due to its numerous advantages including loosening the soil hence increasing soil drainage and root development (Moussa-Machraoui, Errouissi, Ben-Hammouda and Nouira, 2010). Conventional tillage stimulates heterotrophic microbiological activity through soil aeration thus, leading to soil microbial communities dominated by aerobic microorganisms, which accelerate soil organic matter oxidation, decrease soil aggregate stability, aggravate greenhouse gas emission (N_2O, NO, CO_2, etc.) and make the soil vulnerable to soil erosion (Omonode, Smith, Gál and Vyn, 2011), thereby threatening

sustainable crop production (Mathew, Feng, Githinji, Ankumah and Balkcom, 2012). While in no-till soils, microbial compositions are dominated by anaerobic groups and *Clostridium* was the most abundant genus detected (Dorr de Quadros *et al.,* 2012). No till soils were also reported to harbor more diverse soil bacterial communities with higher predicted gene content, although exceptions were observed in the case of some classes of *Proteobacteria*, *Gemmatimonadetes*, and *Chlorobi* (Hariharan, Sengupta, Grewal and Dick, 2017). Bacterial diversity was observed to be higher under No tillage than Conventional tillage (Lupwayi, Lafond, Ziadi and Grant, 2012). Thus, there is still no clear consensus on the overall effect of tillage on bacterial diversity.

For instance, reduced tillage has demonstrated a consistent increase in the abundance of fungi, bacteria, arbuscular mycorrhizal (AM) fungi, and *actinobacteria* in the surface soil. Similar to other reports, conservation tillage practices increase microbial population and activity as well as microbial biomass (Balota, Colozzi-Filho, Andrade and Dick, 2003). This has been the basis why most researchers are now advocating for no till where possible. Contrary to conventional tillage, no-till minimizes soil and nutrient losses through leaching and erosion, increase soil water storage and reduce production costs (Malhi, Grant, Johnston and Gill, 2001).

Grain legumes have long been known to be important components of the traditional cropping systems in the tropics and are also being considered for soil fertility improvement in cereal-based cropping systems in the Nigerian savannas (Yusuf, Abadoo, Iwuafor and Olufajo, 2008). These legumes in symbiosis with specific microbes have the capacity to fix atmospheric nitrogen N_2 that enables them to grow well on nitrogen (N) impoverished soils without adding fertilizer N. Researchers have also shown that legume-cereal rotation can

improve soil fertility principally by increasing organic matter, soil N content and soil N availability as a result of their residue mineralization (Yusuf, Iwuafor, Abadoo, Olufajo, and Sanginga, 2009). Adediran, Akande, Taiwo and Ojo (2010) reported that legumes are credited with supply of a substantial amount of N to the succeeding non – legume crop. The increased N availability to crop may also be due to reduced immobilization because legume crops generally produce higher quality crop residue than cereal crops (Green and Blackmer, 1995).

In most agro-ecosystems, it has been widely established that nutrient addition changes aboveground plant biomass and weeds composition (Suding, Collins, Gough, Clark, Cleland, Gross, … Pennings, 2005).

Since microbial populations play an important role in plant residue decomposition and nutrient cycling, it has been an area of interest to study how fertilizers affect the structure and function of these microbes. Nitrogen fertilization decreased soil pH, and increased soil organic carbon (C) and available N contents. Bacterial taxonomic diversity was observed to decrease by N fertilization alone, but was increased by NPK fertilization. Conversely, soil bacterial diversity is very sensitive to environmental changes, such as those caused by anthropogenic disturbances, including long-term nutrient input (Allison and Martiny, 2008). Clegg, Lovell and Hobbus (2003) showed that nitrogen fertilizer has a significant impact on the total bacterial and actinomycete community structures using polymerase chain reaction- denaturing gradient gel electrophoresis (PCR-DGGE) and phospholipid fatty acid (PLFA) profiling. It was also found that fertilizer amendment altered the abundance of bacterial groups throughout the agriculture soil in Watkisville, Georgia, USA by using both 16S rRNA gene clone libraries and PLFA (Jangid, Williams, Franzluebbers,

Sanderlin, Reeves and Jenkins, 2008). One study showed that γ-*Proteobacteria* were sensitive to fertilization while *Acidobacteria* was unaffected by fertilization (Wu, Qin, Chen, Wu and Wei, 2011). It was found that long-term inorganic nitrogen management influenced the structure of Nitrite-oxidizing bacteria and new ecotypes of non-characterized *Nitrospria* spp. were found in fertilized soil (Freitag, Chang, Clegg and Prosser, 2005). Long-term inorganic nitrogen management was observed to influence the structure of Nitrite-oxidizing bacteria, and new ecotypes of non-characterized *Nitrospria* spp. were found in fertilized soil (Freitag *et al*., 2005)

Soil microorganisms perform critical roles in the decomposition of organic matter, transformation of nutrients, degradation of pollutants, soil structural modification and aggregates stabilization, maintenance of ecosystem sustainability, plant pest control, and regulation of soil productivity or plant growth; which has often served as vital indicator of soil fertility and soil quality (Altieri, 1999; Stockdale and Brookes, 2006). Also, there is dearth of information on the soil microbial diversity that contributes to soil fertility as influenced by long-term tillage under crop rotation and N fertilization in the northern Guinea savanna *Alfisol*. Soil biota is an essential component of soil health; therefore, it constitutes a major fraction of global terrestrial biodiversity (Moreira, Heinrichs and Freitas, 2008).

However, activity and species composition of microbes are generally influenced by many factors including physical and chemical properties of the soil, temperature and vegetation. The dynamics of soil microorganisms have important implications for the response of subsurface soil ecosystems to perturbations. Despite all attempts to measure fluxes and gross microbial pools; the soil and its microbiota still remain a black box because soils form one of the most complex ecosystems teeming with a vast range of microbes with the

identity and functions of a majority of microbes still being investigated (Crecchio, Gelsomino, Ambrosoli, Minati and Ruggiero, 2004; Little, Robinson, Peterson, Raffa and Handelsman, 2008).

1.2 Statement of the Problem

Soil fertility decline is increasingly viewed as critical problem affecting agricultural productivity and environmental welfare in Africa (Smaling, Nandwa and Janssen; Fan, Shen, Yuan, Jiang, Chen, Davies and Zhang, 2011). As soils are exploited, they degrade, especially when continuously cultivated without nutrients and organic matter inputs. The organic matter content of *Alfisols* is easily lost through burning and cultivation. Savanna soils are highly vulnerable to erosion because most of the soils are sandy, low in organic matter and unstable soil aggregates and structure (Odunze, 2003). This deterioration has physical, chemical and biological constituents and it is manifested within individual fields and across entire catchments and landscapes (Bationo, Kimetu, Kimani, Mugendi, Odendo, Silver and Sanginga, 2004). Biological components of degradation manifests when management practices that alter the living and nutrient conditions of soil organisms (such as repetitive tillage or burning of vegetation) result in degradation of soil organisms' microenvironment, hampered biogeochemical processes and ecosystem functioning (FAO, 2005).

Wu and Wang (2007) showed that the diversity index, richness and evenness index of the microbial community of crop rotation were higher than those of mono-cropping. Soil microbial diversity is fundamental to sustainable agriculture because microbes mediate many processes that support agricultural production.

Tillage systems, cropping systems and nitrogen (N) fertilization are factors that can modify soil quality and fertility thereby affecting microbial community composition in an *Alfisol*. There is knowledge gap on how soil microbial diversity is influenced by the interaction of tillage, crop rotation and nitrogen fertilizer in the Nigerian *Alfisols*. It is essential to study microbial diversity not only for basic scientific research, but also to understand the link between diversity and community structure and function.

1.3 Justification of the Study

Agricultural management practices will impact the structure, composition and diversity of microbial communities that will in turn have effects on the ecosystem's productivity. Understanding the microbial dynamics in terms of composition, structure as well as their inter-relations to soil functions is therefore necessary in establishing and integrating management practices that promote sustainable agro-ecosystem functioning. A better understanding of the contribution of microorganisms and impact of management effects would require the characterization of the microbial community shifts under different management practices more so on the long-term basis.

The study of soil microbial diversity can be done using both conventional (culture dependent) and molecular (culture independent) techniques. In the conventional technique which involves normal laboratory isolation and testing their morphological and biochemical characteristics; certain information about the strains of the organisms may be obscured. However, the molecular technique which consists of ribonucleic acid (RNA) or deoxyribonucleic acid (DNA) extraction have provided privilege to characterize these organisms beyond ordinary morphological or phenotypic characteristics; they can be

characterized up to molecular level. Findings from this study will advance the frontiers of knowledge among scientists and suggest efficient agricultural management systems that resource poor farmers can adopt to improve soil quality; and ensure sustainable agricultural production.

1.4 Aim and Objectives

The aim of the research is to evaluate the influence of tillage practices under cereal (*Zea mays* L.) legumes rotation (*Vigna unguiculata* (L.) Walp., and *Glycine max* (L.) Merr.) and nitrogen (N) fertilization on soil microbial diversity and its effects on soil quality. The specific objectives are:

1. To determine the dominant soil microbial groups as influenced by long-term tillage, crop rotation and nitrogen fertilization using conventional and molecular technique.

2. To determine the relative abundance of the different microbial population in the soil.

3. To determine the relationship between selected soil properties and microbial diversity as influenced by tillage, crop rotation and nitrogen fertilization.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Perspectives on Tillage Practices and Soil Microorganisms Dynamics

Current climate change, depletion of natural resources and food security are the main factors driving the need for sustainable agricultural production systems. Conventional agricultural practices employ several principles mainly aimed at maximizing production yields but often at the expense of natural resources and the environment (Hobbs *et al.*, 2008; Powlson *et al*., 2011). These practices include clearing land, deep tillage that incorporates crop residues into the soil often using heavy machinery that compact the soil, and heavy reliance on additional inputs like mineral fertilizers and chemical pesticides. Continuous tilling disrupts the soil structure, incorporates crop residues into the soil leaving the soil surface bare and prone to erosion.

The incorporation of surface residue material into soil through tilling also makes it more accessible to soil microbes and also increases oxygen diffusion into the soil, resulting in greater rates of organic matter decomposition. The increased rates of decomposition result in greater emissions of carbon dioxide (CO2) that further contributes to the greenhouse effect (Six, Frey, Thietand Batten, 2006; Roldan, Salinas-García, Alguacil and Caravaca, 2007). Exhaustion of soil organic matter as well as a decrease in natural biota leads to reliance on mineral fertilizers and chemical inputs for nutrients, pest and disease management. This reliance on chemical inputs can contribute to the buildup of soil toxicity and environmental pollution (Govaerts, Ceja-Navarro, Rivera, Patiño-Zúñiga, Marsch, Vila-Sanjurjo, and Dendooven, 2009). It is in consideration of the above factors that scientists are calling for the development of sustainable farming practices.

The focus of conservation agricultural management principles is to establish management practices that integrate the efficient use of natural resources and external inputs with an aim of improving crop productivity while conserving the environment and maintaining soil quality (Hobbs *et al.*, 2008; Powlson *et al.*, 2011). Conservation agricultural(CA) management practices that include reduced tillage, cover cropping and crop rotation are some of the practices being endorsed by scientists to mitigate soil erosion, minimize the emission of greenhouse gasses, as well as increase soil quality and crop productivity (Hobbs *et al.*, 2008).

The replenishment of soil organic matter (SOM) and the gradual increase in the relative abundance, diversity and activity of soil microbial communities are some of the factors attributed to improved soil quality under CA (Tikhonovich and Provorov, 2011; Mohammadi, Heidari, Khalesro, and Sohrabi, 2011). Reduction in soil disturbance, maintenance of residual material, use of cover crops, and crop rotation have been recognized as factors that lead towards changes in microbial abundance, diversity and activity (Hobbs *et al.*, 2008). These changes call for a strategy to evaluate management practices that can be used depending on the different cropping system, soil type, climatic conditions, as well as potential pests and diseases. It is clear that microbes play an important function in agricultural production, and that different management practices have influence on microbial structure and functions. The questions that arise are which microorganisms and microbial activities are amplified or moderated under different management practices, how do the members of the microbial community symbiotically or competitively interact with each other and how this in turn influences sustainable crop production and ecosystem functions. The goal of CA management practices would be to

achieve a long-run equilibrium of a microbial community structure that would facilitate factors such as increased nutrient capacity, soil structural buildup and aeration, as well as plant disease suppression (Hobbs *et al.,* 2008).

2.2 The Concept of Soil Quality

Alexander (1971) for the first time proposed development of soil quality criteria in the context of agriculture's role in environmental improvement. The soil quality concept per se was introduced by Warkentin and Fletcher (1997) as an approach to facilitate better land use planning for multiple functions. Their concept of soil quality was based on four criteria, upon which future concepts of soil quality were developed. These criteria were that (1) soil resources were constantly being evaluated for an ever-increasing range of uses, (2) several different stakeholder groups were concerned about the state of soil resources, (3) priorities and demand of society were changing, and (4) soil-resource and land-use decisions were made in a human and institutional context. In a broad sense, the concept of soil quality was not introduced until the mid-1980s, wherein emphasis was mainly given to soil resource management, particularly in controlling soil erosion and minimizing its effects on crop productivity (Pierce, Larson and Dowdy, 1984). Later, soil management gradually shifted from minimizing soil erosion to broader issues like sustainable agriculture, environmental health and prevention of soil degradation (Karlen *et al.,* 2003a).

In the 1990s, the pace in soil quality research was further accelerated by the recommendation of the U.S. National Research Council's (NRC) Board on Agriculture that "we conserve and enhance soil quality as a fundamental step toward environmental improvement" and that the concept of soil quality be in principle a guide to agricultural

policies and practices (NRC 1993). Thereafter, many researchers contributed to developing a soil quality concept in the publications entitled, “Defining Soil Quality for Sustainable Environment” (Doran, Coleman, Bezdick and Stewart, 1994) and “Methods for Assessing Soil Quality” (Doran and Jones, 1996).

Soil quality has been defined in several ways including ‘fitness for use’ and dependent upon the extent to which a soil fulfills its destined role (Larson and Pierce, 1994; Singer and Edwig, 2000). In a broad ecological sense, soil quality has been defined as the capacity of a soil to function within ecosystem boundaries to sustain plant-animal productivity, maintain or enhance water and air quality, and support human health and habitation (Karlen*et al.*, 1997). Doran and Safely (1997) further defined soil quality by considering the continuous and dynamic nature of the soil as “the continued capacity of soil to function as a vital living system, within ecosystem and land-use-boundaries, to sustain biological productivity, promote the quality of air and water and maintain plant, animal and human health”. More recently, a healthy soil as part of the soil quality concept is defined as a stable soil system with high levels of biological diversity and activity, internal nutrient cycling and resilience to disturbance (van Bruggen, Semenov, van Diepeningen, de Vos and Blok, 2006). Overall, soil quality is considered as an integrative indicator of environmental quality, food security and economic viability (Herrick, 2000) and therefore, it would serve as a good indicator for monitoring sustainable land management.

The concept developed in this review differs from traditional technical approaches that focus solely on productivity. Instead, soil quality is examined as a holistic concept, recognizing soil as a part of a dynamic and diverse production system with biological,

chemical and physical attributes that relate to the demands of human society (Swift, 1999; Sanchez, Palm and Buol, 2003). Society, in turn, actively adapts soil to its needs, mining it of its nutrients on demand and replenishing these nutrients in times of excess.

2.2.1 Indicators of soil quality

Assessment of soil quality is a great challenge because it is highly dependent on management of soil through resources available in a given agro-ecosystem and the agro-climatic conditions (Karlen, Andrew and Wienhold, 2003b). Common approaches used for assessing the soil quality are either qualitative or quantitative. Qualitative indicators are often sensory descriptors e.g. appearance, smell, feel and taste recorded through direct observations usually made by the growers' (Garlynd, Roming, Harris and Kukakov, 1994; Dang, 2007). Other observations include soil colour, yield response, frequency of ploughing or hoeing, and visual documentation of plant growth, selected weed species, and earthworm casts. The use of indigenous local knowledge and experience of growers provides a simple approach to characterize the status of soil; and to diagnose any change in soil quality (Roming, Garlynd, Harris and Mc Sweeney, 1995; Barrios, Delve, Bekunda, Mowo, Agunda, Ramisch, … Thomas, 2006).

Quantitative assessments of soil quality involve more sophisticated analytical approaches (Harris and Bezdicek, 1994). Generally, soil quality is assessed by the combination of the physical, chemical and biological properties acting as indicators (He, Yang, Baligar and Calvert, 2003), and a large number of different physical, chemical and biological properties of soil are being employed as quantitative indicators to define soil quality (Roming *et al.*, 1995; Dang, 2007). Typical soil physical indicators include texture, bulk density and

infiltration, water holding capacity and retention characteristics, porosity, aggregate stability and soil depth. Organic carbon, pH, electrical conductivity, cation exchange capacity, extractable N, P, K, S are important chemical indicators, and biological indicators include quantity, activity, and diversity of soil fauna and flora and soil enzymes. Several bio-indicators of soil quality have been developed (Trasar-Cepeda, Leiros, Seoane and Gil-Sotres, 2000; Nielsen and Winding, 2002; Anderson, 2003). A number of soil biological properties respond to changes in agricultural practices, showing potential use as indicators of soil quality.

2.3 The Role of Microbial Communities in Agro-ecosystems

Soils form one of the most complex ecosystems teeming with a vast range of microbes with the identity and functions of a majority of these still being unknown (Torsvik and Ovreas, 2002; Fitter, Gilligan, Hollingworth, Kleczkowski, Twyman and Pitchford, 2005; Little *et al.*, 2008). It has been estimated that one gram of soil may contain up to 10 billion microorganisms (Torsvik and Ovreas, 2002). These include a wide range of species of bacteria, fungi, algae and protozoa. The microbial community plays a critical role in the maintenance of soil quality and agro-ecosystem functioning. Soil quality has been defined as “the continued capacity of soil to function as a vital living system, within ecosystem and land use boundaries, to sustain biological productivity, maintain the quality of air and water environments, and promote plant, animal, and human health” (Doran and Zeiss, 2000).

One of the key roles of soil microbial communities is their integral role in regulating soil biogeochemical cycling processes, through decomposition of soil organic matter (Powlson *et al.*, 2011; Tikhonovich and Provorov, 2011). For example, microbes contribute to the

carbon cycle in several ways; firstly, soil microbes are involved in the decomposition of organic matter releasing CO_2 to the atmosphere, and secondly they also act as a carbon sink contributing to the pool of SOC. The balance between these processes and stability of the microbial derived organic matter will determine carbon sequestration; a key goal in sustainability. Another role of soil microbes involves their contribution to soil aggregate formation and soil structure stabilization. Soil microbes have been shown to promote the process of soil aggregate formation and stabilization through different mechanisms that include the mixing and formation of channels within the soil matrix, production of extra-cellular and polymeric substances that coagulate soil particles, degradation and alteration of soil organic matter, and the attachment of their cells to soil particles (Powlson *et al.*, 2011). In particular, fungi are said to facilitate macro-aggregate formation and stabilization as hyphae and mycelium channels through soil (Rillig, 2004; Borie, Rubio and Morales, 2008), while bacteria are said to contribute to micro-aggregate stabilization (Caesar-TonThat, Caesar, Gaskin, Sainju and Busscher, 2007).

