Bacterial diversity of South African soils

AR Sholeye

orcid.org 0000-0003-2109-503X

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Supervisor: Prof OO Babalola

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Student number: 31415695

DECLARATION

I, the undersigned, declare that this thesis submitted to the North-West University for the degree of Master of Science in Biology in the Faculty of Natural and Agricultural Sciences, School of Environmental and Health Sciences, and the work contained herein is my original work with the exceptions to the citations and that this work has not been submitted at any other University partially or entirely for the award of any degree.

Name: Abisola Regina Sholeye

Signature: …….

Date: …….

Co-Supervisor: Dr. Omena Ojuederie

Signature: …….

Date: …….

Supervisor: Professor Olubukola Oluranti Babalola

Signature: …….

Date: …….

DEDICATION

This work is dedicated to the Almighty God the source of life and every good thing.

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Candidate's Contributions: designed the study, managed the literature searches and wrote the first draft of the manuscript.

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Candidate's Contributions: designed the study, managed the literature searches and wrote the first draft of the manuscript.

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LIST OF ABBREVIATIONS

ACE- Abundance Coverage Estimator

BLASTn- Basic Local Alignment Search Tool (nucleotide)

DGGE- Denaturing Gradient Gel Electrophoresis

DNA- Deoxyribonucleic Acid

GHGs- Green House Gases

L.A2RhS- Limpopo Agricultural Rhizosphere Soil

L.ARpS- Limpopo Agricultural Rhizoplane Soil

M.2IFS- Mpumalanga Indigenous Forest Soil site 2

M.IFS- Mpumalanga Indigenous Forest Soil

N.ARpS- North West Agricultural Rhizoplane Soil

N.ARhS- North West Agricultural Rhizosphere Soil

NCBI- National Center for Biotechnology Information

NGS- Next Generation Sequencing

OTU- Operational Taxonomic Unit

PAST- Paleontological Statistics

PCR- Polymerase Chain Reaction

PGPR- Plant Growth Promoting Rhizobacteria

R.A- Relative Abundance

RDPII- Ribosomal Database Project

rRNA- Ribosomal Ribonucleic Acid

GENERAL ABSTRACT

The soil is an essential part of the environment as it provides support for life which comprises microorganisms, plants, animals and humanity. Ecosystem services such as nutrient cycling, food production and temperature regulation are important in maintaining life, all of which can be attributed to the soil; it is therefore of importance that the soil is properly managed.

The soil-dwelling organisms contribute immensely to the general functioning of the soil. Bacteria are numerically abundant and diverse microorganisms present in the soil, are important potential markers of soil health and quality. The abundance, diversity, and richness of bacteria is largely dependent on the organic matter content of the soil. Several studies carried out show that soils rich and abundant in bacteria are good for agricultural purposes as they release important nutrients necessary for plant growth.

South Africa is faced with an increase in land degradation which affects agricultural productivity. Some studies have shown that land degradation affects bacterial diversity, richness and abundance. Soil samples were obtained from specific locations (Mpumalanga indigenous forests, Limpopo agricultural soil and North West agricultural soil), to determine the bacterial diversity in soils possibly undergoing degradation, their causes and indicators of soils currently undergoing degradation. Samples were assessed using High-throughput sequencing.

The bacterial DNA from the soil were extracted using the PowerSoil DNA isolation kit (Mo Bio labs, USA), following the manufacturer's instructions. Data analysis was carried out on the microbiome analyst and the PAST platform.

The bacteria class found to be most abundant in agricultural and forest soils was *Proteobacteria*, with a relative abundance value of 47.3% and 35.5% respectively. The richness of bacterial phyla was higher in the forest (natural/undisturbed) soil than in the Limpopo and North West agricultural soils with richness values of 1969, 1710 and 1663 respectively. The

Limpopo agricultural site had the highest bacterial diversity with a value of 6.6, while the North West agricultural soil and Mpumalanga forest soil had a bacterial diversity of 6.5 and 6.4 respectively. Principal component analysis showed that the class of bacteria that brought about significant differences amongst the soil of Limpopo, the North West and Mpumalanga sites were *Actinobacteria*, *Nitrospirae*, *Proteobacteria* and *Verrucomicrobia*.

It was observed that the most abundant phyla in both indigenous and commercial forest sites were *Proteobacteria, Acidobacteria, Verrucomicrobia, Chloroflexi* and *Actinobacteria*, while the most abundant classes in both the indigenous and commercial forest sites were *Verrucomicrobia*, *Alphaproteobacteria*, *Holophagae*, *Betaproteobacteria*, *Acidobacteria*, *Ktedonobacteria* and *Actinobacteria*. There were notable significant differences ($p=0.03$ using ANOVA) observed in the phyla *Acidobacteria*, *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* and the class *Alphaproteobacteria*, *Betaproteobacteria*, *Holophagae* and *Verrucomicrobia* in the indigenous and commercial forest sites. The soil organic carbon (SOC), nitrate, total carbon (TC), pH, calcium, magnesium, potassium and sodium content showed significant changes ($p<0.05$) from the indigenous sites to the commercial sites, having higher values in the indigenous sites than the forest sites.

Intensive and continuous tillage should be discouraged for best agricultural productivity as this tends to reduce the bacterial abundance, richness, and diversity, all of which are important for healthy plant growth. Bacterial abundance, richness and diversity also help the plants build resistance against sudden environmental changes. The use of cover cropping in agriculture is advised as this help increase the organic matter content of the soil, which is necessary for increased bacterial abundance, richness and diversity.

The effect of the commercialization of natural lands (forests) has not been researched fully. It may have a negative effect on the bacterial composition of the soil which inadvertently affects ecosystem services.

Comparing the bacterial composition of indigenous and commercial forests showed that there is a significant difference in the phyla and class composition of bacteria. From this research the physical and chemical properties had no effect on the bacterial diversity using CCA.

CHAPTER ONE

1.1 General Introduction

The soil is a combination of mineral constituents and is made up of solid, liquid and gaseous phases. It is a habitation for microorganisms as well as a source of support for growing plants. There are various types of soils, these are; the clay, silt, sandy and loam soils (Schoeneberger et al., 2002, Coucheney et al., 2018). The quality of a soil is measured by the ability of that soil to maintain and sustain life and provide a stable conducive environment for living organisms (Bünemann et al., 2018). One of the factors from which the quality of a soil can be detected is the aggregate stability of the soil (Wu et al., 2017). Due to the diversity of the soil ecosystem, opportunities are available for the development of biofuels and new antibiotics (Jansson and Hofmockel, 2018). The soil forms one of the major backbones in the research into biodiversity and it can be used to study past occurrences such as climate change (due to its static nature) that may have occurred at a particular geographic region (Coleman et al., 2018). Soils found in different locations (the forest, rhizosphere of agricultural plants or termite mounds) have peculiar characteristics. For instance, the nature and type of soils present in a forest are highly dependent on the geographical location of the forest.

Forest soils are characterized by trees with large intensive root system, high organic matter layer on the soil surface and recycling of nutrients and organic matter by the soil-inhabiting organisms. Such soils have better agricultural properties due to the availability of increased activities of soil microbiomes (Boyle and Powers, 2013). These soils are affected by human activities such as felling or planting trees. Such activities usually have an adverse effect on the soil microbiome which in turn cause changes in the soil physicochemical properties as well as a reduction in the biological integrity of the soil (Lin et al., 2011).

The soil microbiome carries out important processes that sustain life on the earth which includes the cycling of important chemical elements like carbon and nitrogen, thereby sustaining the growth of vegetation (Jansson and Hofmockel, 2018), and the survival of microorganisms (Pant et al., 2017). In the process of ingesting their secretions, mesofauna and macrofauna provide a controlled and protective environment for the microorganisms present in the soil, and they putrefy plant and animal residues, making it easier for bacteria and fungi to act on the residues (García-Segura et al., 2017, Pant et al., 2017). The presence of the microbial community in the soil plays an important role in plant adaptation to changes in the soil chemical composition (Massenssini et al., 2015). Recent studies conducted on 18 different soil types in Georgia, USA, showed that soils can be differentiated by the relative abundance of bacteria and commonality of specific species of bacteria (Gagelidze et al., 2018). Since bacteria make up the largest group of the soil microbiome in terms of population and species diversity (Gagelidze et al., 2018), their presence in the roots of plants plays important roles; for example, they are important in the cycling of carbon and nitrogen (Sarabia et al., 2018). Continuous studies of these group of organisms have shown a decline in their activity as the soil profile depth increases (Tang et al., 2017). Plant roots (dependent on plant species) release substances (exudates) that determine the type of microbes that will be present in the rhizosphere of the plant (Choudhary et al., 2018). It is therefore easy for plant roots at the rhizosphere to gather useful microbes, especially bacteria, in the cycling of phosphorus and other essential nutrients necessary for plant growth (Castrillo et al., 2017, Chen et al., 2018).

Bacteria in the soil make up the largest fraction of complete DNA constituents due to their wide distribution across the soil (Griffiths et al., 2016), that is about one billion cells in 1g of soil. Other organisms also found in the soil are the fungi, protists, and nematodes which are in smaller quantities, up to one million cells, about one million cells and hundreds of cells respectively (EU, 2010).

The rhizosphere is the region around plant roots and soil where diverse micro and macro-organisms can be found (Philippot et al., 2013). The population and variety of organisms present in the rhizosphere of agricultural soil are dependent on the soil type, the type of plant grown and the exudates released from the plant roots which are capable of promoting or stopping soil organisms, contaminating roots or improving the growth of the plant (Pant et al., 2017). Bacteria in the plant rhizosphere have the ability to control and alter the concentration of heavy metals that could be present in the soil, thereby using plant properties to restore balance to the soil (Deng et al., 2018). They also have the ability to alter their immediate environment and make it suit their physiological processes (Halverson, 2014). Since bacteria are important in controlling the soil habitat, it is therefore essential to study the biological diversity of these bacteria as regards climatic factors and land use pressures which have led to various researches by the scientific community and policymakers (Griffiths et al., 2016).

Due to the complexity of the soil and the diversity of microorganisms present therein, it has been difficult to quantify the composition and function of the different microorganisms present in the soil ecosystem (Xu et al., 2014). Culture methods (liquid or plate) cannot be used to fully study the microbiome of organisms in the soil because they cannot be used to determine the complexity and biotechnological ability of the soil (Nahid et al., 2012). Metagenomics can be used to study the complexity of microorganisms in place of the plate culture methods which were previously used to culture individual microbes (Bouhajja et al., 2016), and also to study the phylogenetic properties of diverse microorganisms and how they adapt in the soil microbiome (Pushpanathan et al., 2014). There are several approaches to high through-put sequencing, such as the 16S amplicon sequencing which has been used to quantify and know the distinctive nature of microbes (Sharpton, 2014) and shotgun metagenomics which is used to characterize microbes functionally (Hiraoka et al., 2016). High through-put sequencing can also be used to study the co-occurrence patterns of microorganisms (Li et al., 2015). High

through-put sequencing methods have been employed by food microbiologists to monitor the changes in the microbial quantity during the storage of fresh foods (Ercolini, 2013). The 16S amplicon sequencing was used to reveal the dominant microorganisms in a thermophilic soil ecosystem in North India (Bhatia et al., 2015). High through-put sequencing was also employed in the first study of biodiversity involving families and genera of bacteria in agricultural soils (Wolińska et al., 2018).

South Africa has a wide and abundant distribution of soil per square meter, but the availability of soil suitable for farming is limited, due to the low rainfall levels (Eijsackers et al., 2017). In a report released by the FAO, South African soils are degrading and there is a reduction in agricultural crop output, the proportion of degraded soil to non-degraded soil is about 2:3 (Sithole et al., 2016). Several factors are responsible for land degradation; these are, anthropogenic activities, land use, climate and other environmental factors. These activities have been known to impact negatively on the diversity of soil microorganisms (Ollivier et al., 2011, Chen et al., 2012, Van der Putten, 2012, Araújo et al., 2014).

Soil microbial activity determines the overall usefulness of the soil (bioremediation), as microorganisms have the ability to withstand sudden environmental and climatic changes that could take place in the soil (Khan et al., 2018). In order to get the consolidated benefits of soil microorganisms, the organisms have to be studied extensively in relation to their functions, their interactions with other biological organisms and their response to the land use practices as well as soil ecosystem (Sarabia et al., 2018).

Knowledge of the bacterial composition could help prevent the prevailing land degradation presently experienced in South Africa. Important findings could be made comparing the bacterial composition of soils from forests and agriculture, this study however focused on indigenous and commercial forests and specific agricultural locations in South Africa.

The study was carried out using the high throughput sequencing method. Data was cleaned using open refine (appendix 1) and data analysis was carried out using the microbiome analyst and PAST platform.

1.2 Problem Statement

There is an ever growing need to seek novel ways to improve agricultural productivity. As the demand for food continues to rise due to a constant increase in population, it is important for researchers to dig deep into the source of this agricultural produce, which is the soil. Since the soil is one of the most diverse ecosystems (comprising of microorganisms, mesofauna, and macrofauna), emphasis should be placed on studying the soil organisms. Bacteria make up the largest percentage of soil microorganisms; research on the peculiarity of bacteria to their environment (land use) is continuous. In addition, the effects of environmental factors (climate change, soil degradation, greenhouse gas (GHG) emissions, nutrient leaching) on these organisms have not been completely studied.

This research uses High throughput sequencing methods to assess and compare bacterial diversity in natural and commercial forests, and agricultural soils. To ascertain soils likely to be degraded in each of these land uses, factors that are responsible for the bacterial abundance, and pin point important bacterial classes that have been discovered, their importance and roles in agricultural production.

1.3 Research Hypothesis

A study of the bacterial diversity of agricultural, commercial and natural lands could give important insight into the factors responsible for possible land degradation and may provide possible solutions to degraded agricultural lands. We hypothesize that the natural/indigenous lands will have more bacterial diversity than the agricultural lands.

1.4 Research Questions

1. Does soil bacterial composition change with land use?

2. Are there peculiar soil bacteria, which could serve as indicators to degrading land?
3. Does soil bacterial diversity give a clear picture of soil health and quality?
4. What are the implications of soil with low bacterial abundance and diversity?
5. What are the factors responsible for bacterial abundance and diversity?

1.5 Research Aim

The research aims to study the bacterial diversity of South African soils obtained from agricultural and forest lands.

1.6 Research Objectives

The research objectives include:

⇒ To determine the bacteria present in the soils obtained from agricultural and forest soils samples using metagenomics;

⇒ To evaluate the abundance and diversity of bacteria obtained from each of the soil samples and how this could infer land degradation;

⇒ Determination of bacterial richness in the different soil samples obtained

Keywords: metagenomics, soil quality, land use, bacterial diversity, soil microbiome

CHAPTER TWO

2.1 Soil Quality Indicators; Their Correlation and Role in Enhancing Agricultural Productivity (Review Paper)

Abstract

Soil with its intrinsic properties is of great importance in ecosystem functioning and stability, restoration of degraded land and sustained food production. Assessing these soil properties is key to maintaining the health of the soil and proper ecosystem functioning. However, the choice of suitable soil assessment instruments has been difficult due to the various factors required for the assessment such as soil type, land use, the analysis involved and cost of the assessment. This review focuses on the different categories of soil quality indicators, the importance of soil quality to ecosystem services, the more suitable and cost-effective indicator, the criteria for choosing the best soil quality indicators and the role of microorganisms, how their diversity can determine the quality of the soil with respect to land degradation. Also, factors that could affect the indicators and the role that soil properties play in assessing soil quality. The interrelatedness of the various soil quality indicators was also reviewed and the best choice of soil quality indicators.

Keywords: soil capacity, soil fertility, land degradation, soil quality indicators, soil quality assessment, soil function

2.2 Introduction

Soil consists of a bulky and intricate microbiome and as a result, can be referred to as a very significant contributor to the earth's microbial distribution. Therefore, soil provides biological services that are necessary for normal life functions on earth. Some of these functions are the provision of arable land suitable for agriculture, where food, feeds, fibre, and biological energy are produced. Soils also help preserve the biodiversity of plants, preserve drinking water through the ultra-filtering process, they serve as protectors from erosion, as well

as carbon-dioxide sinks (Schloter et al., 2018). The soil contributes to human basic needs such as food, water and air (Keesstra et al., 2016). It is therefore imperative to sustain and maintain the quality of soil as this could help solve societal issues such as food security, biodiversity and water resource preservation (Mol and Keesstra, 2012).

Soil quality was in earlier times defined as the ability of soil to sustain crop production, this was however found to be too restrictive as it focused only on the production aspect of soil. Scientists, therefore, incorporated the importance of soil in maintaining the quality of the environment and its importance in sustaining life (Doran and Zeiss, 2000). Soil quality is one of the three major components that contribute to the quality of the environment, while the other two are air and water quality. The quality of water and air are defined majorly by the degree to which they are polluted and how this pollution directly affects humans and animal health and consumption as well as its effects on the natural environment (Bünemann et al., 2018). However, the quality of soil is not limited by the degree to which it is polluted but can be broadly defined as the ability of soil to function within natural or maintained ecosystem confines, to enhance plant and animals output, to sustain and improve the quality of air and water and as well support the health of plants and animals (Doran and Zeiss, 2000). This definition gives a broad perspective of the soil quality. Unlike water and air quality, the quality of soil is composed of the solid, liquid and gaseous phases and more importantly, its usefulness in diverse areas.

Broad soil function can also be seen in the environmental description of soil, which is the ability of soil to enhance plant growth, infiltration and partitioning of precipitation regulation thereby protecting watersheds, acting as buffers to substances that could cause pollution such as chemicals applied during agriculture, organic waste materials and wastes from industries (Bünemann et al., 2018). Predicting soil quality requires taking into consideration the quality and quantity of the biological community components and also taking

into account the different patterns, occurrences, and importance of the environmental processes (Bünemann et al., 2018, Vincent et al., 2018). Soil quality can be assessed for both agricultural ecosystems, where the primary aim is (but not limited to) production, and the natural ecosystem, which is concerned with the preservation of biological diversity and maintaining the quality of the environment.

External influences such as climate change, soil texture and structure, land topography and hydrological processes could affect the soil property values such that it is impossible to establish absolute standard uniform values that are applicable universally. It is therefore imperative that when assessing soil quality, a baseline or reference value be included so that management effect is adequately monitored (Bünemann et al., 2018). The response of soil to environmental changes and management practices is often slow and not easily detected until irreversible damages are done (Nortcliff, 2002).

In order to avert this, it is important when carrying out a soil quality assessment to identify the sensitive soil properties that show the ability of soil to function and are good indicators of soil quality. Soil quality management is limited as it only has short term effects on soil properties such as the soil texture and mineral composition and therefore there is a need for biological soil indicators. The differences between static and manageable soil properties are not well defined and depend on various factors (Schloter et al., 2018). The historical description of soil quality shows that it originated from two different approaches that emphasize either the soil property or on anthropogenic activities (Bünemann et al., 2018). Soil ability is defined as the inherent characteristic of soil to contribute to environmental processes, including the production of biomass (Bouma et al., 2017). Emphasis on intrinsic and static soil properties is connected mainly to soil taxonomy (Bünemann et al., 2018).

Soil degradation refers to the gradual depletion of the soil's biotic and abiotic properties, it has also been defined as the reduction in the overall usefulness of the soil. Soil degradation is influenced by several factors, namely climate, land use and anthropogenic activities. All these factors contribute to the reduction of microbial productivity and diversity in the soil, which influences crop output (Mohamed et al., 2019)

Soil degradation is on the increase due to the increased intensity of anthropogenic activities as a result of agricultural activities and land use practices (Levin et al., 2017). Amongst the six major issues to be addressed by the United Nations is land restoration, which is one of the sustainable development goals (Keesstra et al., 2016). For soils that have high contamination and toxicity risks, remediation is compulsory and monitored by regulatory policies. When the soil contamination is low or moderate, the soil is mainly underutilized and unmanaged (Cundy et al., 2016). As the world population is increasing, there is an increased need for food production, which results in the use of those lands that have previously been underutilized for food and biomass production (Lord, 2015).

Soil quality management has raised a lot of controversy as its earlier definition was focused on the management of soil quality in specific crop productions. From a microbiologist perspective, soil quality management can be identified as the maintenance of the soil's capability of sustaining useful microorganisms in order to meet ecosystem demands, which includes maintaining microbial diversity in the soil, proper water and temperature regulation and balance suitable for microbial diversity. When these qualities are maintained in the soil, it could help prevent soil degradation, thereby improving crop productivity.

Assessment of soil quality provides useful tools required to properly manage the soil resource, taking into consideration how soil products can meet the demands of society. It therefore implies that soil quality and environmental services provided by the soil are related.

Another advantage of soil quality is that it creates awareness and promotes communication between soil users as regards the importance of soil (Karlen and Stott, 1994). The purpose of this review is to identify the terms used to describe and assess soil quality, the approaches to soil quality management, indicators of soil quality and the role of microbial diversity in predicting the quality of soil to avert land degradation.

2.3 Soil Quality Assessment

When describing the soil, various concepts are used. These are soil fertility, soil quality and health and soil capability, all of which are important in soil quality assessment. Man's interest in soil was borne out of the need for food through agriculture and the extraction of minerals. A soil with good quality is said to be capable of maintaining its physical properties even in harsh climatic conditions. These physical properties comprise of the soil aggregate stability, bulk density, water retention and conductivity, soil moisture content and water infiltration (Nouri et al., 2019).

The soil physical properties make up the soil structure which is important in maintaining a sustainable environment, soil productivity, and resilience. However, changes in the soil structure are not immediate and may take a long period of time before effects of changes in climatic factors and anthropogenic activities are observed in the soil. This is because the physical soil properties contribute to the soil resistance to environmental factors by being able to absorb and allow the penetration of water quickly. They also supply the soil water in times of heat thereby regulating the soil temperatures. They therefore, prevent losses that could occur during harsh changes in climatic conditions (Reynolds et al., 2014, Nouri et al., 2019). Although changes in soil structure are not immediate, they can, however, be improved upon. One of the ways in which soil structure has been improved upon is the use of cover cropping. This system helps reduce stress on soil associated with low organic matter input which could cause erosion and compactness, which could eventually cause low crop yields (Nouri et al.,

2018). The use of cover crops also improves the quality of the soil as they preserve water and soil nutrients. They also increase the organic carbon in the soil, which is important in the physical, chemical and biological soil composition (Blanco-Canqui et al., 2011).

Soil quality has broadly been described in terms of the ability of soil to function. The ability of soil to provide the essential nutrients and water capable of supporting plant growth in the absence of toxins is termed soil fertility, such soil is the preferred soil for agricultural productivity (Patzel et al., 2000). Soil quality can be assessed using the soil organic carbon and soil organic matter as indicators. The soil organic carbon and organic matter play an important role in the movement of water and nutrients in the soil and are important in the carbon cycling globally (Keesstra et al., 2016, Van Hall et al., 2017). Major changes in soil physical properties are caused by changes in the soil organic matter, which is also related to the soil aggregate stability, although regions with soils having similar soil organic matter do not necessarily have the same aggregate stability (Zethof et al., 2019).

Soil health has been defined in the context of a living system capable of hosting diverse living organisms which should be conserved and maintained (Doran and Zeiss, 2000). Unlike soil quality and capacity, soil health is concerned with the biological organisms of soil and their functions and the activities they carry out in the soil. These organisms are vital for plant growth and crop development (Bünemann et al., 2018).

The focus of soil quality is more on the output rate, whereas that of soil health is on the soil and factors that could affect the soil's ability to function. This therefore implies that for the soil to function properly, it has to be of good quality. This incorporates the soil organic matter, organic carbon, aggregate stability and all the factors that could possibly maximize the soil output. A good quality soil has the ability to continuously sustain life (Bünemann et al., 2018).

Although the physical properties of soil are important in assessing the soil health and quality, the microbial diversity is of greater importance. The study of their interactions and adaptability to varying environmental changes will give insights to improving agricultural productivity, this can be seen in the feedbacks between the physical properties and soil organisms (Bünemann et al., 2018).

2.4 Importance of Soil Quality to Ecosystem Services

The soil has only been seen in the past as a crop growing medium. The research focus in this area has helped improve understanding of soil importance to ecosystem services (Rinot et al., 2019). Ecosystem services such as water purification, carbon sinks and the preservation of biodiversity (Fig 2.1) are all processes supported by the soil. Thus, the soil is a critical component in maintaining balance in the environment. However, soil is susceptible to various misuses, which can adversely affect its functioning. The use of chemicals for the control of pests and plant growth in agriculture affects the ecosystem negatively and has led to adverse environmental changes, one of which is land degradation. These changes can only be controlled by discontinuing the use of these chemicals (Syed and Tollamadugu, 2019). Bouma et al. (2017) defined soil capability as the ability of soil to meet the ecosystem requirement and in the production of biomass.

Land degradation affects biodiversity negatively and invariably causes a reduction in agricultural productivity which eventually leads to low food production. It is not caused by sudden changes but by the gradual depletion of soil, which eventually causes a loss in soil productivity (Stocking and Murnaghan, 2000). Land degradation could be caused by several other factors such as climate change and the geographical location (Mohamed et al., 2019), all of which affect the ecosystem services. Soil provides human beings with their essential needs which include clean air and water, and also helps in the regulation of climate for sustaining and

supporting communities (Fig 2.1). It is therefore of great importance to sustain and preserve this natural resource from degradation and pollution (Jeffrey and Achurch, 2017).

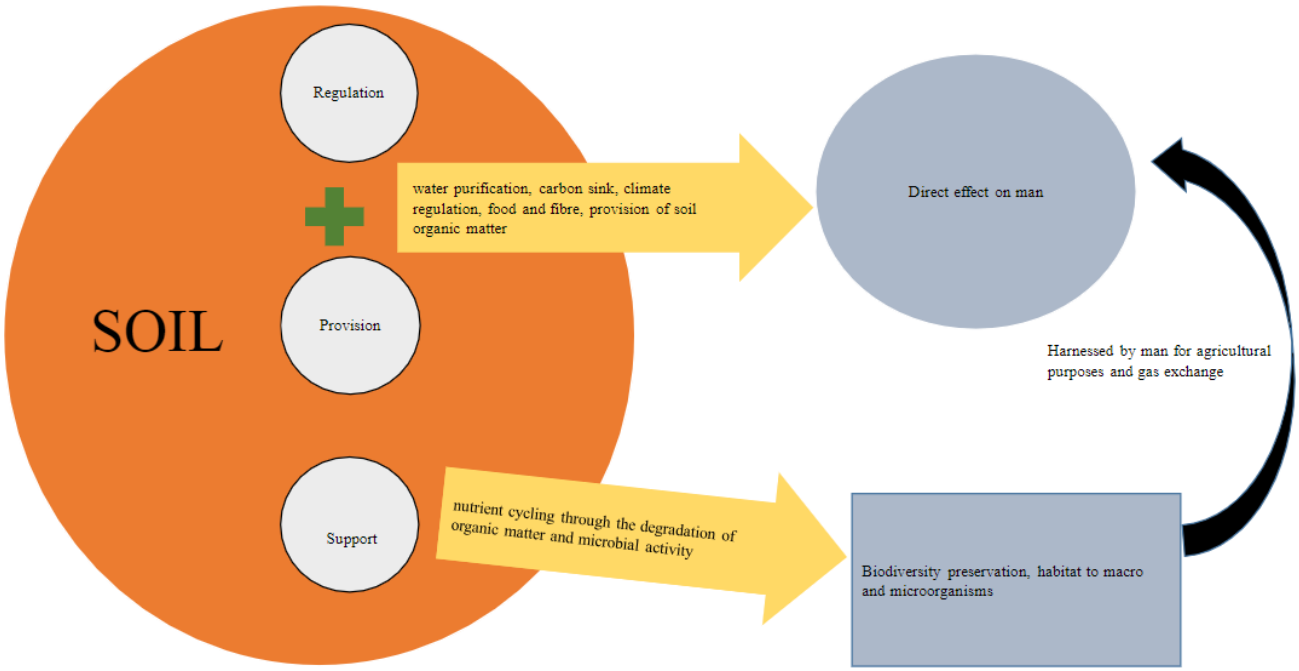

Figure 2.1 Soil Function in the ecosystem
* The soil serves as support, regulation and provision all of which help to maintain ecosystem services.

Ways in which the soil can be protected include, maintaining the soil physical and biological properties, the protection and preservation of microbial diversity, avoiding over tillage and overgrazing, and the use of cover cropping during agriculture (Doran and Zeiss, 2000, Sithole et al., 2016, McBratney et al., 2017).

2.5 Indicators of Soil Quality

Soil indicators are important in ascertaining the quality of the soil. They are picked based on their relevance to soil functions, factors that could affect soil and how they affect ecosystem services. A good soil indicator should be able to detect changes that occur in the soil

as a result of management practices. There are a few limitations when selecting soil quality indicators. They include sensitivity, reliability, ease of measurement and sampling, practical requirements, the capability to differentiate between different soil types and cost of analysis (Idowu et al., 2008, Ritz et al., 2009, Bünemann et al., 2018). Some of these indicators require undisturbed natural environment in order to ascertain their effectiveness, they could also vary as seasons vary. It is therefore important to note the conditions in which soil sample indicators are taken. The indicators can be classified in three main categories, namely physical, chemical and biological indicators.

A lot of studies have been carried out on soil quality indicators, however, none is yet to establish the most effective soil indicator category. This could take a long time as several factors such as seasonal changes, type of plant grown, predominant agricultural practices and any other factors that could possibly affect the soil need to be considered (AbdelRahman et al., 2016). These indicators reflect the changes that occur in the soil due to anthropogenic activities. The physical, chemical and biological indicators interact (Fig 2.2). It is often difficult to separate them due to the fact that they could have direct effects on one another, such as the relationship between the chemical and the biological indicators (Schoenholtz et al., 2000, Ritz et al., 2009).

Since land degradation does not occur as a sudden change but a gradual change in the soil properties as a result of land management practices, a good soil quality indicator should, therefore, be sensitive to slight changes that could occur in the soil. If the indicator is not sensitive enough, there is a chance that changes that could be detrimental to the soil health could occur without any observations made from studying these indicators. They should also be able to provide important information on soil functioning (Paz-Ferreiro and Fu, 2016, Bünemann et al., 2018). Although the most commonly used soil quality indicators are chemical and physical indicators, soil biological indicators have been found to be more sensitive due to

their fast response to environmental disturbances, and are hence better soil quality indicators (Paz-Ferreiro and Fu, 2016).

