BOTSWANA INTERNATIONAL UNIVERSITY
OF SCIENCE & TECHNOLOGY

# INFLUENCE OF SOIL PHYSICO-CHEMICAL PROPERTIES ON INDIGENOUS SOIL BACTERIA IN SELECTED ECOSYSTEMS AT PALAPYE, EASTERN BOTSWANA


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## CERTIFICATION

We the undersigned certify that we have read and hereby recommend for acceptance by the Faculty of Science a thesis titled: *"Influence of soil physico-chemical properties on indigenous soil bacteria in selected ecosystems at Palapye, Eastern Botswana"* in fulfilment of the requirements for the degree of Master of Science in Environmental Science in BIUST.

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## DECLARATION AND COPYRIGHT

I, Modise Mhete, declare that this thesis is my own original work and that it has not been presented and will not be presented to any other university for a similar or any other degree award.

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## DEDICATION

*To my family members for their care, support and belief in me.*

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## ABSTRACT

Advancement of knowledge on soil microbial richness is a key to environmental policy and sustainable land management. In Botswana, there is a dearth of scientific reports on soil bacterial dynamics. The aim of this study is to improve our understanding of how soil physico-chemical properties influence bacterial abundance and distribution under different land-use types (ecosystems). Six ecosystems studied include saline soil, sewage sludge dump, garden, fallow land rainfed and irrigated arable farms. A combination of agar culture and metagenomic approaches via sequencing of PCR amplified 16S rDNA genes from total extracted DNA was used to identify and characterize bacteria communities. Selected soil physico-chemical properties were determined using routine laboratory procedures. The results show that sewage sludge ecosystem had the highest organic matter (59.9 %), cation exchange capacity (8.65 cmol $kg^{-1}$) and clay content (17.83 %), and these three properties mainly influenced the richness and diversity of soil bacterial communities. The soils from bare land, rainfed and irrigated arable farms had the least organic matter and could not sustain enough bacterial communities to produce the required DNA quality for metagenomics analysis. The saline soil had the highest electrical conductivity (EC) (0.716 dS $m^{-1}$), an index of salinity, and had relatively higher DNA concentration (25.8 ng $\mu L^{-1}$). Total counts of culturable bacterial population ranged between $10^7$ and 6 $x10^7$ after using mannitol salt agar and *E. coli* agar. Next generation sequencing (NGS) showed the order of phyla dominance as Proteobacteria > Actinobacteria > Firmicutes > Bacteroidetes > Acidobacteria. Soils in the ecosystems have promising potential to contribute considerably to global carbon and nitrogen geochemical cycling due to the preponderance of Proteobacteria. The presence of unnamed

phyla identified in the ecosystems goes further to support the need for a continued build-up of a comprehensive global soil biodiversity database through extensive research.

**Keywords:** Culture, metagenomics, soil salinity, sewage sludge, ecosystems, bacterial diversity.

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# CHAPTER ONE

## 1.1. INTRODUCTION

Soil is considered as a main pool of bacterial genetic diversity (Robe *et al.*, 2003). The complication of bacterial diversity results from numerous interrelating parameters, which include soil chemical, physical and biological properties.. The total number of microbial cells on Earth is estimated to be $10^{30}$ $g^{-1}$ of soil (Hughes *et al*., 2001; Blay *et al*, 2017). Prokaryotes make up the largest proportion of individual organisms, constituting $10^6$ to $10^8$ separate genospecies (Singh *et al*., 2009). Bacteria are the most copious group of microorganisms in soil (Gans *et al*., 2005). The estimated number of different bacterial genomes varies from 2,000 to 18,000 per gram of soil (Daniel, 2005; Fierer and Jackson, 2007a; Fakruddin and Mannan, 2013).

The biodiversity of bacteria in soil is essential for the conservation of soil health because these microbes are involved in many vital functions including improving soil quality, biogeochemical nutrient cycling, soil formation (pedogenesis) and removal of toxins. Soils also function as a nutrient store for the growth of a range of bacteria which have amazing ability to transform a vast diversity of composite organic compounds due to their metabolic versatility (Haack and Bekins, 2000). Nutrients are taken up readily made and cycled through the ecosystem. Certain species of nitrogen-fixing bacteria mostly from the Actinobacteria phyla are linked with root nodules that supply plants with nitrates (Barrios, 2007). Much of biodegradation of crop remainder and organic matter in soils is done by microbial communities including bacteria and fungi (Gougoulias *et al*., 2014). Bacterial diversity is

reflected as a driver of ecosystem services; specifically those soil ecosystem services are connected with the justifiable agricultural production. These include the principal production of food, fibre and fuel, and water percolation and refining (Hooper *et al*., 2005). The instabilities caused by forest management regularly modifies soil characteristics and in turn impacts on microbial structure and diversity (Doran and Zeiss, 2000; Lin *et al*., 2017).