Microbes play an important role in plant health through control of diseases, and in adaptation to physiological stresses like drought. Studies have shown that different pests can increase, decrease or remain constant after the onset of conservation practices (Garbeva, van Veen and van Elsas, 2004). It is believed that the incorporation of crop rotations and cover crops would have an effect of reducing pest and disease incidences by increasing microbial diversity and in turn increasing competitive advantage of beneficial organisms verses the pathogenic (Patzek, 2008; Govaerts, Ceja-Navarro, Rivera, Patiño-Zúñiga, Marsch, Vila, Sanjurjo and Dendooven, 2009). Microbial diversity has also been

considered a key factor in the development of soil suppressiveness, i.e., the ability of soil to naturally suppress soil borne-diseases (Garbeva *et al.,* 2004). A recent study undertaken to characterize the soil fungal community structure along a disease severity gradient of soil borne pathogen affecting field peas demonstrates that microbial communities differ between soils with diseased plants and healthy plants (Xu, Ravnskov, Larsen, Nilsson and Nicolaisen, 2012).

Garbeva *et al.* (2004) in their review on microbial diversity and soil suppressiveness concluded that understanding shifts in microbial diversity would be necessary towards development of agricultural management practices that would maximize microbial communities that promote building up of soil suppressiveness. Consequently, microbial dynamics in terms of composition, structure as well as their interrelations to soil functions is therefore necessary in establishing and integrating management practices that promote sustainable agro-ecosystem functioning.

2.3.1 Concepts of microbial structure and diversity measures

Diversity, structure and function are some of the descriptors used in characterization of microbial communities. Structural properties mainly aim to describe the microbial community in terms of members who are within a particular community while functional properties, on the other hand, aim to describe how the microbial community behaves in performing various processes (Little *et al.,* 2008). Diversity is a term used to describe the size, distribution and variability within and among communities in terms of structure and function (Torsvik and Ovreas, 2002). In characterization of structural diversity, various components are given consideration. These include the members who are within a

particular community (species composition), their numbers (richness), and the distribution of individuals among species (evenness) (Torsvik and Ovreas, 2002; Nannipieri and Ascher, 2003; Little *et al.,* 2008).

Measures of bio-diversity within a given community referred to as α-diversity, and among the communities referred to as β- diversity can be calculated based on different diversity metrics (Whittaker, 1960; Whittaker, 1972, Ovreas, 2000; Nannipieri and Ascher, 2003).
It is believed that microbial diversity is an attribute that can be used to estimate how well a given ecosystem will perform (Nannipieri and Ascher, 2003) and maintain its function and structure which is termed as its robustness/stability (Little *et al.,* 2008). The robustness of an ecosystems/community refers to its ability to resist change in structure or functioning after a significant perturbation (Nannipieri and Ascher, 2003; Little *et al.,* 2008). Robustness can be looked at in three different ways, temporal stability-how well the community maintains its structure over time; resistance – ability to resist change after a perturbation; and resilience – the ability to return to its native state after significant perturbations/disturbances. It is believed that microbial diversity is directly correlated to ecological stability.

On the other hand, there is still an ongoing debate on whether an increase in community diversity necessarily leads to an increase in functionality and robustness on the basis of functional redundancy (Nannipieri and Ascher, 2003; Little*et al.,* 2008). Functional redundancy has been defined as the ability of one microbial taxon to carry out a process at the same rate as another under the same environmental conditions (Allison and Martiny, 2008). The concept of functional redundancy addresses a challenge to the diversity theory

above because it implies that the loss of diversity/or loss of certain species in a given ecosystem would not necessarily alter the ecosystem function and stability as other species would easily replace its function. The main reason why this would be an important concept to soil microbial ecologists lies in the fact that it would have direct implications on the response of an ecosystem functioning to shifts in microbial composition that may arise due to stress and disturbances.

2.3.2 Characterization of microbial community structure and diversity

Comprehending the dynamics of soil microbial communities and their interacting factors can be a daunting task. First, many of the microorganisms thought to enhance soil quality are difficult to culture or cannot be cultured. It is also difficult to devise experimental designs capable of simulating exact field conditions thus complicating the analysis of interacting environmental effects. In addition, most of the methods are limited in their capabilities to determine microbial composition and linking this to soil functioning (Nannipieri and Ascher, 2003; Six *et al.*, 2006) as well as the fact that most methods that can be used are rigorous and time consuming (Ghazanfar, Azim, Ghazanfar, Anjum and Begum, 2010).

Characterization of the microbial community structure, composition and diversity provides an added avenue of further understanding the role of microbes in influencing key soil ecological functions. The methods for studying microbial community diversity and structure can be categorized into classical, biochemical, and molecular techniques that can be either culture-based or culture-independent (Kirk, Beaudette, Hart, Moutoglis, Klironomos, Lee and Trevors, 2004; Little *et al.*, 2008). The classical approach is the plate count technique that relies on culturing of bacteria or fungi on agar media followed by

identification and quantification of specific taxonomic or functional groups. This technique is, however, limited by the fact that majority of microbes are uncultivable with the estimate being only 1% of microbes in soil can be cultured. The method is also biased towards fast growing microbial groups and is therefore unsuitable in studies geared towards investigating microbial community diversity and structure especially in the environment like soil.

Recent advancement in molecular techniques in the last two decades has revolutionized soil microbiology by providing culture-independent methods that have better resolution in the taxonomic identification of species composition, diversity and functional potential of microorganisms within a given ecosystem. The advent of the next generation sequencing (NGS) platforms has boosted the field of soil microbiology by availing more affordable and faster means of large scale analysis of genetic information from soil microbial communities (Ghazanfar *et al.,* 2010; Doolittle and Zhaxybayeva, 2010; Wooley *et al.,* 2010; Simon and Daniel, 2011).

These mainly involve the extraction and sequencing of nucleic acids from environmental samples directly (metagenomics) or based on specific phylogenetic markers (microbiomics) (Wooley *et al.,* 2010; von Mering *et al.,* 2007; Simon and Daniel, 2011). Several environmental sequencing studies demonstrate the impact this approach has in answering a wide range of ecological diversity and functionality questions in different scientific fields. In a comparative analysis of the microbial communities based on metagenomics from contrasting environments Tringe *et al.* (2005) demonstrated that different environments exhibited a wide range of species complexity. The environments they characterized ranged

from agricultural soils to three deep sea whale carcasses. Not surprisingly, the agricultural soil had a greater species complexity compared to those of the whale carcasses. The applications of environmental sequencing are clearly wide with the potential of having several testable hypotheses from one metagenome/microbiome dataset (Rodriguez-Brito, Rohwer and Edwards, 2006). Nevertheless, the application of environmental sequencing is also faced with some challenges. One challenge involves the isolation and extraction of high-quality DNA that encompasses all the microorganisms found within an environmental sample (Simon and Daniel, 2011). This is due to the fact that many microbial cells may be difficult to lyse using the most common DNA extraction protocols. Extraction of representative DNA is even more challenging for complex soil environments due to the interaction of the microorganisms with the physiochemical properties of soil (Lombard, N., Prestat, van Elsas and Simonet, 2011).

This challenge has been addressed by the development of protocols that allow the isolation of high quality DNA from different environments and thus selection of an appropriate extraction protocol is crucial to obtaining optimum DNA yield (Simon and Daniel, 2011; Lombard *et al.,* 2011). Another challenge involves obtaining a sample that is representative of the particular environment and one that can be utilized in comparative analysis studies. Sequencing based on phylogenetic markers is also stated to have biases mainly due to the PCR amplification steps involved and thus direct sequencing is stated to be ideal in giving the global view of the species composition within a given environment. Concerns regarding soil environmental sequencing are extensively addressed in a review by Lombard *et al.* (2011).

Perhaps one of the more challenging aspects of next generation sequencing relates to data handling and analysis - bioinformatics. The analysis of environmental sequencing is not only faced with the challenge of handling large data sets and short sequence reads but is further hampered by the fact that the sequences originate from a wide range of organisms (Lombard *et al.,* 2011; Tringe *et al.,* 2005). This raises difficulties in analyzing and interpreting the large data output generated. The depth of sequencing, referred to as coverage, varies from one sequencing platform to the other and usually depends on the read length, i.e. the platforms that give longer reads will usually give less depth and vice versa. The different next generation sequencing platforms generate base pairs (bp) sequence read lengths ranging from as short as 35bp to 400 bp (Morozova and Marra, 2008; Glenn, 2011). The platform currently recommended for deeper sequencing is the Illumina Mi/HiSeq which can generate millions of sequence reads lengths ranging from 35-300 bp. The technique used for sequencing, i.e. metagenome or amplicon sequencing, the length of sequences, and depth of coverage are all factors to consider when deciding on bioinformatics program software. This has led to the release of various metagenome/microbiome analysis software platforms that perform processes that include sequence quality control, classification and comparative analysis based on different programming languages and mathematical algorithms. Several open source software applications that are commonly used include QIIME (Caporaso *et al.,* 2010), Mothur (Schloss *et al.,* 2009), and MG-RAST (Meyer *et al.,* 2008).

2.3.3 Assessment of microbial diversity

Microbial diversity viz. structural and functional diversity in soil is increasingly evaluated for measurement of soil health (Visser and Parkinson, 1992). Consequently, this review will only focus on structural diversity and its technique.

2.3.3.1 Structural profiling technique

Structural diversity is defined as the number of parts or elements within a system, indicated by such measures as the number of species, genes, communities or ecosystems (Avidano *et al.,* 2005). Several indices such as species richness and evenness are used to describe the structural diversity of a community (Ovreas, 2000). However, these indices cannot be used for soil microbes as easily as for macro-organisms. Indeed, with the rise of molecular tools in microbial ecology, it became evident that we have described only a very small portion of the diversity in the microbial world. Most of this unexplored microbial diversity seems to be hiding in the high amount of yet uncultured bacteria. New direct methods independent of culturing and based on the genotype and phenotype of microbes allow a deeper understanding of the composition of microbial communities in a soil ecosystem (Amann, Ludwig, and Schleifer, 1995).

Based on molecular studies, it could be estimated that 1 g of soil consists of more than 109 bacteria belonging to about 10,000 different microbial species (Ovreas and Torsvik, 1998) or even much more (Gans, Murray and Dunbar, 2005). This huge level of diversity makes it difficult to employ the microbial community structure as an indicator of soil quality. A widely observed result is that the structural diversity of a bacterial community is often sensitive to environmental changes and exhibits a shift in its composition (Saison *et al.,*

2006). Ovreas and Torsvik (1998) compared the influence of crop rotation and organic farming on microbial diversity and community structure and found higher values for proxies of diversity in soils under organic farming management as compared to conventional practices.

In addition to shifts in community structure, there have been reports that indices of bacterial diversity suggested a reduced diversity in soils contaminated with phenyl-urea herbicides, fumigants etc. (Yang, Yao, Huand Qi, 2000; Ibekwe, Papiernik, Gan, Yates, Yang and Crowley, 2001) Although, these management practices certainly induce change in microbial community, the extent of soil function loss in relation to reduction in microbial diversity is not known. With regard to soil quality assessment, it is also important to note that in addition to examining microbiological effects of various management practices (e.g. herbicides, fungicides, tillage) these changes must also be weighed against chemical- and physical-indicators changes that may also occur in response to these practices (Sharma, Ramesh, Sharma, Joshi, Govaerts, Steenwerth and Karlen, 2011).

2.3.4 Significance of using microorganisms as soil quality indicators

Microorganisms are a component of the 'biological engine of the earth' and provide an integrated measure of soil quality, an aspect that cannot always be obtained with physical and chemical measures and/or analysis of higher organisms (Sharma *et al.*, 2011). Microorganisms are driving many fundamental nutrient cycling processes, soil structural dynamics, degradation of pollutants, various other services (Bloem, de Ruiter and Bouwman,1994) and respond quickly to natural perturbations and environmental stress due to their short generation time and their intimate relation with their surroundings, attributed

to their higher surface to volume ratio. This allows microbial analyses to discriminate soil quality status, and shifts in microbial population and activity could be used as an indicator of changes in soil quality (Kennedy and Smith, 1995; Pankhurst, Kirkby, Hawke and Harch, 2002).

Microbial indicators have been defined as "properties of the environment or impacts that can be interpreted beyond the information that the measured or observed indicator represents itself" (Nielsen and Winding, 2002). Stenberg (1999) listed five different levels at which microorganisms can be studied. These are: (1) as individuals; (2) at population levels (Hill, Mitkowski, Aldrich-Wolfe, Emele, Jurkonie, Ficke, … Nelson, 2000); (3) at the functional group level, including autotrophic nitrification (Stenberg *et al.,* 1998), arbuscular mycorrhiza (AM) (Kahiluoto, Ketoja, Vestberg and Saarela, 2001) and specific soil enzymes; (4) as the whole microbial community studied using genetic or physiological diversity or quantitative methods to enumerate the total community including microbial biomass, basal respiration rate, nitrogen mineralization, denitrification and general soil enzymes (Griffiths, Bonkowski, Roy and Ritz, 2001) and (5) at the ecosystem level which can describe data from all the other levels. It is not possible to use all ecosystems or soil attributes as indicators of soil quality (Karlen and Andrews, 2000) and thus, there is a need to select specific indicators having high discriminating potential and high value to account for actual soil quality status of agricultural systems; an indicator would not be so useful if it is very sensitive to disturbances. In particular, the search for indicator organisms associated with healthy or deteriorated soil requires a unified concept of soil quality.

In this context, microbial indicators can be divided into general, or universal, and specific indicators (Nielsen and Winding, 2002). Universal indicators may include biodiversity,

stability and self-recovery from stress (Parr, Papendick, Hornick and Meyer, 2003). *Rhizobium*, *mycorrhizae* and nitrifying bacteria could be used as specific indicators because of their high sensitivity to agrochemicals (Domsch *et al.,* 1983) or management regimes (Le Roux *et al.,* 2008), and clearly defined roles among soil functions. Specific indicators are dependent on the geographic zone, climate, soil type and land use history.

Although the relationship between soil quality and microbial diversity is not completely understood, a medium to high diversity in agricultural soil is generally considered to indicate a 'good' soil quality (Winding, 2004). This statement is based on the assumption that there is a functional redundancy in a healthy soil, so that soil ecosystem will recover from a stress factor that eliminates part of the microbial community (Yin *et al.,* 2000). In addition, the active microbial pool is serve pool of quiescent microorganisms, which can respond to foreign substances in the soil (Zvyaginstsev *et al.,* 1984). This diverse microbial pool maintains soil homeostasis. The larger the microbial diversity and functional redundancy, the quicker the ecosystem can return to stable initial conditions after exposure to stress or disturbance. This concept is highly debated. Indeed, several removal experiments (in which microbial taxa are successively removed from an innate community through a stressing agent or dilution of the original community) have shown that the functioning and stability of soil microbial communities can be maintained following strong erosion of microbial diversity (Griffiths, 2000; Wertz, Degrange, Prosser, Poly, Commeaux, Guillaumaud and Le Roux, 2007).

Furthermore, some observational studies show some links between soil microbial community structure and functioning (Patra *et al.,* 2006), the shifts in functioning often appear to be linked to key species rather than due to richness. Besides these controversies,

many authors argue that measurements of the structure and activities of specific microbial communities contributing to soil processes has the potential to provide rapid and sensitive means of characterizing changes to soil quality (Waldrop *et al.,* 2000; Bending *et al.,* 2004; Enwall *et al.,* 2005*;* Bressan *et al.,* 2008). In particular, the size and diversity of specific functional microbial groups such as AM fungi and nitrifying bacterial communities have the potential to characterize the effects of management on the sustainability of soil (Chang *et al.,* 2001). Also, a number of features viz. fast growth rate, high degree of physiological flexibility and rapid evolution (mutation) of microorganisms could make microbial communities more resilient to the new environment (Allison and Martiny 2008).

2.4 Influence of Agricultural Management Practices on Microbial Structure and Functions

The microbial community structure and function, is influenced by the interaction of several factors such as the soil physical and chemical properties, climate, crop type, and cultural practices like tillage, crop rotations, cover crops as well as fertilizer and pesticide application (Six *et al.,* 2006; Govaerts *et al.,* 2009). Soil physical and biochemical changes associated with Conservative agriculture (CA) practices have been attributed as factors that would alter the soil microbial ecology (Doran, 1980a; Doran, 1987b; Young and Ritz, 2000; Doran and Zeiss, 2000; Drijber, Doran, Parkhurst and Lyon, 2000). Reduced tillage practices have been associated with greater soil water content and bulk density that promotes greater abundance of anaerobic microbial species (Linn and Doran, 1984). The disturbance of the soil physical framework through tillage has been shown to disrupt fungal hyphae networks, and it's therefore expected that soils under reduced tillage would promote the proliferation of fungi (Young and Ritz, 2000). It is expected that crop residue left on the

soil surface would promote the dominance of saprophytic fungi that are able to breakdown more resistant carbon substrates (Beare, Parmelee and Hendrix, 1992). On the other hand, mixing of surface residue material with soil through tillage not only makes it more accessible to soil microbes, but has been postulated to typically favor the dominance of aerobic bacteria with a greater capacity to breakdown labile substrates (Linn and Doran, 1984; Beare *et al.,* 1992; Simmons and Coleman, 2008).

In a study of cucumber mono-cropping, soil microbial diversity index, richness and evenness were noted to decrease with more years of mono-cropping whereas in rotation cropping soil, the diversity of soil microbial community DNA sequence and evenness were higher than that of mono-cropping soils (Wu and Wang, 2007). This indicates that rotation cropping was propitious to the diversity and stability of soil microbial community.

Surface residue and cover crops not only serve as physical protection from soil erosion but also act as a source of additional organic C to soil and substrates to microbes. The additional C input from cover crops has mostly been shown to correlate with an increase in microbial biomass (Wardle, 1992). The quantity and quality of additional substrates plays an important role in impacting the microbial community structure and activity (Drijber *et al.,* 2000; Bailey *et al.,* 2002; Bending *et al.,* 2002). The plant residue stoichiometry in terms of its C: N: P ratios will have an influence on microbial biomass and activity (Bell *et al.,* 2014). The C: N ratios in plant residue will drive the dynamics of mineralization versus immobilization, while the lignin/cellulose content will drive the decomposition rate as well as the dominance of bacteria versus fungi. The cover crop used for crop rotation would therefore have an influence on microbial dynamics which has been observed in several studies (Acosta-Martínez, Burow, Zobeck and Allen, 2010a; Wortman *et al.,* 2013). For

example, mycorrhizal colonization levels in cotton production have been shown to be greater in crop rotations that included wheat or corn than continuous cotton (Wright *et al.,* 2008; Acosta-Martínez *et al.,* 2010a), supporting the theory that plant species diversity may correlate with microbial diversity.

The inclusion of cover crops with different substrate quality, either high C residue crops and/or leguminous N fixing cover crops on the other hand usually necessitates changing strategies in the application of N based fertilizers (Reiter, Reeves, Burmester and Torbert, 2008). This warrants increasing N-rates when using high C residue crops that would counteract possible immobilization. In contrast, for low C residue crops N-rate would be decreased to compensate for N mineralization. The manipulation of N-rate application introduces another influential factor on microbial community dynamics. Ntrogen (N) addition has been shown to have variable effects on microbial biomass and activity (Wardle, 1992; Treseder, 2008). N can be beneficial by promoting plant growth and thus increasing the quantity of residue that can be returned to soil (Alvarez, 2005).