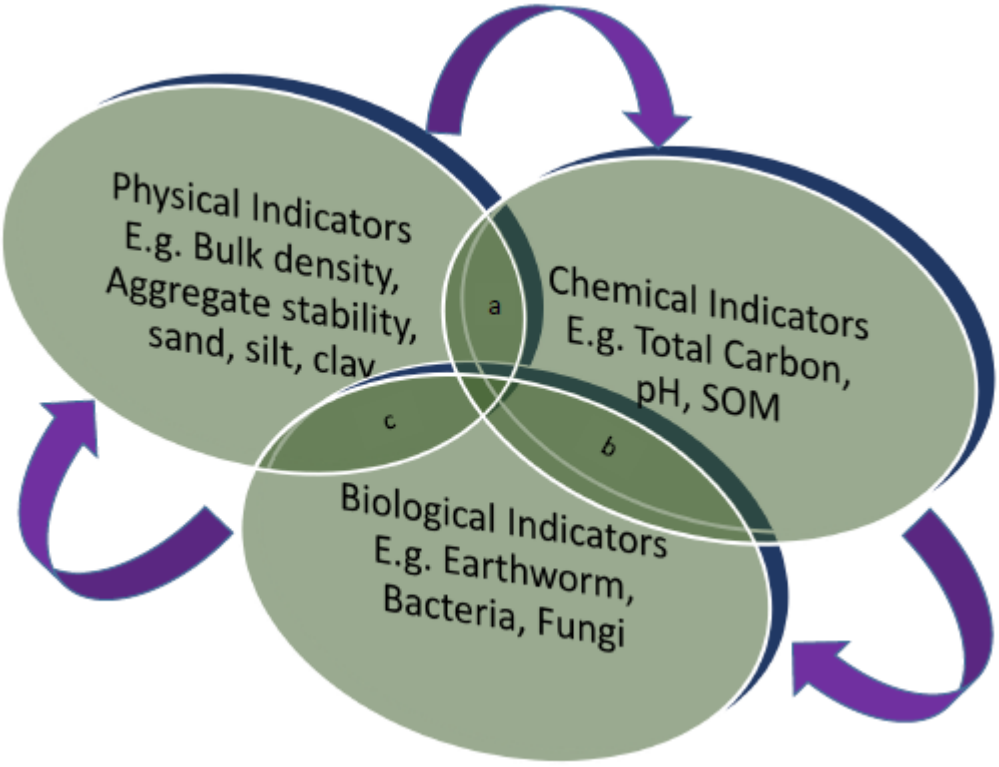

Figure 2.2 Soil Quality Indicators

a- Nutrient composition
b- Soil structure
c- Organic matter content and aggregate stability
* The combination of the physical and chemical indicators (a) determines the nutrient mineralization capacity of the soil as the physical composition of the soil determines the amount of nutrient the soil is capable of accommodating i.e. the more organic matter composition of the soil, the more chemical nutrients the soil is capable of holding. The combination of chemical and biological indicators (b) determines the aggregate stability of the soil. The more biological components the soil contains, the more available the presence of chemical nutrients and the more difficult it is for erosion to take place on the soil as the pores are opened up to retain water. The combination of the soil physical and biological indicators (c) determine the structure of the soil as more biological indicators present in the soil allow for easy water percolation thereby creating more air spaces in the soil.

2.5.1 Physical indicators

Physical soil characteristics such as the promotion of plant root growth, water retention and supply, nutrient recycling, carbon sequestration, exchange of gases and preservation of

biological diversity are important for the growth of plants and trees (Schoenholtz et al., 2000). The addition of organic matter improves the soil physical properties as it makes available key nutrients to the soil which is important for plant growth and ecosystem maintenance (Barus et al., 2019). Physical soil indicators include bulk density, soil type (sand, silt, clay) and the soil aggregate stability. The soil type determines the other physical indicators and is therefore regarded as the most important soil physical indicator (Schoenholtz et al., 2000). A lot of literature shows that the physical indicators are reliable, however, it is not well understood how these indicators show the degree of land degradation (Table 2.1) and the sensitivity of these indicators to slight changes that occur in the environment.

Soils rich in organic matter tend to have better physical soil properties such as increased water retention ability, which is capable of protecting the soil against erosion. The natural lands (forests) tend to have higher aggregate stability than other lands with anthropogenic activities (Table 2.1). This could be as a result of high deposition of organic matter on the soil surface which is acted upon by soil-dwelling organisms (micro and macro-organisms) (Boyle and Powers, 2013).

Table 2.1: Physical soil indicators and results obtained from different land use practices

Soil quality indicator	**Land use description**	**Result**	**Reference**
Dry bulk density (Mgm^{-3})	Natural Forest	1.19	(Tesfahunegn, 2014)
Dry bulk density (Mgm^{-3})	Plantation and protected area	1.35	(Tesfahunegn, 2014)

Dry bulk density (Mgm^{-3})	Grazed land system	1.4	(Tesfahunegn, 2014)
Dry bulk density (Mgm^{-3})	Uncultivated land	1.76	(Tesfahunegn, 2014)
Aggregate stability of soil (%)	Natural forest	54.7	(Tesfahunegn, 2014)
Aggregate stability of soil (%)	Plantation and protected area	53.3	(Tesfahunegn, 2014)
Aggregate stability of soil (%)	Grazed land system	49.0	(Tesfahunegn, 2014)
Aggregate stability of soil (%)	Uncultivated land	26.3	(Tesfahunegn, 2014)
Sand (%)	Agricultural Land	15-60	(AbdelRahman et al., 2016)
Silt (%)	Agricultural Land	1.4-40.1	(AbdelRahman et al., 2016)
Clay (%)	Agricultural land	2.2-60	(AbdelRahman et al., 2016)
Sand (%)	Semi-Natural land	42	(Zethof et al., 2019)
Sand (%)	20 year afforested land	33	(Zethof et al., 2019)
Sand (%)	Cereal Agricultural Land	39	(Zethof et al., 2019)
Silt (%)	Semi-Natural land	43	(Zethof et al., 2019)
Silt (%)	20 year afforested land	49	(Zethof et al., 2019)

Silt (%)	Cereal Agricultural Land	46	(Zethof et al., 2019)
Clay (%)	Semi-Natural land	15	(Zethof et al., 2019)
Clay (%)	20 year afforested land	17	(Zethof et al., 2019)
Clay (%)	Cereal Agricultural Land	15	(Zethof et al., 2019)
Sand ($100g^{-1}$)	Agricultural land	33.0	(Bonfante et al., 2019)
Silt ($100g^{-1}$)	Agricultural land	40.6	(Bonfante et al., 2019)
Clay ($100g^{-1}$)	Agricultural land	26.4	(Bonfante et al., 2019)
Bulk density (Mg m)	Agricultural land (cover cropping system)	0.32	(Nouri et al., 2019)
Bulk density (Mg m)	Agricultural land (no tillage)	<0.00	(Nouri et al., 2019)

2.5.2 Chemical indicators

Due to the interconnectivity of the different categories of soil quality indicators, it is difficult to clearly distinguish the soil quality in terms of its physical, chemical and biological properties. Chemical processes carried out in the soil have an effect on the microbiological processes that take place in the soil; such as the cycling of nutrients, water availability, supply and retention, carbon storage and supply, all of which strongly affect biological activities.

Chemical indicators are most often used when describing soil nutrient availability and can, therefore, be referred to as key components in the supplied nutrient (Powers et al., 1998, Schoenholtz et al., 2000). Soil chemical indicators such as carbon (C), nitrogen (N), potassium (K), phosphorus (P), pH, calcium carbonate ($CaCO_{3)}$, often involve assays which could be costly, although the presence or absence of some of these chemical indicators and their measure could give insights into the overall health of the soil (Schoenholtz et al., 2000). In soils with high organic matter, content tends to be richer in nutrients and more suitable for agricultural purposes. However, persistent tilling of the soil for agricultural purposes could lead to the loss of this organic matter (as seen in Table 2.2). In order to fully understand the effects of these chemical indicators, they must be compared with the biological indicators. This is because it is difficult to understand the effect these chemical indicators could have on the overall health of the soil.

Table 2.2: Chemical soil indicators and results obtained from different land use practices

Soil quality indicator	**Land use description**	**Result**	**Reference**
pH	Agricultural Land (Aeolian plain)	6.81 – 8.23	(AbdelRahman et al., 2016)
$CaCO_3$	Agricultural Land (Aeolian plain)	1.11 – 11.59	(AbdelRahman et al., 2016)
Soil Organic Matter	Agricultural Land (Aeolian plain)	0.2 – 2.5	(AbdelRahman et al., 2016)
pH	Agricultural Land (flood plain)	7.1 – 8.8	(AbdelRahman et al., 2016)
$CaCO_3$	Agricultural Land (flood plain)	0.59 – 4.76	(AbdelRahman et al., 2016)

Soil Organic Matter	Agricultural Land (flood plain)	0.11 – 2.95	(AbdelRahman et al., 2016)
pH	Semi-Natural Land	7.72	(Zethof et al., 2019)
pH	20 year afforested land	7.76	(Zethof et al., 2019)
pH	Cereal Agricultural Land	7.97	(Zethof et al., 2019)
Soil Organic Matter	Semi-Natural Land	58.1	(Zethof et al., 2019)
Soil Organic Matter	20 year afforested land	56.1	(Zethof et al., 2019)
Soil Organic Matter	Cereal Agricultural Land	27.5	(Zethof et al., 2019)
Total Carbon content	Semi-Natural Land	91.6	(Zethof et al., 2019)
Total Carbon content	20 year afforested land	82.2	(Zethof et al., 2019)
Total carbon content	Cereal Agricultural Land	82.5	(Zethof et al., 2019)

2.5.1 Biological indicators

The soil biological indicators are relevant in many ecosystem services carried out in the soil (Fig 2.1) which include nitrogen fixation, nutrient recycling, gas exchange and many processes that are beneficial to people, animals, and plants (García-Orenes et al., 2012). The soil microbiological, biochemical and biological properties can be used to describe the soil efficiently in relation to soil health, function and degradation (Paz-Ferreiro and Fu, 2016). The soil biological indicators give easy room to infer the effect of alterations that must have taken place in the soil, because the abundance and diversity of these communities vary greatly in

different circumstances. The soil organisms are the living components of the soil and are affected by both the physical and chemical indicators. They complement the soil physicochemical characteristics and are hence referred to as the most effective indicators of soil quality (Ritz et al., 2009).

Table 2.3: Biological soil indicators and quantity/counts obtained from different land use practices

Soil quality indicator	Land use description	Quantity/Count	Reference
Earthworm	Natural Forest	11	(Tesfahunegn, 2016)
Earthworm	Trees plantation and protected land	7	(Tesfahunegn, 2016)
Earthworm	Open grazed land	4	(Tesfahunegn, 2016)
Earthworm	Overgrazed land	0	(Tesfahunegn, 2016)
Microbial mass	Vegetative land	34.5 ± 9.1	(Muñoz-Rojas et al., 2016)

Microbial mass	Uncultivated bare land	19.3 ± 1.9	(Muñoz-Rojas et al., 2016)
Fungi	Vegetative land	23.4 ± 8.1	(Muñoz-Rojas et al., 2016)
Fungi	Uncultivated bare land	5.1 ± 1.8	(Muñoz-Rojas et al., 2016)
Actinobacteria	Vegetative land	2.5 ± 0.6	(Muñoz-Rojas et al., 2016)
Actinobacteria	Uncultivated bare land	0.8 ± 0.1	(Muñoz-Rojas et al., 2016)
Pseudomonas	Vegetative land	0.6 ± 0.0	(Muñoz-Rojas et al., 2016)
Pseudomonas	Uncultivated bare land	0.3 ± 0.0	(Muñoz-Rojas et al., 2016)

Soils with increased organic matter content are usually found to be rich in micro, macro and mesofauna as well as microorganisms. These organisms are important for decomposition processes as they are actively involved in the breakdown of complex organic molecules and the release of vital chemicals and nutrients which are useful for plant growth (Barus et al., 2019). Due to the complexity of the adaptive features of the biological indicators, they incorporate different soil processes in unique ways that other soil quality indicators do not. The wide distribution and diversity are one of the ways in which these biological indicators adapt to different soil environments (Doran and Zeiss, 2000, Ritz et al., 2009). The biological indicators can easily be monitored (Table 2.3) and the effect of environmental changes observed, without necessarily incurring too much cost, as they can be measured quantitatively

(Table 2.3) and are identified according to their species, which does not incur high costs of chemical reagents and assays (Tesfahunegn, 2016).

2.6 Roles of Microorganisms in Soil Quality Assessment

Microorganisms are important components of the soil and the ecosystem. They are actively involved in nutrient cycling, degradation of organic matter and protection of plants from harmful chemicals released in the soil.

Microorganisms are sensitive to changes in the soil and are generally diverse in nature. They have been used in the assessment of degraded lands and their diversity and abundances change as the quality of soil changes (Muñoz-Rojas et al., 2016, Li et al., 2019). Microorganisms have different domains the most studied of which are the bacterial, fungal and archaeal domains. Bacteria have the highest biological diversity in the soil, most of which are unculturable. This group of microorganisms grow and multiply at a very fast rate, especially when the soil environment is conducive. They have been found to be richer in soils with high nitrogen (N), phosphorus (P) and soil organic matter (Kaiser et al., 2014). Fungi unlike bacteria, have lower nutrient requirements and have the ability to efficiently use carbon in a substrate with low carbon content (Keiblinger et al., 2010). The differences in the nutrient requirements of bacteria and fungi vary and this can be used when monitoring the overall microbial community. Important inferences have been made from studies carried out on the microbial community with focus on the bacterial and fungal communities such as the effects of increase and decrease in nutrients such as nitrogen (N), Polycyclic aromatic hydrocarbons, organic matter amendment, carbon and phosphorus (Bünemann et al., 2018, Tian et al., 2018, Li et al., 2019, Mohamed et al., 2019)

Some bacterial groups which reside in the rhizosphere of plants have the ability to promote plant growth. These groups are referred to as plant growth promoting rhizobacteria

(PGPR) and they maintain the growth of a plant and are beneficial to plant growth as they contribute to the soil health and quality (Kumar and Verma, 2019). Microorganisms are important when ascertaining the quality of the soil as they play an important role in the degradation of complex soil organic matter, recycling of nutrients, conversion of wastes from plants and animals to useful minerals and making available required nutrients for plant growth (Gagelidze et al., 2018). This is necessary for agriculture for food production. However, in order for these microorganisms to function, there should be the presence of organic matter/litter on which they can act. An increase or decrease in organic matter leads to an increase or decrease in microbial diversity (Wu et al., 2008, Ponge et al., 2013). This means the organic matter composition directly affects the microbial composition of the soil, which in turn affects the soil quality.

Since microorganisms are important in the cycling of essential plant nutrients, the adaptation of plants to sudden environmental changes and in fighting pathogens that could affect plants, they can help boost agricultural productivity if the soil favours their proliferation. This can help curb the use of fertilizers, which has been known to have an adverse effect on soil when applied continuously. Also, instead of using pesticides on agricultural soil, microorganisms have been found to prevent certain plant diseases (Tsiafouli et al., 2015, Li et al., 2017a).

In addition, the study of the microorganisms present in the soil gives important insights into the overall health of the soil and its ability to function adequately. A reduction in microbial diversity reduces the ability of soil to function normally, as well as its resistance to major environmental changes such as erosion (Aksoy et al., 2017).

2.7 Conclusion

In measuring the quality of soil, the physical, chemical and biological indicators are of great importance. However, the biological indicators are most important and more studies should be carried out on the factors that could help understand the changes that could occur among the community composition of these biological indicators in the soil.

2.8 Acknowledgements

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CHAPTER THREE

3.1 Bacterial Diversity and its effect on Agricultural Productivity (Review Paper)

Abstract

The soil is of great economic importance in the terrestrial biosphere as it is actively involved in many processes of life and most importantly, maintaining a balance in the ecosystem. The soil has the most diverse microbiome, bacteria being the most abundant and widely distributed microbe. Bacterial diversity is an important factor when determining soil quality as it helps retain the stability and functionality of the soil. Bacteria possess beneficial characteristics which can be useful as biofertilizers, biocontrol and bioremediation agents. However, these qualities have not been fully harnessed. Development and advancement in technology such as the High-throughput sequencing methods, has helped in understanding the functions and patterns of interaction observed in the soil microbiome. This review focuses on how factors such as soil texture and structure, physiochemical properties, termite activities, and land use practices affect microbial diversity in the soil, with emphasis on bacteria and the most suitable methods for bacterial diversity analysis.

Keywords: Bacteria evolution, Deforestation, Metagenomics, Microbial diversity, Soil microbiome, Phylogenetic analysis

3.2 Introduction

Soil may be defined as a complex and biologically diverse ecosystem capable of supporting life (Delgado and Gómez, 2016). Its surface and sub-surface regions promote the survival and prevent the extinction of most organisms living on the planet (Doran and Zeiss, 2000). It can be referred to as a 'living system' (Delgado and Gómez, 2016) whose function is not just the production of food and fibre, but it is also of great importance in maintaining environmental quality locally, as well as on a regional and global scale (Doran and Zeiss, 2000).

It is a living ecosystem because it is home to about a quarter of all species of living organisms on the planet (Aksoy et al., 2017). These organisms, the microfauna, macrofauna and mesofauna, are important components of the soil, they are involved in processes such as soil aeration, nutrient cycling, and organic matter degradation. The soil microfauna make up an integral part of the soil complex with bacteria being the largest by total population and diversity (Gagelidze et al., 2018). The quality and health of soil depend on the abundance and diversity of this microfauna which can often be influenced by the soil texture and other environmental factors. The evolution of these soil organisms is only possible due to the favourable ambient environment provided by the soil. Therefore, it can be inferred that some soil organisms may not be found in any other regions except those that promote their multiplication and are suitable for their habitation.

In agriculture, soil bacteria can be used to determine and predict the fertility and productivity of the soil; these organisms are concentrated in the rhizosphere of plants and the upper 0-20cm of the soil surface. Several studies carried out on soil microbial populations have shown that these organisms carry out important functions such as the cycling of nutrients, providing support for plant growth, purification of water and generally maintaining a balance in the soil ecosystem (Vos et al., 2013).

Bacteria being the most abundant and diverse microorganisms in the soil is of great importance, as studies on certain strains of bacteria, called plant growth promoting rhizobacteria (PGPR), have shown that these organisms are capable of promoting plant growth and preventing certain plant diseases caused by harmful microbes that could be found in the soil (Babalola, 2010). However, these groups of organisms are largely underexplored due to poor knowledge of how they interact with other organisms, their functions, and factors that could limit or promote their presence. Although high-throughput sequencing methods have been used to study bacteria, very little is known of the effects of environmental factors and soil

properties on these organisms. Owing to their abundance and diversity, bacteria form different communities whose functions vary according to the species present and their abundance. These communities can be formed in response to soil pH, texture, organic matter presence, activities of other organisms and the type of plant present in the soil. However, certain changes in the soil microhabitat can affect these bacterial communities, which in turn could affect the overall functioning of the soil; this can be referred to as soil degradation. These changes capable of causing variation and reduction in the microbial community composition must be persistent and be able to cause changes in the individual organisms present in the soil. Not all disturbances in the soil affect the microbial communities, because there is usually an increased number of individual species performing the same function in diverse communities which are interrelated (Jurburg et al., 2018).

Soil degradation is a gradual process that takes place when the microbial population and diversity of the soil reduces due to changes caused by poor human management practices such as intensive grazing, use of pesticides, industrial pollution and climatic factors (Aksoy et al., 2017).

This review discusses the evolution of bacteria, and the importance of bacterial diversity in sustainable agriculture, as well as the effects of soil texture, soil physicochemical properties and land use on bacterial diversity, and how all these are linked to land degradation and agricultural productivity. Also, the methods of improving soil microbial population and diversity for agricultural purposes are discussed as a possible means of providing a conducive environment void of human disturbances and providing the needed bacterial population and diversity suitable for agricultural food production.

3.3 Soil Microbiome

The soil microbiome or microbiota is an organized group of microbial populations performing various functions. These have been used in recent times to refer mainly to the members of the bacterial domain, owing to recent developments in library generation of databases and computerized tools, the widespread and evolutionary gene pointers. In contrast to the above statement, the soil microbiome consists of all microorganisms present in the soil (Knight, 2016) in relation to their environment. It is an important aspect of the soil as it comprises all the microorganisms that can be found in the soil. These microorganisms comprising different species, work in close collaboration as part of different communities to perform various tasks. Some of these tasks include nutrient cycling (carbon, nitrogen, phosphorus) which help to sustain the growth of plants. Sadly, many of these community's beneficial and important functions are threatened by fluctuations due to climate change, soil degradation and poor land use practices (Amundson et al., 2015).

Microorganisms coexist and the interest of researchers has increased in recent times, which can be attributed to the constant growth and improved technologies in sequencing DNA which has made the study of the evolutionary characters of these microorganisms possible (Alori et al., 2017). This advancement in technology has caused a rapid decrease in the cost of microbe DNA sequencing, which has helped scientists expand knowledge regarding evolutionary traits, unlike in the past when these studies were expensive (Knight, 2016). In recent times, the soil microbial community has been employed to reinstate the functions of the ecosystem (Calderón et al., 2017). In order to fully harness the potentials of the soil microbiome, it is important to understand the fundamental factors responsible for their interactions and how these interactions are affected by variable environmental conditions.

Despite technological advancements, gaps as to how microorganisms interact and can be controlled are yet to be filled. Therefore, studies on their molecular patterns and roles are

yet to be fully understood (Jansson and Hofmockel, 2018). The soil microbial ecosystems work in close association with plants and are important determinants of atmospheric carbon in the form of carbon dioxide (CO_2) and methane (CH_4) and their presence or absence in the soil (Crowther et al., 2016). The soil microorganisms, unlike other microorganisms of other ecosystems (water, human digestive tract), evolved in response to stable chemical processes and safe physical environments (Jansson and Hofmockel, 2018). The richness of the soil microbiome gives room for scientists to explore and exploit the potentials of the metabolic processes carried out by the soil microorganisms for biofuels (Ling et al., 2015) and other biologically produced products useful as substitutes for chemical processes.

3.4 Insights into the Soil Microbiome

Various studies have been carried out in order to ascertain the coexistence of microbes in the soil, one of which is the study of the genomic component of the microbe, significant changes in their diversity and population (Ling et al., 2015, Knight, 2016). An example of these insights into the soil microbiome is the experiment carried out using uncultured bacteria for antibiotic purposes to annihilate pathogens with antibiotic resistance traits (Ling et al., 2015). Also, a study carried out in China on the soil microbiome was done through the amplification of the 16S rRNA, inferences were made from the interaction and interconnectivity between microorganisms when organic matter and chemical fertilizers were used and the resultant effects of these interactions between microorganisms on the crop yield were measured (Wu et al., 2018). These insights have led to various observations and discoveries such as factors that could affect microbial succession; some of which are pH, soil texture and type, climatic factors and land use practices. These factors have also been known to affect the distribution and diversity of these soil microbes.

3.5 Evolution and Distribution of Bacteria

Awareness of the importance of soil bacteria has increased in recent times and has developed more interest among researchers on this group of organisms. Due to the dynamic and novel characteristics observed in widely studied species of bacteria, researchers are yet to fully understand the correlation of their abundance, functions, and factors that could influence their evolution. One of the greatest problems faced by researchers is the diversity and population of these organisms (most of which are unculturable) in the soil. Several predictions have been made on the relationship between the evolution of bacteria, their prevailing environment, interactions with other soil organisms and their reproducibility. One of the agriculturally important group of bacteria (heterotrophs) are found to evolve together with algae (Ramanan et al., 2016). Previous studies on bacterial evolution suggest that organisms with shorter evolutionary time reproduce faster than those with longer evolutionary times. This could be attributed to the number of DNA replications that takes place within the cell (Weller and Wu, 2015). The Firmicutes, a bacteria phylum, has been researched, and arguments have arisen on the impact on the spore formation of some of the bacterial species and the rate of evolution. Some scientists believe that the spore formation affects the rate of evolution, which implies that it would take a longer time for the spore-forming species to evolve (Maughan, 2007, Weller and Wu, 2015). However, the evolution of microorganisms is only made possible when the surrounding factors are favourable and could also encourage their abundance and distribution.

3.6 Importance of Microbial Diversity in the Soil

Microbial diversity is the measure of the total number of genes with varying functions present in the soil together with their frequency of occurrence and abundance (Aksoy et al., 2017). Microorganisms make up the largest number of living organisms present in the soil, a gram of soil could contain billions of microbial cells (Torsvik and Øvreås, 2002). Only a few

of these cells have been fully explored and utilized. The efficiency and sustenance of plants and animals largely depends on the diversity and dynamism of the microbial community (Doran and Zeiss, 2000, Alori et al., 2017) because these organisms break down organic matter to release useful nutrients in the soil which plants need to grow, and they are also involved in plants' resistance to diseases (Babalola, 2010). Bacteria help the soil retain its stability and functionality when there are sudden environmental changes and/or pollution. The health of soil largely depends on the diversity of microorganisms. Bacteria, one of the key nutrient cycling agents in the soil, are composed of diverse organised communities (Torsvik et al., 1996) which function independently and are undisturbed by sudden (short term) environmental changes (Gagelidze et al., 2018). The abundance, distribution, and diversity of bacteria are largely dependent on the soil texture among other factors.

The ability of soil to provide room to diverse microorganisms could be used as a tool to predict the soil health and quality which in turn could help policymakers and agricultural ecologists seek better ways to preserve the soil habitat. Better management strategies need to be carried out when the soil microbial diversity declines, hence there is a need to further investigate the factors responsible for this decline in microbial diversity.

3.7 Factors Affecting Microbial Distribution in the Soil

3.7.1 Soil texture and structure

Microbial populations are formed in response to the soil characteristics and composition (Chau et al., 2011), these soil characteristics can be found in the soil texture which is a useful determinant of the microbial population while the soil composition can be described as the soil structure. Soil texture covers processes involved in nutrient uptake and chemical element composition while the soil structure represents the layout of organic matter and mineral content of the soil (Delgado and Gómez, 2016). Microorganisms thrive in soils that have a high

capacity to retain water and a large surface area (Hendrix et al., 1998). Studies carried out on the effect of soil texture on bacterial diversity have shown that soils with a higher capacity to retain water had higher bacterial populations due to substrate and water availability (Torsvik and Øvreås, 2002).

The coarseness of soil determines its overall ability of the soil to retain water, that is, the clay, sand, and loam particle content composition. Studies have shown that there is a relationship between the soil moisture and the mud composition with the nutrient interactions (C: N, N: P) of the soil (Tian et al., 2018). Soils with more clay content tend to have higher microbial populations than those with higher sandy particles in all soil types from different regions and land uses (Hendrix et al., 1998).

3.7.2 Soil physicochemical properties

Healthy soil is of great importance in maintaining a balanced ecosystem, but this requires a balanced soil microbiota. One of the ways of regulating this soil microbiota is by determining the prevailing physicochemical properties of the soil (Li et al., 2017a). This is because soil chemical elements are in constant interaction with the microbial constituents of the soil. The physicochemical properties regulate the soil enzymatic activities, respiration and soil biodiversity of the soil microorganisms and are stable over a long period of time (about 10 years) before they are influenced by changes due to land use practices (Liu et al., 2018a). Recent studies carried out on the physicochemical properties of soil have shown that bacteria and archaea showed more sensitivity to chemical changes in the soil than fungi and algae (Massenssini et al., 2015). Also, persistent fertilizer application on soil has been shown to reduce bacterial richness and cause an imbalanced soil microbiota (Li et al., 2017a).

Bacteria communities are actively involved in the cycling of chemicals such as carbon (C), nitrogen (N), and phosphorus (P) which are essential nutrients for plant growth, through

the decomposition of organic compounds found on the soil surface (Uzoh and Babalola, 2018). This could account for the overall nutrient composition of the soil. Therefore, the availability of diverse bacteria in the soil is primarily dependent on the available carbon (C), nitrogen (N) and phosphorus (P) contents (Delgado-Baquerizo et al., 2017). Although all the chemical compounds available and useful for plant growth in the soil are recycled by bacteria, bacterial diversity was detected to be more sensitive to the direct or indirect impact of the increase or decrease in pH (Table 3.1), soil organic carbon, nitrogen and phosphorus more than other soil chemical compounds (Huang et al., 2016, Wakelin et al., 2016).

Table 3.1The effect of pH on the abundance of bacteria in different locations

Bacteria	Abundance (%)	pH	Location	Ref
Proteobacteria	28	above 5.75	*China*	(Jiang et al., 2016)
Bacillus	28	5.3	Western Georgia	(Gagelidze et al., 2018)
Pseudomonas	20	5.3	Western Georgia	(Gagelidze et al., 2018)
Rhodococcus	52	5.3	Western Georgia	(Gagelidze et al., 2018)
Bacillus	32	7.56	Eastern Georgia	(Gagelidze et al., 2018)
Pseudomonas	37	7.56	Eastern Georgia	(Gagelidze et al., 2018)
Rhodococcus	31	7.56	Eastern Georgia	(Gagelidze et al., 2018)
Acidobacteria	28	<2.0	Koiliaris, Greece	(Tsiknia et al., 2014)

Actinobacteria	0.0013	<2.0	Koiliaris, Greece	(Tsiknia et al., 2014)
Alphaproteobacteria	0.02	<2.0	Koiliaris, Greece	(Tsiknia et al., 2014)
Bacillus	42	7.4	Eastern Georgia	(Gagelidze et al., 2018)
Actinobacteria	0.548	0.56	Czech Republic	(Sagova-Mareckova et al., 2015)
Acidobacteria	19.1	3.6-3.7	Natural Chaecyparus Forests	(Lin et al., 2011)

3.7.3 Land use practices

Africa's sub-Saharan region is faced with increased food insecurity due to the high degradation of the earth's surface (Sithole et al., 2016). Soil degradation is a process involved in the gradual reduction in soil properties caused by processes which deplete the biotic and abiotic soil components. Various studies have been carried out on the effect of different land use practices (deforestation, intensive grazing and agricultural practices) (Fig 3.1) and results obtained from research have shown that land use practices strongly affect the soil properties if the land is a natural or managed forest (Moghimian et al., 2017). These processes affect the microbial composition, soil physicochemical properties and soil structure over a long period of time. An example of this can be seen in the studies carried out on sustainably managed land and intensely managed land (Jurburg et al., 2018), in which the intensely managed land was found to be more prone to drought while the sustainably managed land was not. In another research on the impacts of conservation agriculture and conventional tillage, the conservation

agricultural practice reduced the pressure on agricultural land caused by intensified soil tilling while the conventional tilling system was observed to cause soil degradation over time (Sithole et al., 2016).