Given the microscopic size of the effects of individual microbes they are very small with respect to total ecosystem functioning. However, on a community level, bacteria are vital, and their functioning has major impacts on the landscape level and beyond. Essentially, the activities of microbes are crucial for supporting all terrestrial life, with major roles in the global cycling of carbon, nitrogen, sulfur and other elements (Schimel and Bennett, 2004; Schmidt, 2006; Ranjard and Richaume, 2001). Plant growth can also be promoted by bacteria; for example, N fixation as well as through root-colonizing rhizobacteria producing phytohormones and volatiles stimulating plant growth and protecting plant roots against pathogens (Sturz and Nowak, 2000).

Many bacteria are taken out from soils and used in industrial production of various things including food processing and production, creation of biocides, bio control agents, medicines and other natural products. Pharmaceutical companies use millions of money yearly in testings soil and litter for useful microorganisms so as to produce medicine (Banat *et al*., 2000). The soil bacterial biomass is the principal components of decomposer system regulating nutrients cycling, energy flow and ultimately plant and ecosystem productivity. The ability of bacteria to decompose pesticides and other organic compounds is an important benefit to modern agriculture that relies on the use of toxic chemicals, which when used persistently will cause intolerable environmental health risks (Topp, 2003). Few universal

principles exist about the patterns of temporal variability and their temporal dynamics are likely to be very crucial in deciding the level released from immobilized unbalanced nutrients for other components of the ecosystem (Wardle, 2002).

Some strains of Azospirillum and Pseudomonas are also capable of indole acetic acid (IAA) production and production of other metabolites that inhibit some fungal pathogens (Mehnaz and Lazarovita, 2006). However, present studies have discovered that the P-solubilizing capability of some bacteria isolated based on the traditional method using tricalcium phosphate, could not be re-introduced instantly to the field, where the environmental conditions might be very unique from those imposed for the selection (Mehnaz and Lazarovita, 2006; Bashan et al., 2013 a,b).

### 1.2. Statement of Problem

While the significance of bacteria for ecosystem functions and sustaining soil quality in agriculturally managed systems have long been known, the effect of land use type and management type on soil bacterial communities is poorly addressed as little or no research is being done (Kibblewhite *et al*., 2008). In Botswana, studies have not been done to characterize the indigenous soil microbes in relation to soil properties. So far studies around the world have only focused on the chemical and physical properties of the soil (Schoenholtz *et al*., 2000; Glaser *et al*., 2002; Tejada *et al*., 2006; Pouyat *et al*., 2007). The knowledge on the effects of land use on the biological properties of the soils is very limited in the country. It is a well-known policy in Botswana that farmers are advised by agricultural demonstrators to submit soil samples from their fields to research centers under the Ministry of Agriculture for analysis of soil quality and fertility. The tests done on the soils, however, are limited to the physicochemical properties of the soils while little or no attention is given to the biological

properties and this is the subject of this study. The study investigate both aspects of soil physicochemical and biological properties and it will help in reaching a conclusion of whether certain types of agricultural practices affect bacterial communities (an agricultural practice consistently associated with reduction in abundance and diversity of soil could imply biological degradation). Knowledge of these biological parameters will give us a starting point in coming up with prevention and mitigation measures anticipated during production. The outcome of this study would be useful to farmers, environmentalists and policymakers who are the key stakeholders in soil quality management.

### 1.3. Objectives

The main aim of this study was to identify and characterize, where possible the bacterial populations present in six different ecosystems in Eastern Palapye using both culture dependent and genomic approaches with particular focus on how land use and selected soil physico-chemical properties affect the bacterial loads and interactions in soils.

The specific objectives were:

1. To determine the physical and chemical properties of soil under different ecosystems and management
2. To characterize the bacterial communities across the different ecosystems in Palapye.
3. To examine the relationships between bacterial communities and physicochemical properties of soil

### 1.4. Research Questions

1. What are the physico-chemical properties of soils under different land uses and their effects on abundance of bacterial species?
2. Does the bacterial community in the soil differ across different ecosystems?