Nitrogen fertilization was observed to increase the relative abundance of *Proteobacteria* and *Actinobacteria*, but reduced the abundance of *Acidobacteria*, consistent with the general life history strategy theory for bacteria. The positive correlation between N application rate and the relative abundance of *Actinobacteria* indicates that increased N availability favored the growth of *Actinobacteria* (Dai *et al.,* 2018). Similarly, the Kabete long-term trial in Kenya revealed that bacterial community structure and diversity was negatively affected by inorganic NP fertilizer, and that microbial communities in the soil with organic input clustered away from the soil with inorganic input (Kamaa *et al*., 2011). Chu *et al*. (2007) found that organic manure fertilizer promoted the population of *Bacillus*

spp. in the soil, compared to inorganic fertilizer. In contrast, Chen *et al*. (2010) reported that organic and inorganic fertilizer had a similar effect on the composition of soil denitrifying communities.

The added residue then acts as an additional source of C substrate to soil microbes that may promote their proliferation and diversity. On the other hand, N can change the osmotic potential and soil chemistry creating conditions that can be toxic to soil microorganisms. For example, high levels of N can lead to acidic conditions, which in turn limit availability of magnesium and calcium, and increase aluminium solubility, which can be toxic to microbes (Treseder, 2008).

Furthermore, Yao Wang, Kang, Wang, Zhang, Hou and Guo (2018) revealed that fertilizations had no significant influence on the richness and diversity of the bacteria and fungi. However, the abundance of individual bacterial or fungi phylum or species was sensitive to fertilizations. Fertilization, particularly the phosphorus fertilizer, influenced more on the abundance of the AMF species and colonization.

Several studies have shown that tillage, soil type, crop species, and residue management can alter the diversity, structure and distribution of soil microbial community, microbial activity, as well as soil quality parameters (Lupwayi *et al.,* 1998; Feng *et al.,* 2003; Roldan *et al.,* 2007; Reganold *et al.,* 2010; Reeve *et al.,* 2010). Reeve *et al.* (2010) investigated the effects of soil type and farm management on various microbial activities that included microbial respiration, enzyme assays and ecological functional genes. By correlating microbial activities to gene functions, their study showed that management had an influence on functional activity and diversity of the microbial community, with soils that were managed organically having a greater microbial diversity relative to conventionally

managed soils. Based on their results, management method was indicated to have a more significant effect on microbial activity compared to the soil type. Spedding, Hamel, Mehuys and Madramootoo (2004) compared different tillage techniques i.e. minimum tillage, conventional tillage and No-till with and without crop residue. Their results showed that residue had a greater impact on microbial dynamics compared to the different tillage systems, with the plots that included retained residue having greater microbial biomass C and N and was greater by 61 and 96 %, respectively.

Lupwayi *et al.* (1998) and Feng *et al.* (2003) both did studies looking at the microbial community structure and diversity under conventional tillage and no till systems based on substrate utilization patterns. Lupwayi *et al.* (1998) investigated the microbial community structure and diversity under wheat in no till and conventional tillage with or without crop rotation. Their results showed that the diversity and distribution of bacteria species was significantly reduced in plots under tillage. On the other hand, the diversity was significantly greater in fields under crop rotation of wheat with clover in comparison to fields under continuous wheat.

2.5 *Alfisols* and Northern Guinea Savanna of Nigeria (Samaru-Zaria)

Alfisols are soils with a clayey B horizon and exchangeable cations (Ca^{2+}, Mg^{2+}, K^{+} and Na^{+}) saturation greater than 50% calculated using acetic acid determination method (NH_4OAC-CEC) at pH 7 and are found majorly in the savanna and drier forest zones of humid and sub-humid tropics (FAO, 1992). The less leached slightly acidic (5.5 to $\leq$ 7.0); *Alfisols* derived from Pre-Cambrian crystalline basement complex rocks are the most dominant in the Guinea and Derived savanna zones of Nigeria (Harpstead, 1973).

Ogunwole *et al.* (2001) have reported that Samaru *Alfisols* were Aeolian drift of varying thickness. *Alfisols* are similar to *Ultisols* except for a considerably higher natural fertility status (Eswaran *et al.*, 1995) and higher base saturation. The main soil subgroup of Samaru is Typic Haplustalf under USDA soil taxonomy (Soil Survey Staff, 2003) or Orthic Acrisol in the FAO-UNESCO legend.

The rainfall distribution pattern for Samaru is monomodal, usually reaching its peak between July and August (Uyovbisere and Lombin, 1991). The mean annual rainfall is about 1060 mm (Owonubi *et al.*, 1991), falling almost entirely within six months, from May to October. Recently, Odunze (2011) have reported that the long mean annual rainfall of Samaru is 986.5 mm and is concentrated between May and October with a peak in August. The mean daily air temperature ranges between 15^{o}C and 38^{o}C representing the minimum and maximum temperature of Samaru respectively (Oluwasemire and Alabi, 2004). *Alfisol*s are widely distributed in the sub-humid and semi-arid tropical regions of Africa, including large areas of Western, Eastern, Central and South-Eastern Africa (FAO, 1992).

FAO (1992) reported that *Alfisols* supports a wide variety of cereal crops (maize, rice, sorghum, millet), root and tuber crops (yam, cassava, cocoyam, sweet potato), and grain legumes (soybean, cowpea, groundnut, pigeon peas, chicken peas). However, their productivity is limited by their low structural stability and susceptibility to surface crusting, soil compaction and erosion (FAO, 1992). They have low water retention capacity and are subject to drought (Kang and Juo, 1993). Also, deficiencies of N and P are common while deficiencies of K, Mg, S, Fe and Zn occur under intensive cultivation (Cottenie *et al.*,

1981). They have been reported to be inherently low in P due to their acidic nature and high level of Fe and Al oxyhydroxides which have high affinity for P (Ugbaje and Agbenin, 2009). However, FAO (1992) have reported that *Alfisols* have low P-fixation and high residual effects from applied P and in addition, mycorrhizal symbiosis is common and effective on these soils particularly with root crops, resulting in a low P requirement for crop production.

Scientific evidence have shown that *Alfisols* of the Nigerian savanna are inherently infertile, highly weathered, extensively leached, acidic, poorly buffered and generally low in organic matter and nutrient status (Agbenin, 2003). Because of their low buffering capacity, *Alfisols* acidify rapidly under continuous cultivation, particularly with the high rate of nitrogenous fertilizers (Kang and Juo, 1993). Chude *et al.,* (2012a) reported that the soils are generally shallow, coarse textured, yellowish grey or yellowish brown in the surface layer and have red or reddish brown subsurface and lower horizon with higher clay content. For purpose of management, *Alfisols* are grouped as low activity clays (LAC) soils having a low effective cation exchange capacity (ECEC) of 16meq/100 g clay in the subsoil (Juo and Adams, 1986). Odunze (2003) has reported that the soils are low in inherent fertility, organic matter, cation exchange capacity (CEC) and dominated by low activity clays. Observations have revealed that majority of the LAC soils in West Africa have an especially low ECEC of < 8 meq/100 g soil.

The organic matter content of the surface soils in the entire zone is very low, the value being less than 2% and the total N content never exceeding 0.1% (Chude *et al.*, 2012a). The low organic matter has been attributed to seasonal bush burning and rapid humification of

sparse litter and the low polymerization of humid compounds into stable complexes with soil clays (Ojanuga, 1977). As the clay fraction of these soils are composed mainly of kaolinites, halloysite, and oxides of Fe and Al, the soil ECEC depends solely on the soil organic matter level, which controls nutrient absorption and release (FAO, 1992). In West African *Alfisols*, soil organic matter (SOM) accounts for 80% of CEC and available phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) and CEC have been said to be highly correlated with soil organic matter (SOM) levels (Agboola, 1994).

CHAPTER THREE

3.0. MATERIALS AND METHODS

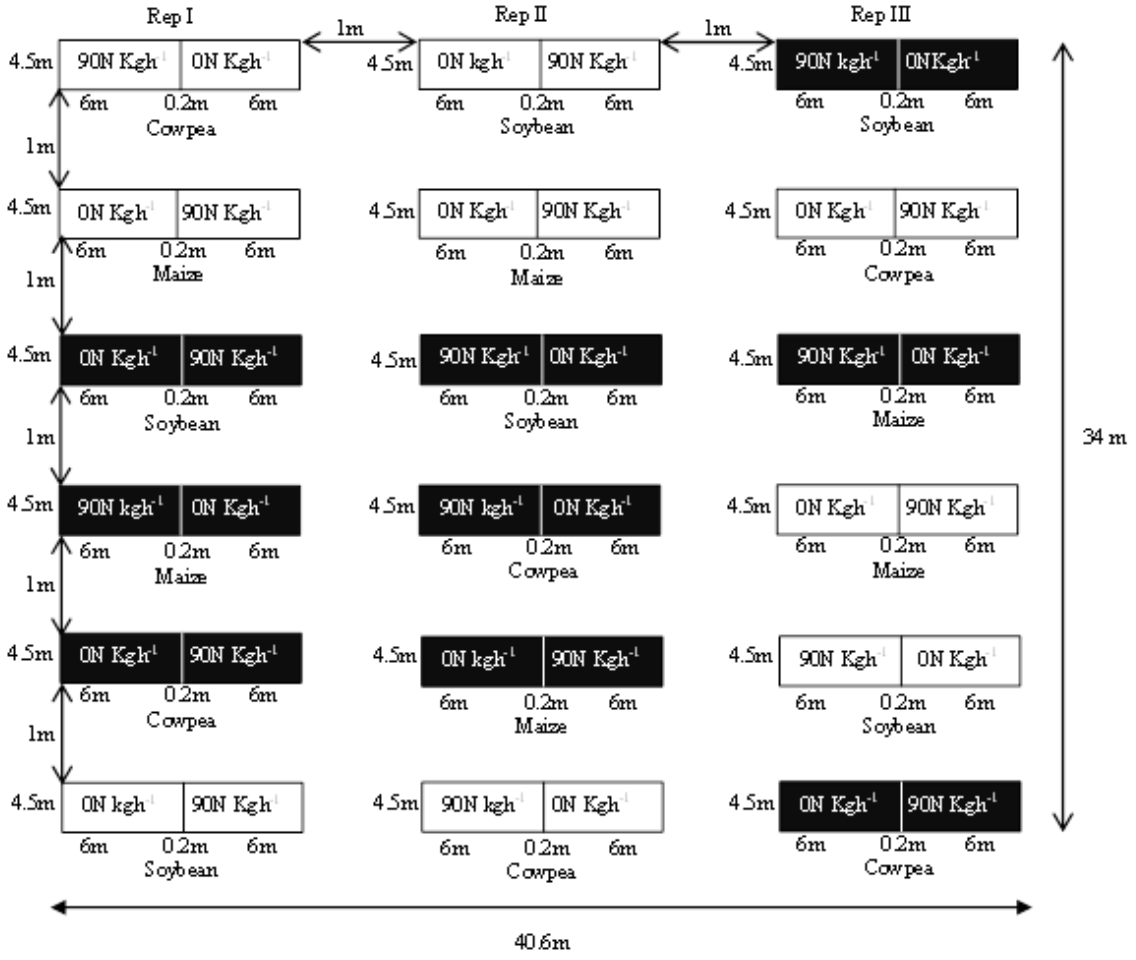
3.1. Experimental Site

The soil samples used for the study were collected from one of the research fields (ROD) of the Institute for Agricultural Research (IAR), Ahmadu Bello University, Zaria; located in northern Guinea savanna zone within latitude 11^0 10'N and longitude 07^0 36'E, an altitude of 686 m above sea level with an annual rainfall of 1033 mm distributed between May and October. The rainfall pattern is monomodal. Samaru has a mean temperature of 19.88^{o}C (minimum), 35.07^{o}C (maximum) (IAR Meteorological Station, 2017). The vegetation is characterized by fire – tended and fire – tolerant trees with an understory of shrubs and grasses (Omeke *et al.*, 2014).The soil is low in inherent fertility; organic matter, cation exchange capacity and dominated by low activity clays (Odunze, 2003). Farming is the major socio - economic activity of the study area (Omeke *et al.*, 2014). The main soil sub – group is Typic Haplustalf (*Alfisols*) USDA Soil Taxonomy (Ogunwole *et al.*, 2001); or Orthic Acrisol in the FAO-UNESCO Legend (Valette and Ibanga, 1984) derived from Pre – Cambrian Crystalline basement complex rocks with some Quaternary Aeolian deposits.

3.2 Description of the Study

The study was based on a 15-year old field trial, which began in 2003 with two tillage practices (reduced and conventional tillage), three rotation crops (cowpea, maize and soybean) and two nitrogen fertilizer rates (0 and 90 kg N ha^{-1}); laid down in a randomized complete block design in split-split plot arrangement with three replications. Tillage systems were in the main plot, crop rotations were in sub-plot and N fertilizer rates were in the sub-sub plot. The conventional tillage (CT) involved the use local hoe to make ridges,

when it was time to weed, hoes were used to remove the weed and then earthen-up four weeks after the first weeding. While in reduced tillage (RT), ridges were not made cutlass or hoes were used to tilt the soil when sowing the seeds and herbicides were used to control weeds. Cowpea (IT89 KD – 288), soybean (TGx 1448 – 2E) and maize (SAMMAZ 14) were planted in the first phase (first year) of the rotation, in which the legumes received 40 kg $P_2O_5ha^{-1}$ and20 kg K_2Oha^{-1} only; whereas the maize received N fertilizer rates stated, in addition to uniform application of 60kg $P_2O_5ha^{-1}$and 60kg K_2Oha^{-1} respectively. In the second phase, maize was grown in all the plots (second year). Each plot was divided into two during the maize phase to accommodate the N fertilizer rates (see experimental layout). The rotation system was therefore cowpea – maize, soybean – maize and continuous maize (maize – maize).



Key

Conventional tillage

Reduced tillage

Area per plot = 4.5m by 6.0 = 27m^2

Area of experimental field = 34m by 40.6m = 1 380.4m^2

Figure 1: Experimental Layout

3.3 Soil Sampling

Soil samples were taken in October during 2018 rainy season. Five soil samples were randomly collected in each plot at 0 – 15 cm depth using an auger. The samples were bulked to form a composite sample and divided into two sets. One set of the samples was transported immediately to the laboratory and stored in the refrigerator at $4^{o}C$ for microbial analysis. The second set of samples was air-dried under shed. Plant residues and pebbles were removed from the samples and gently grounded in a porcelain mortar with pestle which were sieved through 2 mm and 0.5 mm mesh sieves and subjected to routine soil analysis according to the standard procedure described in IITA (1989).

3.4 Laboratory Analysis

The sieved air-dried samples were subjected to physical and chemical analysis which were analysed at Soil Science Laboratory, Department of Soil Science, Ahmadu Bello University, Zaria. Particle size distribution was determined using hydrometer method, described by Gee and Bauder (1986); while USDA textural triangle was used to obtain textural classes for the samples. Soil pH was determined by the use of a pH meter connected to a glass electrode in ratio 1:2.5 soil to water and soil to 0.01 M $CaCl_2$ (Hendershot *et al.*, 1993). The modified Walkley and Black procedure as described by Nelson and Sommers (1982) was to determine organic carbon. Total nitrogen was determined by micro – Kjeldahl method involving digestion and distillation method as described by Bremner and Mulvaney (1982). Available phosphorus was determined by Bray No. 1 method (Bray and Kurtz, 1945). Basic cations (K^{+}, Ca^{2+}, Mg^{2+} and Na^{+}) were extracted in 1.0 M ammonium acetate (NH_4OAc) at pH 7 (IITA, 1989).

3.5 Microbial Analysis

3.5.1 Soil serial dilution

Population of bacteria and fungi were measured using the methods described by Xu and Zheng (1986) and Lin (2010).Numbers of colony forming units (cfu) (g^{-1}) of fresh soil were measured in triplicate using serial dilutions. Conventional dilution spread-plating was performed to assess the culturable bacterial and fungal colony forming units (cfu). Ten grams (10 g) of soil sample was weighed and added to 90 mL of deionized water. The suspension was shaken and labelled as "A". Before the soil settles, 1 ml of the suspension was measured with a sterile pipette and transferred to a test tube containing 9-ml deionized water blank which was vortexed thoroughly and labelled as "B". Similar dilution steps were repeated for the remaining test tubes labeled sequentially C, D, E, F, G and H. This resulted in serial dilutions of 10^{-1} through 10^{-8} grams of soil per ml.

3.5.2 Spread plate technique for culturing bacteria

Nutrient agar powder was prepared by measuring 28 g of the powdered agar and suspended in 1000 ml of distilled water, and heated in a microwave for few minutes to homogenize the suspension. The homogenized solution was autoclaved for 15 minutes at 121^{o}C and allowed to cool to about 40 – 45 ^{o}C; precisely 25ml of the agar solution was poured on each plate. To grow bacterial colonies, five pre-prepared nutrient agar plates were taken and labeled as D, E, F, G and H. Samples in the labeled test tubes D, E, F, G and H were vortexed, 0.1 ml of the suspension was pipetted onto each plate. This increases the dilution value further, by a factor of ten (D = 10^{-4}, E = 10^{-5}, F = 10^{-6}, G = 10^{-7}, H = 10^{-8}). After the

plates were plated, the bacteria plates were inverted and incubated at room temperature for one (1) day.

3.5.3 Spread plate technique for culturing fungi

Viable fungus propagules or colony forming units (cfu) were determined using sabouraud dextrose agar. Sabouraud dextrose agar powder was prepared by measuring 65 g of the powdered agar and suspended in 1000 ml of distilled water, and heated in a microwave for few minutes to homogenize the suspension. The homogenized solution was autoclaved for 15 minutes at 121^{o}C and allowed to cool to about 40 – 45 ^{o}C; precisely 25ml of the agar solution was poured on each plate. To grow fungi, five pre-prepared sabouraud dextrose agar plates were taken and labelled as C, D, E, F, G. Using the techniques described previously. The lower dilutions are used because fungi loads are less than that of the bacterial population (C =10^{-3}, D=10^{-4}, E =10^{-5}, F =10^{-6}, G =10^{-7}).The plated fungi plates were inverted and incubated at room temperature for three (3) days.

Final counts were expressed as the number of CFU per 1 g of fresh soil.

$$\text{Number of CFU per g moist soil} = \frac{1}{(\text{dilution factor})} \times \text{number of colonies}$$

3.6 DNA Extraction

The DNA was extracted from the soil samples using ZymoBiomics Kit (InqabaBiotec. South Africa). For each sample, DNA was isolated from 0.25 g of soil using the ZymoBIOMICS™ DNA Miniprep Kit (D4300) according to the manufacturer's protocol (ZymoBiomics, 2018) which was performed at the Central Research Laboratory, Faculty of Veterinary Medicine, Usmanu Danfodiyo University, Sokoto.

3.6.1 Amplification and polymerase chain reaction (PCR) of 16S rRNA

The 16Small-subunit ribosomal (16S rRNA) region was amplified using universal primers 8F5'-AGAGTTTGATCCTGGCTCAG-3' (Zhou *et al.*, 1997) and 926R 5'-CCGTCAATTCATTTGAGTTT-3' (Muyzer*et al.*, 1995). PCR was performed in 25µl volume containing 12.5µl master mix, 1 µl each of 515 Forward and 1391 Reverse primers, 3 µl of DNA template and 7.5µl molecular grade water. The reaction mix was transferred to an Applied Biosystem 9700 (GeneAmp®PCR System 9700) Thermocycler and subjected to initial denaturation at 94^{o}C for 3 minutes, followed by 35 cycles of denaturation at 94^{o}C for 30 seconds, annealing at 50^{o}C for 11 seconds and extension at 72^{o}C for 60seconds followed by a final extension at 72^{o}C for 7 minutes (Ranjard *et al.*, 2000).