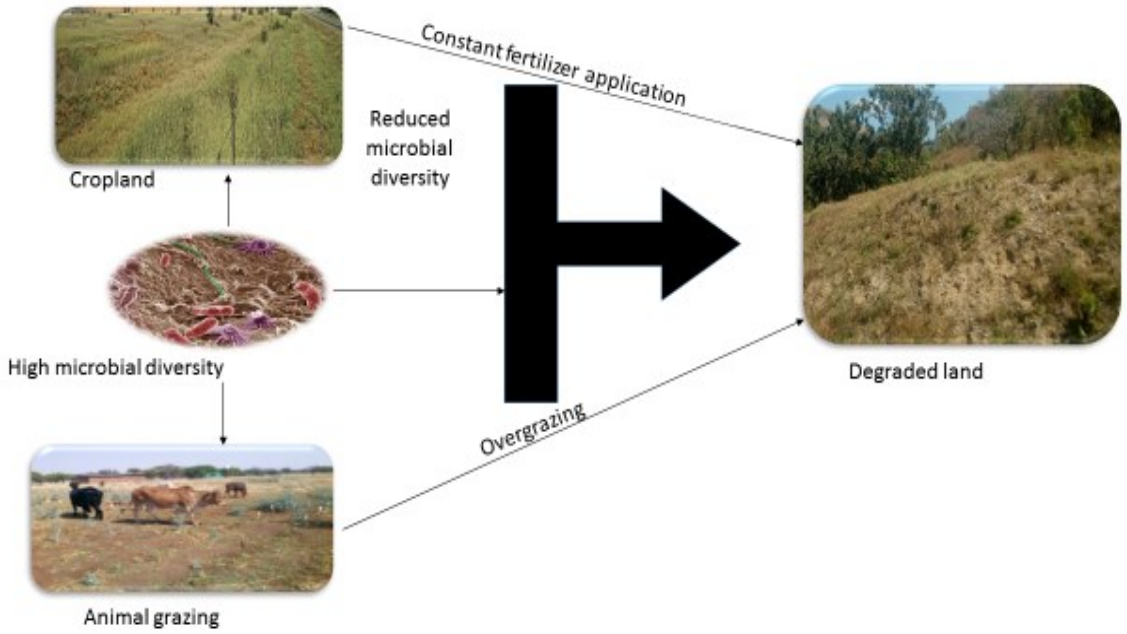


Figure 3.1 The effects of excessive grazing and constant fertilizer application on the soil microbial diversity and land. A land high in microbial diversity has more nutrients which promote the growth of crops and can be used for agriculture. However, with poor land management practices (constant fertilizer application and overgrazing), there can be a reduction in the microbial diversity which could eventually lead to land degradation.

In a bid to provide food capable of sustaining the rapid increase in population, several processes have been included in agriculture in order to increase crop yield and output. Soil health and quality is of great importance in agriculture, soil biota are involved in various processes (such as nutrient cycling and decomposition of organic matter, they are also important in protecting the plants from diseases and sudden changes which may harm the plant's productivity thereby maintaining the stability in the ecosystem which can be used to determine the overall quality of the soil (Ponge et al., 2013). However, these human perturbations increase the stress imposed on the soil habitat which can be experienced over a long period of time (Chakraborty et al., 2011). The soil ecosystem is often directly and

sometimes inadvertently affected by human activities which alter the bacterial community diversity and occurrence (Wu et al., 2008). Processes involving the application of manure, organic matter amendments and application of fertilizers, (Fig 3.1) have been shown in various studies to cause a change in the natural soil microfauna, for example the study carried out by (Wu et al., 2008) showed a decline in the bacterial diversity when an organically managed land was compared to a perennial land. Also, processes such as tillage and grazing animals were found to affect fungal diversity (Wu et al., 2007). Ploughing, intensive tilling, and overgrazing adversely affect the soil structure which gives room to erosion, soil degradation, and reduced soil fertility (Doran and Zeiss, 2000, Abiven et al., 2009, Sithole et al., 2016, Wakelin et al., 2016). Several methods have been adopted to ascertain the effects of human activities on the soil microbial population and diversity (Fig 3.1) and how such factors affect also the abiotic components of the soil over time. Effort to reduce the effects of these disturbances caused by human activities are ongoing. The fingerprinting method was used in analysing the bacterial diversity before, during and after the soil disturbance, the result was a reduction in the microbial population which became more significant with time (Wu et al., 2008, Chakraborty et al., 2011). In order to restore and maintain important microorganisms, it is important to protect the natural environment.

3.7.3.1 Forest soils and their importance

Forest soils differ according to their geographic region, and the type of forest depends on the prevalent soil properties. Changes in the soils of the forest are continuous due to activities such as root decay, decomposition of plant and animal residues and the activities of macro, micro, and mesofauna (Fig 3.2) (Boyle and Powers, 2013), hence forests are referred to as reservoirs of carbon, since the activities of these organisms make available deposits of carbon from decomposition (Lladó et al., 2017b). The trees of the forest are often characterised by their extensively deep roots, high litter composition of the soil surface and high organic

matter content which differentiates them from lands used for agriculture and pasture lands (Boyle and Powers, 2013). They are often disturbed by insect outbreaks, fires, blowdowns and some anthropogenic activities (poor management systems, environmental pollution, and climate change). Decomposition is carried out by microorganisms such as bacteria and fungi whose diversity and evenness is highly dependent on the nature of the topsoil (Amoo and Babalola, 2017) (Fig 3.2). Factors which determine the abundance of these organisms are the pH and C: N ratio of the soil (Högberg et al., 2007, Gauthier et al., 2015). Bacteria are said to be more susceptible to low pH ranges while fungi can survive low pH (Högberg et al., 2007).

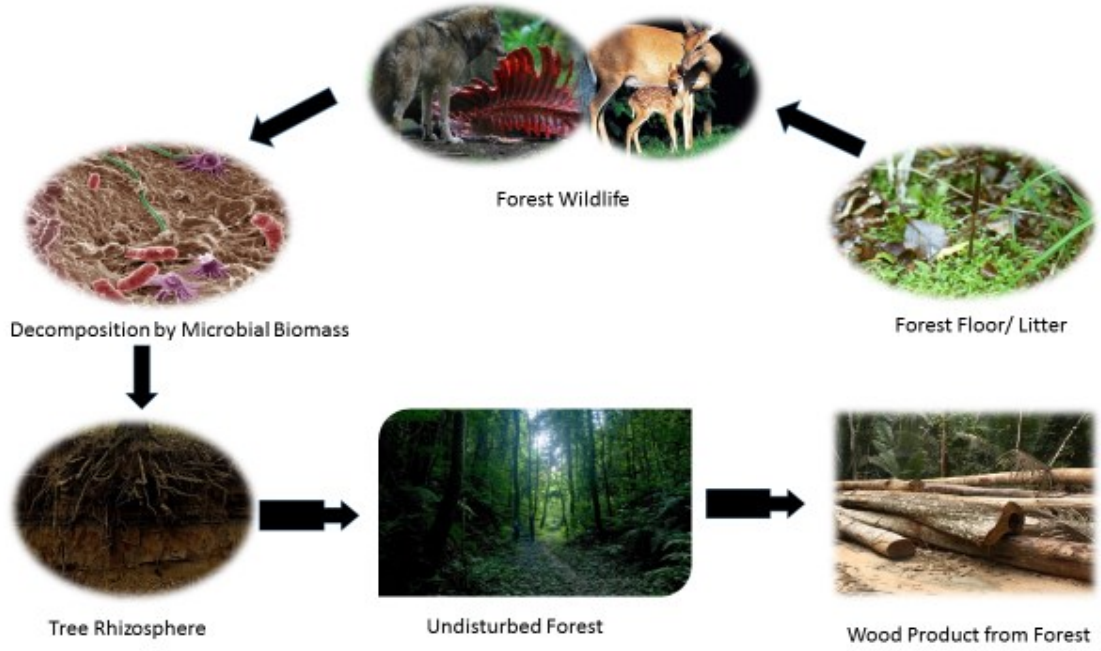


Figure 3.2 The Interaction in the forest biome. Grasses on the forest floor (forest floor/litter) are consumed by herbivores, these herbivores are preyed on by carnivores (forest wildlife), microorganisms decompose dead animal residues and leaf litters thereby making nutrients available in the tree rhizosphere which makes the trees grow extensively high and healthy. These trees are then harnessed by human beings for wood.

3.7.3.2 Effects of deforestation on soil microorganisms

The forest habitat is one of the most important habitats because of its ability to provide protection to biologically diverse organisms, and the soil and it is also a useful source of timber (Uroz et al., 2016) which can be used for various purposes. Due to the high organic matter

content present in the forest from leaf and litter decomposition, the forest soil tends to be rich in carbon and nitrogen components. In addition, the microbial populations tend to be higher and more diverse in the forest habitat due to the undisturbed nature of the habitat. These microhabitats play an important role in the overall functioning of the forest due to activities such as decomposition of organic matter and nutrient recycling (Colombo et al., 2016, Lladó et al., 2017b). However, activities such as the felling of trees and conversion of forests to agricultural lands often affect the microbial composition and diversity. The total carbon lost during the conversion of forest to agricultural land in Ethiopia was about 50% loss in Mg per hectare (Kassa et al., 2017). Deforestation also affects the microbial diversity in the soil, leaving a lasting effect that cannot be easily repaired. An example was recorded in the study carried out by Kassa et al. (2017) in which a deforested land still showed a decline in soil fertility and carbon and nitrogen contents even after 20-25 years. Since bacteria are known to be of great importance in the forest habitat, and in any soil ecosystem at large, any change that alters their diversity also alters the habitat in general. Common bacteria phyla found in the forest microbiome are Actinobacteria, Proteobacteria, Bacteroidetes, Firmicutes, and Acidobacteria (Lladó et al., 2017b).

3.7.4 Influence of termite activity on microbial diversity

Termites are diverse macroorganisms which can be found on the soil surface, they belong to the phyla Isoptera and are of different species which perform various functions, some of which are; the *Hypotermes sp.* (fungus growing termites) capable of constructing mound and soil sheetings (Harit et al., 2017) and the *Odontotermes sp*., *Macrotermes sp*. and the *Reticulitermes sp*. which build mounds only (Harit et al., 2017, Li et al., 2017b). Termites feed on wood and are capable of decomposing dry plant matter in the form of lignocellulose. Their ability to break down this form of cellulose is as a result of the microbial composition of their gut (Auer et al., 2017), they form a symbiotic relationship with their gut microbes such as

bacteria and archaea communities and together with eukaryotic organisms present in the soil, are able to degrade these lignocellulosic matter into acetate, methane and an intermediate product of hydrogen with the aid of these organisms (Ambele et al., 2018). They are important components of the carbon cycle because of their ability to decompose lignocellulosic matter, they are equally important in the cycling of nitrogen due to their ability to mineralize the humus content in certain termite species that feed on soil (Brune, 2014, Manjula et al., 2016).

Termites construct mounds as a need to sustain themselves constantly in humid environments (Haverty and Sunden-Bylehn, 2000) and maintain these mounds by getting soil from the depth of the soil profile structure and soil surface structure and also wood materials (Ali et al., 2016). Studies have shown that these termites are able to improve the soil biological and physicochemical properties thereby increasing the fertility (due to the high diversity of microorganisms) of the soil and surrounding areas of activity (Harit et al., 2017, Jembere et al., 2017). Their mounds can, therefore, be a good source of biofertilizers (Manjula et al., 2016) in agriculture. Also, these lignocellulose biomasses are sources of biofuels and as such, the mounds are useful sources of biofuels. Therefore, the microbial biomass is an essential component in the mounds of termite which when studied extensively could lead to more important discoveries.

3.8 Methods used for Microbial Diversity Analysis

Since the environment plays an important role in the evolution of microorganisms, any study concerned with knowing the activities of microorganisms and their interactions should also take into consideration the natural habitat of such organisms. Over the years, scientists have sought to know more about the unseen (micro) living creatures present in the environment. This has led to various experiments carried out in the laboratory. The soil harbours the largest and most diverse group of microorganisms, some of which have been cultured and found very

useful to man. However, cultural methods have been very limited due to the population of these organisms which are not culturable. Culture methods (liquid or plate) are limited because they cannot be used to fully study the microbiome of organisms (the complexity and biotechnological ability), and over time scientists have established that the population of unculturable bacteria in the soil is much higher than the culturable ones (Nahid et al., 2012). For example, when the laboratory culture method was adopted for the study of microorganisms present in an arid land, it was observed that there were much less diverse microbes than when the same samples were tested using high throughput sequencing methods (Zhang et al., 2012). This supports the theory that culture method of soil microbiome analysis can only account for less than 1% of microbial activity and presence. High throughput sequencing, also known as next generation sequencing (NGS) methods, have been used in the study of bacterial species that are unculturable. This technological advancement has also increased knowledge on the functions and patterns observed in microorganisms (Hiraoka et al., 2016).

Metagenomics uses high throughput sequencing methods that can be used to study the complexity of microorganisms without culturing them in the laboratory. It is the whole genome analysis of organisms and has been used in place of the plate culture methods which were previously used to culture individual microbes (Bouhajja et al., 2016). The phylogenetic properties of diverse microorganisms and how they adapt in the soil microbiome can be analyzed using metagenomics (Pushpanathan et al., 2014). Metagenomic studies can either involve whole genome analysis or the study of individual species. Several approaches have been applied in this study, one of these is the 16S amplicon sequencing, a species level approach which can been used to quantify and know the distinctive nature of microbes (Sharpton, 2014). This has been applied in the study of the relationship between enzymes and large populations of diverse bacteria present in an arid soil, using the Denaturing Gradient Gel Electrophoresis (DGGE) method (Zhang et al., 2012). Furthermore, 16S amplicon has also

been applied in revealing the dominant microorganisms in a thermophilic soil ecosystem in North India (Bhatia et al., 2015). Another approach to metagenomics is the shotgun metagenomics, a whole genome level approach which can be used to characterize microbes functionally (Hiraoka et al., 2016). Metagenomic analysis can also be used to study the co-occurrence patterns of microorganisms (Li et al., 2015). The use of metagenomics is a major breakthrough in research, as the results obtained from metagenomic analysis have led to novel enzyme discoveries, which have been used to study changes in microbial abundance and have also been applied in the provision of bioremediation agents (Ercolini, 2013, Bao et al., 2017). The application of Illumina, a high-throughput sequencing method, was used to analyse the functions and genes of organisms in the whole genome state present in an oil-contaminated soil, this led to the discovery of a wide range of microbial communities and enzymes (Bao et al., 2017). Metagenomics has proven to be a better method of analysis as it was also employed in the first study of biodiversity involving families and genera of bacteria in agricultural soils (Wolińska et al., 2018). The best method for the study of functional diversity of microorganisms is the whole genome sequencing, since studies carried out on individual species cannot fully give insights into how the organisms function in the soil habitat. This is why researchers have employed the use of high-throughput sequencing methods. These methods develop daily and are becoming more cost-effective and available to researchers.

3.9 Conclusion

Bacteria are the most abundant microorganisms found in the soil and are of great importance to human beings due to their usefulness as biofertilizers, biocontrol and bioremediation agents. Further studies are required on the evolutionary trend of bacteria and the factors that could promote or mar their population and distribution. This information will be useful in the identification of the most suitable environment for their proliferation, which could help resolve the prevailing soil degradation and enhance agricultural productivity while

maintaining soil fertility. Metagenomics is the most suitable method for understanding the diversity and functionality of soil microorganisms.

3.10 Acknowledgements

This review was supported by NWU Postgraduate Bursary awarded to A.R.S. by North-West University, South Africa for her master's degree. Researches in the Microbial Biotechnology Laboratory of O.O.B. are supported by the National Research Foundation of South Africa, grants UID81192, UID86625 and UID105248.

CHAPTER FOUR

4.1 Land Use and Its Influence on Soil Bacterial Diversity (Research Article)

Abstract

Reduction in the organic matter content of the soil has been recognised to influence soil fertility, hence farmers apply fertilizer to boost crop production. This is one of the major factors affecting microbial abundance in the soil. The soil microorganisms perform important functions in maintaining the balance in the ecosystem, as well as the fertility of the soil. Thus, in this study, the effect of land use on soil microbial diversity in two agricultural sites and one native indigenous forest in South Africa was determined. It is hypothesized that the forest land would be more biologically diverse with microorganisms due to reduced anthropogenic activities. High-throughput sequencing method was used. The results obtained from the study showed that the agricultural lands were abundant in the classes *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Acidobacteria* with relative abundance values of 47.3%, 17%, 8.2% and 7.1% respectively while the indigenous native forests were abundant in the classes *Proteobacteria*, *Verrucomicrobia*, *Acidobacteria* and *Actinobacteria* with relative abundance values of 35.5%, 27.8 %, 23.7%, and 5.8% respectively. The species richness using the observed richness estimator, was 1969, 1710 and 1663 for the Limpopo agricultural soil, the North West agricultural soil and the Mpumalanga forest soils respectively. However, Limpopo agricultural soil had a higher diversity index than the other sites, with an average Shannon Diversity index of 6.6 as against 6.5 and 6.4 observed in the North West agricultural soil and Mpumalanga forest soil respectively, this shows the Limpopo site is a more suitable site for agriculture. A principal component analysis was carried out on all the soil samples and it was observed that the classes *Actinobacteria*, *Nitrospirae*, *Proteobacteria* and *Verrucomicrobia* brought about the significant differences observed amongst the sites. It is therefore suggested that the application of fertilizers and chemicals on agricultural soil should be amended with

organic matter application in order to enhance beneficial soil microorganisms and consequently increase agricultural output and maximize food production.

Keywords: Soil fertility, agricultural soil, soil biodiversity, land degradation, DNA sequencing, bioinformatics analyses

4.2 Introduction

The soil is an anchor to agricultural productivity (Rees et al., 2018) and it contains mineral constituents and is made up of solid, liquid and gaseous phases. It also serves as a habitation for microorganisms as well as a source of support for plant growth (Certini and Ugolini, 2013). Soil fertility is important for food, and it is dependent on the complex inter-relations between the biological, chemical and physical soil components (Johnston et al., 2009). Due to the high importance of soil in food-producing organizations, it is therefore important to preserve soil qualities to uphold profitable, ecological and manufacturing outcomes in the agricultural sector. In response to an increasing world population, the need for the production of food has also increased. This has led to a search for alternative ways of improving food production. Two major ways have helped in achieving this. The first is the application of nitrogen-based fertilizers, from the nineteenth century, which was further expedited in the late nineteenth century, because nitrogen is an important nutrient for plant growth (Rees et al., 2018). Although the atmosphere comprises of abundant atmospheric nitrogen, this nutrient can only be made available to plants through specific organisms present in the soil. The second alternative was sought in the improvement of food production by the conversion of natural forests to agricultural lands (Rees et al., 2018).

Agricultural activity is the most common interaction between people and the surrounding environment, where people fully utilize their natural resources, in comparison with other ecological processes (Barrios et al., 2018). One method adopted by food manufacturers

is exhaustive tilling and the application of fertilizers to boost productivity on agricultural land, but this has a negative impact on the ecosystem and the health of human consumers (Tilman et al., 2002, Wall et al., 2015). The exhaustive tilling of the ground for food production coupled with the application of fertilizers to enhance productivity has led to a loss of profitable and natural ecological properties because these processes often lead to losses in biodiversity (Barrios et al., 2018). It is estimated that the human population will increase by more than 10% in the years 2001-2030. It is therefore expected that agricultural output, in order to meet the dietary demands of the growing population, should increase by at least 30-40%. Many vital roles that support ecological processes depend mainly on biodiversity, abundance and the actions of soil-dwelling microorganisms. This biodiversity changes in relation to the operational taxonomic units (OTUs), relative-abundance and spreading of these organisms depending on the type of soil, climate, and land cover and use (Rees et al., 2018).

Changes in the vegetation cover or land use and the related loss of ecological properties are a resultant effect of anthropogenic activities and natural occurrences and several studies have shown that increased changes since the late nineteenth century are linked to increasing human population, exhaustive use of vegetation cover and losses in natural environment (Falcucci et al., 2007). Furthermore, agricultural activities have been regarded as one of the major factors responsible for biodiversity losses (Norris et al., 2010). Soil biodiversity is of great importance as it helps the soil retain stability when there are sudden changes in the natural soil ecosystem, due to the activities of different microorganisms which serve as bio-buffers (Singh and Gupta, 2018). Joining effective agricultural practices with the conservation of biodiversity has been challenging. In order to curb loss in biodiversity, the United Nations set goals to restore ecological systems and degraded lands by 15% by the year 2020 (Tscharntke et al., 2012).

In addition, land degradation hampers biological efficiency and affects the ecosystem, community, and ecological balance. Degraded lands cause a reduction in bacterial taxonomic units, richness, and bio-diversity (Araújo et al., 2014). Land degradation is currently on the increase, due to increase in land usage, sometimes in the form of exhaustive agricultural practices, and also due to climate change (Tscharntke et al., 2012, Singh and Gupta, 2018).

Soil microorganisms play an important role in maintaining the value of soil as they serve as markers of major soil changes. They are mostly richer and more diverse in soils with better quality and these microorganisms are more densely populated in the rhizosphere of a plant (Hermans et al., 2017). They contribute to the supply of nutrients and serve as biological control of plant diseases (Lugtenberg, 2014). Natural lands (forests) have been found to possess higher biodiversity than other land uses (Moghimian et al., 2017). In order to restore degraded land, it is therefore important to seek ways of restoring soil microbial diversity. This study was therefore conducted to determine the bacterial abundance and diversity in indigenous natural forest and two agricultural sites in South Africa, and to infer the effect of agricultural practices on the bacterial population as it relates to land degradation.

4.3 Materials and Methods

4.3.1 Description of soil sample collection sites:

Thohoyandou is located in the Vhembe district of Makwarela, South of Venda, in the Limpopo Province, South Africa (latitude: 22,878541; longitude: 30,481845). The region's mean annual rainfall is 462 mm with a mean annual temperature of 23ºC. This region is one of the major suppliers of agricultural produce in the province and the nation as a whole. Irrigation systems are used for farming in this region and efforts are constantly put in place to improve the agricultural systems, one of which is climate-smart agriculture. This agricultural system supports the conservation of biodiversity and agroforestry. The second agricultural site, is the North-West University farm, Molewane (latitude: 25,789149; longitude: 25,618401),

Mafikeng North West province. This region has a mean annual rainfall between 300 -700 mm and a mean annual temperature between 22ºC and 34ºC (http://www.weathersa.co.za/). The forest soil sample was obtained from the indigenous forests Tweefontein and Witklip in the Mpumalanga province, which is one of the largest afforested areas of South Africa. This region comprises of about 0.6 million hectares of commercial and indigenous forest (Fig. 4.1)

4.3.2 Soil sample collection

Rhizosphere and rhizoplane soil samples (2 samples each) of Bambara groundnut plant (*Vigna subterranea*) were collected from agricultural soils located in the North West and Limpopo provinces of South Africa. Forest soil samples were collected (2 samples) at a depth of 0-10 cm deep. The soil samples were conveyed to the laboratory in an ice box and stored at a temperature of 4 ºC for further analyses. Fifty grams (50g) of the soil sample was immediately stored at a temperature of -20 ºC for DNA extraction.

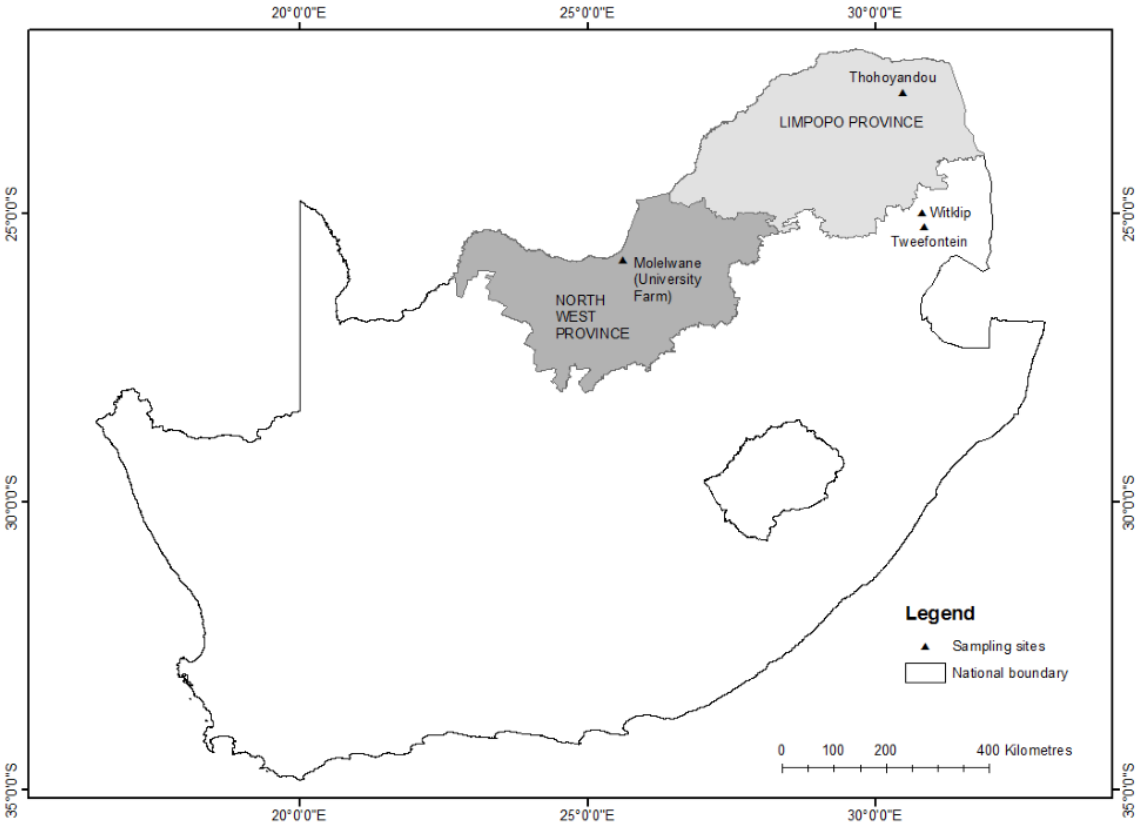


Figure 4.1 Sampling sites: Rhizosphere and rhizoplane soil samples of Bambara groundnut plant were collected from agricultural soils located in North West and Limpopo provinces of South Africa. Indigenous forest soils were collected from Mpumalanga in South Africa.

4.3.3 DNA extraction and PCR amplification of bacteria from soil samples

DNA extraction was performed using the Mo-Bio PowerSoil Isolation Kit (Mo Bio labs, USA) according to the instructions stated by the manufacturer. Forty microliters (40 µl) of extracted DNA samples were amplified on the 16S rDNA at the V4 region using 515/806 PCR primers coupled with specific barcode on the forward primer in 5 cycles of PCR (the HotStarTaq plus Master Mix Kit was used under the following conditions; (Qiagen, USA)) at a temperature of 94 ºC, this was allowed to continue for 3 min, and then for another 30-35 cycles at a temperature of 94 ºC for 3s, it was then allowed to run at an annealing temperature of 53 ºC, for 40s, then 72 ºC for 1 min, followed by an extension at a temperature of 72 ºC for 5 min. The PCR products were checked on 2 % agarose gel. The PCR sample products with similar weight and DNA concentration were pooled together.

4.3.4 Illumina DNA sequencing and data analysis

The pooled DNA samples were further purified using Ampure XP beads and prepared for Illumina DNA sequencing. Sequencing was carried-out at MR DNA on a MiSeq, adhering to the manufacturer's instructions.

The sequenced data was processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA) http://www.mrdnalab.com/. Sequences were joined, barcodes were removed, and sequences with reads less than 150 base-pairs and with large base-calls were removed. Sequences were then denoised, Operational Taxonomic Units (OTUs) were generated and chimeras removed. The OTUs were selected by clustering at a 3% value of divergence. The OTUs were then classified taxonomically using the BLASTn against the RDPII and NCBI databases. Data were cleaned using Open refine, then, using Microbiome Analyst platform which uses R codes, a total of 3,026 low abundance features were removed

based on prevalence and 266 low variance features were removed based on the interquartile range. The number of features remaining after the data filtering step was 2,387.

4.3.5 Data analysis

Alpha diversity analysis, Chao1 richness, Observed richness, Abundance-based Coverage Estimator (ACE) and Shannon index were determined. Kruskal Wallis test for equal variance was used at a significance level of $\alpha = 0.05$. In order to determine the differences in bacterial diversity between sample sites (beta-diversity), the Bray-Curtis and Weighted Unifrac Distance was used. Spearman's Correlation coefficient was used to analyse the relationship/correlation between the relative abundance of the OTU/Genus/Class using the value of $p < 0.05$ as the level of statistical significance in accordance to the test of difference in the least significance.

4.4 Results

4.4.1 Bacterial composition/structure

After the sequence was quality filtered and the chimeras removed, the total sequences remaining from the agricultural and forest soils was 408,612. The total OTUs obtained after clustering were 13,116 which was picked at 97% based on a similarity between sequences. A total of 17,721 Operational Taxonomic Units (OTUs) (phyla, class, family, genus and species) were obtained. The forest soil had the highest OTU and taxonomic group totalling 13,035 while the agricultural soils had a total of 4,686 OTUs and taxonomic groups. The predominant class obtained in the agricultural soil were *Proteobacteria*, *Actinobacteria*, *Bacteroidetes* and *Acidobacteria* with relative abundance (R.A) values of 47.3%, 17.0%, 8.2%, and 7.1% respectively (Fig 4.2A). Conversely, the most abundant class in forest soils were *Proteobacteria*, *Verrucomicrobia*, *Acidobacteria* and *Actinobacteria* with relative abundance (R.A) values of 35.5%, 27.8%, 23.7%, and 5.8% respectively (Fig 4.2B). The R.A of *Proteobacteria* in the agricultural soil was higher than that of the forest soil. However, the

forest soil contained *Verrucomicrobia* with higher levels of *Acidobacteria* when compared with the agricultural soil. The richness of the soil samples was tested using the Observed richness, ACE and Chao 1 indexes, all of which showed no significant difference. Box plot analysis showed that the sample reads and abundance was similar enough to be compared meaningfully (Fig 4.3).

The community analysis shows the distribution of the North West agricultural soil samples, Limpopo agricultural soil and the Mpumalanga forest soil samples (Fig. 4.4). The values for the samples were scaled from -200 (minimum value) to 250 (maximum value). The scaling was done uniformly in order not to affect the relative difference of values within a single sample or between the samples. The median lines within the plot for the samples lie close to each other indicating the distribution of abundance count between the samples.

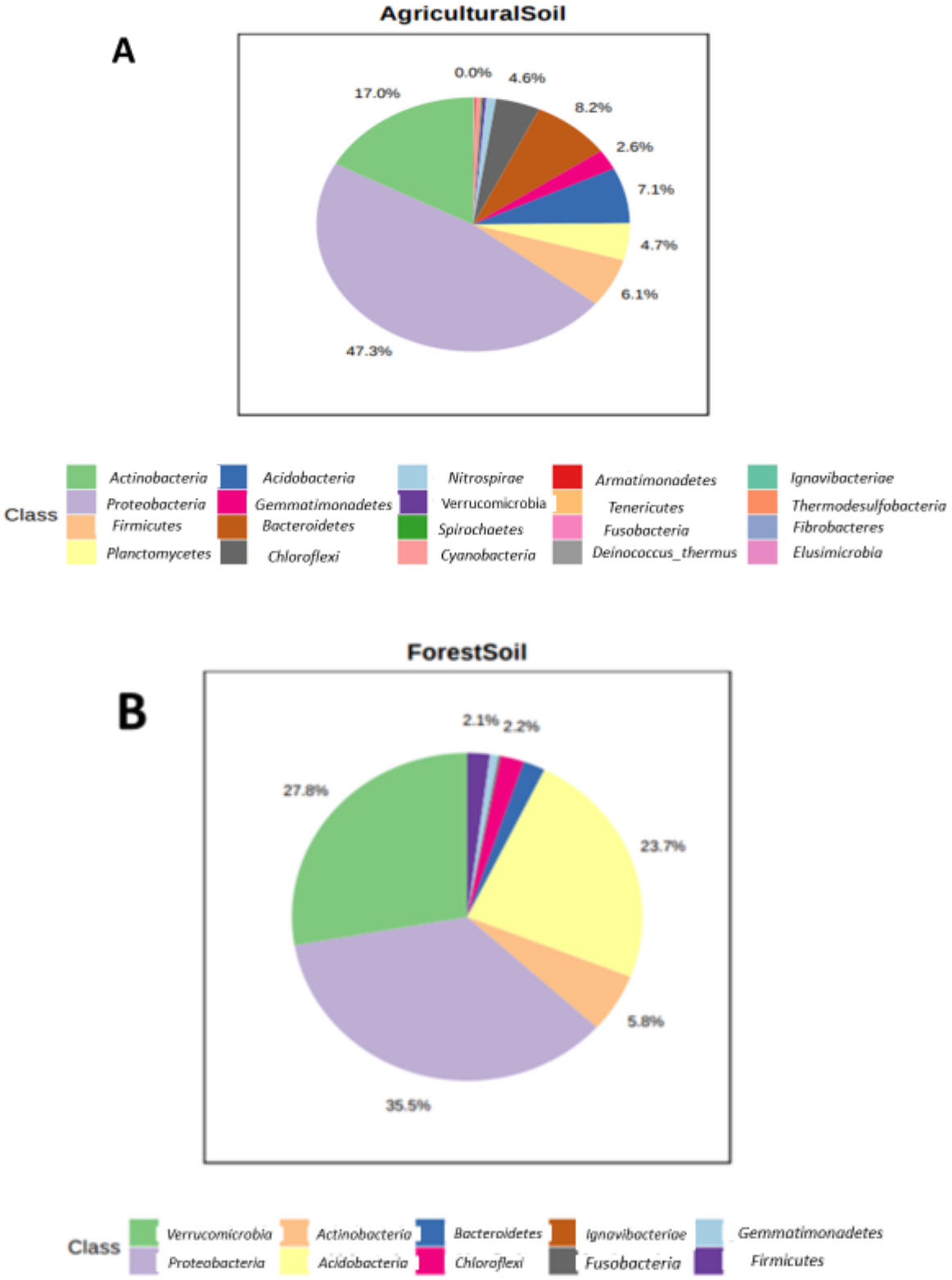


Figure 4.2 Pie-charts showing the abundances of bacteria in forest and agricultural soils A) Class of bacteria present in the agricultural soils. The total classes of bacteria present in the agricultural soil as presented by the microbiome analyst are 20, the most abundant being the *Proteobacteria* and *Planctomycetes* with the least abundance. B) The total classes of bacteria present in the forest soil as presented by the microbiome analyst are 10, the most abundant being the *Proteobacteria* and *Firmicutes* with the least abundance.

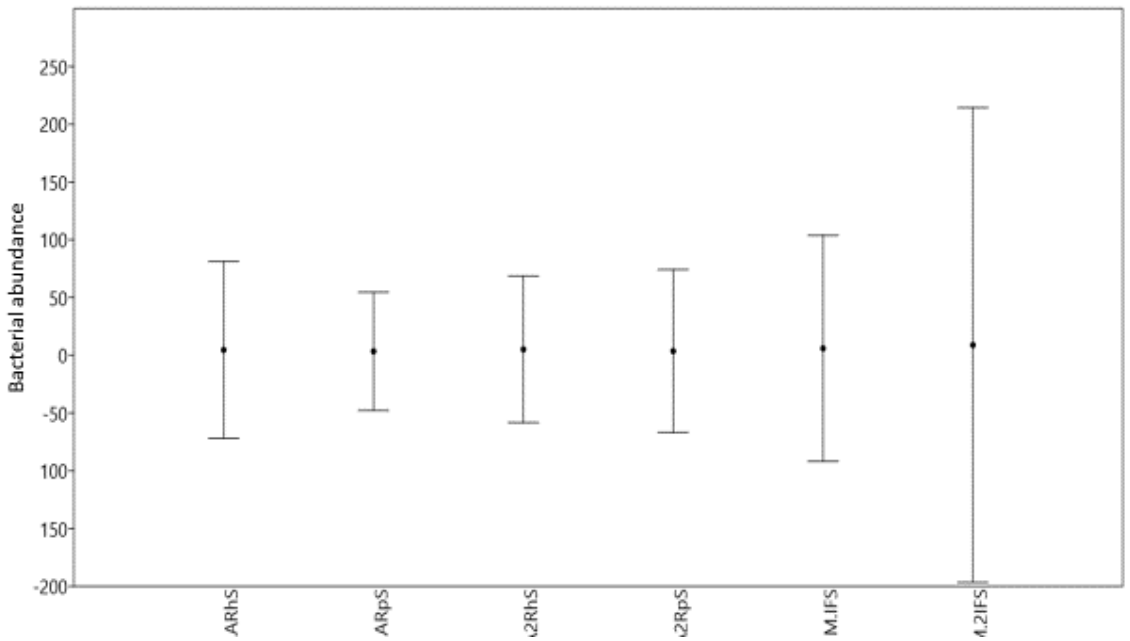


Figure 4.3 A community plot showing the abundance count distribution of the agricultural soil and forest soil samples (N.ARhS= North West agricultural Rhizosphere Soil, N.ARpS= North West agricultural Rhizoplane Soil, L.A2RhS= Limpopo Agricultural Rhizosphere Soil, L.ARpS= Limpopo Agricultural Rhizoplane Soil, M.IFS= Mpumalanga Indigenous Forest Soil, M.2IFS= Mpumalanga Indigenous Forest soil site 2)

4.4.2 Rarefaction Analysis

A rarefaction analysis curve was used to assess the richness of the bacteria community and the sampling competence in the North West and Limpopo Bambara nut agricultural rhizosphere and rhizoplane soils, as well as the Mpumalanga forest soil (Fig 4.4). The results showed that the rarefaction curves for each group of samples have attained saturation, which represents the true bacterial diversity of samples.

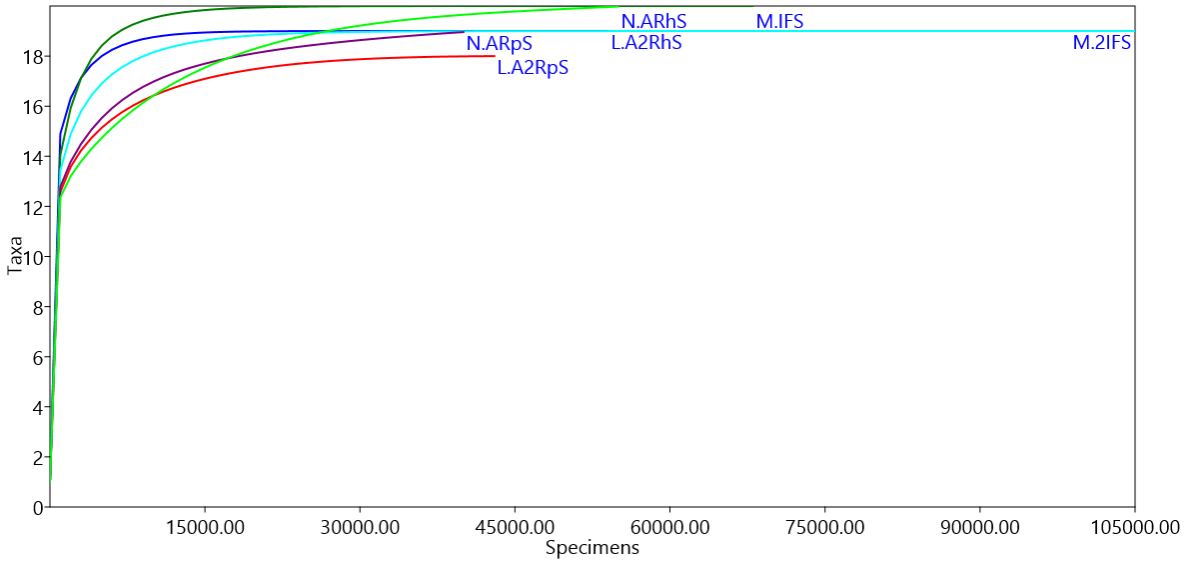


Figure 4.4 A Rarefaction curve used to estimate richness in the agricultural and forest soil samples and the sampling effort. The vertical axis shows the taxa abundance expected after sampling the read counts as shown in the horizontal axis. (N.ARhS= North West agricultural Rhizosphere Soil, N.ARpS= North West agricultural Rhizoplane Soil, L.A2RhS= Limpopo Agricultural Rhizosphere Soil, L.ARpS= Limpopo Agricultural Rhizoplane Soil, M.IFS= Mpumalanga Indigenous Forest Soil, M.2IFS= Mpumalanga Indigenous Forest soil site 2)

4.4.3 Alpha and Beta Diversity Indexes

Bacterial diversity

The differences observed between the different sites were analyzed using the principal component analysis and a double hierarchical dendrogram. The differences in bacterial diversity of the different locations, using a principal component analysis, revealed that the following classes of organisms; *Actinobacteria*, *Nitrospirae*, *Gemmatimonadetes*, *Verrucomicrobia*, and *Proteobacteria* contributed much to the observable variation among the various sites (Fig 4.5). Using the Kruskal Wallis test of an equal median at a significant level of $p < 0.05$, these variations observed using the principal component analysis were insignificant, with a p-value of 0.6247. In order to ascertain the evolutionary relationship between sites, a phylogenetic tree was constructed.

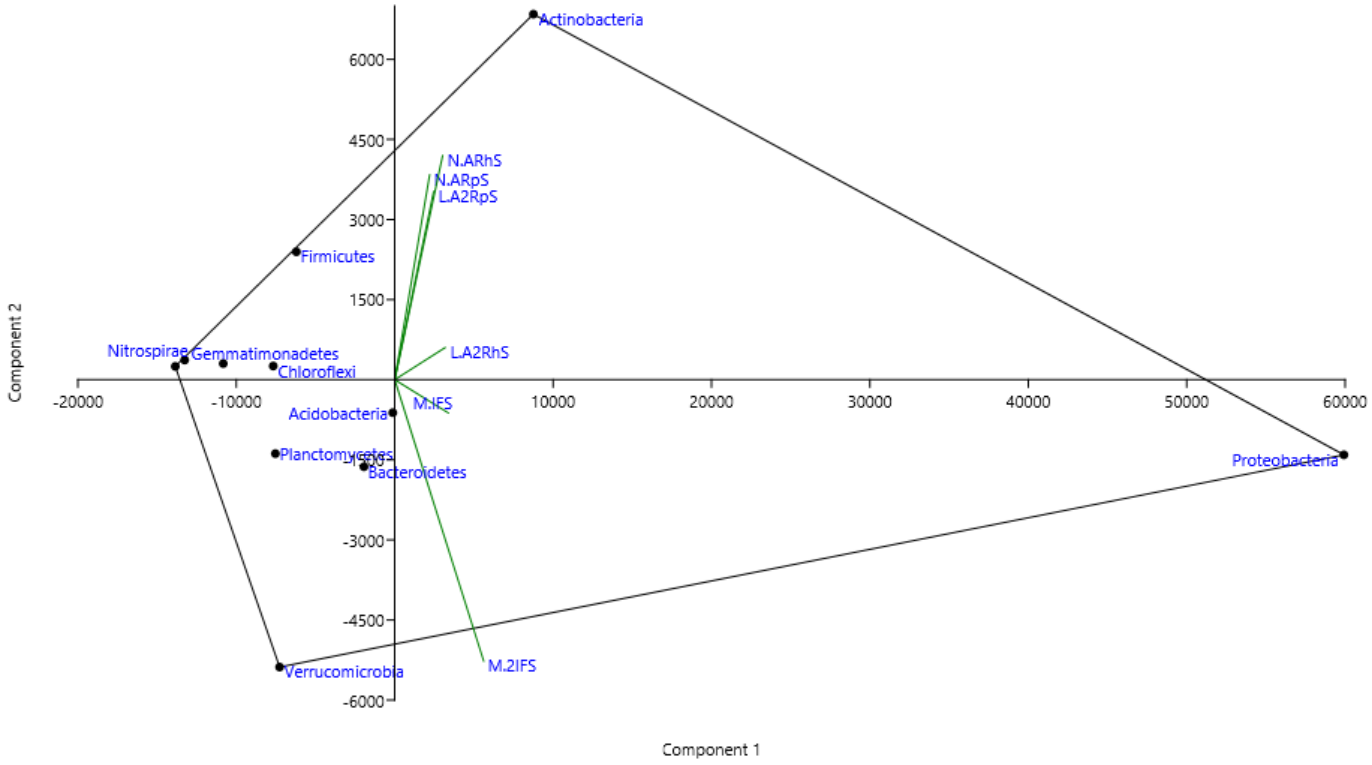


Figure 4.5 Principal component analysis of the bacterial classes among the agricultural and forest sites. Sites were projected at different angles and abundant classes in the direction of the site where they are found. (N.ARhS= North West agricultural Rhizosphere Soil, N.ARpS= North West agricultural Rhizoplane Soil, L.A2RhS= Limpopo Agricultural Rhizosphere Soil, L.ARpS= Limpopo Agricultural Rhizoplane Soil, M.IFS= Mpumalanga Indigenous Forest Soil, M.2IFS= Mpumalanga Indigenous Forest soil site 2)

Alpha diversity was measured using OTUs, phylum, class, order, family, genus and species level with observed richness, Chao 1 richness, and abundance coverage-based estimator (ACE) (Table 4.1). The richness values showed that the OTU level had the highest mean values of richness and the least was the phylum level which had richness mean values ranging from 0-3 in all 3 sites. The Mpumalanga forest site had the highest richness mean value (1969) which was followed by the Limpopo agricultural site (1710) and then the North West agricultural site (1663) at both the OTU level and phylum level. Shannon diversity index, Simpson and Pielou's evenness were used to measure the diversity and evenness (Table 4.2). The bacterial community diversity indices including the Shannon, Simpson and the Evenness were higher in the sites L.A2RhS, N.ARpS, M.IFS and least in the sites N.ARhS, L.A2RpS and M.2IFS (Table 4.2) although using the Kruskal Wallis test for an equal median, the differences observed were not significant (p=0.6247). The heat map was further used to ascertain the differences in

the microbial composition among the different sites at various levels. The abundances varied among organisms in the various sites (appendix 2). *Verrucomicrobia* and *Bacteroidetes* were more abundant in M.IFS, *Ignavibacteriae*, *Acidobacteria*, *Planctomycetes* were abundant in MIFS, the class *Actinobacteria* and *Armatimonadetes* were more abundant in N.ARpS, *Gemmatimonadetes* were most abundant in N.ARhS, *Chloroflexi* was equally abundant in the sites N.ARhS and L.A2RpS *Spirochaetes, Fusobacteria, Nitrospirae, Tenericutes* were most abundant in L.A2RhS, *Cyanobacteria* were equally abundant in N.ARpS and L.A2RhS, *Firmicutes, Deinococcus_thermus*, and *Proteobacteria* were abundant in the site L.A2RhS Fig 4.6A. The heat map for the phylum, class, order and family levels were carried out as shown in Figs 4.6A, 4.6B and 4.6C. The heat map depicts the percentage of each bacterial class (variables on the vertical axis) within each sample (horizontal axis). The relative Euclidean distance values are depicted by the red and blue colours, indicating low and high abundance respectively, correlating with colours shown in the legend at the side of the figure. Clusters based on the size of the distance of samples along the horizontal axis and the bacterial classes along the vertical axis are indicated at the top of the figure. Observed, Chao 1 and ACE are non-parametric richness estimators that take into account the total number of variables without repetition. They have different levels of sensitivity however. The mean standard error is used to reduce the variations between sample observations. The more the reduced standard error, the closer the samples are or the more reduced the variations between them.

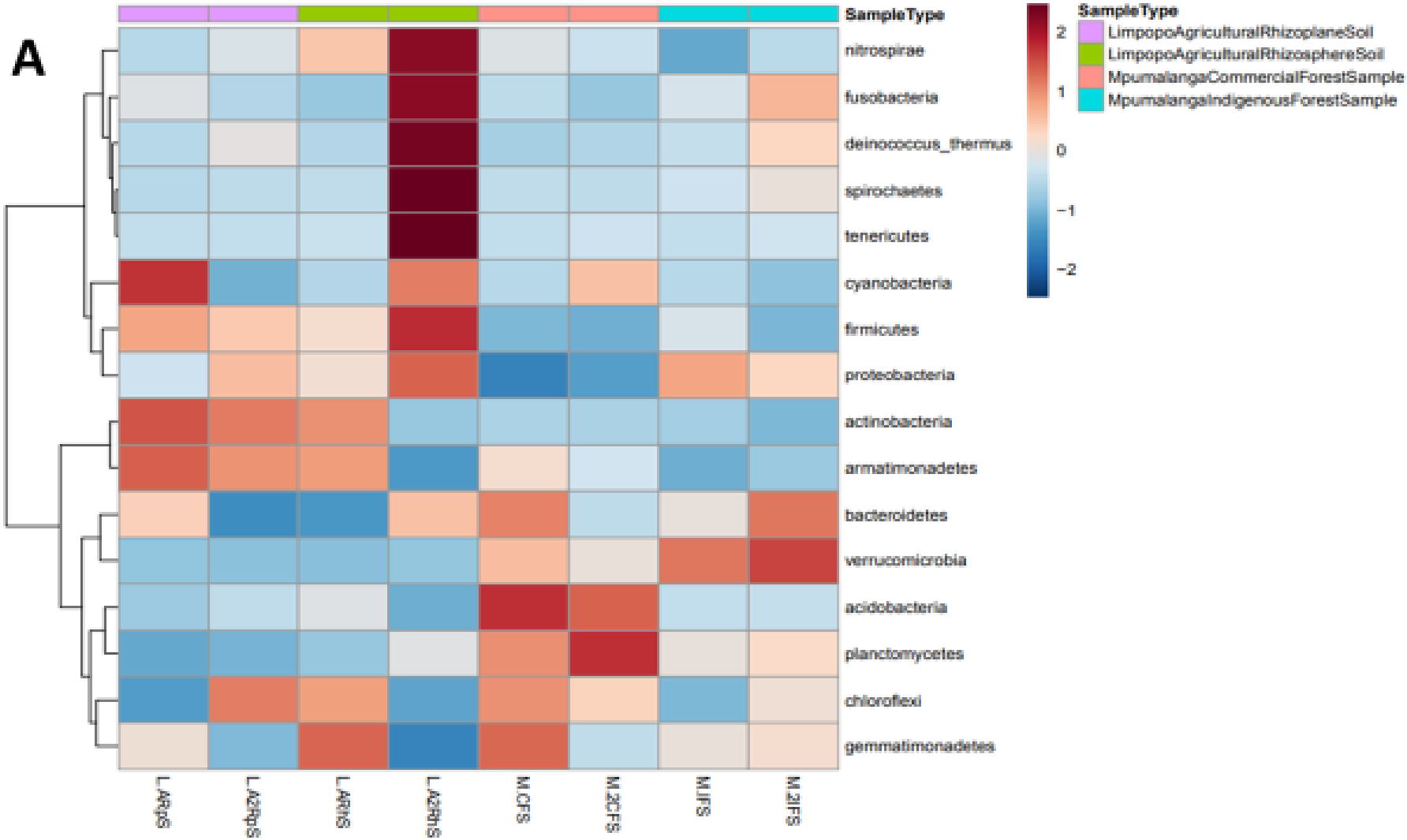
Table 4.1: Richness of samples from the OTU level to the species level using Chao 1, Observed and ACE indices

Location		North West Agricultural Soil			Limpopo Agricultural Soil			Mpumalanga Indigenous Forest Soil		
		Chao 1	Observed	ACE	Chao 1	Observed	ACE	Chao 1	Observed	ACE
OTU	Mean Value	1800.516	1663	1755.699	1835.8905	1710	1790.0355	2175.241	1969	2076.5895
Level	Mean Standard Error	25.75151	-	20.40503	24.551605	-	20.626215	36.55626	-	22.38892
	Mean Value	1	1	-	1	1	-	2	2	-
Phylum	Mean Standard Error	-	-	-	-	-	-	-	-	-
	Mean Value	19.5	19.5	19.883125	18.5	18.5	18	29.5	29.5	29.5
Class	Mean Standard Error	0.365336	-	-	0	0	1.763834	0	-	1.503872
	Mean Value	47.1	47	47.65127	47.25	47.984215	47	70	70	70
Order	Mean Standard Error	0.433977	-	2.9366195	0.7297055	-	2.5935445	0	-	2.71884
	Mean Value	94.966665	92	95.57184	94.125	93	94.243625	127.5	127.5	127.5
Family	Mean Standard Error	6.1355985	-	4.0049785	2.008143	-	3.732268	0	-	3.527508
	Mean Value	194.9154	187.5	195.30765	201.6	189.5	196.9601	256.5	255	255.8913
Genus	Mean Standard Error	5.7567835	-	5.8205285	9.349072	-	5.8205285	2.2297545	-	6.0101505
	Mean Value	382.3	371.5	378.19475	398.7011	385.5	396.0054	488.60415	476	482.1744
Species	Mean Standard Error	6.063857	-	9.1742745	7.3545455	-	8.9986835	7.9553095	-	9.7142505

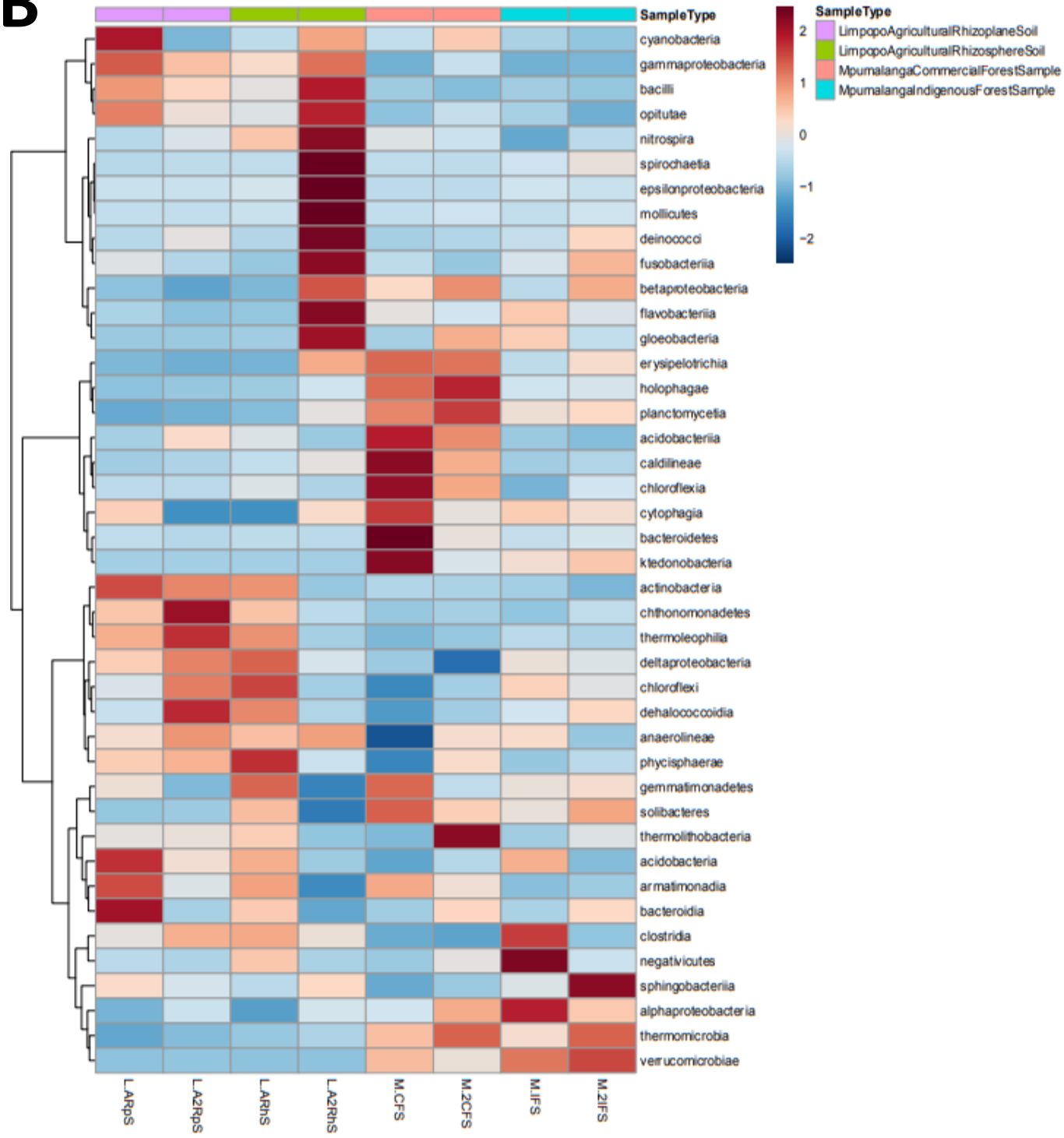
Table 4.2 Bacteria community diversity among different sites

Sample Sites	Diversity Index		
	Simpson	Shannon	Evenness
N.ARhS	0.9945	6.427	0.1865
N.ARpS	0.9955	6.483	0.2091
L.A2RhS	0.9969	6.867	0.2559
L.A2RpS	0.9924	6.298	0.1761
M.IFS	0.9946	6.641	0.1510
M.2IFS	0.9893	6.217	0.0963

N.B: The Simpson diversity index measures the degree of concentration when individual observations are classified into types, Shannon diversity index measures the average proportion of an individual observation from the total number of observations while the evenness measures the abundances that make up the richness of an area. The higher the diversity index, the higher the evenness of the observation.