3.6.2 Agarose gel electrophoresis

To prepare agarose gel, 1g of 1% agarose gel powder was weighed and poured into an already weighed 100ml 1x TBE (Tris Borate EDTA) buffer, microwave for 1 – 2 minutes for it to homogenize; 10µl of ethidium bromide was added. The cast was set with the comb inserted while the melted agarose was poured into the cast and allowed to solidify. Then 5µl of the PCR products were mixed with 1µl loading dye (ethidium bromate) and violin dye. The whole contents were loaded into the wells. The gel was placed into the tank with the TBE buffer filled to the precise mark and run at 90volts for 1 minute. The product was subsequently subjected to agarose gel electrophoresis with an expected amplicon size of 965bp (base pairs). The gel was dried and viewed via BioRad Gel DOC Imager (BioRad) (Sambrook and Russel, 2001).

3.6.3 Sequencing and basic local alignment search tool (BLAST)

The amplicons (PCR products) were packaged and sent to Inqaba Bioscience, South Africa for Sanga Sequencing (single phase sequencing using 926R 5'–CCGTCAATTCATTTGAGTTT–3'primer). The sequenced results were for phylogenetic analysis using MEGA version X Software. A FastA format of the sequence obtained was copied to the BLAST to compare the sequence with already deposited sequence in the GenBank (Altschul *et al.,* 1990). Reference sequence was downloaded from the GenBank (Maidak *et al.,* 1994) database (National Centre for Biotechnology Information [NCBI]) considering the sequence identified and query over. Cluster W tool was used for sequence augmentation.

Furthermore, due to inadequate funds, we were unable to amplified or run PCR and sequenced the internal transcribed spacer genes (ITS) region for fungi characterization.

3.6.4 Phylogenetic tree

Phylogenetic and molecular evolutionary analyses were conducted using MEGA X (Kumar *et al.,* 2018). A Neighbour joining method with 1000 bootstrapping was used to construct the phylogenetic tree of sequences obtained with reference sequence NCBI.

3.6.5 Soil microbial diversity

Soil microbial diversity was estimated by the Shannon-Weiner diversity formula;

$H' = -\sum_{i=1}^{n} pi * LnPi$ (Shannon and Weiner, 1963)

H' is the diversity index, *Ln*=natural logarithm, $Pi = {ni}/{Ni}$ where pi is the ratio in decimal of individual species; *ni* is the number of individuals in each group, and *Ni* is the total number of individuals.

3.7 Statistical Analysis

Data obtained from the trial were subjected to analysis of variance (ANOVA) using the Proc ANOVA programme of SAS package (SAS 9.1, 2010). Where significant F – values were obtained, treatments means were separated using Duncan Multiple Range Test (DMRT) at $P \leq 0.05$ (Gomez and Gomez, 1984). Correlation analysis was established between selected soil properties and soil microbial groups in the treatments.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Soil Properties

The characteristics of soils at the experimental site are presented in Table 1. The result shows that the soils texture was generally sandy loam soil. The tillage practices, crop rotation and N fertilizer rates are predominantly sandy loam in texture. The higher fraction of sand found in soil explained the relatively low clay content of soil due to the parent material. Malgwi *et al.* (2000) and Voncir *et al.* (2008) reported that the dominance of sand contents in Northern Nigerian soils is as a result of sorting of materials by clay eluviations and surface wind erosion. The fact that clay content of the soil was low could be due to sorting of soil material by biological activities, clay migration down the profile or surface erosion by run-off or a combination of these factors (Odunze, 2006). It implies that such soils are well drained, relatively stable and are suitable for arable crop production.

The soil pH in H_2O ranges from 5.58 – 6.00 which was slightly acidic and moderately acidic in pH 0.01 M $CaCl_2$ (4.80 to 5.15) though not significant (Table 1). Total nitrogen, organic carbon and exchangeable sodium were not significant. The crop rotation treatment had significantly ($P \leq 0.05$) higher available phosphorus, exchangeable bases (K, Ca, Mg) and CEC which is at variance with the findings of Odunze (2006) who reported available P., exchangeable cations and CEC. These results may be partly attributed to lower leaching losses of the bases due to the litter on the soil surface, influence of residual effect of fertilizer applied and improvement on long-term impact of cropping system on the stated soil properties; specifically continuous maize (M/M) depicted the highest values of exchangeable bases and CEC compared to other crop rotation, higher available P was

Table 1: Effects of Long-term Tillage, Crop Rotation and N-fertilizer Rates on Soil Properties at the Experimental Site

Treatments	Sand	Silt	Clay	Textural Class	pH in H_20	pH in $CaCl_2$	OC	TN	Avail. P	Exchangeable Bases				CEC
	g kg^{-1}						g kg^{-1}		mg kg^{-1}	Cmol g kg^{-1}				Cmol g kg^{-1}
										K	Na	Ca	Mg	
Tillage (T)														
CT	563.22	315.56	121.11	SL	5.82	5.02	8.00	0.46	21.48a	0.21	0.20	5.58	1.50	8.32
RT	531.67	333.89	134.44	SL	5.76	4.95	7.99	0.42	17.83b	0.21	0.18	5.09	1.40	7.65
SE±	1.724	1.940	0.938		0.032	0.042	0.044	0.029	1.156	0.013	0.015	0.209	0.085	0.251
CR														
C/M	546.59	310.08	143.33	SL	5.83	5.03	8.03	0.04	18.70ab	0.21b	0.18	4.80b	1.28b	7.25b
M/M	551.67	323.33	125.00	SL	5.80	5.04	8.05	0.04	17.56b	0.26a	0.22	6.03a	1.71a	9.00a
S/M	545.00	340.00	115.00	SL	5.74	4.88	7.92	0.05	22.70a	0.18b	0.18	5.17b	1.36b	7.71b
SE±	2.111	2.375	1.148		0.040	0.052	0.054	0.004	1.428	0.015	0.019	0.255	0.104	0.308
N-Rate (kg ha^{-1})														
N0	531.11	347.78	121.11	SL	5.79	5.00	8.00	0.42	20.14	0.23	0.21	5.13	1.88	7.79
N1	563.89	301.67	134.44	SL	5.79	4.97	8.00	0.46	19.16	0.20	0.17	5.53	1.52	8.18

SE±	1.724	1.940	0.938	0.032	0.042	0.442	0.003	1.156	0.013	0.015	0.209	0.085	0251
Interactions				NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T * CR	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T * NR	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
CR * NR	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
T * CR * NR	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	**	**	*

CT = Conventional tillage, RT= Reduced tillage, NR= Nitrogen fertilizer rate, SL = Sandy loam, C/M = Cowpea-Maize Rotation, M/M = Continuous Maize, S/M = Soybean-Maize Rotation, N0 = 0 kg N ha^{-1}, N1 = 90 kg N ha^{-1}, TN = Total Nitrogen, OC = Organic carbon, Aval. P = Available Phosphorus, K = Potassium, Na = Sodium, Ca = Calcium, Mg = Magnesium, CEC = Cation Exchange Capacity, * = Significant at $P<0.05$, ** = Significant at $P<0.01$, NS = Not significant at either $P<0.05$ or $P<0.01$.

observed in soybean/maize rotation, followed cowpea/maize rotation while the least value was shown in continuous maize (Table 1). The moderately acidic nature of soil reflected the true characteristic of a savanna *Alfisol,* which was due to uptake of basic cations by plant through cultivation and, partly, to the overall removal of crop residues by farmers after harvest as well as leaching. Available phosphorus was significant ($p \leq 0.05$) which depicted soybean-maize rotation with the highest values followed by cowpea-maize rotation while continuous maize has the least value (Table 1). This disagrees with the findings of Chude *et al.* (2012) who reported medium value. The very high value of available phosphorus might be due to improve management practices, residual effect of P fertilizer applied to maize and legumes which is often immobile (Basamba *et al.,* 2006). The low organic carbon, total nitrogen and exchangeable Na was in agreement with the report of Odunze, (2003) who reported low OC, TN and exchangeable Na in the same agro-ecological zone; which could be attributed to intensive cultivation, total removal of crop residues after harvest by farmers used for fencing, fuel, feeding livestock, surface run-off, leaching and the short period of fallow for natural regeneration of organic matter.

Interaction of long-term tillage practices, crop rotation and N-fertilizer rates (T*CR*NR) were depicted in Table 2. Conventional tillage, crop rotation and N-fertilizer rates indicated sandy loam compared to reduced tillage, crop rotation and N- fertilizer rates that almost revealed loamy soil texture. The loamy texture observed in reduced could be as due to the presence of crop residues on the soil surface which aid in reducing soil erosion, improve organic matter and soil aggregation. Influence of T*CR*NR on available P in RTSMN0 treatment was ranked the highest (28.75 mg kg^{-1}) followed by RTSMN1, RTCMN0 and RTMMN (23.85 mg kg^{-1}, 23.85 mg kg^{-1} and 23.36 mg kg^{-1}) while the least rank was

Table 2: Interactions of Long-term Tillage, Crop Rotation and N-fertilizer Rates on Soil and Microbial Properties

Soil Properties and units	Conventional Tillage (CT)						Reduced Tillage (RT)						SE±
	C/M		M/M		S/M		C/M		M/M		S/M		
	N0	N1	N0	N1	N0	N1	N0	N1	N0	N1	N0	N1	
Sand g kg^{-1}	523.3	536.7	543.3	596.7	550.0	630.0	603.3	520.0	483.3	583.3	483.3	516.7	NS
Silt g kg^{-1}	366.7	320.0	306.7	286.7	366.7	246.7	260.0	296.7	400.0	300.0	386.7	360.0	NS
Clay g kg^{-1}	110.0	143.3	150.0	116.6	83.3	123.3	136.7	183.3	116.7	116.7	130.0	123.3	NS
Textural Class	SL	SL	SL	SL	SL	SL	SL	L	L	SL	L	L	
pH in H_2O	6.00	5.85	5.86	5.84	5.60	5.80	5.79	5.72	5.77	5.75	5.80	5.74	NS
pH in $CaCl_2$	5.14	5.04	5.15	5.04	4.80	4.90	4.91	5.03	4.98	4.96	5.00	4.81	NS
TN g kg^{-1}	0.39	0.47	0.36	0.36	0.47	0.46	0.40	0.50	0.46	0.50	0.46	0.50	NS
OC g kg^{-1}	7.70	8.50	8.00	8.30	7.20	8.30	7.78	8.20	8.40	7.40	8.80	7.30	NS
Avail. P mg kg^{-1}	21.72bc	16.01cd	14.54d	16.50cd	19.27bc	18.95bcd	13.23d	23.85b	23.36b	15.84d	28.75a	23.85b	2.855
Exch. Bases													
K Cmol g kg^{-1}	0.22	0.22	0.28	0.22	0.18	0.15	0.22	0.16	0.29	0.23	0.18	0.19	NS
Na Cmol g kg^{-1}	0.21	0.17	0.27	0.21	0.20	0.17	0.18	0.16	0.22	0.18	0.17	0.17	NS
Ca Cmol g kg^{-1}	3.80cde	5.00c	7.40a	6.00bc	5.07c	6.33b	6.00bc	4.53cd	4.33cd	6.47b	4.27cd	5.00c	0.511
Mg Cmol g kg^{-1}	1.02de	1.31bcd	2.18a	1.57bc	1.24d	1.67b	1.47bc	1.32bcd	1.19d	1.88b	1.16d	1.37bcd	0.207

CEC Cmol g kg^{-1}	6.07cde	7.30c	10.87a	8.83b	7.67c	9.20b	8.60bc	7.03cd	6.93cd	9.40b	6.60cd	7.37c	0.615
Bacteria cfu g^{-1} moist soil	0.86 x 10^{10}	4.74 x 10^{10}	2.51 x 10^{10}	12.80 x 10^{10}	0.87 x 10^{10}	4.80 x 10^{10}	6.25 x 10^{10}	3.82 x 10^{10}	1.34 x 10^{10}	4.74 x 10^{10}	1.40 x 10^{10}	0.60 x 10^{10}	NS
Fungi cfu g^{-1} moist soil	1.67 x 10^{9}	6.20 x 10^{9}	0.94 x 10^{9}	1.60 x 10^{9}	2.10 x 10^{9}	1.81 x 10^{9}	1.30 x 10^{9}	1.95 x 10^{9}	2.95 x 10^{9}	3.53 x 10^{9}	4.06 x 10^{9}	1.18 x 10^{9}	NS
Bacterial/fungal ratio	8.03	7.87	29.00	120.45	11.37	24.88	68.82	17.94	11.83	13.75	4.51	4.91	NS

C/M = Cowpea-Maize Rotation, M/M = Continuous Maize, S/M = Soybean-Maize Rotation, N0 = 0 kg N ha^{-1}, N1 = 90 kg N ha^{-1}, SL = Sandy loam, L = Loam, TN = Total Nitrogen, OC = Organic carbon, Aval. P = Available Phosphorus, Exch. = Exchangeable, K = Potassium, Na = Sodium, Ca = Calcium, Mg = Magnesium, CEC = Cation Exchange Capacity, Means with similar letters across the same at *P<0.05* using DMRT, NS = Not significant at either P<0.05 or P<0.001.

observed in RTSMN0 (13.23 mg kg^{-1}) (Table 2). Exchangeable bases (Ca and Mg) dominated the exchange sites of the soils. Interaction of tillage, crop rotation and N-fertilizer rates on exchangeable Ca which ranges from low to moderate were revealed CTMMN0 (7.40 Cmol kg^{-1}) was ranked the highest followed by CTSMN1 (6.33 Cmol kg^{-1}) and RTMMN1 (6.47 Cmol kg^{-1}), next were CTMMN1 and RTCMN0 (6.00 Cmol kg^{-1}) while the lowest ranked exchangeable Ca depicted 3.80 Cmol kg^{-1} (Table 2).

Exchangeable Mg was observed to followed similar trend like exchangeable Ca ranging from medium to high with CTMMN0 (2.18 Cmol kg^{-1}) ranked the highest followed by CTSMN1 and RTMMN1 (1.67 Cmol kg^{-1} and 1.88 Cmol kg^{-1}) while the lowest ranked value was depicted in CTCMN0 (1.02 Cmol kg^{-1}) (Table 2). Cation exchange capacity was observed to follow similar pattern like the exchangeable cations which ranges from moderate to high with CTMMN0 (10.87 Cmol kg^{-1}) being the highest followed by CTMMN1, CTSMN1 and RTMMN1 (8.83 Cmol kg^{-1}, 9.20 Cmol kg^{-1} and 9.40 Cmol kg^{-1}) respectively. The influence of T*CR*NR on bacterial population, fungal population and bacteria/fungi ratio was insignificant.

4.2 Effect of Tillage, Crop Rotation and N-Fertilizer Rates on Specie Richness

Effect of tillage system, crop rotation and N fertilization on dominant microbial groups is presented in Table 3. The results showed that bacterial population in soils was significantly ($P < 0.01$) influenced by crop rotation and N fertilizer. Although significant effects were found on the bacterial populations due to these treatments, there was no significant difference in the main treatment effects including tillage practice on fungal population (Table 3). This was clearly demonstrated by the total number of bacteria load recorded on tillage which showed that CT (4.428×10^{10} cfu g^{-1} moist soil) have more population than RT (3.025×10^{10} cfu g^{-1} moist soil); though, not significant. The higher number of bacteria compared to fungi in the soil suggests a normal arable soil condition for the studied site. This could be due to availability of more arable soil resources that support growth of bacteria in cultivated soil than in forest soils that conform with the findings of Omeke *et al.* (2016). Isirimah *et al.* (2010) reported that fungi are more in population in forest soils than arable soils. Thus, our finding is at variance with the reports of Lupwayi *et al.,* 2012 who stated that no tillage has more bacteria load than conventional tillage. This could be due to insufficient soil mixed the trial field.

Crop rotation and N fertilizer rates have remarkable effects on bacteria load which was significant ($p<0.01$). The mean separation revealed that continuous maize has the highest specie richness (5.348×10^{10} cfu g^{-1} moist soil), followed by cowpea/maize rotation (3.917×10^{10} cfu g^{-1} moist soil) and lastly soybean/maize rotation (1.917×10^{10} cfu g^{-1} moist soil) (Table 3). The result is in disagreement with Wu and Wang (2007) who showed that the diversity index, richness and evenness index of the microbial community of crop rotation

Table 3: Effect of Tillage, Crop Rotation and N-Fertilizer on Microbial Richness at Samaru during 2018 Rainy Season.

Treatments	Bacteria (cfu g^{-1} moist soil)	Fungi (cfu g^{-1} moist soil)
Tillage (T)		
Conventional Tillage	4.428×10^{10}	2.372×10^{9}
Reduced Tillage	3.026×10^{10}	2.494×10^{9}
SE±	0.5483×10^{10}	0.2849×10^{9}
Crop Rotation (CR)		
Cowpea/Maize	3.917×10^{10}ab	2.783×10^{9}
Maize/Maize	5.348×10^{10}a	2.253×10^{9}
Soybean/Maize	1.917×10^{10}b	2.263×10^{9}
SE±	0.6716×10^{10}	0.3489×10^{9}
N-Fertilizer Rate (kgha^{-1})		
0	2.205×10^{10}b	2.154×10^{9}
90	5.249×10^{10}a	2.711×10^{9}
SE±	0.5483×10^{10}	0.2849×10^{9}
Interactions		
T * CR	**	**
T * NR	**	*
CR * NR	*	**
T * CR * NR	NS	NS

CT = Conventional tillage, RT= Reduced tillage, NR= Nitrogen fertilizer rate, means followed by the same letters in the column are not significant ($P<0.05$), cfu= colony forming unit, * = Significant at $P<0.05$, ** = Significant at $P<0.01$, NS = Not significant either at $P<0.05$ or $P<0.01$.

were higher than those of mono-cropping however, our results is in conformity with the report of Vieira and Nahas (2005) who stated that higher numbers of total bacterial and fungal colony forming units (cfu) were observed in cereals cropped soils. This could be due the availability of substrates that were properly mixed that support the growth of heterotrophic bacteria.

Nitrogen fertilizer rates on bacteria population was significant at $p<0.01$. Bacteria population with N fertilizer rates (90 kg N ha^{-1}) was ranked higher (5.249 x 10^{10} cfu g^{1} moist soil) than 0 kg N ha^{-1} (2.205 x 10^{10} cfu g^{-1} moist soil) (Table 3). The result is in consonance with Clegg *et al*., (2003) who stated that nitrogen fertilizer has a significant impact on the total bacterial community structures but in contrast with Farmer *et al.* (2017) who reported that long-term fertilizer addition greatly reduced the population of bacteria as well as its richness in a brown soil. The variation observed could be due to the fact that the N fertilizer applied within the rhizosphere stimulated growth of gram-negative bacteria more than fungi in the soil. Bacterial growth was favoured by the crop-root rhizo-deposition, rich in amino acids (Vinzke *et al.*, 2004) and soluble sugars (Jensen, 1996). Influenced by these factors, bacteria multiply faster in population in soil under N fertilizer application; though N rates used in this study are modest and may not cause significant shift in soil microbial equilibrium since P and K were adequately supplied in all the treatments.