A
SampleType
nitrospirae
fusobacteria
deinococcus_thermus
spirochaetes
tenericutes
cyanobacteria
firmicutes
proteobacteria
actinobacteria
armatimonadetes
bactercidetes
verrucomicrobia
acidobacteria
planctomycetes
chloroflexi
gemmatimonadetes
LA4RpS
LA2RpS
LA4RhS
LA2RhS
M.4CFS
M.3CFS
M.4FS
M.3FS
SampleType
LimpopoAgriculturalRhizoplaneSoil
LimpopoAgriculturalRhizosphereSoil
MpumalangaCommercialForestSample
MpumalangaIndigenousForestSample
2
1
0
−1
−2

B


SampleType
cyanobacteria
gammaproteobacteria
bacilli
opitutae
nitrospira
spirochaetia
epsilonproteobacteria
mollicutes
deinococci
fusobacteriia
betaproteobacteria
flavobacteriia
gloeobacteria
erysipelotrichia
holophagae
planctomycetia
acidobacteriia
caldilineae
chloroflexia
cytophagia
bacteroidetes
ktedonobacteria
actinobacteria
chthonomonadetes
thermoleophilia
deltaproteobacteria
chloroflexi
dehalococcoidia
anaerolineae
phycisphaerae
gemmatimonadetes
solibacteres
thermolithobacteria
acidobacteria
armatimonadia
bacteroidia
clostridia
negativicutes
sphingobacteriia
alphaproteobacteria
thermomicrobia
verrucomicrobiae
LARpS
LA2RpS
LARhS
LA2RhS
M.CFS
M.2CFS
M.IFS
M.2IFS
SampleType
2
1
0
−1
−2
SampleType
LimpopoAgriculturalRhizoplaneSoil
LimpopoAgriculturalRhizosphereSoil
MpumalangaCommercialForestSample
MpumalangaIndigenousForestSample

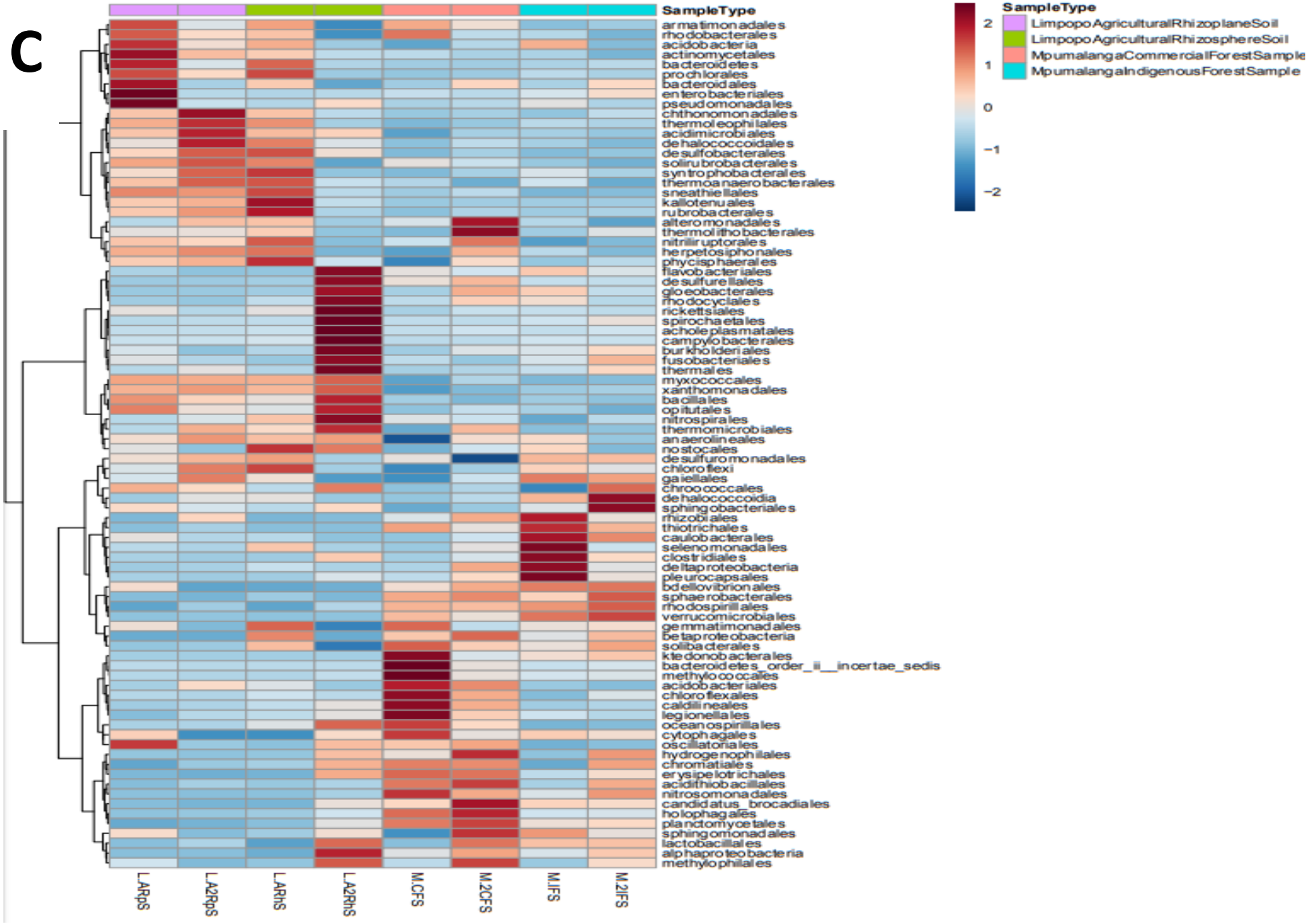


Figure 4.6 Heat maps showing the relative abundances of bacteria at the agricultural and forest sites. Using different colours, where the colour with the highest intensity red shows higher abundances than the ones with lower intensity (blue) A) Shows the R.A at the class level, B) Shows the R.A at Order level C) Shows R.A at Family levels from agricultural and forest sites.

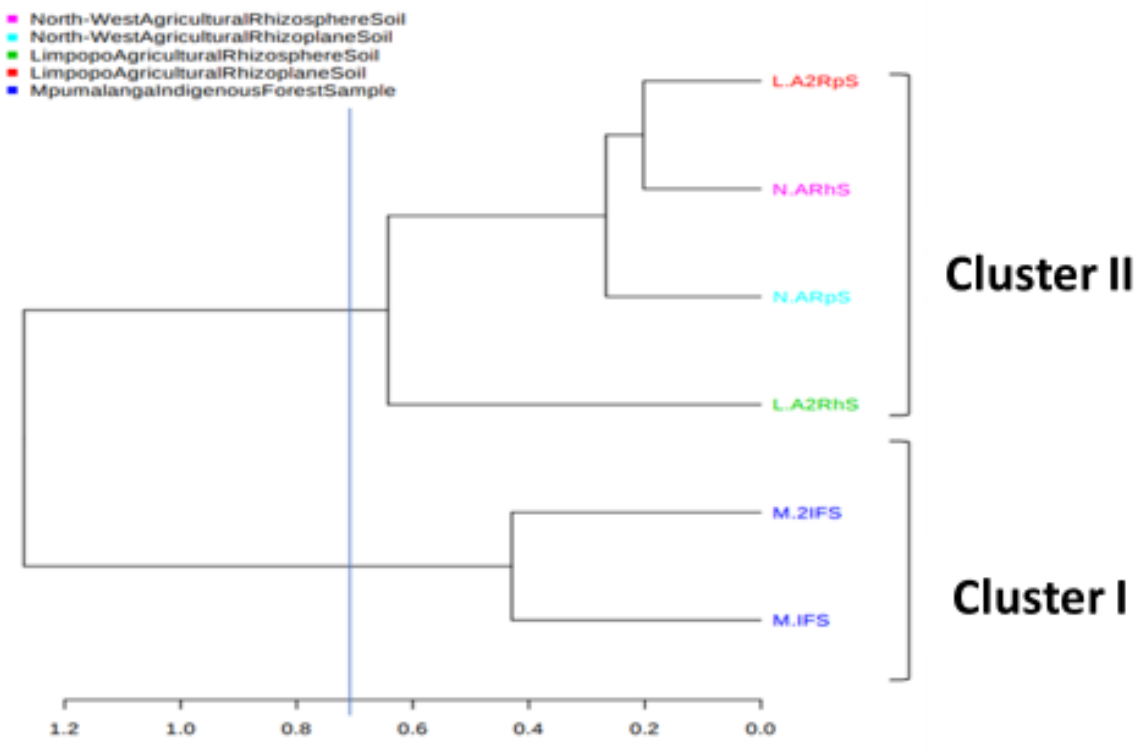
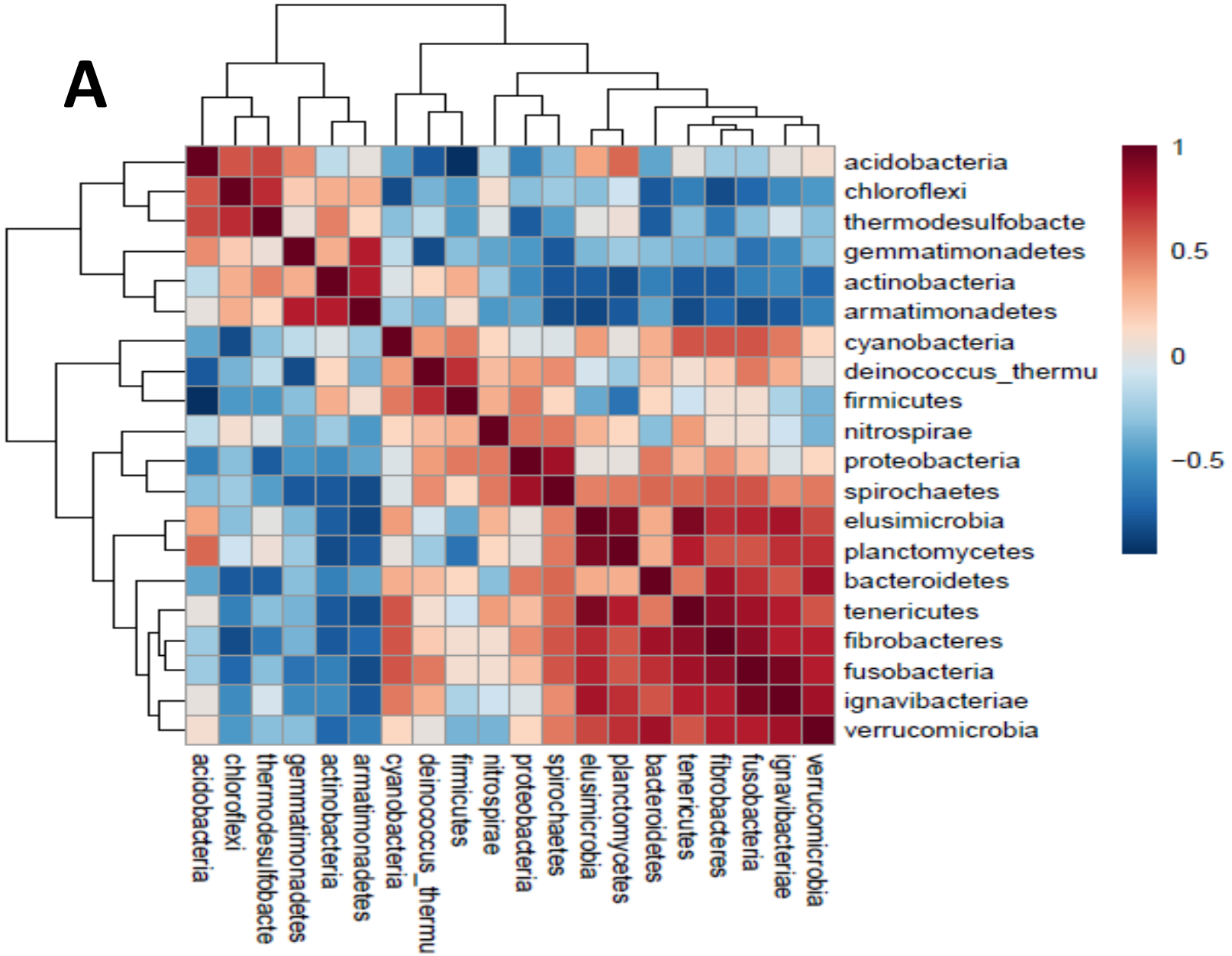


Fig 4.7 Phylogenetic tree showing the evolutionary similarities of microorganisms among all sampled sites using the microbiome analyst online platform.

4.4.4 Correlation Analysis

Correlation analysis was carried out on the various samples in order to partition points into groups based on their similarities. This analysis was carried out on the class (Fig 4.8A) and order (Fig 4.8B) using the Spearman rank correlation as the distance matrix, and analysis was carried out using the microbiome analyst online platform. In order to observe the relationship among organisms at different levels, a correlation analysis was carried out using the Spearman metric distance. *Verrucomicrobia* correlated to the organisms *Spirochaetes*, *Elusimicrobia*, *Planctomycetes*, *Bacteroidetes*, *Tenericutes*, *Fibrobacteres*, *Fusobacteria*, *and Ignavibacteriae*. The red colour intensity was higher with *Bacteroidetes*, however, it still showed close to zero correlation with *Cyanobacteria* and *Proteobacteria*. *Ignavibacteriae* correlated with *Spirochaetes*, *Elusimicrobia*, *Planctomycetes*, *Bacteroidetes*, *Tenericutes*, *Fibrobacteres*, *Fusobacteria and Ignavibacteriae* with the exception of *Proteobacteria* with a correlation colour of blue, which indicates that the correlation is below zero. It, however, showed a higher correlation with *Cyanobacteria* and *Deinococcus-thermus*. *Fusobacteria* showed high correlation with *Spirochaetes*, *Elusimicrobia*, *Planctomycetes*, *Bacteroidetes*, *Tenericutes*, *Fibrobacteres*, *Fusobacteria*, *Ignavibacteriae*, *Cyanobacteria*, and *Deinococcus-thermus*. *Fibrobacteres* shows high correlation with *Spirochaetes*, *Elusimicrobia*, *Planctomycetes*, *Bacteroidetes*, *Tenericutes*, *Fibrobacteres*, *Fusobacteria*, *Ignavibacteriae*, *Cyanobacteria* and *Deinococcus-thermus*. *Tenericutes* shows close correlation with *Spirochaetes*, *Elusimicrobia*, *Planctomycetes*, *Bacteroidetes*, *Nitrospirae*, *Fibrobacteres*, *Fusobacteria*, *Ignavibacteriae* and *Cyanobacteria*. *Fibrobacteres* shows high correlation with *Spirochaetes*, *Elusimicrobia*, *Planctomycetes*, *Bacteroidetes*, *Tenericutes*, *Fibrobacteres*, *Fusobacteria*, *Ignavibacteriae*, *Cyanobacteria* and *Deinococcus-thermus*.


A
acidobacteria
chloroflexi
thermodesulfobacte
gemmatimonadetes
actinobacteria
armatimonadetes
cyanobacteria
deinococcus_thermu
firmicutes
nitrospirae
proteobacteria
spirochaetes
elusimicrobia
planctomycetes
bacteroidetes
tenericutes
fibrobacteres
fusobacteria
ignavibacteriae
verrucomicrobia
1
0.5
0
−0.5

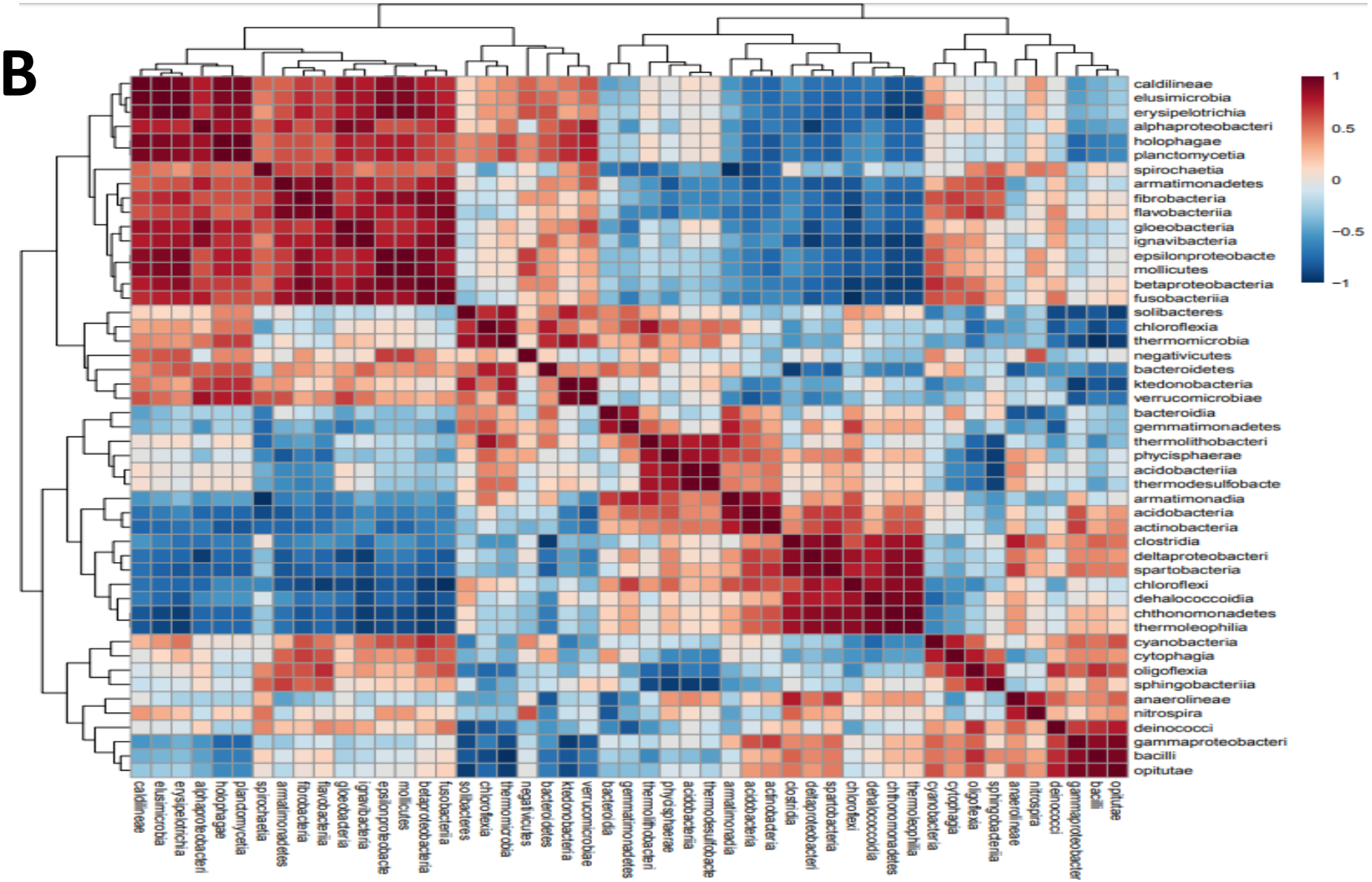


Figure 4.8 Correlation analysis of bacteria at the A) Class level, B) Order level Red showed that the microorganisms are closely correlated and blue showed that the correlation among microorganisms is less, as indicated by the number key on the right-hand side of the graph. Also, a phylogenetic tree shows the evolutionary trend of the microorganisms on the top horizontal axis and left vertical axis. The colour intensity shows the percentage correlation, that is, the more it tends towards one, the more closely correlated they are.

4.5 Discussion

The use of Next Generation Sequencing for DNA samples allows for an understanding of ambiguous microbial diversity in close association with different environments. Metagenomic analysis of diverse bacteria using amplicon sequencing is dependent on the hypervariable regions of 16S rDNA. The V3 or V6 hypervariable regions are widely used for phylogenetic affiliation because of their greater discriminating power and efficient phylogenetic assignment of reads to different taxa (Manjula et al., 2016).

The diversity indices used to ascertain the differences that could occur within the sites were the Shannon, Simpson and Pielou's Evenness which will be significantly higher in more fertile soil than in less fertile soil (Wu et al., 2018).

Rhizosphere microorganisms are important in maintaining chemical balance in the soil and are also important in increasing crop yield and production (Bakker et al., 2012, Wu et al., 2018)In this study, the Limpopo agricultural site had higher bacterial abundance and diversity than the North West agricultural site. This could be as a result of healthier agricultural practices in the region as opposed to the North West agricultural site. However, the forest soil was found to have the highest richness, which could be due to the various activities that take place in the forest such as the death and decay of animals and the activities of macro and microorganisms during decomposition (Lladó et al., 2017b). The high bacterial diversity seen in the Limpopo agricultural site suggests that this site is a healthy agricultural site capable of producing high yield crops. This is due to the fact that bacterial diversity, when maintained over time allows the maintenance of a relatively stable ecosystem with the rhizosphere soil suitable for the cycling of nutrients (Wu et al., 2018).

Differences observed in the microbial composition of the soils obtained from the different sites are evidence of variation in chemical composition and nutrient availability in the

soil. This is due to the release of chemical components by the roots into the soil, which inadvertently determines the type of microbe found in certain soil types and the extent of their diversity. The results obtained from the class level of bacteria present showed that all the bacteria found at class level in the forest sample were also found in the agricultural soil. However, two classes *Gemmatimonadetes* and *Verrucomicrobia* were more abundant in the forest soils than the agricultural soils. The class *Gemmatimonadetes* have been found to be adapted to regions of low moisture (DeBruyn et al., 2011), only very few of this class have been cultured, while a vast majority still remain unculturable. The *Verrucomicrobia* class have been found to be very useful in the degradation of cellulose, fixation of nitrogen and the breakdown of complex polysaccharides to acetate (Wertz et al., 2012, Lladó et al., 2017b), all of which are quintessential in the growth of plants.

The presence of these organisms could be responsible for the extensive root system of trees in the forests (Lladó et al., 2017b). Since some of these classes of organisms present in the forest soil have been found to be of benefit to plant production (Lladó et al., 2017b), it is important to incorporate them in agricultural systems through agroforestry. The reduction in the microbial diversity in soil has been seen as one of the major characteristics of soil degradation, and this diversity needs to be restored. Efforts should be made to harness organisms of beneficial importance to agriculture from the forest microbiome. This could be helpful in improving agricultural productivity and improving plant resistance to diseases. As seen from the results obtained using the Chao 1, ACE and observable richness, the forest site was richer in bacterial composition than both agricultural sites.

Both agricultural sites had the same crop planted on them; however, they had different climatic conditions. Although the more favourable climatic condition was the North West region, this region, however, had less bacterial diversity than the Limpopo agricultural site. This could be as a result of poor farming practices. The Limpopo agricultural site practices

climate-smart agriculture which incorporates some level of agroforestry. The use of microorganisms to destroy harmful pollutants and in the restoration of degraded soil is on the increase (Tripathi et al., 2017). The pie chart (Fig 4.2) showed the predominance of the class *Proteobacteria* in both agricultural sites as well as the forest sites. This class has been known to be abundant in soil which is rich in nutrients (Trivedi et al., 2013). The correlation analysis plot (Fig 4.8) showed that some of the organisms are interrelated, hence the relationship observed on the phylogenetic tree shows that their functions vary widely. The bacteria phyla found in most abundance, the *Acidobacteria, Actinobacteria, Proteobacteria, Bacteroidetes* and *Firmicutes* (Lladó et al., 2017b), were found in all three sites, but were more abundant in some sites than others. Using the heat map, the forest soil had a higher abundance of the phyla *Acidobacteria* and *Bacteroidetes.* This implies that the bacteria produce carbon from cellulose degradation. This could be of great benefit in agriculture as carbon is an important component required by plants for photosynthesis. The North West agricultural site only showed abundance in *Actinobacteria,* organic matter has been found to be rich in the phyla *Bacteroidetes* and *Proteobacteria*. This infers that the forest site has these phyla in abundance due to the presence of organic matter. This can be incorporated into agricultural sites that have lower crop yield as these phyla are associated with nutrient availability in the soil (Lladó et al., 2017b). The North West agricultural site showed signs of degradation, as the microbial diversity and abundance is reduced.

4.6 Conclusion

Land use greatly influences the bacterial distribution, abundance, and diversity in soil. Microbial diversity and abundance can be a good indicator of degraded land. Efforts should be made to improve on microbial diversity through the conservation of natural lands. In addition, the use of organic matter on agricultural farms should be encouraged instead of persistent use of chemical fertilizers, this is because chemical fertilizers have long term adverse effects on

soil productivity. Intensive tilling of agricultural lands should be discouraged, as it causes a negative long term adverse effect on microbial population and diversity of the soil which could eventually lead to degradation. Farmers should adopt the cover cropping method as this improves the presence of diverse bacteria necessary for nutrient cycling.

4.7 Acknowledgements

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CHAPTER FIVE

5.1 Forest Commercialization Impacts on Bacterial Diversity (Research Article)

Abstract

Forest commercialization may have negative impacts on soil bacterial diversity. However, how it affects bacterial abundance and diversity is still unknown. The aim of this study was to investigate the effect of forest commercialization on bacteria diversity and structure. Comparison was carried out between two sites in the most afforested regions in South Africa, both sites having commercial and indigenous forests. The soil was analyzed using the metagenomics. Differences in bacterial composition at the sites was done using analysis of variance (ANOVA) and non-metric multidimensional scaling (NMDS), also, a canonical correspondence analysis (CCA) was carried out to show the relationship between the sample sites, environmental factors and bacterial composition. The most abundant phyla in all forest sites were *Proteobacteria, Acidobacteria, Verrucomicrobia, Chloroflexi* and *Actinobacteria* with relative abundance values of 33%, 25%, 20%, 6% and 5% respectively, while the most abundant classes in all forest sites were *Verrucomicrobia*, *Alphaproteobacteria*, *Holophagae*, *Betaproteobacteria*, *Acidobacteria*, *Ktedonobacteria* and *Actinobacteria*, with corresponding relative abundant values of 19%, 17%, 14%, 11%, 7%, 6%, and 5%. All sites had equal numbers of operational taxonomic units (OTUs). Significant differences were observed in the phyla composition of the commercial and indigenous forests in both sites. The physical and chemical properties had no significant effect on bacterial composition and diversity in the forest soil evaluated.

Keywords: bacterial abundance, forest conservation, indigenous forest, land use, soil nutrients

5.2 Introduction

Terrestrial environments are undergoing constant and continuous changes as a result of land use. Many of these changes have caused loss of important ecosystem functions. Some of these functions are seen in nutrient cycling and microbial diversity (Foley et al., 2005, Ayala-Orozco et al., 2018, Meng et al., 2019). Changes in the ecosystem can be caused by the conversion of forests either to commercial forests, agricultural lands or deforestation. These changes affect the soil properties and thereby influence the microbial composition of the soil (Hartmann et al., 2014, Bruun et al., 2015, Guan et al., 2015, Meng et al., 2019).

The soil possesses the largest diversity of microorganisms in the ecosystem which are important in the transport of nutrients among biotic and abiotic components (Lin et al., 2017, Liu et al., 2017, Tyc et al., 2017). Recent studies carried out on the forest ecosystem showed that forest conversions led to variations in the microbial structure (Cundy et al., 2016, Guo et al., 2016a, Guo et al., 2016b) and, also, the land cover influenced the composition of bacteria in the soil (Chim Chan et al., 2008).

Soil bacteria, one of the major contributors to the biogeochemical processes that take place in the soil, is more inclined to certain nutrient elements than others which changes as the forest composition changes, whereas other microorganisms do not show a specific inclination to specific nutrient elements. For example, in a recent study, bacterial abundance showed a significant correlation to organic carbon (OC), nitrogen (N), and phosphorus (P) elements (Zhang et al., 2016). Plants require nitrogen as it is an essential element for growth. However, plants can only access nitrogen in its mineralized forms (ammonia and nitrate) which can only be made available through the action of certain groups of bacteria such as ammonia oxidizing bacteria (AOB)(Amoo and Babalola, 2017).

Leaf litter fuels the mineralization of nitrogen (Stokdyk and Herrman, 2014), therefore, soils rich in litter may also have higher concentrations of N-mineralizing bacteria. Since fungi and bacteria are the key microorganisms in the cycling of essential nutrients and as such are expected to be directly affected by the presence or absence of these chemical substances required by plants (Camenzind et al., 2018), it is therefore important to understand the ecosystem factors responsible for their abundance, composition and structural diversity. Microorganisms are actively involved in nutrient distribution in the soil and the degree to which they take up these nutrients is dependent on the fertility of the soil. Soil nutrients such as potassium, phosphorus, calcium and magnesium are readily available in un-weathered rock and deplete as the rocks weather and soil development takes place (Walker and Syers, 1976, Vitousek and Sanford Jr, 1986).

In order to ascertain the relationship between soil properties and soil microorganisms, various methods have been developed. In recent times, the use of High-throughput sequencing methods have been used and this method allows comprehensive analyses of microorganisms on a wide range of samples (Knight et al., 2012). Owing to this development, it is easy to observe changes in microbial diversity due to changes in environmental conditions (Liu et al., 2018b). Soil microbial environments are a crucial part of the soil and are therefore major contributors to ecosystem services, thus it is important to note the physical and chemical drivers of their composition and diversity. This can be done by using the 16S and illumina targeted sequencing (ITS).

Recent studies show that forest management systems are vital for proper maintenance of ecosystem services, as forests are store houses for large organic matter above ground. Mismanaged forests could lead to biodiversity losses and consequently land degradation (Crowther et al., 2014, Bastida et al., 2015, Lladó et al., 2017a).This study was therefore conducted to determine the effects of indigenous and commercial soil physical and chemical

properties on bacterial distribution and diversity in South Africa. Although studies have been carried out on the factors that could impact bacterial distribution (Lin et al., 2017, Liu et al., 2017, Lladó et al., 2017a), there is no known research that has been carried out on South African soil. Such a study is important as ecosystem services need to be maintained to prevent land degradation. It is hypothesized that bacteria will be more abundant in indigenous forest soils than in commercial soils. Soils rich in organic matter and nutrients tend to have a higher microbial abundance than soils with less nutrients (Crowther et al., 2014), also bacteria present in these regions will be adapted to the soil properties.

5.3 Materials and Methods

5.3.1 Sample collection sites

Samples were collected from Witklip commercial forest latitude [-25°12'50.0245"]; longitude [-30°56'47.0327"] Tweefontein commercial forests latitude [-25°3'8.8998"]; longitude [30°47'23.0316"] as well as Witklip indigenous forest latitude [-25°13'24.7744"] longitude [30°51'14.7258"] and Tweefontein indigenous forest latitude [-24°58'42.6909"] longitude [30°48'57.5057"] in Mpumalanga province, South Africa. The commercial forests were characterised by coniferous trees while the indigenous forests were characterised by deciduous trees. The Mpumalanga region has the largest forested areas in South Africa, which covers an area of 0.6 million hectares.

5.3.2 Sample collection

Soil samples were collected at a depth of 0-10cm after clearing the top of debris and leaf litter. A soil core was used to obtain the samples from the soil. The samples were labelled and put in sampling bags, and placed in an ice box at a temperature of 4°C for further analysis. Fifty grams (50 g) of the samples was stored at -20°C for DNA extraction.

5.3.3 Soil physical and chemical analyses

Two grams (2 g) of soil was mixed in 10ml of deionised water in order to carryout the pH test using the Jenway 3520 pH meter (Cole-Parmer Instruments Staffordshire, UK). The soil was dried at a temperature of 105 ºC for 24 hrs in the oven to obtain the soil moisture content (Colombo et al., 2016). Determination of the total nitrogen and carbon content was done using the dry combustion analysis method and organic carbon was done using the Walkley Black analysis (Walkley and Black, 1934).

5.3.4 Extraction of bacterial DNA, PCR amplification and illumina sequencing

DNA extraction was carried out using Mo-Bio PowerSoil Isolation kit (MO Bio labs, USA) strictly adhering to the manufacturer's instructions. Amplification of the 16SrRNA was carried out using the V4 region with universal bacteria PCR primers 515F/806R. HotStarTaq plus Master Mix was used to couple specific primers. In order to ascertain the presence of the DNA products, samples were run in 2% agarose gel. Samples having similar weights and concentration were pooled together. The Ampure XP beads were used to purify the DNA products ready for illumina and DNA sequencing. DNA sequencing was carried out at Molecular Research (MR DNA) Shallowater, Texas, USA on a MiSeq, strictly adhering to the instructions stated by the manufacturer.

MR DNA pipeline was used to process the sequenced data. Sequences were joined, barcodes removed and sequences which had reads less than 150bp and large base calls were removed. OTUs which clustered at 3% divergence were selected. Taxonomic classification was carried out using BLASTn from the RDPII and NCBI databases.

5.3.5 Data analyses

Analysis of variance (ANOVA) and t tests (David and Gunnink, 1997)were used to measure the differences observed between the soil physical and chemical and bacterial

composition. To ascertain the differences in bacterial composition between sites the permutation analysis of variance (PerMANOVA) (Anderson, 2001) was used. The nonmetric multidimensional scaling (NMDS) was used to measure the composition of the data similar to that carried out by (Meng et al., 2019). Open refine was used to clean the data with typographical errors and repeated values. Also, the Microsoft excel, microbiome analyst, PAST v3 (Hammer et al., 2001) were used in the analysis. Relationship between the bacteria kingdom at all levels and the physical and chemical properties of the soil was done using the Canonical Correspondence Analysis (CCA) which tests the relationship between the environmental factors and the taxa. Data filtering was carried out on the microbiome analyst with 2,067 low abundance features removed, 300 low variance features were removed and there were 2,692 features remaining for analysis.

5.4 Results

5.4.1 Soil physical and chemical properties

In the Tweefontein site, there were no significant changes in the total nitrogen (TN) content. However, the soil organic carbon (SOC), nitrate, total carbon (TC), pH, calcium, magnesium, potassium and sodium content showed significant changes from the indigenous sites to the commercial sites, having higher values in the indigenous sites than the forest sites. The phosphorus content was higher in the commercial forest than the indigenous sites. The Witklip commercial forest site had non-significantly higher concentrations of soil organic carbon (SOC), total carbon (TC) and total nitrogen (TN), than the Witklip indigenous forest site. They also showed a significantly higher concentrations of nitrate, pH, calcium (Ca), Magnesium (Mg) and Sodium (Na) than the Witklip indigenous forest site. However, the concentration of potassium was significantly higher in the Witklip indigenous forest than the Witklip commercial forest site (Table 5.1).

The Tweefontein commercial forest had significantly higher clay and silt particles than the indigenous site, while the indigenous site had a significantly higher concentration of sand particles than the commercial site. At the Witklip site, the amount of clay particles was significantly higher in the indigenous site than the commercial site. The commercial site showed higher values of silt and sand particles than the Witklip indigenous site (Table 5.1).

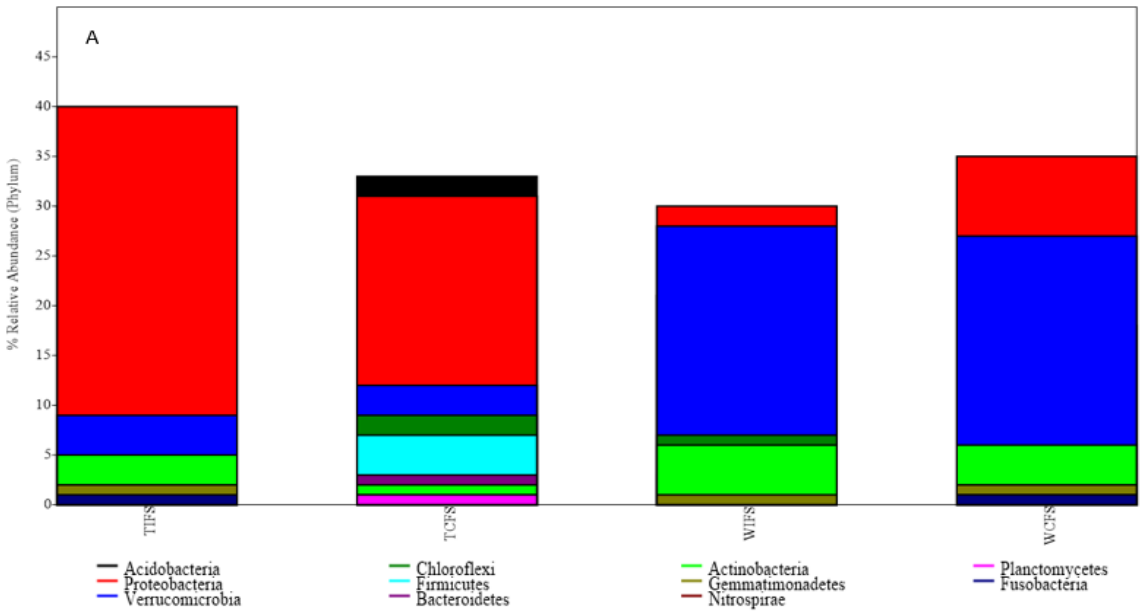
Table 5.1: Soil physical and chemical properties of Tweefontein and Witklip commercial and indigenous forest sites.
SOC: Soil Organic Carbon content; NO_3^-: Soil Nitrate content; TC: Total Carbon; TN: Total Nitrate; P: Phosphorus; Ca: Calcium; Mg: Magnesium; Na: Sodium
Values in parenthesis are standard deviation (SD) where n=2. TIFS- Tweefontein indigenous forest, TCFS- Tweefontein commercial forest, WIFS-Witklip indigenous forest, WCFS- Witklip commercial forest

Sites	Sand (2.0-0.05mm)	Silt (0.05-0.002mm)	Clay (<0.002mm)	SOC (%)	NO_3^- (mg/kg)	TC (%)	TN (%)	pH	P (mg/kg)	Ca (mg/kg)	Mg (mg/kg)	K (mg/kg)	Na (mg/kg)
TIFS	59.85 (3.25)	35.2 (2.10)	9 (0.60)	4.08 (0.07)	65.13 (0.01)	4.58 (0.02)	0.02 (0.01)	4.78 (0.11)	2.33 (0.02)	234.5 (1.50)	95.6 (0.10)	70.3 (0.10)	15.35 (0.15)
TCFS	23.5 (1.40)	50.8 (1.60)	19 (0.10)	3.21 (0.06)	63.38 (0.16)	3.97 (0.05)	0.01 (0.00)	4.28 (0.01)	3.16 (0.00)	15.85 (0.05)	52.35 (0.15)	54 (0.10)	11.6 (0.30)
WIFS	55.1 (0.60)	16.95 (0.45)	25.05 (0.75)	2.67 (0.07)	5.91 (0.03)	2.91 (0.03)	0.001 (0.00)	5.1 (0.01)	5.03 (0.01)	164.5 (1.50)	97.65 (0.45)	92.2 (0.10)	15.5 (0.20)
WCFS	55.15 (1.95)	17.45 (0.75)	23.45 (1.05)	2.97 (0.05)	22.01 (0.19)	3.04 (0.00)	0.01 (0.00)	5.31 (0.03)	3.21 (0.08)	320.5 (1.50)	117 (1.00)	69.95 (0.35)	16.6 (0.30)

5.4.2 Bacterial distribution and diversity

OTUs were picked at a 97% similarity and the total numbers of OTUs were 13,035. The predominant phyla were *Proteobacteria, Acidobacteria, Verrucomicrobia, Chloroflexi* and *Actinobacteria* with relative abundance values of 33%, 25%, 20%, 6% and 5% respectively. In the class level, the predominant classes were *Verrucomicrobia*, *Alphaproteobacteria*, *Holophagae*, *Betaproteobacteria*, *Acidobacteria*, *Ktedonobacteria* and *Actinobacteria* with relative abundance values of 19%, 17%, 14%, 11%, 7%, 6%, and 5% respectively (Figure 5.1).

ANOVA results showed that there were significant differences in the phylum level, ($p < 0.05$) which occurred between *Acidobacteria, Proteobacteria* and *Verrucomicrobia*. Also, at the class level, significant differences ($p < 0.05$) were observed in *Alphaproteobacteria*, *Betaproteobacteria, Holophagae* and *Verrucomicrobia* (Table 5.2)



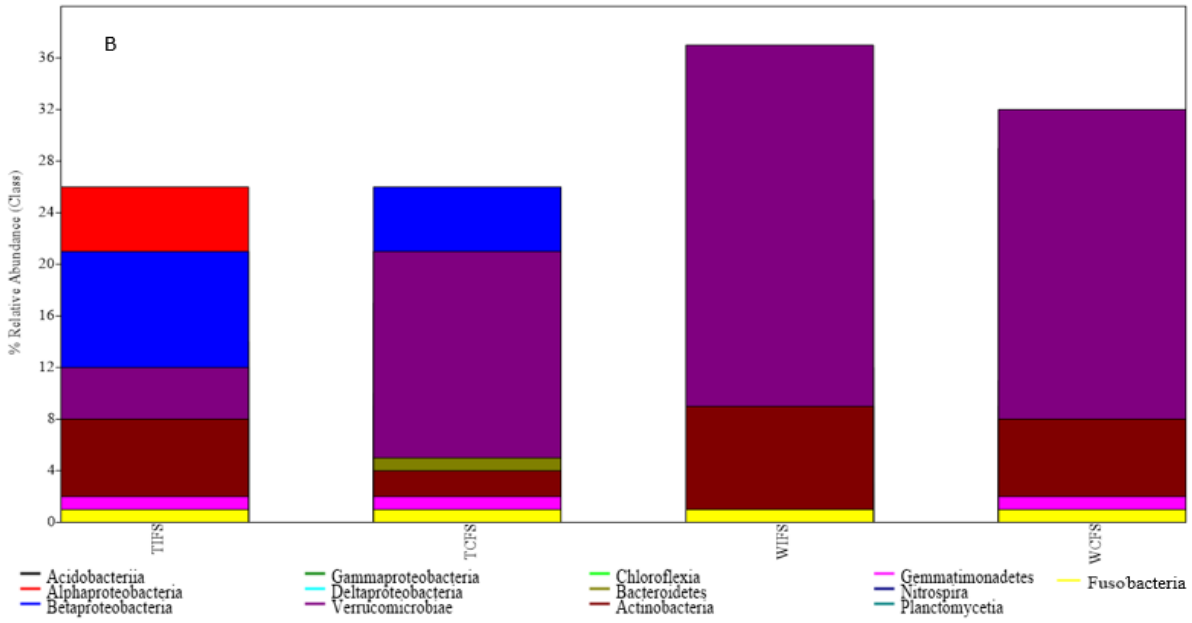


Figure 5.1 Relative abundances of bacteria at A)phyla and B)class level

Table 5.2 ANOVA results for the forest soils obtained from Tweefontein and Witklip indigenous and commercial forests (phylum level)

Phylum	TIFS	TCFS	WIFS	WCFS
Acidobacteria	5.092*	5.125*	4.395*	5.059*
Actinobacteria	0.008*	0.041*	0.689*	0.024*
Alphaproteobacteria	7.155**	7.181**	6.607**	7.129**
Bacteroidetes	0.417	0.384	1.114	0.449
Betaproteobacteria	4.326**	4.352**	3.778**	4.300**
Candidatus_Sacchaibacteria	1.328	1.295	2.025	1.361
Chlamidiae	1.340	1.307	2.037	1.373
Chloroflexi	0.289	0.322	0.408	0.257

Cyanobacteria	1.335	1.302	2.032	1.368
Elusimicrobia	1.340	1.306	2.037	1.372
Firmicutes	0.306	0.272	1.003	0.338
Gemmatimonadetes	1.030	0.996	1.727	1.062
Holophagae	5.616**	5.642**	5.069**	5.590**
Ignavibacteriae	1.336	1.303	2.033	1.369
Nitrospirae	1.277	1.244	1.974	1.309
Others	1.289	1.257	1.960	1.321
Planctomycetes	1.187	1.155	1.858	1.218
Proteobacteria	7.460*	7.492*	6.789*	7.428*
Spirochaetes	1.289	1.257	1.960	1.321
Verrucomicrobia	3.932*,**	3.964*,**	3.261*,**	3.901*,**

*indicates significant differences at phylum level, **indicates significant differences at class level, *,** indicates significant differences in both phyla and class

5.4.3 Differences in bacterial structure and composition among sites

There were no significant differences observed at the phylum and class levels using perMANOVA analysis between the forest types. Non-metric multidimensional scaling (NMDS) had a stress value of 0 (Fig 5.2A and 5.2B). Using Bray-Curtis similarity measure, the distance between the Tweefontein commercial and Tweefontein indigenous forests at the class and phylum levels was considerable as shown on NMDS 1 with $p < 0.05$, while the distance between the Witklip commercial and Witklip indigenous forests was not distinct with $p > 0.05$. Also, along the NMDS axis 2 the distance between the Tweefontein commercial and

indigenous forests at the class and phylum levels, was distinct. At the Witklip sites, the distance between the commercial and indigenous forests was not distinct with $p > 0.05$ at class level (Table 5.3). In the phylum level, plotting the NMDS, the Tweefontein commercial and indigenous forests showed significant difference on both axes while the Witklip commercial and indigenous forests showed no significant differences on both axes.

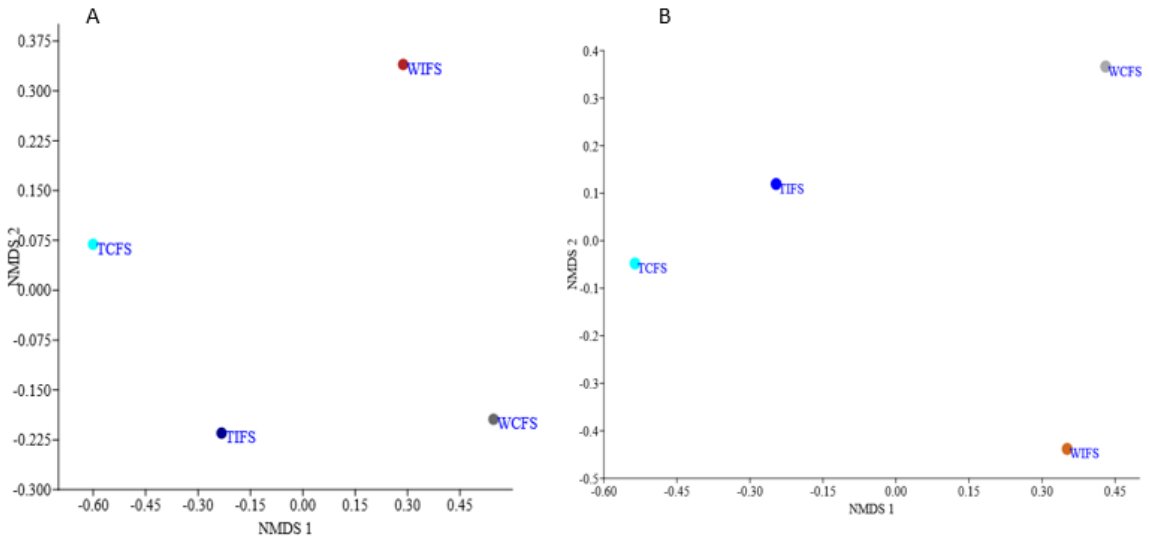


Figure 5.2 Non-metric Multidimensional Scaling (NMDS) of the forest sample sites at A) Class level, B) Phylum level: TIFS- Tweefontein Indigenous Forest Site, TCFS- Tweefontein Commercial Forest Site, WIFS- Witklip Indigenous Forest Site, and WCFS- Witklip Commercial Forest Site.

Table 5.3 Axis scores for the forest sites at class level.

Sites	NMDS Axis 1	NMDS Axis 2
Tweefontein Indigenous Forest Soil	-0.233	-0.219
Tweefontein Commercial Forest Soil	-0.601	0.069

Witklip Indigenous Forest Soil	0.287	0.340
Witklip Commercial Forest Soil	0.546	-0.194

5.4.4 Influence of soil properties on bacterial diversity

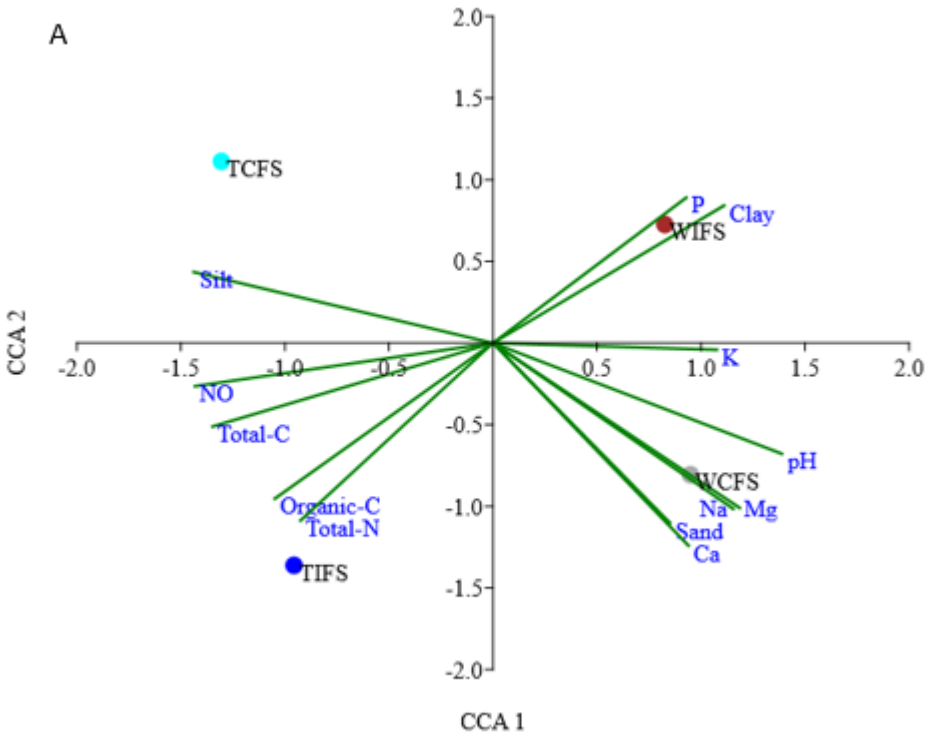
The soil from the Witklip indigenous forest was found to have the highest levels of phosphorus and clay, the Tweefontein commercial forest soil was found to have the highest silt content, the Tweefontein indigenous forest soil had highest levels of total carbon, total nitrogen, and nitrate and the Witklip commercial forest soil had the highest levels of magnesium, sodium, calcium, sand, and also had the highest pH (Fig 5.3A). There were no significant differences between the environmental variables and the bacterial levels ($p > 0.05$) from the phylum to the species level. The bacterial levels were seen to cluster at regions with closely related physical and chemical properties (Fig 5.3 B-G).

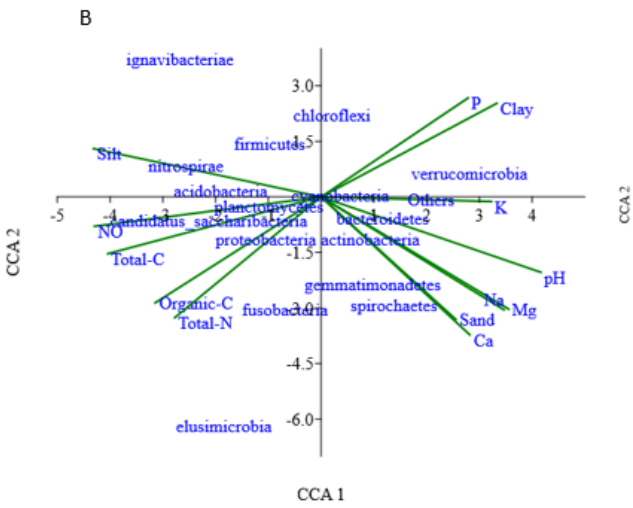
The phylum *Verrucomicrobia* was found in regions with high clay content. *Proteobacteria* was found in a region with high levels of organic carbon and total nitrogen. *Actinobacteria* was found in regions with high calcium, sand, magnesium, sodium and pH, *Acidobacteria* was found in regions high in total nitrogen, organic carbon and silt, *Chloroflexi* was found in between regions high in phosphorus, clay and silt (Fig 5.3B).

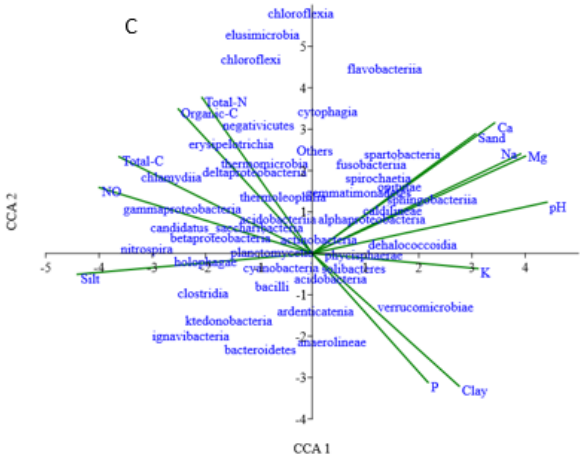
At the class level, *Verrucomicrobia* was observed to be in-between regions with high levels of phosphorus and clay. *Alphaproteobacteria* was seen in regions with high levels of sand, calcium, sodium and magnesium, *Holophagae* was on the same plane as the higher level of silt, *Ktedonobacteria* was seen in-between regions with high levels of silt and phosphorus,

Actinobacteria was found across regions with high levels of total carbon, organic carbon, total nitrogen, sand, calcium, sodium, magnesium and high pH (Fig 5.3C).

Bacteria clustered along regions between high levels of calcium and total nitrogen, organic carbon and total carbon, at order level (Fig 5.3D). At the family level, clustering was more towards the central point where almost all the physical and chemical properties were least in concentration (Fig 5.3E). At the genus level, clustering increased in regions with high levels of total nitrogen, organic carbon, sand, calcium, magnesium, sodium, phosphorus, clay, nitrate, total carbon, but was more at the centre (Fig 5.3F). At the species level, clustering increased at regions with high levels of phosphorus and clay, although it was higher at regions with high level of magnesium, sodium, sand, calcium and pH, high clusters were also seen in regions between calcium and total nitrogen, also at regions with high nitrate and total carbon (appendix 3).




B
ignavibacteriae
chloroflexi
firmicutes
Silt
nitrospirae
acidobacteria
planctomycetes
candidatus_saccharibacteria
NO
Total-C
proteobacteria
actinobacteria
Organic-C
Total-N
fusobacteria
bacteroidetes
Others
verrucomicrobia
gemmatimonadetes
spirochaetes
P
Clay
K
Na
Mg
Sand
Ca
pH
elusimicrobia
CCA 1
CCA 2


C
chloroflexia
elusimicrobia
chloroflexi
flavobacteriia
Total-N
Organic-C
cytophagia
negativicutes
erysipelotrichia
Others
Total-C
chlamydiia
deltaproteobacteria
thermomicrobia
fusobacteria
spartobacteria
spirochaetia
NO
gammaproteobacteria
thermoleophilia
candidatus_saccharibacteria
betaproteobacteria
nitrospira
planctomycetia
holophagae
cytophagia
Silt
clostridia
bacilli
ktedonobacteria
ignavibacteria
bacteroidetes
anaerolineae
ardenticatenia
verrucomicrobiae
P
Clay
Ca
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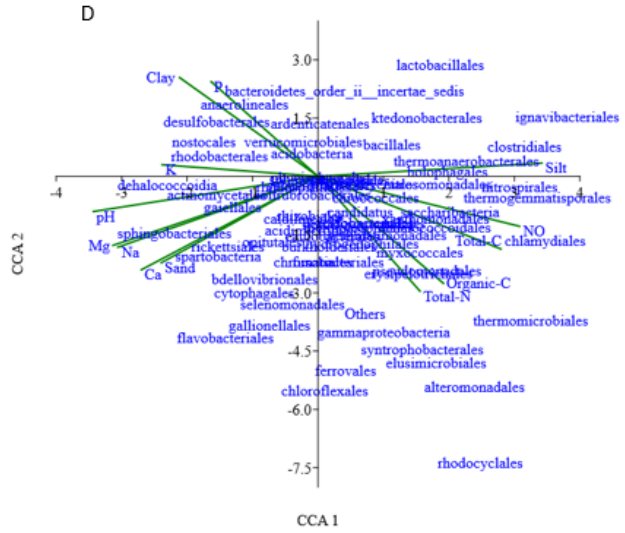
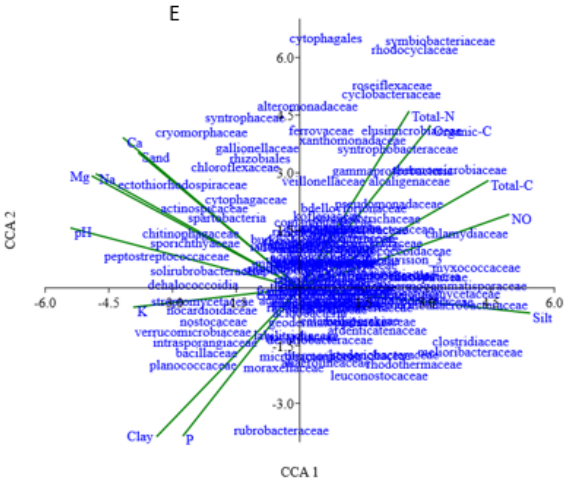
K
CCA 1
CCA 2


D
lactobacillales
Clay
P
bacteroidetes_order_ii__incertae_sedis
anaerolineales
desulfobacterales
ardenticatenales
ktedonobacterales
ignavibacteriales
nostocales
verrucomicrobiales
bacillales
clostridiales
rhodobacterales
acidobacteria
thermoanaerobacterales
Silt
K
dehalococcoidia
nitrospirales
thermogemmatisporales
pH
gaiellales
sphingobacteriales
Mg
Na
Ca
Sand
spartobacteria
rickettsiales
NO
Total-C
chlamydiales
myxococcales
bdellovibrionales
cytophagales
Organic-C
Total-N
selenomonadales
Others
thermomicrobiales
gallionellales
flavobacteriales
gammaproteobacteria
syntrophobacterales
elusimicrobiales
ferrovales
chloroflexales
alteromonadales
rhodocyclales
CCA 1
CCA 2


E
cytophagales
symbiobacteriaceae
rhodocyclaceae
roseiflexaceae
cyclobacteriaceae
alteromonadaceae
syntrophaceae
Total-N
cryomorphaceae
ferrovaceae
elusimicrobiaceae
Organic-C
xanthomonadaceae
gallionellaceae
syntrophobacteraceae
Ca
Sand
rhizobiales
chloroflexaceae
Mg
Na
ectothiorhodospiraceae
verrucomicrobiaceae
alcaligenaceae
Total-C
actinomycetales
cytophagaceae
sphingobacteriaceae
NO
pH
chitinophagaceae
chlamydiaceae
sporichthyaceae
peptostreptococcaceae
myxococcaceae
solirubrobacteridae
dehalococcoidia
nocardioidaceae
K
Silt
nostocaceae
verrucomicrobiaceae
intrasporangiaceae
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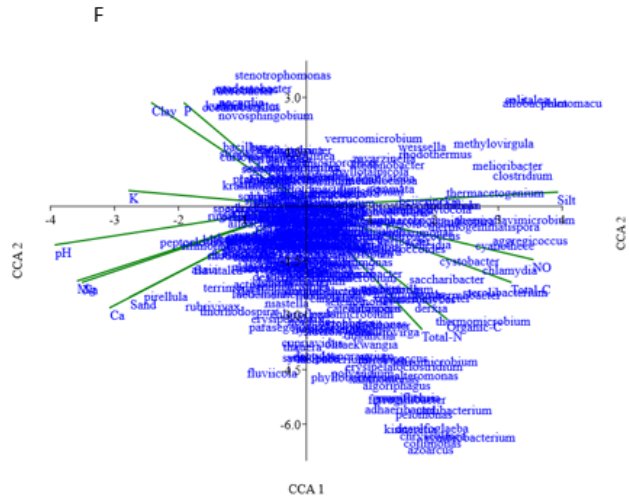
bacillaceae
planococcaceae
moraxellaceae
leuconostocaceae
rubrobacteraceae
Clay
P
CCA 1
CCA 2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Clay
P
novosphingobium
verrucomicrobium
weissella
methylovirgula
rhodothermus
melioribacter
clostridium
K
Silt
thermacetogenium
flavimicrobium
pH
cytobacter
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thermomicrobium
Total-N
fluviicola
CCA 1
CCA 2

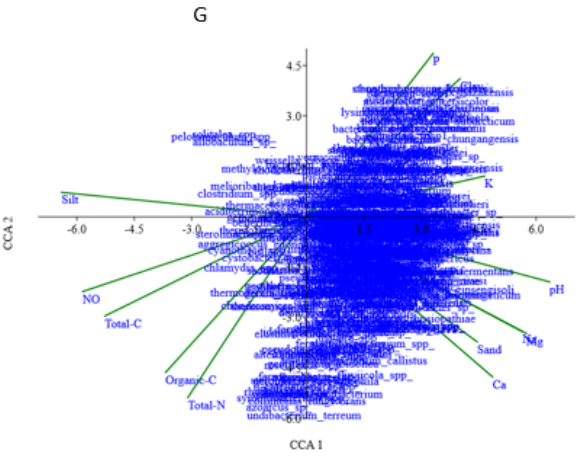
G
P
Silt
NO
Total-C
pH
Mg
Sand
Ca
Organic-C
Total-N
CCA 1
CCA 2

Figure 5.3 Canonical Correspondence Analysis (CCA) of environmental variables and bacteria at the A) Sample sites, B) Phylum, C) Class, D) Order, E) Family, F) Genus and G) Species levels. Close clustering shows the point at which the physical and chemical properties were close to being equally distributed. Clusters observed at levels with almost equal physical and chemical properties.

5.5 Discussion

Change in land use as well as the conversion of indigenous forests to more productive forests greatly impacts bacterial composition and soil properties (da C Jesus et al., 2009, Guo et al., 2016a). Differences in land use (i.e. commercial and indigenous) had no effect on the phyla or classes, as bacteria phyla and classes of both commercial and indigenous forests were the same. Also, the most abundant phyla and classes were similar to those observed from previous studies (Colombo et al., 2016, Meng et al., 2019). The commercial sites showed higher levels of *Acidobacteria, Actinobacteria* and *Alphaproteobacteria*. These differences may have occurred due to preferences for soil with certain physical properties such as low pH. Research carried out on these bacterial classes showed that *Actinobacteria* and *Acidobacteria* preferred soil with lower pH values (Lauber et al., 2009, Hermans et al., 2017, Meng et al., 2019). However, our studies showed that although these bacterial classes were abundant in the commercial forest soils, these soils did not have the lowest pH values, with pH values of 4.28 and 5.31 at the Tweefontein and Witklip regions respectively.

Soils that have similar history have been observed to be similar in bacterial composition especially when the types of crop grown in the soil are the same for a long period of time. This therefore suggests that land use is a major determinant of soil bacterial community (Moghimian et al., 2017). Although the types of trees grown in the indigenous and commercial forests were different, they had the same total number of OTUs, similar bacterial phyla and classes. The four forests sites showed a lot of similarity in the bacterial composition, this supports previous studies that showed that land use has a significant influence on bacterial composition and structure (Norris et al., 2010, Moghimian et al., 2017, Meng et al., 2019).