The effects of tillage on fungal loads were not significant (Table 3). The findings disagree with the report of several researchers who stated that reduced tillage favours the growth of fungi (Feng *et al.*, 2003; Helgason *et al.*, 2009; Mathew *et al.*, 2012). Although, not statistically significant, the most abundant fungi loads was observed in cowpea/maize

rotation (2.783 x 10^9 cfu g^{-1} moist soil) followed by soybean/maize rotation (2.263 x 10^9 cfu g^{-1} moist soil) and the least was noticed in continuous maize (2.253 x 10^9 cfu g^{-1} moist soil). The high fungi $cfug^{-1}$ moist soil is related to the findings of Meliani *et al.* (2012) who reported high fungi load in a field with cover crops. Also, the effects of N fertilizer rates on fungi population were not significant which implies that they are unaffected by the application of N-fertilizer.

However, the interaction between tillage and crop rotation (T*CR) on both bacteria cfu and fungi cfu were highly significant (p<0.01), tillage and N fertilizer rates (T*NR) was also highly significant for bacteria loads (p<0.01) and significant for fungi (p<0.05), and crop and N fertilizer rates (CR*NR) were observed to be significant for both bacteria (p<0.05) and fungi (p<0.01) richness (Table 2).

The interactions among the factors on the microbial groups and their means separation (ranking) were presented in the following Figures 2 – 7.

Interaction of tillage and crop rotation (Figure 2) revealed that bacterial population significantly increased under continuous maize and soybean-maize rotation with conventional tillage (p<0.01) but was higher under cowpea-maize rotation with reduced tillage; the opposite is the case for fungal population except cowpea-maize rotation (p<0.01) with conventional tillage that had higher fungal population (Figure 3). This is due to the fact that conventional tillage releases the nutrients more quickly because the residues are crushed and mechanically incorporated to soil, which stimulates microorganisms that degrade organic matter while in no-tillage conditions, the nutrient releasing potential is progressive, since the residues remain on the soil surface and therefore decomposition is

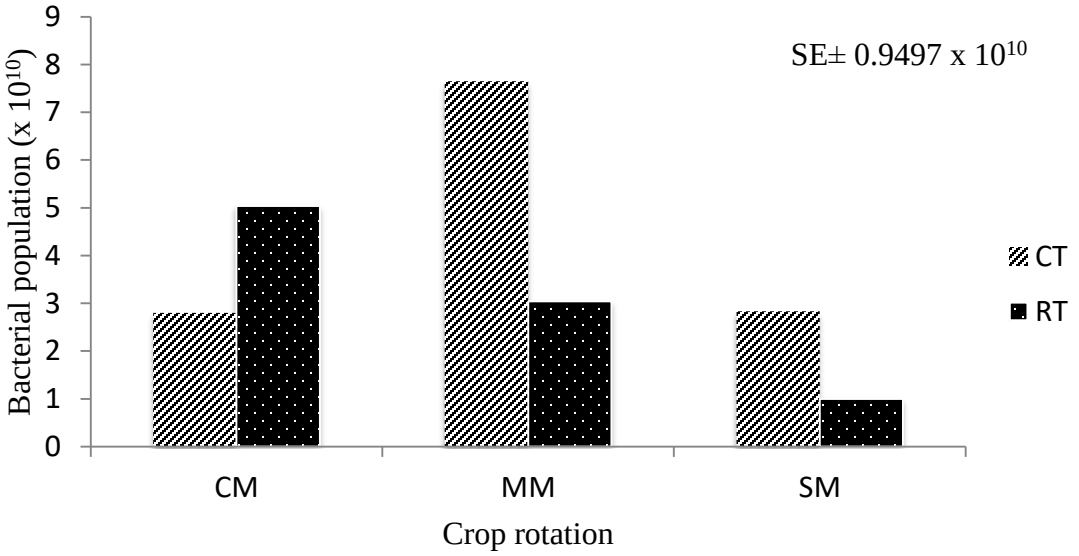


Figure 2: Interaction of Tillage (T) and Crop Rotation (CR) on Bacterial Population

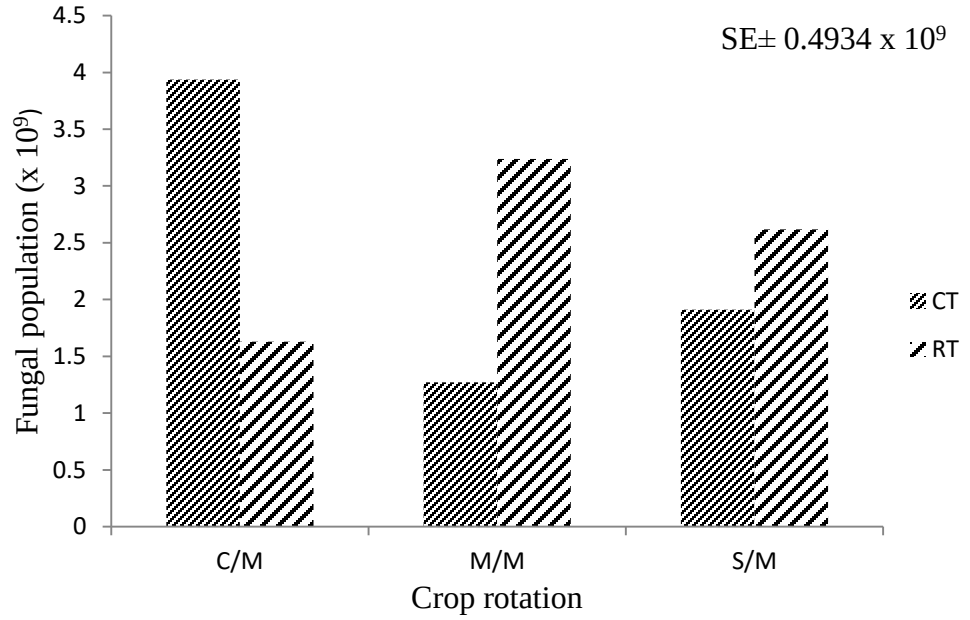


Figure 3: Interaction of Tillage (T) and Crop Rotation (CR) on Fungal Population

slower. Our result is at variance with the reports of Smith (2016) who noted that crop type and tillage have negligible effects on the number and diversity of species instead affect species composition. It could be due to the duration of the tillage and crop rotation practices in the trial field (15 years) while that of Smith's field is two years crop rotation; also the environment where the studies was conducted differ likewise the time of soil samples collection.

The interaction between tillage practices and N fertilizer rates revealed bacterial population (Figure 4) in conventional tillage and 90 kg N ha^{-1} to be significantly higher followed by reduced tillage with 0 kg N ha^{-1} as well as 90 kg N ha^{-1} (p<0.01). The highest bacterial load could be as a result of mixing of surface residue material with soil through tillage in collaboration with residual N rates which not only make it accessible to soil microbes, but also favor the dominance of aerobic bacteria with a greater capacity to breakdown labile substrates. Fungal population ranked the highest in conventional tillage with 90 kg N ha^{-1} and higher in reduced tillage with 0 kg N ha^{-1} (p<0.05) (Figure 5). Although there has been mixed report as to whether reduced tillage practices have a greater impact on bacterial or fungal populations (Helgason *et al.*, 2009). This report is at variance with the findings of Yao *et al*. (2018) who revealed that fertilizations had no significant influence on the richness and diversity of the bacteria and fungi. However, the abundance of individual bacteria or fungi species was sensitive to fertilizations. The differences observed might be due to the time the samples were collected and the method used in enumerating microbial load. We used spread plate method whereas most of studies used quantitative PCR (qPCR) values to determine microbial population (Justin *et al*., 2012).

Also, the significant fungi population observed in reduced tillage with 0 kg N $ha^{-1}$is consistent with the report of Balota *et al*. (2003) who noted that reduced tillage practices increase microbial population, activity as well as microbial biomass especially of fungi, bacteria, arbuscular mycorrhizal (AM) fungi, and *actinobacteria* in the surface soil.

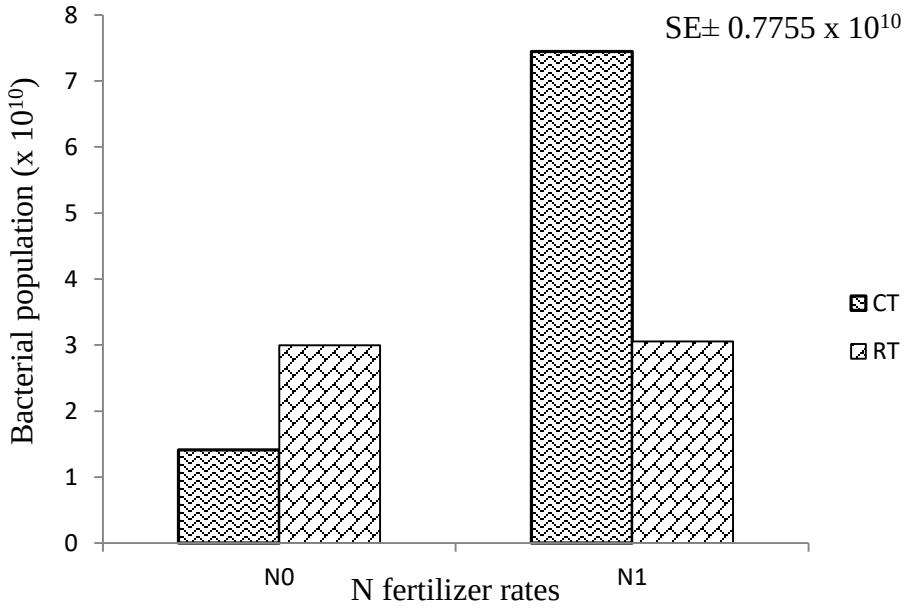


Figure 4: Interaction of Tillage (T) and N-Fertilizer Rates (NR) on Bacterial Population

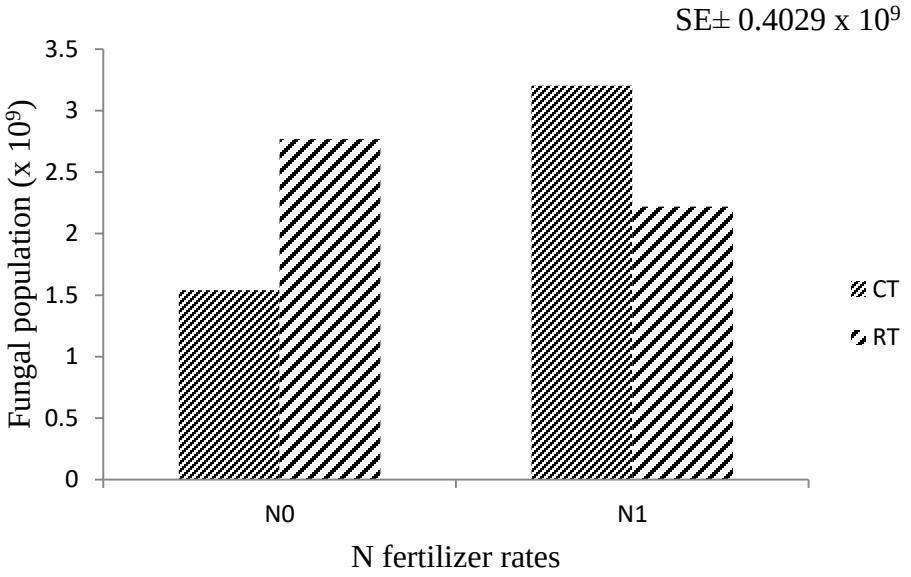


Figure 5: Interaction of Tillage (T) and N-Fertilizer Rates (NR) on Fungal Population

The interaction of crop rotation and N fertilizer rates on bacterial and fungal population were shown (Figure 6 and Figure 7). Bacterial population under continuous maize (M/M) and N1 (90 kg N ha^{-1}) was significantly higher followed by cowpea-maize (C/M) rotation with neither 0 kg N ha^{-1} (N0) nor 90 kg N ha^{-1} (N1) (Figure 6). In contrast, C/M rotation with N1 depicted a significantly higher fungal population (Figure 7) followed by soybean/maize rotation with N0 (0 kg N ha^{-1}) as well as continuous maize (MM) under N1 (90 kg N ha^{-1}). The mixed results may lie not only in the cropping system and N fertilizer rates but also in the microbial characterization methods that have been used. Most of this research to date has been conducted using techniques such as plate counts, community level physiological profiles, fatty acid methyl ester analysis (FAME), denaturing gradient gel electrophoresis (DGGE) and phospholipid fatty acid analysis (PLFA) (Justin *et al.*, 2012).

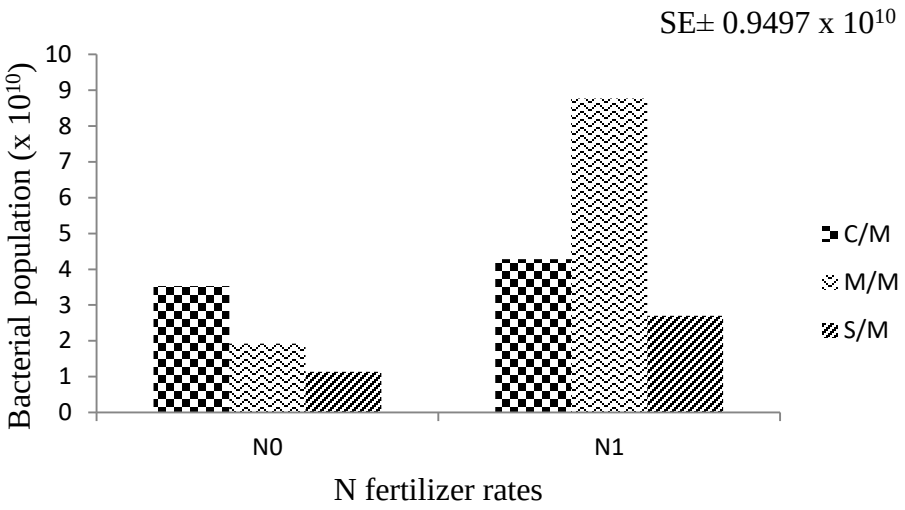


Figure 6: Interaction of Crop Rotation (CR) and N-Fertilizer Rates (NR) on Bacterial

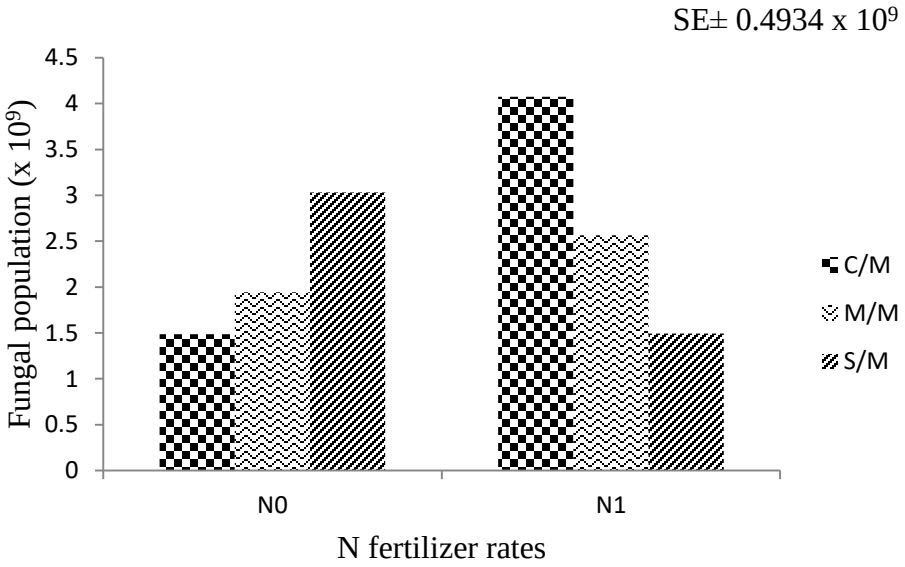


Figure 7: Interaction of Crop Rotation (CR) and N-Fertilizer Rates (NR) on Fungal Population

4.2.1 BioRad gel image of the PCR products of 16S rRNA obtained from the treatments

The BioRad gel image shown in Plate 1 of the electrophoresis gel image of the amplicons in each sample labeled A, B, C, D, E, F, G, H, I, J, K and L revealed that DNA of bacteria was successfully isolated from the soil samples and separated them based on size as depicted by the DNA bands in the image. Small molecular weight DNA flows through the gel with little resistance while the large molecular weight DNA migrate through the gel with great resistance which led to the bands separation as shown in the gel image. The letter "N" (second column in the image) serves as negative control which is blank and implies there is no DNA in the well.

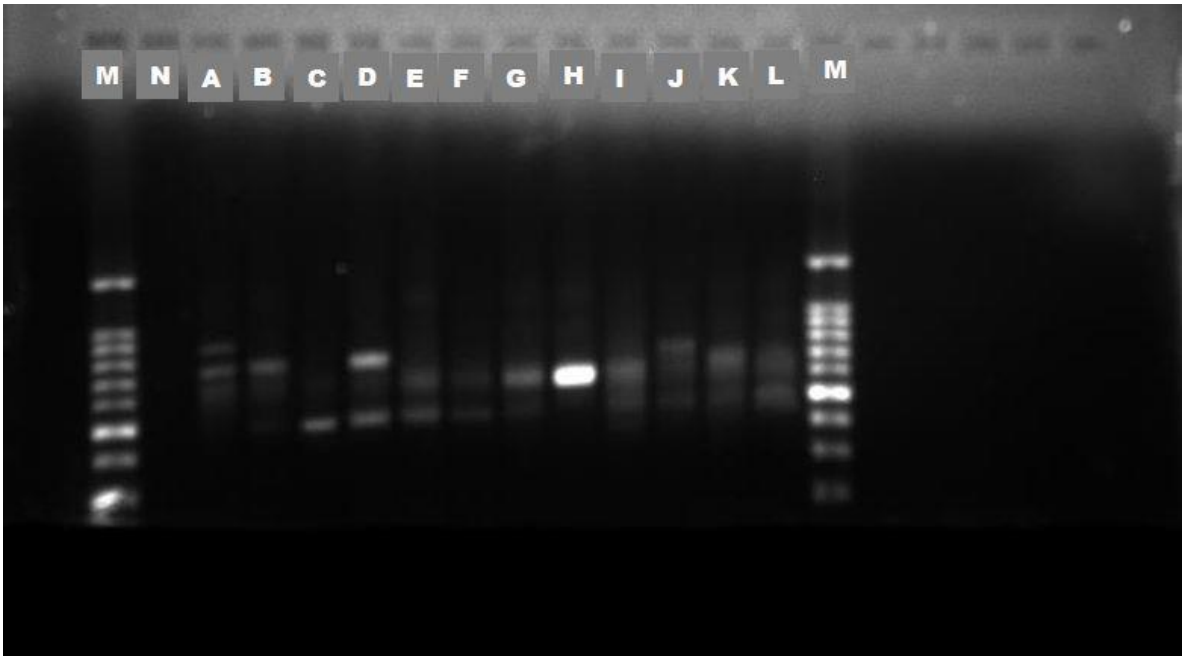


Plate 1: Agarose Gel Image of the PCR of Bacteria Isolates of the Treatments.