Leaf litter composition also plays an important role in bacterial diversity as it affects the top soil composition (Li et al., 2019), since bacteria are mostly found in soil regions with high nutrient and organic matter. In forest soils, they will be found mostly in regions where

leaf litter is present, therefore, the type of bacteria found will be dependent on the type of nutrient since they are the major decomposers of litter. We hypothesized that bacteria will be more abundant in the indigenous forests than the commercial forests. Although the indigenous forests had higher abundance in Acidobacteria, Actinobacteria, Bacteroidetes, Proteobacteria and Verrucomicrobia bacterial phyla, higher Betaproteobacteria and Holophagae bacterial class, the distribution of these abundances must have been affected by the type of tree planted and the season in which samples were obtained as found in a previous study (Zhang et al., 2016).

Plant species have been found to play an important role in shaping the bacterial community and abundance (Barrios et al., 2018, Nouri et al., 2019). Plants constantly compete for water and nutrients. They therefore release exudates through their roots which attract beneficial bacteria. Trees also influence the structural composition of bacteria and modify the type of bacteria that will be found in the soil (Lejon et al., 2005). The differences in the tree types may be the cause of differences in bacterial abundance in the indigenous and commercial soils. However, significant differences existed between phyla of indigenous and commercial forests in the same region, this showed that the tree species could be responsible for the differences observed between the forest types.

Soil physical and chemical properties can be determined by the plant species present as previous studies have proved (Li et al., 2017a, Tang et al., 2017, Tian et al., 2018). However, in the present study, there was no significant effect of the soil physical and chemical properties on the bacterial abundance. The Eigenvalues from the phylum to the species level was above 70%, which shows that the CCA was able to effectively predict the patterns between the environmental variables and the bacteria levels.

However, the CCA analysis of the distribution of bacterial phyla to species, showed some of these bacterial groups were found in specific regions. For example, *Verrucomicrobia* was observed in regions with high clay content at the phylum and class levels.

5.6 Conclusion

In conclusion, land use, tree type and species are important determinants of the bacterial structure and composition. Forest conversion to commercial forests should therefore be discouraged as this could lead to a reduction in bacterial abundance and consequently cause land degradation.

5.7 Acknowledgements

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CHAPTER SIX

6.1 General Conclusion and Recommendation

Land use, tree type and species are important determinants of the bacterial structure and composition. Maintaining natural lands should be of top priority, as the opposite could lead to loss of biodiversity and consequently loss of major ecosystem services. The natural lands were observed to be richer in bacteria than both agricultural lands. In order to maintain and sustain existing lands, methods such as re-afforestation, which can enhance microbial, and particularly bacterial diversity, should be encouraged.

Although there are no specific bacteria that show a degrading land, the absence or reduced presence of bacteria phyla such as *Acidobacteria, Bacteroidetes, Actinobacteria and Proteobacteria* could mean the land would likely be degrading because these phyla play important role in the cycling of plant nutrients from organic matter decomposition.

The soil biological indicators are a good measure of the soil health and quality, as observed in the Limpopo and forest sites, which had higher bacterial diversity and richness than the North West agricultural sites. Although there are no set standards for diversity and richness measurements, it is important that the soil has high abundances and richness of these phyla, as seen in the study.

Comparing the two agricultural sites, the Limpopo agricultural site had a higher bacterial diversity measurement than the North West agricultural site. This showed that the North West agricultural site is closer to being a degraded land than the Limpopo agricultural site. The unperturbed land (forest site) also had a higher bacterial diversity than the North West agricultural site, as a result of the organic matter composition of the soil due to litter composition and animal decomposition that normally take place in such sites.

Although we hypothesized that the natural lands would have more bacterial diversity, we discovered that this was not the case for the lands under study. It may have been as a result of the variation in the tree/plant type, organic matter present, soil type or seasonal variation.

Further research should be carried out on analysing the effects of different types of organic matter on the soil-dwelling organisms that are important for agricultural productivity. As seen in the Limpopo agricultural site, the bacterial diversity is much higher than the forest site. This could give a better understanding of factors that could boost bacterial diversity and help in the revival of degraded land.

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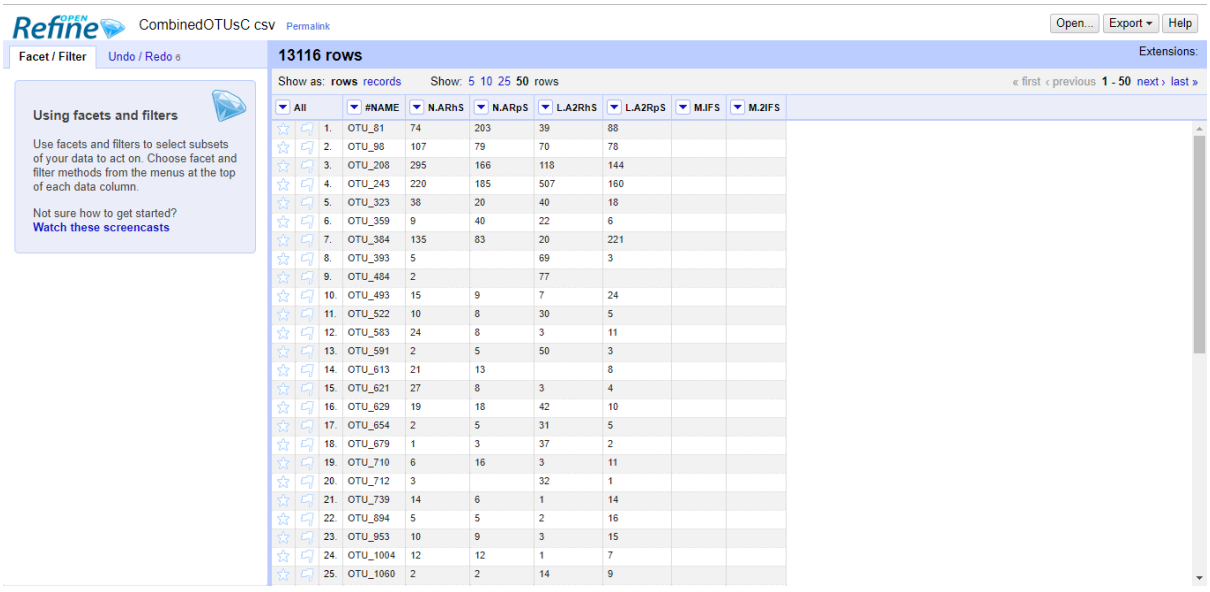
APPENDICES

Appendix 1: Open refine is an open source offline web tool used for analysing and cleaning messy data. This tool was used to clean, sort and combine data obtained from the extracted samples without human or machine errors. This tool is specifically useful in visualizing huge datasets for the purpose of editing and correcting errors in names, to filter off unwanted observations.



All	#NAME	N.ARhS	N.ARpS	L.A2RhS	L.A2RpS	M.IFS	M.2IFS
1.	OTU_81	74	203	39	88		
2.	OTU_98	107	79	70	78		
3.	OTU_208	295	166	118	144		
4.	OTU_243	220	185	507	160		
5.	OTU_323	38	20	40	18		
6.	OTU_359	9	40	22	6		
7.	OTU_384	135	83	20	221		
8.	OTU_393	5		69	3		
9.	OTU_484	2		77			
10.	OTU_493	15	9	7	24		
11.	OTU_522	10	8	30	5		
12.	OTU_583	24	8	3	11		
13.	OTU_591	2	5	50	3		
14.	OTU_613	21	13		8		
15.	OTU_621	27	8	3	4		
16.	OTU_629	19	18	42	10		
17.	OTU_654	2	5	31	5		
18.	OTU_679	1	3	37	2		
19.	OTU_710	6	16	3	11		
20.	OTU_712	3		32	1		
21.	OTU_739	14	6	1	14		
22.	OTU_894	5	5	2	16		
23.	OTU_953	10	9	3	15		
24.	OTU_1004	12	12	1	7		
25.	OTU_1060	2	2	14	9		

Every step is trackked, can be exported and reused.

Appendix 2: Metadata and abundance tables for class to species level.

Metadata

#NAME	Sample Type
N.ARhS	North-West Agricultural Rhizosphere Soil
N.ARpS	North-West Agricultural Rhizoplane Soil
L.A2RhS	Limpopo Agricultural Rhizosphere Soil
L.A2RpS	Limpopo Agricultural Rhizoplane Soil
M.IFS	Mpumalanga Indigenous Forest Sample
M.2IFS	Mpumalanga Indigenous Forest Sample

Abundance table for class

	L.A2RpS	L.A2RhS	M.IFS	M.2IFS	N.ARpS	N.ARhS
Cyanobacteria	124	415	436	413	284	219
Verrucomicrobia	174	358	2536	9247	203	178
Nitrospirae	346	977	483	693	255	582
Gemmatimonadetes	937	1033	1594	2535	1080	1665
Planctomycetes	985	2367	5149	4773	820	1568
Chloroflexi	2254	1813	3129	4613	1458	2845
Bacteroidetes	2546	4738	4845	9609	3298	3179
Firmicutes	3001	5338	2602	3827	3128	3483
Acidobacteria	3499	3234	9948	8940	2851	4985
Actinobacteria	9627	6770	9123	11888	9746	11832
Proteobacteria	20580	27888	29815	49805	18413	25534

Abundance Table for order

	L.A2RpS	L.A2RhS	M.IFS	M.2IFS	N.ARpS	N.ARhS
Acidobacteria	255	157	257	225	485	439
Acidobacteriia	1868	1441	4167	2733	1152	2017
Actinobacteria	8999	6654	9025	11630	9367	11247
Alphaproteobacteria	8467	10983	15691	22998	7280	9534
Anaerolineae	439	546	568	653	324	540
Armatimonadetes	1	6	4	39	2	0
Armatimonadia	21	1	47	37	44	49
Bacilli	1810	4047	1310	2062	2223	1972
Bacteroidetes	15	33	213	181	32	38
Bacteroidia	164	172	424	711	402	347
Betaproteobacteria	1064	4919	5334	7581	1340	1665
Caldilineae	46	82	182	133	36	74
Chloroflexi	239	108	149	306	158	390
Chloroflexia	409	444	1154	1102	400	631
Chthonomonadetes	37	6	4	12	18	27
Clostridia	1144	1204	998	1595	858	1412

Cyanobacteria	120	388	397	378	283	219
Cytophagia	960	1844	2131	3367	1412	1218
Dehalococcoidia	1027	586	632	1543	470	1084
Deinococci	15	32	16	17	13	3
Deltaproteobacteria	8528	8084	5563	15430	6838	11411
Elusimicrobia	0	15	35	11	0	1
Epsilonproteobacteria	2	48	29	20	4	5
Erysipelotrichia	0	57	90	75	2	1
Fibrobacteria	0	11	9	19	5	3
Flavobacteriia	101	797	251	456	123	109
Fusobacteriia	6	52	39	43	12	5
Gammaproteobacteria	2517	3794	3195	3770	2943	2917
Gemmatimonadetes	937	1033	1594	2535	1080	1665
Gloeobacteria	4	27	39	35	1	0
Holophagae	344	852	3100	1758	272	478
Ignavibacteria	4	31	43	42	8	4
Ktedonobacteria	9	2	116	410	0	4
Mollicutes	2	85	40	20	2	5
Negativicutes	4	20	24	19	4	29
Nitrospira	346	977	483	693	255	582
Oligoflexia	2	60	3	6	8	2
Opitutae	125	314	153	124	187	146
Phycisphaerae	249	241	411	388	208	447
Planctomycetia	736	2126	4738	4385	612	1121
Solibacteres	1032	784	2424	4224	942	2051
Spartobacteria	8	1	4	6	2	5
Sphingobacteriia	1306	1892	1826	4894	1329	1467
Spirochaetia	14	317	19	114	5	14
Thermodesulfobacteria	4	0	17	0	1	5
Thermoleophilia	628	116	98	258	379	585
Thermolithobacteria	43	10	180	76	41	69
Thermomicrobia	85	45	328	466	70	122
Verrucomicrobiae	41	43	2379	9117	14	27

Abundance table for family

	L.A2RpS	**L.A2RhS**	**M.IFS**	**M.2IFS**	**N.ARpS**	**N.ARhS**
Acholeplasmatales	2	74	10	20	1	5
Acidimicrobiales	301	227	213	246	182	289
Acidithiobacillales	14	19	105	103	8	18
Acidobacteria	255	157	257	225	485	439
Acidobacteriales	1868	1441	4167	2733	1152	2017
Actinomycetales	5009	4899	5831	7751	6396	6165
Alphaproteobacteria	13	197	221	180	15	13
Alteromonadales	445	291	1060	332	263	552
Anaerolineales	439	546	568	653	324	540

Armatimonadales	21	1	47	37	44	49
Armatimonadetes	1	6	4	39	2	0
Bacillales	1766	3875	1114	1888	2164	1931
Bacteroidales	164	172	424	711	402	347
Bacteroidetes	3	1	0	3	14	15
Bacteroidetes incertae_sedis	12	32	213	178	18	23
Bdellovibrionales	2	5	60	78	17	3
Betaproteobacteria	0	4	18	15	1	10
Burkholderiales	786	3039	1875	3280	1029	1185
Caldilineales	46	82	182	133	36	74
Campylobacterales	2	48	29	20	4	5
Candidatus_brocadiales	6	94	326	210	11	7
Caulobacterales	193	196	351	1029	259	246
Chloroflexales	349	423	1079	1046	349	536
Chloroflexi	239	108	149	306	158	390
Chromatiales	181	462	631	886	129	242
Chroococcales	34	97	43	161	36	30
Chthonomonadales	37	6	4	12	18	27
Clostridiales	440	785	804	1303	408	532
Cytophagales	960	1844	2131	3367	1412	1218
Dehalococcoidales	782	397	368	263	316	767
Dehalococcoidia	245	189	264	1280	154	317
Deltaproteobacteria	12	14	72	52	2	22
Desulfobacterales	156	100	54	60	86	206
Desulfovibrionales	4	41	20	24	4	7
Desulfurellales	0	65	38	14	0	1
Desulfuromonadales	5423	5099	3456	12981	4549	7285
Elusimicrobiales	0	15	35	11	0	1
Enterobacteriales	13	14	31	149	191	8
Entomoplasmatales	0	11	30	0	1	0
Erysipelotrichales	0	57	90	75	2	1
Fibrobacterales	0	11	9	19	5	3
Flavobacteriales	101	797	251	456	123	109
Fusobacteriales	6	52	39	43	12	5
Gaiellales	364	134	373	849	207	319
Gallionellales	0	8	8	23	1	0
Gammaproteobacteria	0	10	13	19	1	0
Gemmatimonadales	937	1033	1594	2535	1080	1665
Gloeobacterales	4	27	39	35	1	0
Halanaerobiales	0	7	15	0	0	0
Herpetosiphonales	46	16	69	48	37	61
Holophagales	344	852	3100	1758	272	478
Hydrogenophilales	116	846	1819	1971	128	183
Ignavibacteriales	4	31	43	42	8	4
Kallotenuales	14	5	6	8	14	34
Ktedonobacterales	9	2	116	410	0	4
Lactobacillales	44	172	196	174	59	41
Legionellales	30	75	99	91	23	29
Methylococcales	1	28	29	31	6	0
Methylophilales	8	74	88	67	22	8

Myxococcales	1591	2555	1465	1608	1532	1975
Natranaerobiales	0	1	4	0	1	4
Nitriliruptorales	24	10	50	21	22	47
Nitrosomonadales	57	208	1081	1885	54	74
Nitrospirales	346	977	483	693	255	582
Nostocales	4	36	20	4	15	50
Oceanospirillales	10	65	34	41	9	20
Oligoflexales	2	60	3	6	8	2
Opitutales	125	314	153	124	187	146
Oscillatoriales	55	204	257	128	177	66
Phycisphaerales	249	241	411	388	208	447
Planctomycetales	730	2032	4412	4175	601	1114
Pleurocapsales	1	19	43	49	3	2
Prochlorales	26	32	34	36	52	71
Pseudomonadales	124	357	151	126	810	96
Rhizobiales	6840	7336	11630	16324	5487	7383
Rhodobacterales	226	194	268	345	305	347
Rhodocyclales	97	740	445	340	105	205
Rhodospirillales	874	1191	2127	3958	655	874
Rickettsiales	112	1479	320	443	257	363
Rubrobacterales	910	391	492	709	677	1674
Selenomonadales	4	20	24	19	4	29
Sneathiellales	13	4	31	5	12	18
Solibacterales	1032	784	2424	4224	942	2051
Solirubrobacterales	2391	993	2066	2054	1883	2753
Spartobacteria	8	1	4	6	2	5
Sphaerobacterales	20	24	240	426	26	49
Sphingobacteriales	1306	1892	1826	4894	1329	1467
Sphingomonadales	196	386	743	714	290	290
Spirochaetales	14	317	19	114	5	14
Syntrophobacterales	1340	205	398	613	648	1912
Thermales	15	32	16	17	13	3
Thermoanaerobacterales	704	411	175	292	449	876
Thermodesulfobacteriales	4	0	17	0	1	5
Thermoleophilales	628	116	98	258	379	585
Thermolithobacterales	43	10	180	76	41	69
Thermomicrobiales	65	21	88	40	44	73
Thiotrichales	7	6	44	104	1	0
Verrucomicrobiales	41	43	2379	9117	14	27
Xanthomonadales	1692	2467	998	1888	1502	1952

Abundance table for genus

	L.A2RpS	**L.A2RhS**	**M.IFS**	**M.2IFS**	**N.ARpS**	**N.ARhS**
Acetobacteraceae	151	275	618	1499	114	156
Acholeplasmataceae	2	74	10	20	1	5
Acidimicrobiaceae	255	161	126	205	145	233
Acidithiobacillaceae	14	19	105	103	8	18
Acidobacteria	255	157	251	221	485	439

Acidobacteriaceae	1872	1442	4121	2735	1153	2024
Acidothermaceae	11	10	9	26	13	12
Actinomycetaceae	74	100	417	63	72	92
Aerococcaceae	0	1	2	0	6	15
Alcaligenaceae	1	37	19	77	1	6
Alicyclobacillaceae	5	41	16	22	2	9
Alphaproteobacteria	13	201	221	184	15	13
Alteromonadaceae	413	285	1044	271	243	519
Anaerolineaceae	439	546	568	653	324	540
Anaeromyxobacteraceae	669	825	327	436	572	801
Anaplasmataceae	53	227	155	273	89	164
Armatimonadaceae	21	1	47	37	48	53
Armatimonadetes	1	6	4	39	2	0
Bacillaceae	1436	3160	249	1103	1346	1381
Bacillales	36	83	127	370	548	49
Bacteroidaceae	154	154	143	350	230	333
Bacteroidales	0	0	91	86	4	1
Bacteroidetes	3	1	0	3	14	15
Bartonellaceae	37	0	6	6	1	3
Bdellovibrionaceae	2	5	60	78	17	3
Beijerinckiaceae	9	5	80	99	16	15
Betaproteobacteria	0	4	18	15	1	10
Bogoriellaceae	0	1	33	14	2	0
Bradyrhizobiaceae	3600	1465	2343	1181	1934	3222
Brucellaceae	19	65	105	89	22	39
Burkholderiaceae	280	1328	606	604	240	385
Burkholderiales	81	471	1015	898	157	158
Caldilineaceae	46	82	182	133	36	74
Candidatus_brocadiaceae	6	94	326	210	11	7
Candidatus_midichloriaceae	14	2	21	11	0	1
Carnobacteriaceae	12	7	11	10	19	25
Catenulisporaceae	0	0	10	19	0	0
Caulobacteraceae	193	196	351	1029	259	246
Chitinophagaceae	1036	1463	1120	4389	1107	1240
Chloroflexaceae	283	377	754	601	275	361
Chloroflexi	231	108	145	292	154	394
Chromatiaceae	94	402	99	83	83	126
Chroococcales	34	97	43	161	36	30
Chthonomonadaceae	37	6	4	12	18	27
Clostridiaceae	83	348	308	542	92	87
Clostridiales_incertaesedis	300	284	179	346	269	392
Colwelliaceae	1	4	12	53	1	0
Comamonadaceae	160	790	188	1659	257	237
Conexibacteraceae	1018	488	738	689	757	1253
Corynebacteriaceae	88	174	173	246	81	194
Coxiellaceae	11	51	76	53	6	6

Cryomorphaceae	50	178	164	266	51	40
Cryptosporangiaceae	4	1	12	15	4	3
Cystobacteraceae	315	475	135	236	314	547
Cytophagaceae	966	1850	2135	3368	1420	1232
Dehalococcoidaceae	782	397	368	263	316	767
Dehalococcoidia	245	189	264	1280	154	317
Deltaproteobacteria	12	14	72	52	2	22
Dermabacteraceae	24	40	40	11	37	47
Desulfobacteraceae	37	43	1	1	14	16
Desulfobulbaceae	119	57	53	59	72	190
Desulfovibrionaceae	3	37	20	24	3	2
Desulfurellaceae	0	65	38	14	0	1
Desulfuromonadaceae	1	45	50	273	2	1
Ectothiorhodospiraceae	87	58	527	798	44	116
Elusimicrobiaceae	0	15	35	11	0	1
Enterobacteriaceae	13	14	31	149	191	8
Erysipelotrichaceae	0	57	90	81	2	5
Erythrobacteraceae	74	165	33	40	104	102
Eubacteriaceae	5	14	128	51	11	17
Fibrobacteraceae	0	11	9	19	5	3
Flavobacteriaceae	51	619	87	190	72	69
Frankiaceae	421	187	865	1257	288	527
Fusobacteriaceae	6	52	39	43	12	5
Gaiellaceae	363	129	373	841	206	318
Gallionellaceae	0	8	8	23	1	0
Gammaproteobacteria	0	10	13	19	1	0
Gemmatimonadaceae	954	1039	1600	2537	1091	1675
Geobacteraceae	500	716	1621	1361	452	768
Geodermatophilaceae	1026	327	1337	527	1075	1471
Gloeobacterales	4	27	39	35	1	0
Glycomycetaceae	17	3	67	176	5	3
Hahellaceae	10	14	30	29	9	18
Halanaerobiaceae	0	7	15	0	0	0
Halomonadaceae	0	51	4	12	0	2
Helicobacteraceae	3	95	29	21	2	9
Heliobacteriaceae	8	4	46	10	1	4
Herpetosiphonaceae	46	16	69	48	37	61
Holophagaceae	344	852	3109	1762	272	478
Holosporaceae	1	1	10	10	1	0
Hydrogenophilaceae	116	846	1819	1971	128	183
Hyphomicrobiaceae	1013	2273	4048	6055	930	1048
Hyphomonadaceae	95	73	88	156	89	140
Iamiaceae	46	66	87	41	37	56
Ignavibacteriaceae	1	17	6	24	2	0
Intrasporangiaceae	3	18	15	14	17	13
Jiangellaceae	6	1	4	4	14	22

Kallotenaceae	14	5	6	8	14	34
Kineosporiaceae	0	0	5	9	0	0
Kofleriaceae	112	383	524	348	98	129
Ktedonobacteraceae	9	2	109	365	0	4
Lachnospiraceae	1	19	2	6	0	1
Lactobacillaceae	3	13	25	48	12	0
Legionellaceae	19	24	23	38	17	23
Leptospiraceae	10	19	12	96	4	14
Leuconostocaceae	0	22	5	0	0	0
Melioribacteraceae	3	14	37	18	6	4
Methylobacteriaceae	984	1644	3002	4800	1201	1674
Methylococcaceae	1	28	29	31	6	0
Methylocystaceae	32	121	110	159	21	49
Methylophilaceae	8	74	88	67	22	8
Microbacteriaceae	44	267	40	74	153	56
Micrococcaceae	172	525	152	892	570	362
Micromonosporaceae	705	667	485	676	705	885
Moraxellaceae	19	44	16	4	598	19
Moritellaceae	4	0	3	4	13	0
Motilibacteraceae	3	1	8	8	1	2
Mycobacteriaceae	331	537	37	51	241	331
Myxococcaceae	67	58	80	42	47	58
Nakamurellaceae	21	7	2	8	17	22
Nannocystaceae	8	77	51	89	12	18
Natranaerobiaceae	0	1	4	0	1	4
Nitriliruptoraceae	24	10	50	21	22	47
Nitrosomonadaceae	57	208	1081	1885	54	74
Nitrospiraceae	346	977	483	693	255	582
Nocardiaceae	51	62	89	58	56	44
Nocardioidaceae	238	590	348	588	448	350
Nocardiopsaceae	48	33	2	7	41	65
Nostocaceae	2	14	10	1	10	27
Oligoflexaceae	0	6	1	4	2	0
Opitutaceae	125	314	153	124	187	146
Oscillatoriales	55	204	257	128	177	66
Oscillochloridaceae	0	1	3	4	5	13
Others	0	0	4	5	0	0
Oxalobacteraceae	264	413	47	42	374	399
Paenibacillaceae	50	174	23	45	94	73
Pasteuriaceae	239	404	313	288	174	419
Pelobacteraceae	4922	4340	1788	11344	4096	6511
Peptococcaceae	6	21	43	26	12	6
Peptoniphilaceae	10	0	5	5	9	1
Phaselicystidaceae	57	302	10	33	113	64
Phycisphaeraceae	249	241	411	388	208	447
Phyllobacteriaceae	510	592	585	1294	557	825

Planctomycetaceae	725	2030	4421	4178	601	1112
Pleurocapsales	1	19	43	49	3	2
Polyangiaceae	351	373	323	320	367	353
Porphyromonadaceae	7	10	37	154	52	10
Prochlorococcaceae	26	32	34	36	52	71
Promicromonosporaceae	3	59	109	109	33	15
Propionibacteriaceae	87	45	13	15	162	237
Pseudomonadaceae	105	313	135	122	212	77
Pseudonocardiaceae	563	341	621	1150	1033	573
Psychromonadaceae	27	2	1	4	6	33
Rhizobiaceae	359	555	580	1999	567	236
Rhizobiales	125	247	88	87	114	118
Rhodobacteraceae	126	113	179	184	207	199
Rhodobiaceae	100	275	126	234	66	108
Rhodocyclaceae	97	740	445	340	105	205
Rhodospirillaceae	671	799	1391	2351	500	683
Rhodospirillales	52	117	124	112	41	35
Rhodothermaceae	12	32	213	178	18	23
Rickettsiaceae	37	1217	117	138	151	164
Rickettsiales	7	32	17	11	16	34
Rikenellaceae	3	8	153	121	116	3
Rivulariaceae	1	22	6	3	2	18
Roseiflexaceae	66	45	322	441	69	162
Rubrobacteraceae	910	391	492	709	677	1674
Ruminococcaceae	1	0	39	169	2	0
Sandaracinaceae	12	48	14	91	6	5
Saprospiraceae	35	212	350	150	22	18
Scytonemataceae	1	0	4	0	3	5
Sinobacteraceae	1359	1095	560	1461	1105	1662
Sneathiellaceae	13	4	31	5	12	18
Solibacteraceae	1032	784	2420	4218	942	2051
Solirubrobacteraceae	1373	505	1332	1395	1126	1500
Spartobacteria	8	1	4	6	2	5
Sphaerobacteraceae	20	24	240	426	26	49
Sphingobacteriaceae	235	217	356	355	200	209
Sphingomonadaceae	122	221	710	674	186	188
Spirochaetaceae	4	298	7	18	1	0
Spiroplasmataceae	0	11	30	0	1	0
Sporichthyaceae	735	82	346	415	435	551
Staphylococcaceae	0	13	1	10	0	0
Streptococcaceae	29	129	153	116	22	1
Streptomycetaceae	268	690	336	537	794	230
Streptosporangiaceae	43	78	36	135	61	35
Symbiobacteriaceae	17	67	17	25	7	12
Syntrophaceae	387	120	30	56	195	548
Syntrophobacteraceae	953	85	368	557	453	1364

Syntrophomonadaceae	9	32	47	123	5	12
Thermaceae	15	32	16	17	13	3
Thermoactinomycetaceae	0	0	256	80	0	0
Thermoanaerobacteraceae	700	409	175	292	447	872
Thermodesulfobacteriaceae	4	0	17	0	1	5
Thermoleophilaceae	628	116	98	258	379	585
Thermolithobacteraceae	43	10	180	76	41	69
Thermomicrobiaceae	65	21	88	40	44	73
Thermomonosporaceae	23	50	308	657	38	20
Thermosporotrichaceae	0	0	7	45	0	0
Thioalkalispiraceae	0	2	5	5	2	0
Thiotrichaceae	7	6	44	104	1	0
Veillonellaceae	4	20	24	19	4	29
Verrucomicrobia	8	7	287	484	3	9
Verrucomicrobiaceae	33	36	2088	8664	11	18
Vulgatibacteraceae	0	14	1	13	3	0
Xanthobacteraceae	46	81	548	276	52	44
Xanthomonadaceae	327	1357	432	427	392	281

Abundance table for species level

	L.A2RpS	**L.A2RhS**	**M.IFS**	**M.2IFS**	**N.ARpS**	**N.ARhS**
Acetivibrio	1	0	39	169	2	0
Acidimicrobium	86	44	63	115	48	86
Acidisphaera	0	3	46	122	0	0
Aciditerrimonas	153	101	63	90	84	118
Acidithiobacillus	14	19	105	103	8	18
Acidobacterium	1738	1340	3141	2042	1073	1843
Acidothermus	11	10	9	26	13	12
Acidovorax	100	489	25	33	139	154
Acinetobacter	19	44	16	4	598	19
Actinoallomurus	0	30	261	541	2	8
Actinokineospora	32	10	178	318	36	19
Actinomadura	23	20	47	116	36	12
Actinomyces	74	100	417	63	72	92
Actinophytocola	92	46	47	63	218	81
Actinoplanes	10	42	0	0	18	19
Adhaeribacter	164	53	295	922	281	367
Aeromicrobium	7	33	37	3	11	5
Aetherobacter	1	2	10	26	0	0
Afifella	58	72	56	170	45	56
Afipia	0	0	8	10	0	0
Agrobacterium	3	16	1	0	33	3
Agromyces	14	36	10	22	40	6
Alcaligenes	1	37	10	72	1	6

Alistipes	3	8	153	121	116	3
Alkalilimnicola	1	31	62	5	1	0
Alkalispirillum	2	15	9	4	1	0
Alkanibacter	3	22	49	36	10	4
Alterococcus	16	6	2	6	9	21
Alteromonas	390	236	1004	242	215	494
Ammoniphilus	1	10	3	0	2	7
Amycolatopsis	4	12	99	3	3	9
Anaerococcus	10	0	5	5	9	1
Anaerolinea	56	65	52	72	44	55
Anaeromyxobacter	669	825	327	436	572	801
Anaplasma	6	0	54	0	10	2
Aquicella	6	46	76	52	3	3
Aquimonas	11	36	35	15	18	4
Archangium	0	6	15	1	2	0
Aridibacter	116	114	146	182	213	161
Armatimonas	21	1	47	37	48	53
Arthrobacter	170	480	138	882	565	362
Azoarcus	73	674	131	186	81	141
Azohydromonas	0	0	9	5	0	0
Azorhizobium	5	22	28	16	4	9
Azospirillum	9	90	344	94	3	7
Bacillus	1432	3137	239	1095	1284	1370
Bacteroides	154	154	143	350	230	333
Bartonella	37	0	6	6	1	3
Bauldia	0	8	9	12	0	0
Beggiatoa	0	6	6	2	1	0
Bellilinea	171	210	383	50	63	199
Bifissio	3	1	0	3	14	15
Blastocatella	11	33	14	25	10	14
Blastococcus	150	151	65	62	330	288
Blastomonas	0	35	57	46	0	0
Blastopirellula	14	69	289	421	16	28
Bosea	12	26	95	8	9	11
Brachybacterium	24	40	40	11	37	47
Bradyrhizobium	442	614	356	430	316	331
Brevibacillus	16	9	3	2	11	13
Brevundimonas	3	8	0	1	8	0
Brucella	6	16	69	5	10	6
Burkholderia	135	447	478	412	67	148
Byssovorax	9	111	102	148	22	12
Caedibacter	0	9	4	5	0	0
Caldanaerobacter	33	34	16	23	8	23
Caldilinea	46	82	182	133	36	74
Calothrix	1	22	6	3	2	18
Candidatus_armantifilum	0	0	91	86	4	1

Candidatus_babela	0	7	1	4	0	1
Candidatus_blochmannia	2	0	11	10	0	0
Candidatus_captivus	1	1	10	10	1	0
Candidatus_carsonella	0	51	4	12	0	2
Candidatus_chloracidobacterium	128	10	91	14	262	264
Candidatus_cryptoprodotis	34	98	19	22	146	99
Candidatus_isobeggiatoa	7	0	38	102	0	0
Candidatus_koribacter	134	102	875	349	80	181
Candidatus_kuenenia	6	89	102	39	11	7
Candidatus_midichloria	14	2	21	11	0	1
Candidatus_nardonella	0	5	1	10	1	0
Candidatus_nasuia	0	0	14	15	0	10
Candidatus_nitrotoga	0	8	8	23	1	0
Candidatus_odyssella	7	23	13	6	16	34
Candidatus_pelagibacter	0	175	84	21	2	1
Candidatus_phytoplasma	2	74	10	20	1	5
Candidatus_rhodoluna	1	30	9	3	0	0
Candidatus_scalindua	0	5	224	171	0	0
Candidatus_solibacter	1032	784	2420	4218	942	2051
Candidatus_zinderia	0	113	4	11	0	0
Carboxydothermus	1	18	9	26	0	0
Carnobacterium	12	7	11	10	19	25
Catellatospora	10	35	2	6	14	12
Catenulispora	0	0	10	19	0	0
Catenuloplanes	194	18	106	100	124	153
Caulobacter	109	82	326	974	112	154
Cellulosimicrobium	0	23	22	4	4	7
Cellvibrio	0	45	2	3	5	0
Cetobacterium	0	38	3	5	0	0
Chelatococcus	6	1	0	0	6	8
Chitinimonas	0	10	23	32	1	0
Chitinophaga	236	547	232	401	173	222
Chloroflexus	283	377	754	601	275	361
Chloronema	0	1	3	4	5	13
Chondromyces	334	138	193	135	336	328
Chroococcidiopsis	1	19	43	49	3	2
Chryseobacterium	0	10	1	5	2	0
Chthoniobacter	8	1	4	6	2	5
Chthonomonas	37	6	4	12	18	27
Citricoccus	2	45	14	10	5	0
Clostridium	75	333	299	499	84	74
Comamonas	1	23	1	21	0	1
Conexibacter	1018	488	738	689	757	1253
Corynebacterium	86	21	40	43	80	193
Coxiella	5	5	0	1	3	3
Craurococcus	9	2	4	5	13	11

Cristispira	0	277	4	13	1	0
Croceibacter	2	7	0	1	4	17
Crocinitomix	12	24	1	9	15	12
Cronobacter	11	14	20	139	191	8
Crossiella	18	12	0	1	27	39
Cryocola	11	25	20	43	26	17
Cryptosporangium	4	1	12	15	4	3
Cupriavidus	87	709	14	22	102	99
Curvibacter	29	105	108	201	58	48
Cyanobacterium	0	14	4	5	2	0
Cystobacter	315	469	120	235	312	547
Cytophaga	295	687	874	869	441	294
Dechloromonas	16	8	228	15	9	36
Defluviicoccus	0	5	0	12	0	0
Dehalococcoides	782	397	368	263	316	767
Dehalogenimonas	245	189	264	1280	154	317
Denitratisoma	8	58	86	139	15	28
Desulfobacca	0	5	0	6	0	0
Desulfobulbus	0	13	25	3	0	0
Desulfocaldus	12	7	71	48	2	21
Desulfoglaeba	13	20	0	1	2	2
Desulfomonile	5	23	7	31	2	6
Desulforegula	37	43	1	1	14	16
Desulfotalea	119	44	28	56	72	190
Desulfotomaculum	4	0	16	5	11	6
Desulfovibrio	3	37	20	24	3	2
Desulfovirga	45	3	32	72	22	34
Desulfurella	0	65	38	14	0	1
Desulfuromonas	1	45	50	273	2	1
Devosia	147	441	627	474	233	116
Dongia	507	447	51	48	250	327
Duganella	0	0	10	7	0	0
Dyella	3	16	15	22	2	7
Dysgonomonas	1	2	29	36	32	4
Ectothiorhodospira	14	4	6	4	6	15
Edaphobacter	0	0	23	28	0	0
Ehrlichia	0	131	45	95	3	2
Elusimicrobium	0	15	35	11	0	1
Endozoicomonas	0	4	28	10	0	0
Ensifer	27	18	32	10	57	17
Erysipelothrix	0	56	4	7	0	1
Erythrobacter	7	32	0	6	15	30
Eubacterium	5	11	14	11	11	17
Exiguobacterium	36	83	127	370	548	49
Facklamia	0	1	2	0	6	15
Ferrimicrobium	16	16	0	0	13	29

Ferruginibacter	30	29	30	19	9	13
Fibrobacter	0	11	9	19	5	3
Fictibacillus	2	13	5	5	39	6
Filimonas	2	85	29	42	3	2
Filomicrobium	14	102	5	11	10	6
Fimbriimonas	1	6	4	39	2	0
Flavisolibacter	203	128	150	366	254	383
Flavitalea	55	23	2	0	33	43
Flavobacterium	49	597	84	179	66	52
Flexibacter	2	535	78	141	4	8
Fluviicola	33	113	152	249	20	17
Frankia	421	187	865	1257	288	527
Fusobacterium	6	14	36	38	12	5
Gaiella	363	129	373	841	206	318
Gemmata	163	408	990	732	144	218
Gemmatimonas	954	1039	1600	2537	1091	1675
Geoalkalibacter	32	83	64	85	28	36
Geobacter	468	633	1557	1276	424	732
Geodermatophilus	869	151	1253	460	728	1161
Georgenia	0	1	33	14	2	0
Gloeobacter	4	27	39	35	1	0
Gluconobacter	43	61	95	32	20	56
Glycomyces	17	3	67	176	5	3
Granulibacter	18	48	1	8	6	8
Granulicella	0	0	12	17	0	0
Hahella	10	10	2	19	9	18
Haliangium	96	315	395	311	88	117
Haliea	0	7	1	6	1	1
Haliscomenobacter	35	203	342	146	22	17
Haloactinopolyspora	6	1	4	4	14	22
Halocella	0	7	15	0	0	0
Helicobacter	3	95	29	21	2	9
Heliobacterium	8	4	46	10	1	4
Herbaspirillum	152	125	14	4	178	305
Herpetosiphon	46	16	69	48	37	61
Hirschia	0	10	6	3	2	4
Holophaga	344	852	3109	1762	272	478
Hymenobacter	13	14	12	180	43	43
Hyphomicrobium	23	89	102	70	14	25
Hyphomonas	25	52	18	47	30	59
Iamia	46	66	87	41	37	56
Ideonella	25	61	0	11	51	41
Ignavibacterium	1	17	6	24	2	0
Inquilinus	9	14	20	44	10	5
Intrasporangium	2	8	15	14	6	6
Isosphaera	17	18	268	245	7	10

Kaistia	0	6	54	108	0	2
Kaistobacter	17	14	46	7	17	34
Kallotenue	14	5	6	8	14	34
Kibdelosporangium	21	6	6	18	77	15
Kofleria	16	68	129	37	10	12
Kouleothrix	231	108	145	292	154	394
Krasilnikovia	23	22	0	0	23	49
Kribbella	53	40	60	116	65	45
Ktedonobacter	9	2	109	365	0	4
Labrys	32	38	316	29	41	30
Lachnoclostridium	1	19	2	6	0	1
Lacibacterium	1	9	35	12	0	0
Lactobacillus	3	13	25	48	12	0
Lautropia	4	3	0	0	8	27
Lechevalieria	138	65	132	210	321	105
Legionella	19	24	23	38	17	23
Leifsonia	1	30	0	0	8	4
Leptolyngbya	18	24	30	12	24	0
Leptospira	10	19	12	96	4	14
Lewinella	0	5	4	1	0	0
Limnobacter	12	13	1	2	22	45
Longilinea	150	222	48	31	183	255
Luedemannella	22	63	37	47	6	26
Luteolibacter	0	0	44	200	0	0
Lutispora	8	15	9	43	8	13
Lysobacter	90	223	206	133	106	80
Magnetospirillum	15	6	65	116	35	44
Maricaulis	70	11	64	106	57	77
Massilia	99	161	19	20	154	82
Melioribacter	3	14	37	18	6	4
Mesorhizobium	510	592	585	1294	557	825
Methylibium	10	62	91	134	8	42
Methylobacillus	6	55	11	25	19	5
Methylobacterium	173	335	137	131	225	175
Methylocapsa	0	0	48	93	0	0
Methylococcus	0	5	22	23	1	0
Methylocystis	3	22	14	109	0	3
Methylohalomonas	0	5	12	9	0	0
Methylomicrobium	1	23	7	8	5	0
Methylophilus	2	19	77	42	3	3
Methylorosula	3	4	32	6	10	7
Methylosinus	29	99	96	50	21	46
Micavibrio	11	20	135	163	10	5
Microbacterium	17	146	1	6	79	29
Microbispora	6	12	3	88	5	15
Microcoleus	7	52	149	20	61	7

Microlunatus	87	45	13	15	162	237
Micromonospora	156	206	13	89	163	260
Microvirga	811	1309	2865	4669	976	1499
Modestobacter	7	25	19	5	17	22
Moorella	547	226	74	115	339	706
Moritella	4	0	3	4	13	0
Motilibacter	3	1	8	8	1	2
Mucilaginibacter	18	28	34	58	9	3
Mycobacterium	331	537	37	51	241	331
Myroides	0	5	2	5	0	0
Myxococcus	67	58	80	42	47	58
Nakamurella	21	7	2	8	17	22
Nannocystis	4	58	30	53	9	7
Natronoanaerobium	0	1	4	0	1	4
Neorhizobium	27	34	9	10	74	30
Niastella	253	279	518	3188	304	346
Nitriliruptor	24	10	50	21	22	47
Nitrobacter	194	144	1791	535	184	300
Nitrosococcus	93	390	83	16	74	107
Nitrosomonas	7	48	2	137	6	10
Nitrosospira	35	65	64	188	25	43
Nitrosovibrio	15	95	1015	1560	23	21
Nitrospira	346	973	474	692	255	582
Nocardia	3	3	58	26	2	0
Nocardioides	178	517	251	469	372	300
Nonomuraea	36	49	28	47	48	19
Nordella	125	239	79	75	114	118
Nostoc	2	14	10	1	10	27
Novispirillum	0	7	2	12	0	0
Novosphingobium	47	68	234	123	58	63
Oceanibacterium	0	0	4	4	0	3
Oceanibaculum	2	27	74	35	2	10
Ochrobactrum	7	45	36	84	9	23
Odoribacter	1	5	2	3	16	1
Ohtaekwangia	387	502	788	1048	533	398
Oligoflexus	0	6	1	4	2	0
Opitutus	109	308	151	118	178	125
Orientia	0	0	10	19	1	5
Ornatilinea	44	6	11	7	8	13
Oscillatoria	0	20	44	72	3	0
Others	0	4	12	10	1	0
Owenweeksia	4	2	8	8	11	9
Paenibacillus	33	155	17	43	81	53
Pandoraea	2	6	0	53	5	3
Parabacteroides	5	3	6	115	4	5
Paracoccus	88	70	167	166	103	106

Paracraurococcus	16	21	107	185	20	17
Parasegetibacter	39	35	47	26	72	51
Pasteuria	239	404	313	288	174	419
Pedobacter	46	56	78	92	52	45
Pedomicrobium	55	132	132	116	74	63
Pedosphaera	8	7	287	484	3	9
Pelobacter	4922	4340	1788	11344	4096	6511
Pelomonas	7	26	4	1	37	2
Pelotomaculum	0	7	11	9	0	0
Phaselicystis	57	302	10	33	113	64
Phenylobacterium	81	100	25	35	138	91
Phormidium	4	89	21	15	19	14
Phycisphaera	249	241	411	388	208	447
Pirellula	332	903	1474	994	260	569
Piscinibacter	44	170	95	489	94	73
Planctomyces	109	372	784	965	76	142
Planktothrix	7	14	4	2	35	7
Plantactinospora	36	44	0	1	38	60
Plesiocystis	4	19	21	36	3	11
Polaromonas	23	147	50	1403	23	32
Polyangium	7	122	18	11	9	13
Pontibacter	30	34	64	77	18	26
Porphyrobacter	67	133	33	34	89	72
Prochlorococcus	26	32	34	36	52	71
Promicromonospora	2	20	81	98	20	6
Prosthecobacter	32	11	2013	8447	9	18
Pseudochrobactrum	6	4	0	0	3	10
Pseudolabrys	5	10	204	231	5	3
Pseudomonas	105	268	133	119	207	77
Pseudonocardia	258	190	159	537	351	305
Pseudoramibacter	0	3	114	40	0	0
Pseudoxanthomonas	1	8	0	0	26	2
Psychromonas	27	2	1	4	6	33
Quadrisphaera	0	0	5	9	0	0
Ralstonia	40	140	90	83	35	63
Reyranella	52	117	124	112	41	35
Rheinheimera	0	2	6	59	8	17
Rhizobium	51	258	78	208	129	58
Rhizomicrobium	2	48	31	22	3	0
Rhodobacter	0	7	1	0	8	1
Rhodobium	33	159	69	61	18	37
Rhodocista	7	4	145	570	18	19
Rhodococcus	48	46	29	28	52	44
Rhodocytophaga	53	19	12	77	84	74
Rhodomicrobium	79	75	11	0	79	107
Rhodopirellula	47	93	299	621	37	55

Rhodoplanes	693	1386	3140	5362	517	731
Rhodopseudomonas	2952	681	93	198	1425	2580
Rhodospirillum	0	5	214	627	1	0
Rhodothermus	12	32	213	178	18	23
Rhodovastum	0	0	21	20	0	0
Rhodovulum	5	18	6	16	5	12
Rickettsia	3	1119	88	97	4	60
Roseiflexus	66	45	322	441	69	162
Roseomonas	14	23	13	38	17	20
Rubellimicrobium	33	17	4	2	87	75
Rubrobacter	910	391	492	709	677	1674
Rudanella	1	0	0	3	5	6
Rufibacter	6	2	2	6	7	7
Rugosimonospora	0	0	5	11	0	0
Saccharophagus	23	42	39	23	27	24
Sandaracinus	12	48	14	91	6	5
Saprospira	0	4	4	3	0	1
Scytonema	1	0	4	0	3	5
Segetibacter	29	20	81	206	57	66
Selenomonas	0	4	10	0	0	0
Singulisphaera	29	38	24	34	46	57
Sinorhizobium	251	223	406	1663	274	126
Skermanella	115	182	435	780	179	271
Smaragdicoccus	0	13	2	4	2	0
Sneathiella	13	4	27	1	12	15
Solimonas	4	14	43	39	2	8
Solirubrobacter	1373	505	1332	1395	1126	1500
Solitalea	5	6	1	0	0	0
Sphaerisporangium	1	17	5	0	8	1
Sphaerobacter	20	24	240	426	26	49
Sphingobacterium	166	127	243	205	139	161
Sphingobium	21	11	6	1	23	30
Sphingomonas	26	78	367	496	77	40
Sphingosinicella	11	15	0	1	11	21
Spirochaeta	4	21	3	5	0	0
Spiroplasma	0	11	30	0	1	0
Sporichthya	735	82	346	415	435	551
Sporocytophaga	15	4	10	45	4	9
Sporomusa	4	7	13	15	4	29
Staphylococcus	0	13	1	10	0	0
Starkeya	4	11	0	0	2	2
Stella	51	117	331	1089	38	44
Steroidobacter	1352	1059	468	1386	1093	1650
Streptococcus	29	129	153	116	22	1
Streptomyces	268	690	336	537	794	230
Subaequorebacter	2	6	2	0	3	7

Symbiobacterium	17	67	17	25	7	12
Synechococcus	34	83	39	156	34	30
Syntrophobacter	895	62	336	484	429	1328
Syntrophomonas	9	32	47	123	5	12
Syntrophus	382	92	23	19	193	542
Telmatobacter	0	0	8	20	0	0
Tepidamorphus	9	44	1	3	3	15
Terriglobus	0	0	62	279	0	0
Terrimonas	189	317	31	141	202	114
Tetracoccus	0	1	1	0	4	5
Tetrasphaera	1	10	0	0	11	7
Thalassobacillus	2	10	5	3	23	5
Thalassomonas	1	4	12	53	1	0
Thalassospira	6	3	6	1	2	0
Thermaerobacter	300	284	179	346	269	392
Thermanaerothrix	18	43	74	493	26	18
Thermincola	2	14	16	12	1	0
Thermoanaerobacter	119	131	76	128	100	143
Thermobifida	48	33	2	7	41	65
Thermodesulfatator	4	0	17	0	1	5
Thermodesulfovibrio	0	4	9	1	0	0
Thermoflavimicrobium	0	0	256	80	0	0
Thermoleophilum	628	116	98	258	379	585
Thermolithobacter	43	10	180	76	41	69
Thermomicrobium	65	21	88	40	44	73
Thermomonas	206	983	137	128	212	166
Thermosporothrix	0	0	7	45	0	0
Thermus	15	32	16	17	13	3
Thiobacillus	116	846	1819	1971	128	183
Thiobacter	2	178	829	264	4	2
Thiocystis	1	10	10	8	1	2
Thiohalomonas	70	8	450	785	36	101
Thiohalophilus	0	2	5	5	2	0
Trichodesmium	19	5	9	7	35	38
Tumebacillus	5	41	16	22	2	9
Turicella	2	153	133	203	1	1
Turicibacter	0	1	86	74	2	4
Undibacterium	13	14	0	0	42	12
Vampirovibrio	2	5	60	78	17	3
Veillonella	0	9	1	4	0	0
Verrucomicrobium	1	25	31	17	2	0
Verrucosispora	177	117	57	43	212	170
Virgisporangium	77	120	265	379	107	136
Vulgatibacter	0	14	1	13	3	0
Wandonia	1	39	3	0	5	2
Weissella	0	22	5	0	0	0

Wolbachia	47	96	56	178	76	160
Woodsholea	0	6	0	19	1	1
Xanthomonas	16	91	35	124	28	22
Xylanimicrobium	1	16	6	7	9	2
Zavarzinella	14	129	293	166	15	33

Appendix 3

Metadata

#NAME	**Sample Type**
TIFS	Tweenfontein Indigenous Forest Soil
TCFS	Tweenfontein Commercial Forest Soil
WIFS	Witklip Indigenous Forest Soil
WCFS	Witklip Commercial Forest Soil

Appendix 4

One-way ANOVA

	Sum of sqrs	df	Mean square	F	p(same)
Between groups:	4.52E+09	20	2.26E+08	4.719	4.06E-08
Within groups:	5.51E+09	115	4.79E+07		
Total:	1.00E+10	135			
omega^2:	0.3536				

For more information on the forest soil data, kindly send an email to: abisola.sholeye@yahoo.com

Appendix 5: p-value results at phylum level

	TIFS	TCFS	WIFS	WCFS	acidobacteria	proteobacteria	verrucomicrobia	chloroflexi	firmicutes	bacteroidetes	actinobacteria	gemmatimonadetes	nitrospirae	planctomycetes	fusobacteria	ignavibacteriae	candidatus_saccharibacteria	cyanobacteria	chlamydiae	elusimicrobia
TIFS		1	1	1	0.05882	0.000375	0.4786	1	1	1	1	1	1	1	1	1	1	1	1	1
TCFS	0.03324		1	1	0.05492	0.000355	0.4614	1	1	1	1	1	1	1	1	1	1	1	1	1
WIFS	0.697	0.7303		1	0.2108	0.001822	0.8234	1	1	1	1	0.9997	0.9982	0.9986	0.9991	0.9973	0.9975	0.9973	0.9973	0.9973
WCFS	0.03245	0.06569	0.6646		0.06285	0.000397	0.4955	1	1	1	1	1	1	1	1	1	1	1	1	1
acidobacteria	5.092	5.125	4.395	5.059		0.9945	1	0.1038	0.03055	0.02379	0.0598	0.005418	0.002884	0.003176	0.003768	0.002478	0.002529	0.002486	0.002454	0.002458
proteobacteria	7.246	7.28	6.549	7.214	2.155		0.6484	0.000679	0.000236	0.000212	0.000381	0.000164	0.00016	0.000161	0.000162	0.00016	0.00016	0.00016	0.00016	0.00016
verrucomicrobia	3.784	3.817	3.087	3.752	1.308	3.462		0.6312	0.3299	0.2826	0.4828	0.1017	0.06261	0.06758	0.0771	0.05543	0.05635	0.05556	0.05498	0.05506
chloroflexi	0.2891	0.3223	0.4079	0.2567	4.803	6.957	3.495		1	1	1	1	0.9999	1	1	0.9999	0.9999	0.9999	0.9999	0.9999
firmicutes	0.3055	0.2723	1.003	0.338	5.397	7.552	4.09	0.5946		1	1	1	1	1	1	1	1	1	1	1
bacteroidetes	0.4167	0.3835	1.114	0.4492	5.509	7.663	4.201	0.7058	0.1112		1	1	1	1	1	1	1	1	1	1
actinobacteria	0.008117	0.04136	0.6889	0.02433	5.084	7.238	3.776	0.281	0.3136	0.4248		1	1	1	1	1	1	1	1	1
gemmatimonadetes	1.03	0.9964	1.727	1.062	6.121	8.276	4.814	1.319	0.7241	0.6129	1.038		1	1	1	1	1	1	1	1
nitrospirae	1.277	1.244	1.974	1.309	6.369	8.523	5.061	1.566	0.9714	0.8602	1.285	0.2473		1	1	1	1	1	1	1
planctomycetes	1.239	1.206	1.936	1.272	6.331	8.486	5.023	1.528	0.9337	0.8225	1.247	0.2096	0.03774		1	1	1	1	1	1
fusobacteria	1.173	1.14	1.87	1.206	6.265	8.42	4.957	1.462	0.8676	0.7564	1.181	0.1435	0.1038	0.0661		1	1	1	1	1
ignavibacteriae	1.336	1.303	2.033	1.369	6.428	8.583	5.121	1.625	1.031	0.9196	1.344	0.3067	0.05939	0.09713	0.1632		1	1	1	1
candidatus_saccharibacteria	1.328	1.295	2.025	1.361	6.42	8.575	5.113	1.617	1.023	0.9116	1.336	0.2987	0.05141	0.08916	0.1553	0.00797		1	1	1
cyanobacteria	1.335	1.302	2.032	1.368	6.427	8.582	5.119	1.624	1.03	0.9184	1.343	0.3055	0.05821	0.09595	0.1621	0.001172	0.006798		1	1
chlamydiae	1.34	1.307	2.037	1.373	6.432	8.587	5.124	1.629	1.035	0.9235	1.348	0.3106	0.06329	0.101	0.1671	0.003907	0.01188	0.005079		1
elusimicrobia	1.34	1.306	2.037	1.372	6.431	8.586	5.124	1.629	1.034	0.9228	1.348	0.3099	0.06259	0.1003	0.1664	0.003204	0.01117	0.004376	0.000703	
spirochaetes	1.34	1.307	2.037	1.372	6.432	8.586	5.124	1.629	1.034	0.9231	1.348	0.3102	0.0629	0.1006	0.1667	0.003516	0.01149	0.004688	0.000391	0.000313

Appendix 6: p - value results at class level

	TIFS	TCFS	WIFS	WCFS	acidobacteria	actinobacteria	bacteroidetes	candidatus_saccharibacteria	chlamydiae	chloroflexi	cyanobacteria	elusimicrobia	firmicutes	fusobacteria	gemmatimonadetes	ignavibacteriae	nitrospirae	Others	planctomycetes	proteobacteria	spirochaetes	verrucomicrobia
TIFS		1	1	1	0.03223	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4.24E-05	1	0.4117
TCFS	0.03206		1	1	0.02976	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3.91E-05	1	0.3949
WIFS	0.6709	0.703		1	0.1416	1	1	0.9992	0.9991	1	0.9992	0.9991	1	0.9998	0.9999	0.9992	0.9995	0.9991	0.9996	0.000371	0.9991	0.7748
WCFS	0.03119	0.06325	0.6397		0.0348	1	1	1	1	1	1	1	1	1	1	1	1	1	1	4.60E-05	1	0.4284
acidobacteria	5.264	5.296	4.593	5.233		0.03957	0.01303	0.000807	0.000777	0.07634	0.000789	0.000778	0.01765	0.001332	0.002103	0.000786	0.000953	0.000778	0.001076	0.9959	0.000777	1
actinobacteria	0.08411	0.1162	0.5868	0.05292	5.18		1	1	1	1	1	1	1	1	1	1	1	1	1	5.31E-05	1	0.4572
bacteroidetes	0.3488	0.3167	1.02	0.38	5.613	0.4329		1	1	1	1	1	1	1	1	1	1	1	1	2.17E-05	1	0.2471
candidatus_saccharibacteria	1.278	1.246	1.949	1.309	6.542	1.362	0.9289		1	0.9999	1	1	1	1	1	1	1	1	1	1.51E-05	1	0.03685
chlamydiae	1.29	1.258	1.961	1.321	6.554	1.374	0.941	0.0121		0.9999	1	1	1	1	1	1	1	1	1	1.51E-05	1	0.03578
chloroflexi	0.3704	0.4025	0.3005	0.3392	4.894	0.2863	0.7192	1.648	1.66		0.9999	0.9999	1	1	1	0.9999	1	0.9999	1	0.000157	0.9999	0.618
cyanobacteria	1.285	1.253	1.956	1.316	6.549	1.369	0.9358	0.006927	0.005175	1.655		1	1	1	1	1	1	1	1	1.51E-05	1	0.03623
elusimicrobia	1.289	1.257	1.96	1.32	6.553	1.373	0.9403	0.01139	0.000717	1.66	0.004459		1	1	1	1	1	1	1	1.51E-05	1	0.03584
firmicutes	0.2355	0.2034	0.9064	0.2667	5.5	0.3196	0.1133	1.042	1.054	0.6059	1.049	1.054		1	1	1	1	1	1	2.56E-05	1	0.2956
fusobacteria	1.119	1.087	1.79	1.151	6.384	1.204	0.7707	0.1582	0.1703	1.49	0.1651	0.1696	0.884		1	1	1	1	1	1.52E-05	1	0.05359
gemmatimonadetes	0.9733	0.9413	1.644	1.005	6.237	1.057	0.6245	0.3044	0.3165	1.344	0.3113	0.3158	0.7378	0.1462		1	1	1	1	1.54E-05	1	0.07446
ignavibacteriae	1.286	1.254	1.957	1.317	6.55	1.37	0.937	0.008121	0.003981	1.656	0.001194	0.003264	1.05	0.1663	0.3125		1	1	1	1.51E-05	1	0.03613
nitrospirae	1.225	1.193	1.896	1.256	6.489	1.309	0.8765	0.05239	0.06449	1.596	0.05932	0.06378	0.9898	0.1058	0.252	0.06051		1	1	1.51E-05	1	0.0418
Others	1.289	1.257	1.96	1.321	6.553	1.373	0.9405	0.01162	0.000478	1.66	0.004698	0.000239	1.054	0.1698	0.316	0.003503	0.06401		1	1.51E-05	1	0.03582
planctomycetes	1.187	1.155	1.858	1.218	6.451	1.271	0.8381	0.09085	0.1029	1.557	0.09777	0.1022	0.9514	0.06736	0.2135	0.09897	0.03846	0.1025		1.52E-05	1	0.0458
proteobacteria	7.46	7.492	6.789	7.428	2.196	7.376	7.808	8.737	8.749	7.089	8.744	8.749	7.695	8.579	8.433	8.745	8.685	8.749	8.646		1.51E-05	0.6367
spirochaetes	1.289	1.257	1.96	1.321	6.554	1.374	0.9406	0.0117	0.000398	1.66	0.004777	0.000319	1.054	0.1699	0.3161	0.003583	0.06409	7.96E-05	0.1025	8.749		0.03581
verrucomicrobia	3.932	3.964	3.261	3.901	1.332	3.848	4.281	5.209	5.222	3.561	5.216	5.221	4.167	5.051	4.905	5.218	5.157	5.221	5.119	3.528	5.221	