M= Ladder

N= Negative control

A=CTCN1 (Conventional tillage cowpea 90kg N ha^{-1})

B=CTCN0 (Conventional tillage cowpea 0kg N ha^{-1})

C=CTMN1 (Conventional tillage maize 90 kg N ha^{-1})

D=CTMN0 (Conventional tillage maize 0 kg N ha^{-1})

E=RTSN1 (Reduced tillage soybean 90 kg N ha^{-1})

F=RTSN0 (Reduced tillage soybean 0 kg N ha^{-1})

G=RTMN1 (Reduced tillage maize 90 kg N ha^{-1})

H=RTMN0 (Reduced tillage maize 0 kg N ha^{-1})

I=RTCN1 (Reduced tillage cowpea 90 kg N ha^{-1})

J=RTCN0 (Reduced tillage cowpea 0 kg N ha^{-1})

K=CTSN1 (Conventional tillage soybean 90 kg N ha^{-1})

L=CTSN0 (Conventional tillage soybean 0 kg N ha^{-1}).

4.2.2 Phylogenetic Trees of the Sequenced PCR Products obtained for the Treatments.

The Phylogenetic tree inferred from the bacteria sequences (16S rRNA) revealed three cluster groups prior to BLAST of the sequences of all treatments. These phylogenetic trees gave insight on the relationship of species in each of the treatments, which the microbial count was unable to detect (Figure 8):

Group A: CTCN0, RTCN1, RTMN0, CTCN1 and RTMN1
Group B: CTSN1, RTSN0, RTSN1 and CTSN0
Group C: CTMN0, CTMN1 and RTCN0.

Group A treatments were observed to have recently emerged due to some divergence in their traits. This suggests why they tend to cluster together which indicated that the organisms in the treatments share a recent common ancestor that differ from those of "Group B and Group C". Though, treatment CTSN1 (Group B) was found to have a common ancestor with treatments in Group A.

Group B treatments were noted to have organisms that evolutionary emerged earlier than organism in Group A treatments. In other words, organisms found in Group B are older than and/or existed before the organisms found in Group A. Similarly, they shared a common ancestor with taxa in CTMN0 (conventional tillage maize/maize rotation 0 kg N/ha) found in Group C (Figure 8).

Organisms depicted in Group C treatments were revealed to have evolutionary emerged earlier than organisms found in both Groups A and B.

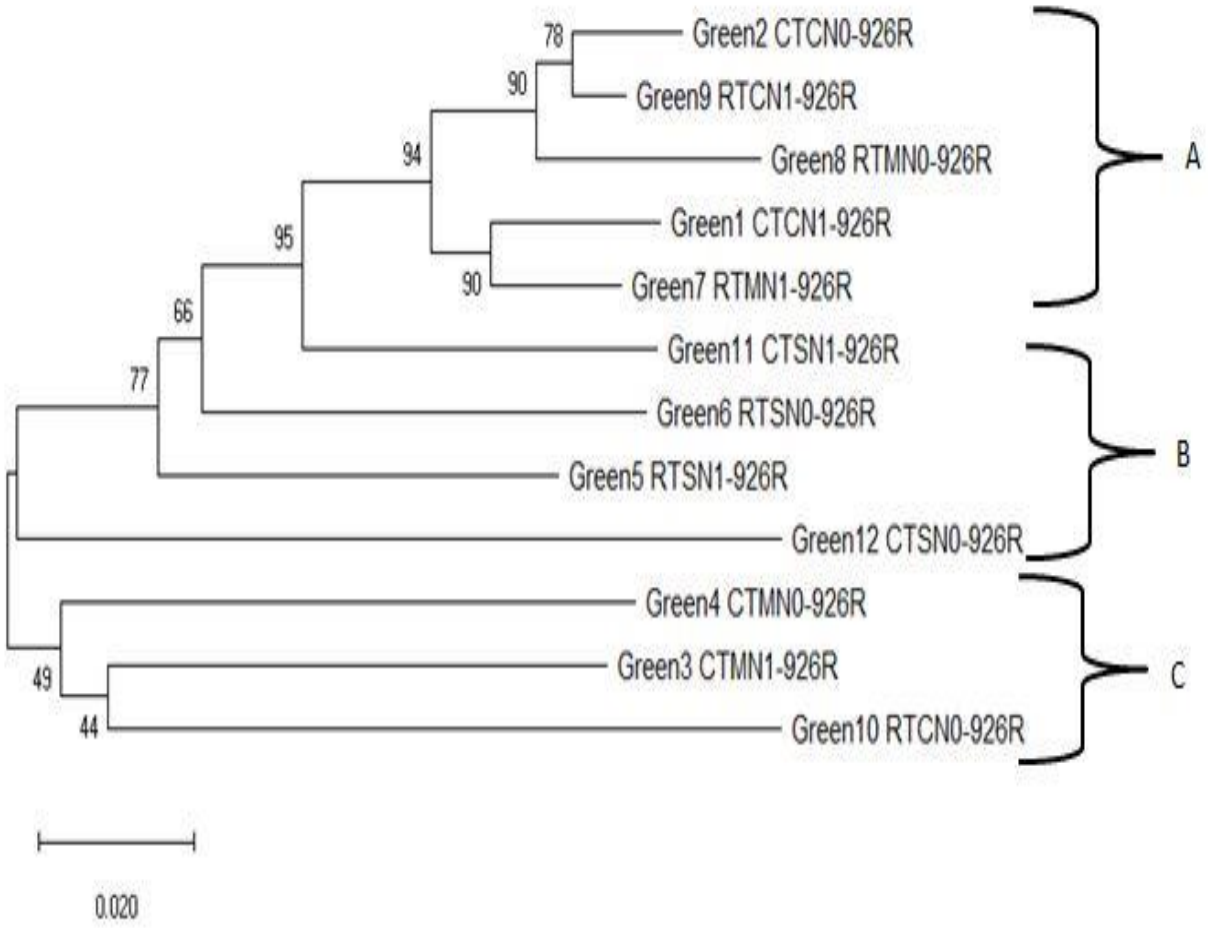


Figure 8: A Phylogenetic Tree of the Sequences of the Treatments before BLAST

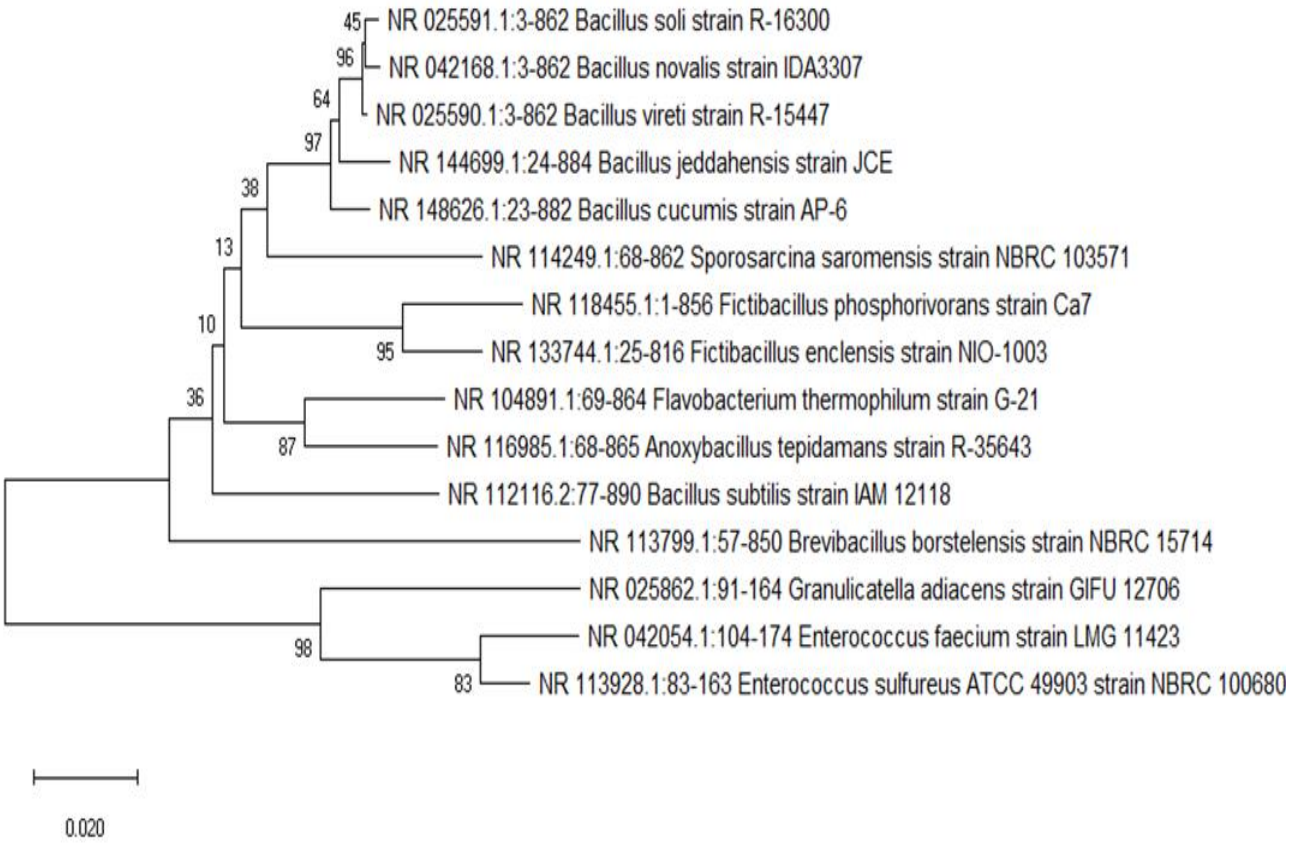
Consequently, the phylogenetic trees were grouped base on the effect of crop variety on the treatments which revealed how organisms that are similar cluster together (Figure 8a–8c). Continuous maize as well as cowpea/maize treatments Figure 8a (five phylogenetic trees) and Figure 8c (three phylogenetic trees) show the identity of bacteria with the closest similarity with the obtained sequence. The findings revealed that it is the similarity in the identities (strains) that resulted in the clusters. Perhaps, the identified organisms with the closest relationship with the samples are:

1. CTCN0- *Bacillus cucumis*
2. RTCN1- *Bacillus cucumis*
3. RTMN0- *Bacillus thioparans and Bacillus jeotgali*
4. CTCN1- *Bacillus soli and Bacillus drentensis*
5. RTMN1- *Anoxybacillus contaminans*
6. CTSN1- *Anaerobacillus alkalilacustris* and *Polygonibacillus indicireducens*
7. RTSN0- *Planococcus rifietoensis*
8. RTSN1- *Bhargavaea cecembensis*
9. CTSN0- *Geobacter metallireducens*
10. CTMN0- *Paenibacillus sp*
11. CTMN1-*Bacillus simplex* and *Joetgacibacillus marinus*
12. RTCN0-*Bacillus thermocloacae*, *Chelativorans composti*, *Sinorhizobium* and *Rhizobiales bacterium*

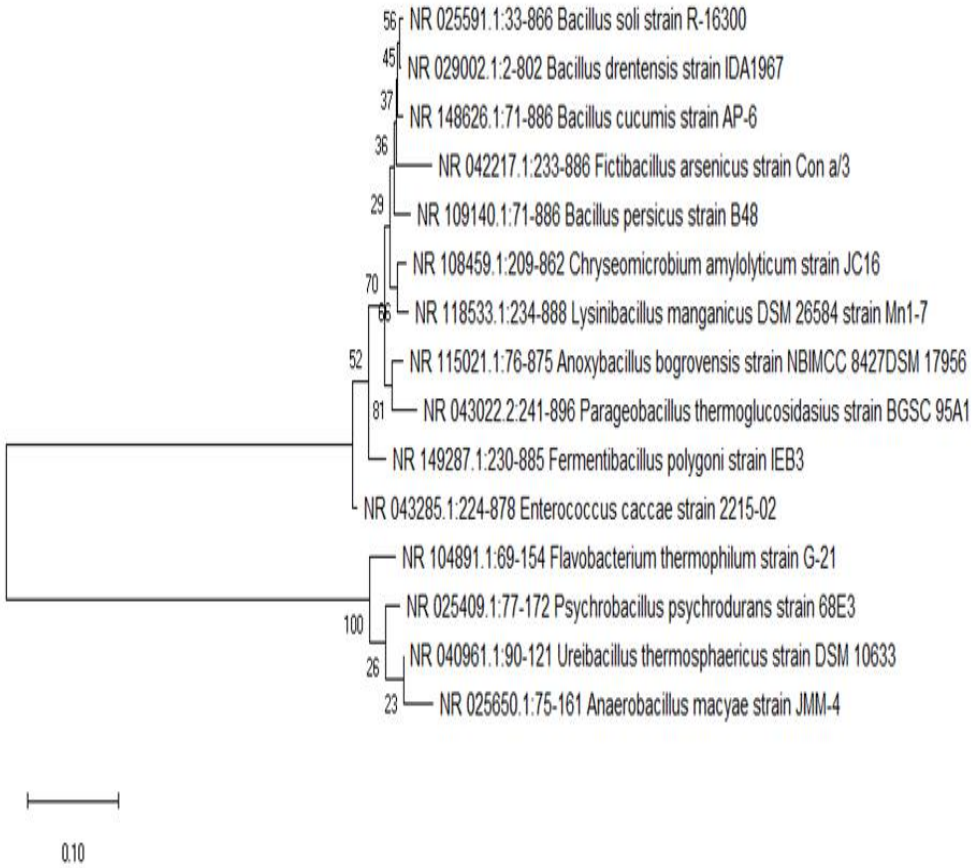
Overall, the tillage and nitrogen levels seem not to be affecting the bacterial diversity as much as the crop variety. The continuous maize and cowpea-maize rotation treatments are generally dominated by *Bacillus* and are separate from the soybean-maize rotation treatments, which are characterized by the presence of other genera. *Bacillus* spp was

shown to be the dominant genus which is similar to the report of Meliani *et al.* (2012) who stated that *Bacillus* and *Pseudomonas* genera predominate in agricultural and forest ecosystems.

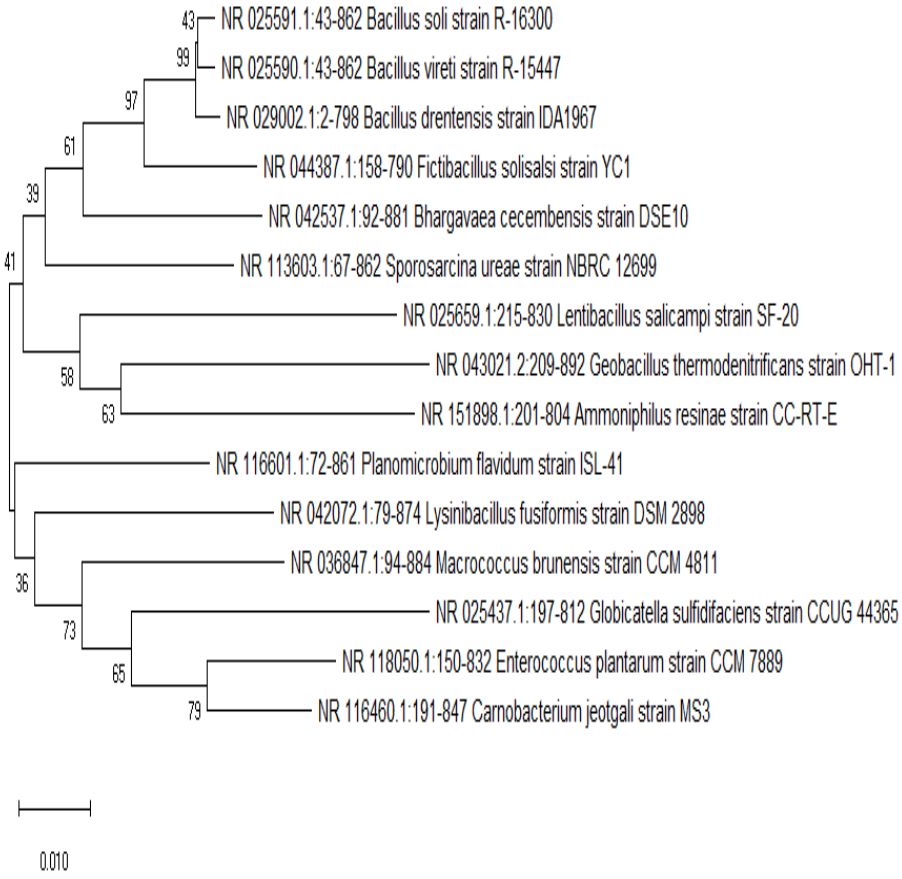
Most of the taxa found in the samples have been reported to be involved in degradation of pesticides, phosphorus and nitrogen cycling (Meliani *et al.*, 2012).



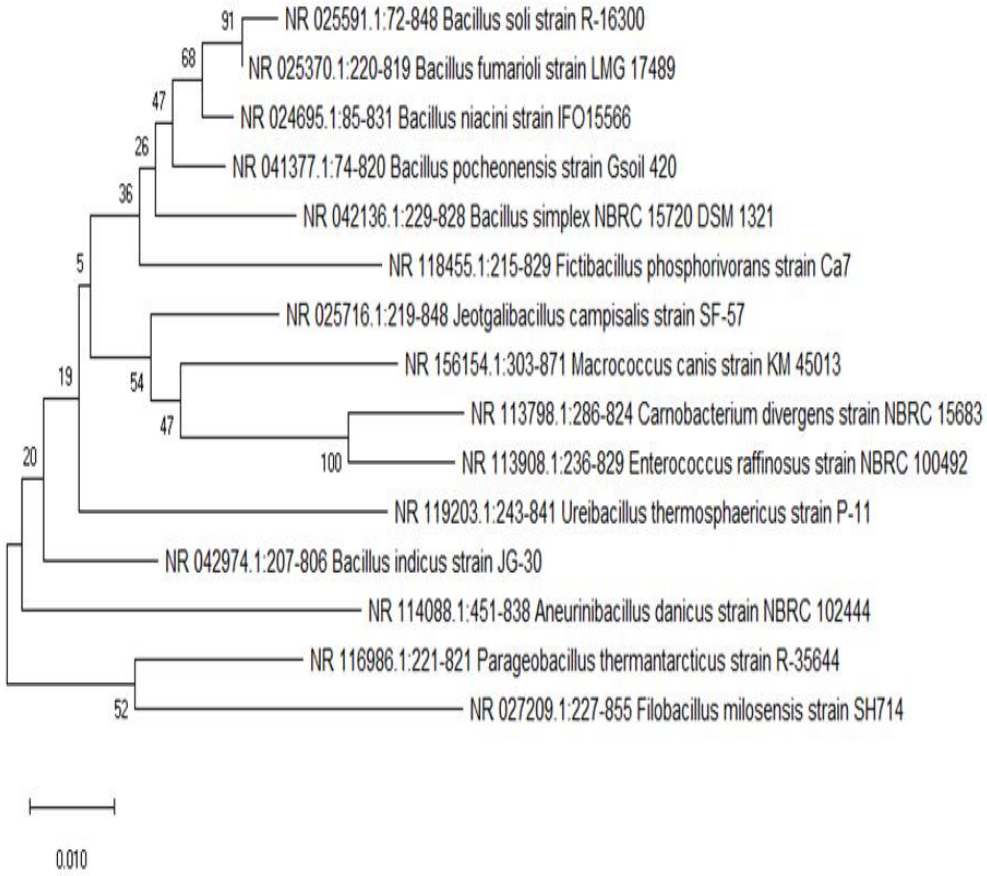
Phylogenetic tree 1. Green2_CTCN0-926R



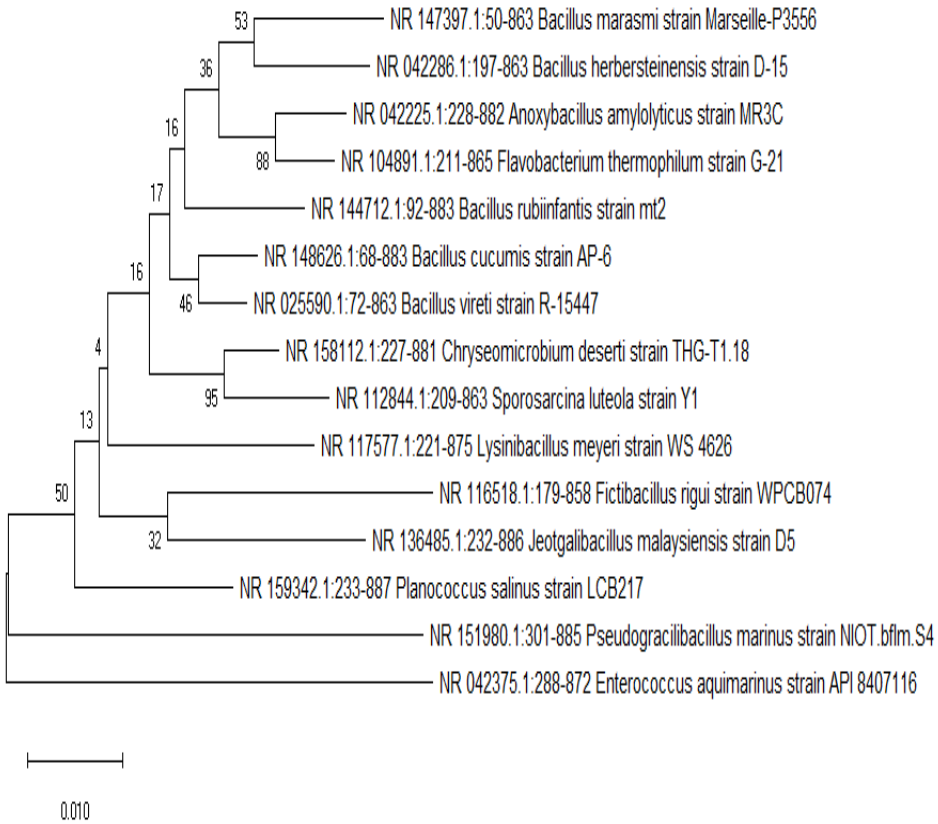
Phylogenetic tree 2.Green9_RTCN1-926R



Phylogenetic tree 3. Green8_RTMN0-926R

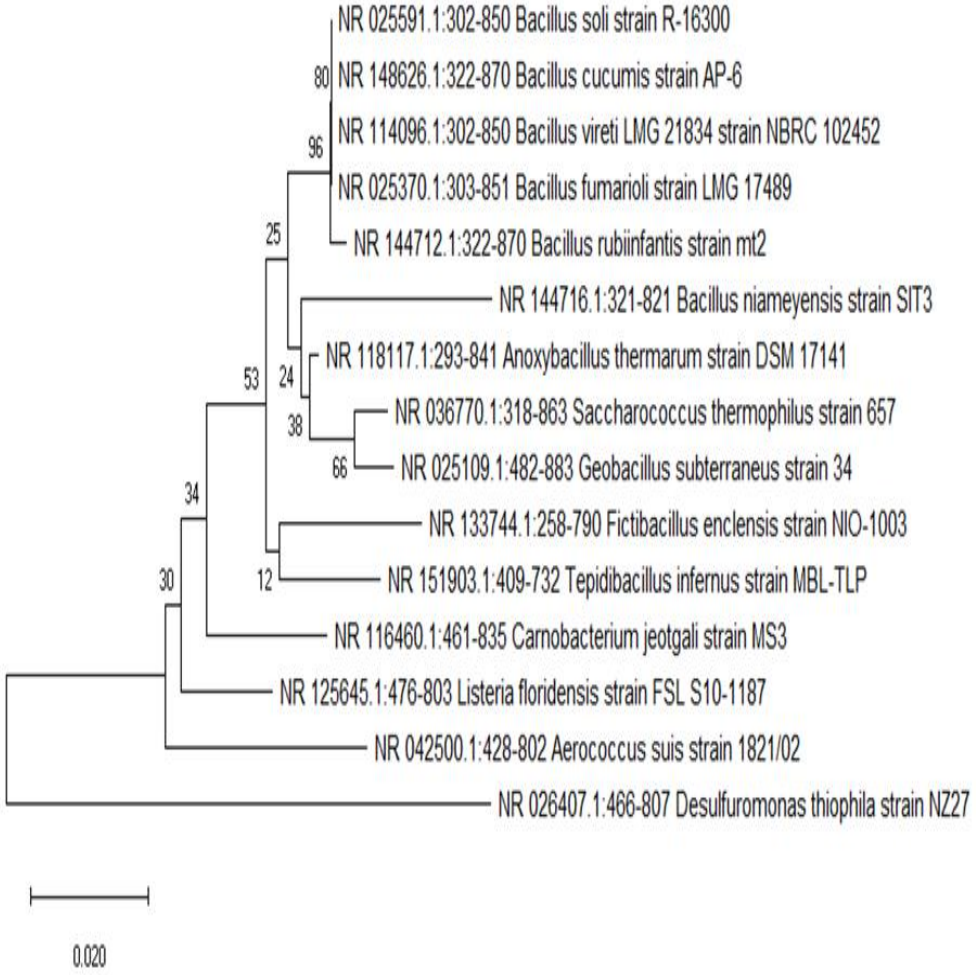


Phylogenetic tree 4. Green1_CTCN1-926R

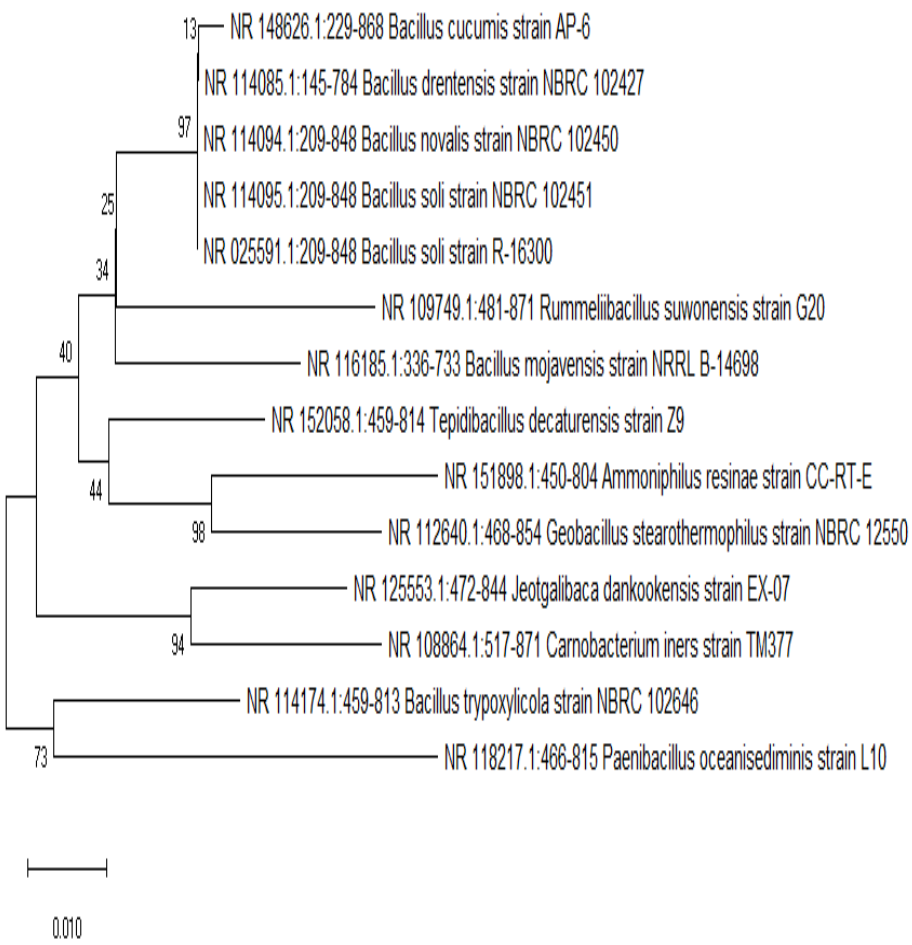


Phylogenetic tree 5. Green7_RTMN1-926R

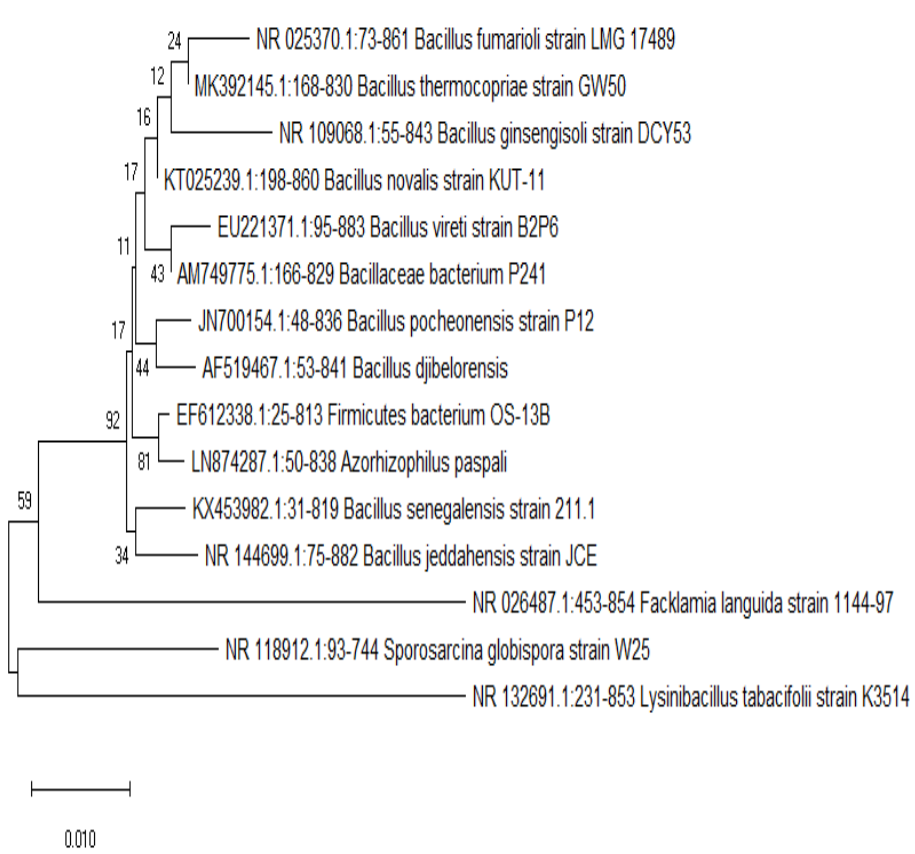
Figure 8a: Group A treatments depict Taxa (Genera) and their Evolutionary Relationships that are influenced by Continuous Maize and Cowpea/Maize Rotations



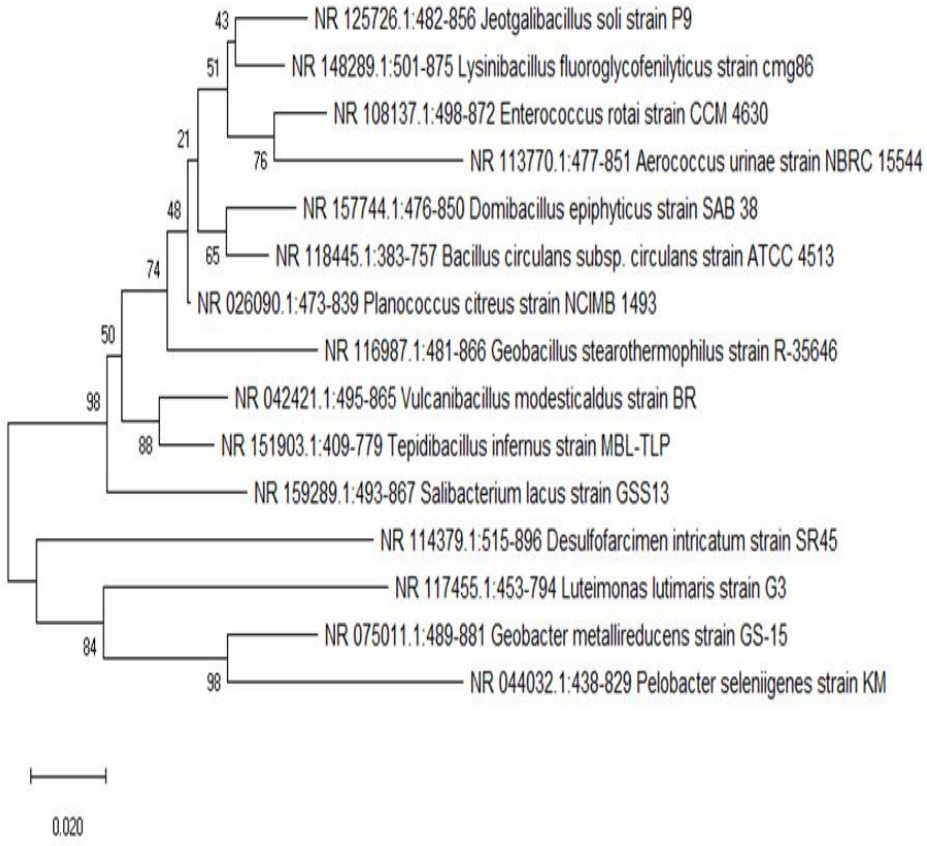
Phylogenetic tree 6 Green11_CTSN1-926R



Phylogenetic tree 7 Green6_RTSNO-926R

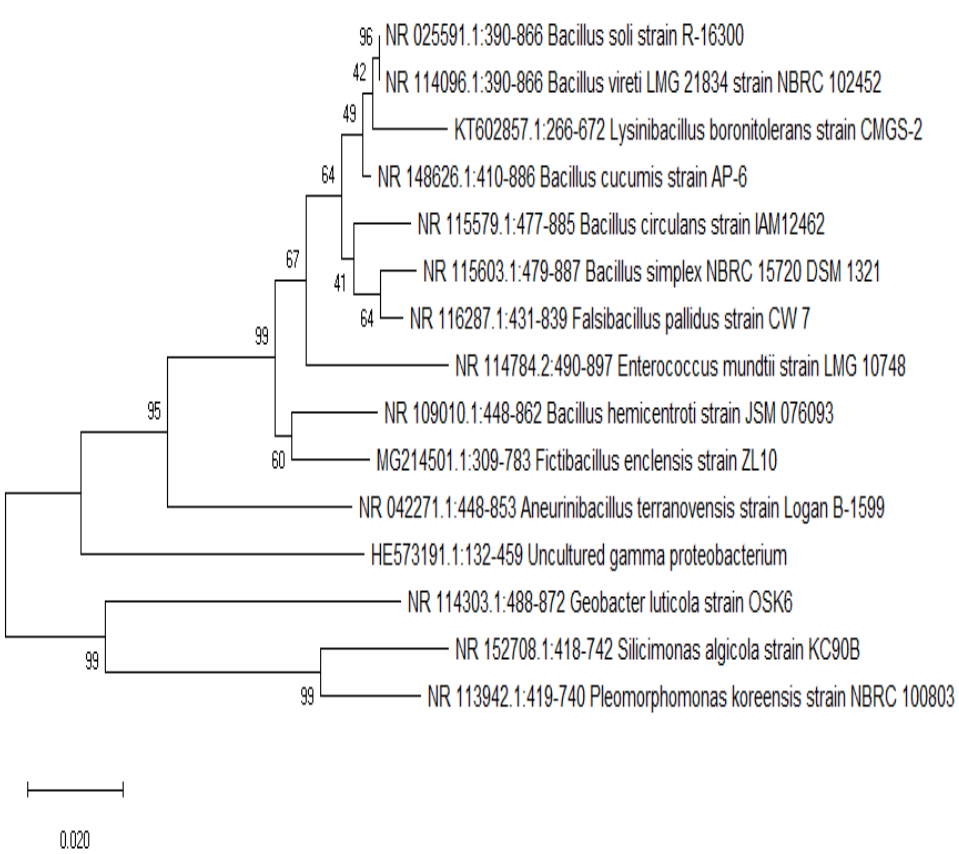


Phylogenetic tree 8 Green5_RTSN1-926R

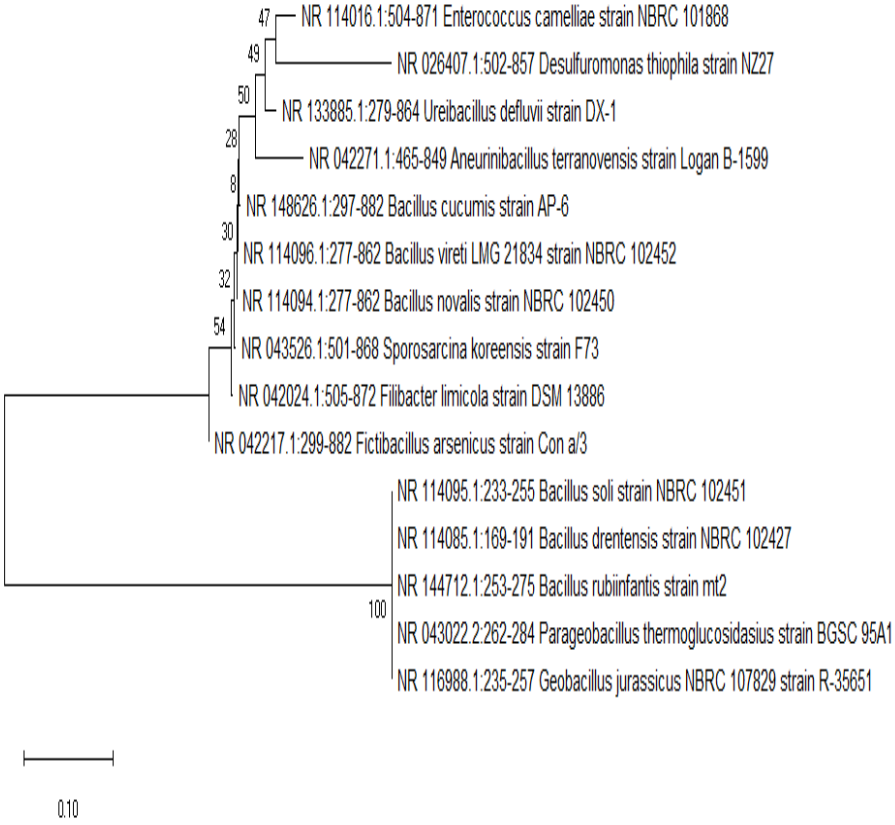


Phylogenetic tree 9 Green12_CTSNO-926R

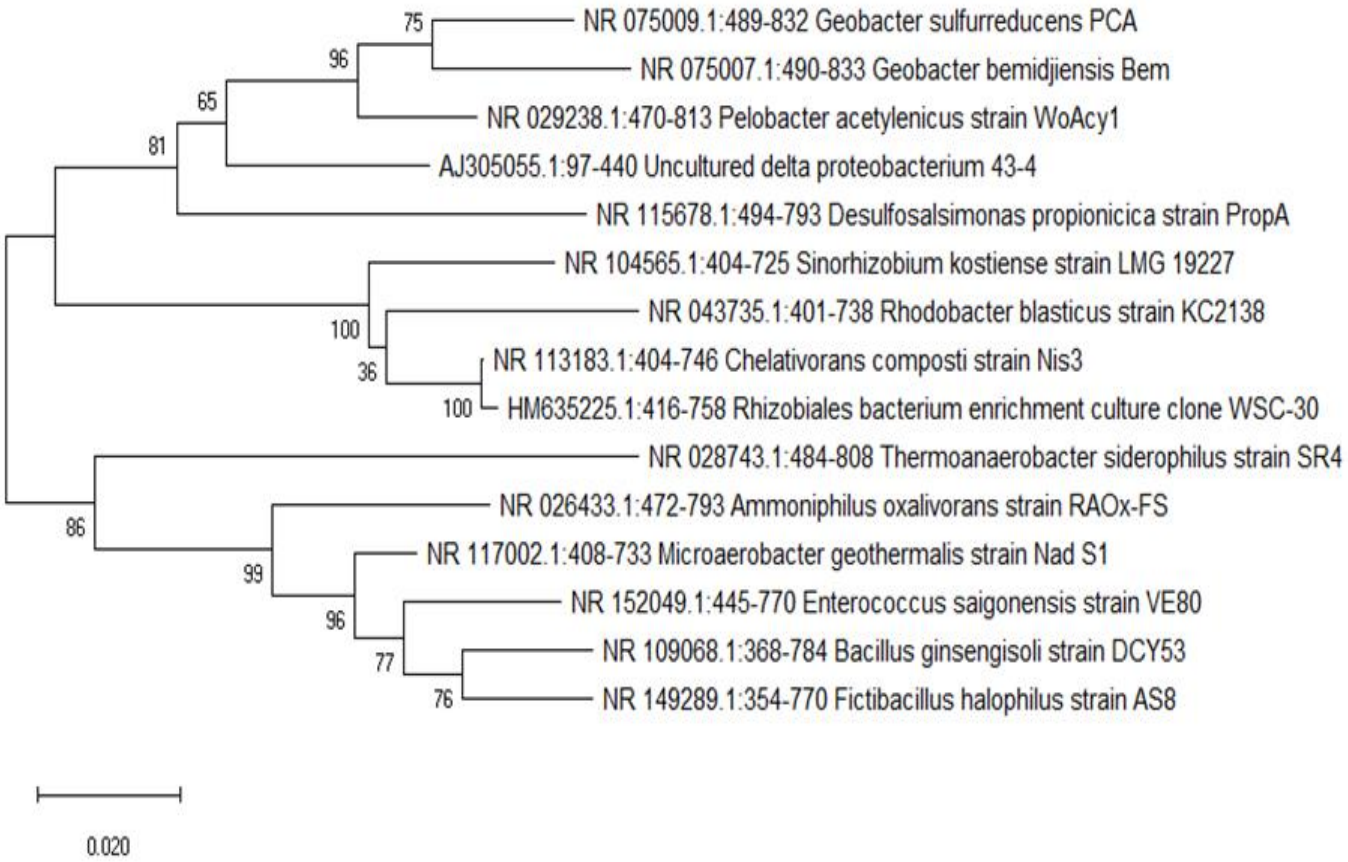
Figure 8b: Group B treatments reveal Taxa (Genera) and their Evolutionary Relationships that are influenced by Soybean/Maize Rotation



Phylogenetic tree 10 (Green4_CTMN0-926R)



Phylogenetic tree 11 Green3_CTMN1-926R.



Phylogenetic tree 12 Green10_RTCNO-926R

Figure 8c: Group C Treatments show Taxa (Genera) and their Evolutionary Relationships that are influenced by Continuous Maize and Cowpea/Maize Rotation

4.3 Effect of Tillage, Crop Rotation and N Fertilizer Rates on the Relative Abundance of Microbial Groups

Results showed that bacterial/fungal ratios are different in soils under tillage, crop rotation and N fertilizer rates (Table 4). The results presented on Bcfu/Fcfu ratio in the treatments were statistically the same which agrees with the reports of Lauber *et al.* (2008) who stated that bacterial/fungal ratios were not significantly different across the land-uses. Although, the results showed that bacterial/fungi ratio (Table 4) was high which is in agreement with the report of Isirimah *et al.* (2010) that arable soil support more bacteria growth than fungi. This suggests that the tilling of the soil makes crop residues vulnerable to decomposition by fast growing bacteria. It implies that such soils can rapidly restore its stability and maintain ecosystem functions after perturbation due to soil management practices. Also, plots with CT have high Bcfu/Fcfu ratio (33.600) compared to RT treatment (20.290). This is in agreement with Simmons and Coleman (2008) findings, who revealed that soil mixed with surface residue material through tillage not only makes it more accessible to soil microbes, but has been postulated to typically favor the dominance of aerobic bacteria with a greater capacity to breakdown labile substrates.

The effect of crop rotation on bacterial/fungal ratio was significant ($P<0.05$). Continuous maize was ranked the highest value for the Bcfu/Fcfu ratio (43.760) whereas the least value was observed in soybean/maize rotation (11.420); the effect of N fertilizer rates was not significant.

The interaction of tillage and N fertilizer rates was observed to be significant ($P<0.05$). It revealed that tillage and N fertilizer rates have remarkable influence on bacteria/fungi ratio in the study area (Figure 9).

Table 4: Effect of Tillage, Crop Rotation and N-Fertilizer on Bacterial/Fungal Ratio

Treatments	Bcfu: Fcfu
Tillage (T)	
Conventional Tillage (CT)	33.60
Reduced Tillage (RT)	20.29
SE±	7.685
Crop Rotation (CR)	
Cowpea/Maize (C/M)	25.68ab
Cowpea/Maize (C/M)	43.75a
Soybean/Maize (S/M)	11.41b
SE±	10.869
N-Fertilizer Rate (kg ha^{-1})	
0	22.27
90	31.63
SE±	7.685
Interactions	
T * CR	NS
T * NR	*
CR* NR	NS
T * CR*NR	NS

Bcfu= Bacteria colony forming unit, Fcfu = Fungi colony forming unit, * = Significant at $P<0.05$, NS = Not significant at $P<0.05$.

Interactions of tillage and N fertilizer rates on bacterial-fungal ratio revealed significant (Figure 9) increase under conventional tillage and N1 (90 kg N ha^{-1}) as well as higher bacterial-fungal ratio in reduced tillage and N0 (0 kg N ha^{-1}). This could be due to availability of substrates and residual nitrogen that favour the growth of fast growing bacteria and agreed with the report of Clegg *et al*., (2003) who noted that tillage and nitrogen fertilizer have a significant impact on the total bacterial community structures.

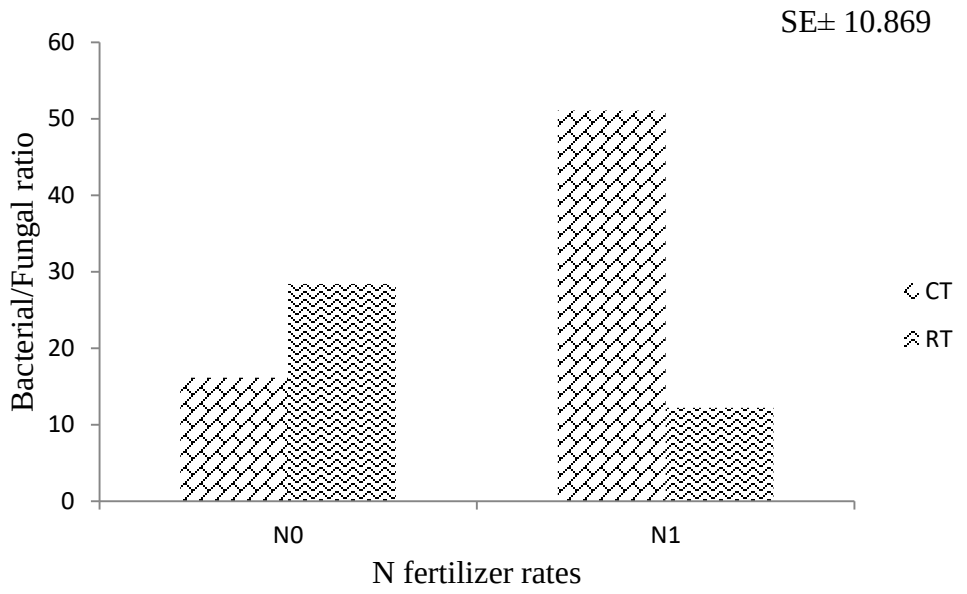


Figure 9: Interaction of Tillage (T) and N-Fertilizer Rates (NR) on Bacterial/Fungal Ratio

4.4 Correlation Analysis between Soil Microbial Diversity and Selected Soil Properties

The results of correlation analysis between selected soil properties and soil microbial diversity of bacterial and fungal Shannon Wiener diversity indices are presented in Table 5. Available P was moderately (negatively) correlated with exch. Ca, and Mg, CEC and Shannon Weiner Diversity Index for bacterial (SWDB) ($P<0.01$). However, as available P decreases, SWDB, Ca, Mg and CEC increase though this process is not constant because of the heterogeneous nature of soil.

SWDB was weakly and negatively correlated with Sodium (Na) ($P<0.05$). It suggests that as Na content in soil decreases, SWDB increases. This variation could be due to management practice being performed on the soil and methods of determining microbial diversity; we determined diversity using soil serial dilution (spread-plate technique) to obtain microbial population while other studies used DNA sequence (quantitative PCR) (Rousk *et al.*, 2010; Dai *et al.*, 2018).

However, TN, Available P and OC revealed positive correlation with Shannon Weiner Diversity Index for fungal (SWDF) but were not significant. The results revealed that SWDF were not affected by soil chemical properties which conforms with the findings of Rousk *et al.* (2010) who reported that SWDF are unaffected by soil chemical properties.

Table 5: Correlation Coefficient (r) between Selected Soil Properties and Soil Microbial Diversity

Soil Properties	pH H_2O	pH $CaCl_2$	TN	OC	Avail. P	Exchangeable bases ($Cmolkg^{-1}$)				CEC	SWDB
			$g\ kg^{-1}$	%	$mgkg^{-1}$	K	Na	Ca	Mg	Cmol/kg	
pH $CaCl_2$	0.786**										
TN	-0.053	0.088									
OC	0.408*	0.306	-0.003								
Avail. P.	0.379	0.424**	0.244	0.420							
K	0.183	0.329	-0.117	0.081	-0.160						
Na	0.375*	0.561**	-0.163	0.151	0.070	0.736**					
Ca	-0.291	-0.223	-0.127	-0.221	-0.655**	0.174	0.113				
Mg	-0.338	-0.181	-0.063	-0.173	-0.594**	0.204	0.133	0.932**			
CEC	-0.307	-0.204	-0.129	-0.241	-0.668**	0.218	0.148	0.986**	0.947**		
SWDB	-0.174	-0.230	0.069	0.060	-0.485**	-0.211	-0.340*	0.274	0.265	0.280	
SWDF	-0.042	-0.011	0.053	0.016	0.056	-0.018	-0.141	-0.068	-0.099	-0.125	0.000

SWDB= Shannon Weiner Diversity index Bacterial, SWDF = Shannon Weiner Diversity index Fungi, TN= Total nitrogen, OC= Organic carbon, Avail. P. Available phosphorus, CEC= Cation exchange capacity, *= Significant at 5%, **= Significant at 1%

4.4.1 Correlation between soil microbial diversity and particle size distributions

The correlation between particle size distribution and Shannon Weiner diversity index for bacterial (SWDB) and Shannon Weiner diversity index for fungal (SWDF) was shown in Table 6. Shannon Weiner diversity index for bacterial (SWDB) was strongly (positively) correlated with sand (P<0.01). On the contrary, SWDB was observed to be strongly (negatively) correlated sand (P<0.01) (Table 6). This disagree with the reports of several researchers who noted that bacteria tend to be positively correlated with clay and demonstrated that fine texture promoted bacterial growth (Bonneau and Souchier 1994; Meliani *et al.*, 2012). This is attributed to the fact that clay fraction retain substrates, moisture required by bacterial and also protect them from predators (Bonneau and Souchier, 1994). This difference could be due to the type of soil (textual class), our trial field depicted a sandy loam textural while that of Meliani's field was silty clay loam. Other variations include the environment where the study was conducted; ours was conducted in Nigeria whereas that of Meliani was done in Algeria. SWDF was unaffected by particle size distribution.

Table 6: Correlation Coefficient (r) between Soil Microbial Diversity and Particle Size Distributions

Soil properties	Sand	Silt	Clay	SWDB
Silt	-0.901**			
Clay	0.082	-0.509**		
SWDB	0.543**	-0.590**	0.275	
SWDF	-0.109	0.088	0.016	0.000

SWDB = Shannon Weiner diversity index Bacteria, SWDF = Shannon Weiner diversity index Fungi, * = Significant at 5%, ** = Significant at 1%

CHAPTER FIVE

5.0 Summary, Conclusion and Recommendations

5.1 Summary

In summary, results for soil characteristics revealed soil texture which ranges from sandy loam to loamy soil. Soil pH in water is slightly acidic and pH in 0.01 M $CaCl_2$ is moderately acidic. Similarly, Ca, Mg, CEC, available P was moderate among the treatments but reduced tillage soybean-maize rotation and 0 kg N ha^{-1}revealed the highest content. Soil organic carbon, total nitrogen, potassium and sodium were low which is typical of an *Alfisol*. Also, microbial population for both bacterial and fungal were high. The microbial diversity results suggest a better resilience that could revive soils that have been disturbed either by tillage or other agricultural management practices.

Bacterial count under cropping systems was significant (P<0.05)and depicted continuous maize to have the highest specie richness (5.348 x 10^{10} cfu g^{-1} moist soil), followed by cowpea/maize rotation (3.917 x 10^{10} cfu g^{-1} moist soil) and lastly soybean/maize rotation (1.917 x 10^{10} cfu g^{-1} moist soil). Similarly, Bacterial population in 90 kg N ha^{-1} (5.249 x 10^{10} $cfug^{-1}$ moist soil) was ranked higher than 0kg N ha^{-1} (2.205 x 10^{10} $cfug^{-1}$ moist soil). The effect of interactions of tillage and crop rotation, tillage and N fertilizer rates, and crop rotation and N fertilizer rates were significant for both bacteria and fungi loads (P<0.05).

Phylogenetic trees were grouped according to the effect of crop variety which revealed *Bacillus* to be the most dominant genera in continuous maize, cowpea/maize (group A) as well as soybean/maize (group B) treatments. Other genera revealed in the group C are*Rhodobacter*, *Rhizobiales*, *Sinorhizobium*, *Thermoanaerobacter*, uncultured

Deltaproteobacterium, Baccillaceae, Domibacillus, Facklamia, Firmicutes, Tepdibacillus and *Vulcanibacillus*. Most of the organisms are involved in nutrient cycling, degradation of pesticides, etc.

Available P as well silt were observed to be negatively correlated with Shannon Weiner Diversity Index for Bacteria (SWDB) (P<0.01) while sand depicted positive correlation with SWDB (P<0.01).

5.2 Conclusion.

It was concluded that long-term tillage, cropping systems and N fertilizer rates improve soil fertility as shown in the values of Ca, Mg, soil pH, CEC and available P which were significant (P<0.05) at the experimental site. The significant difference recorded in the interaction of tillage and crop rotation on bacteria load (P<0.05) and bacteria/fungi ratio were remarkable.

Our results indicate that long-term cropping rotations and N fertilizer rates can impact soil microbial populations, diversity, richness, and community composition. Interestingly, cropping sequence had a larger impact on bacterial community composition and relative levels of bacteria and fungi than did the tillage treatment.

SWDB also positively correlated with sand (P<0.01) which disagreed with the findings of Meliani *et al.* (2012) who revealed that bacteria was positively correlated to clay. Available P. was also negatively correlated with SWDB (P<0.01); SWDF was unaffected by soil chemical properties.

5.3 Recommendations

The results therefore enable us to suggest the following:

1. Long-term tillage practices crop rotation and N-fertilizer rates improve some soil chemical properties (Ca, Mg, CEC and Avail. P) among the treatments

2. Maize-maize and Cowpea-maize rotation depicted higher specie richness thus; it should be encouraged among farmers because it leads to increase in microbial diversity; improve ecosystem functioning, soil fertility and ensure sustainable crop production.

3. Further studies should focus on internal transcribed spacer genes (ITS) for fungi characterization.

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APPENDICES

Appendix I: Fertility Class Rating for Topsoil (0 – 15cm) in Nigeria

Parameter	Very Low	Low	Moderate	High	Very High
Total N (g kg^{-1})	0.3 – 0.5	0.6 – 1.0	1.1 – 1.5	1.6 – 2.0	2.1 – 2.4
Bray I P (mg kg^{-1})	<3	3 – 7	7 – 20	≥20	
Exchangeable K(Cmol kg^{-1})	0.12 – 0.2	0.21 – 0.3	0.31 – 0.6	0.61– 0.73	
Exchangeable Ca (Cmol kg^{-1})	0.65 – 2	2.01 – 5	5.01 – 5.42		
Exchangeable Mg (Cmol kg^{-1})	0.24 – 0.3	0.31 – 1.0	1.01 – 3.0	3.01 – 3.55	
CEC (Cmol kg^{-1})	2.72 – 5.0	5.01 – 7.0	7.01 – 10.01	10.01– 12.0	12.01 – 21.4
Organic C (g kg^{-1})	<4.0	4.0 – 10	10 – 14	14 – 20	≥20

Source: National Special Programme for Food Security (NSPFS) 2005

Appendix II: Rating for Soil Fertility Classes

Nitrogen (Total N %)	Ranges
Very Low	0.3 – 0.5
Low	0.6 – 1.0
Moderately Low	1.1 – 1.5
Medium	1.6 – 2.0
Moderately high	2.1 – 2.4
Phosphorus (Bray – 1) (mg/kg)	
Very Low	<3
Low	3 – 7
Moderate	7 – 20

High	>20
Potassium (Exchangeable K) (Cmol/kg)	
Very Low	0.12 – 0.2
Low	0.2 – 0.3
Moderate	0.31 – 0.6
High	0.61 – 0.73
Organic Carbon (%)	
Very Low	<4.0
Low	4.0 – 10
Moderate	10 – 14
High	14 – 20
	>20
Very High	

Source: Chude*et al.*, 2012

Appendix III: Ratings for Soil Fertility Classes

parameters	Very Low	Low	Medium	High	Very High	Units
Zn	<1.0	1.0 – 1.5	1.6 – 3.0	3.1 – 5.0	>5.0	mg/kg
Cu	1.0	1.0 – 2.0	2.1 – 4.0	4.1 – 6.0	>6.0	mg/kg
Fe		<2.5	2.5 – 5.0	>5.0		mg/kg
Mn	<1.0	1.0 – 2.0	2.1 – 3.0	3.1 – 5.0	>5.0	mg/kg
N		<1.5	1.5 – 2.0	>2.0		mg/kg
P		<10	10 – 20	>20		g/kg
K		<0.15	0.15 – 0.30	>0.30		Cmol/kg
Ca		<2	2 – 5	>5.0		Cmol/kg
Mg		<0.3	0.3 – 1.0	>1.0		Cmol/kg
Na		<0.2	0.2 – 0.3	>0.3		Cmol/kg
OC		<10	10 – 15	>15		g/kg

Source: Esu, 1991