3. Which groups of bacteria are dominantly present in agricultural soils?

### 1.5. Significance of the study

Bacterial assessment is an essential step in understanding microorganism processes in soil health in relation to agricultural production. Regardless of our knowledge of the tremendous usefulness of soil microbes, very little is known of soil bacterial diversity in Botswana. Currently, there is no study in Botswana that has been carried out to investigate the role of bacterial diversity in agricultural soils using both the culture dependent and culture independent method. This study will provide an insight into the impacts of different physico-chemical properties on bacterial diversity and how different bacterial phyla are useful in the soil. Rapid and accurate identification of bacterial species in soil samples is a critical need in agriculture because bacterial diversity are reflected as drivers of ecosystem services, in specific those soil ecosystem services connected with justifiable agricultural production. Information derived from this study will have positive effects on our understanding of the role of microbial processes in soil health in relation to agricultural production. The outcome of the study will provide essential baseline data that future research can build on. This study will help in clearly understanding the type of bacterial phyla existing in soil as the degree of the diversity of bacteria in soil is seen to be vital in sustainment of soil health and quality (Doran and Zeiss, 2000). It will help in addressing the problem of Botswana soil health and quality, for increased crop production since a wide range of bacteria are involved in biogeochemical cycles and as plant growth promoters. Overall, this study will contribute knowledge about the occurrence of bacteria in soil required for the maintenance of soil health and quality. For the first time in the semi- arid soils of Botswana, this study seeks to: i) combine metagenomics and phylogenetic approaches to produce high resolution characterisation of bacterial

abundance and diversity; ii) explore the influence of soil physico-chemical properties on bacterial communities in the ecosystems.

### 1.6. Outline of the thesis

Chapter ONE: General introduction

Chapter TWO: Review of relevant and contemporary literature

Chapter THREE: Materials and methods used in this study

Chapter FOUR: Research findings.

Chapter FIVE: Discussion of results

Chapter SIX: Conclusions, recommendations and directions for future research.

# CHAPTER TWO

# REVIEW OF LITERATURE

## 2.1. Soil quality properties

The examining of different soil properties under dissimilar long term management practices provides an improved insight of the mutual relationship of microbial communities and soil biochemical properties in ensuring soil functions and result in a complete judgement of soil quality. Considering soil quality signals based on site particular components and their connection to specific placeable ecosystem services has been projected as an assumed method to monitor and assess alterations in soil functions and quality (Sojka *et al*., 2003; Andrews *et al*., 2004; Zobeck *et al.,* 2008). These soil properties include soil carbon and nitrogen, bulk density, soil pH, electrical conductivity, and extractable crop nutrients like nitrogen, phosphorus and potassium (Arshad and Martin, 2002; Arias *et al*., 2005). These soil quality indicators are chosen based on how they add to crop productivity, nutrient cycling and environmental quality. Soil microbial community structure and diversity have been regarded to be among the biological indicators for evaluating soil quality. Additionally it is evident that the eccentric of plant species, soil type, soil texture, and nitrogen accessibility can impact bacterial community structure, but elaborated data on the regarded bacterial groups and level of these regulations is still a deficit (Nacke *et al*., 2011).

A special group of bacteria known as *Rhizobia* reside in nodules of leguminous plants where they fix nitrogen and supply the plant with nitrogen for development. Reciprocally, the plant supply the bacteria with organic substrates for growth. The *Rhizobium*-legume symbiosis is characterized by high host specificity (Dazzo and Hubbel, 1975). Populations of *Rhizobium*

have previously been projected as indicator of healthy soil (Visser and Parkinson, 1992) based on the sensitivity of the organism to pesticides (Chaudri *et al*., 1993). The abundance of *Rhizobium* has been included in the UK Sewage Sludge Network as a microbial indicator of heavy metal pollution in agricultural soils (Chambers *et al*., 1999). While soil type has remained a crucial factor influencing bacterial species (Brockett *et al*., 2011), soil pH has been reported as the top predictor of soil microbial diversity (Nicol *et al*., 2008, Rousk *et al*., 2010). The pH is frequently associated with vital environmental factors manipulating the microbial community, including nutrient accessibility, heavy metal toxicity, and plant community structure. However, the inter-relationship between community structure and other factors such as soil structure and soil type make it difficult to attribute bacterial ecology to pH alone (Fernández-Calvino *et al*., 2011). Other studies also suggest that soil properties are significant pioneers of soil bacterial community structure, but soil pH emerges as the major element influencing community composition (Tscherko *et al*., 2004; Chaparro *et al*., 2012). This shaping of soil pH has been distinguished at coarse levels of taxonomic resolution.

Numerous studies proposed that influences of important drivers of soil organic mineralization such as soil temperature, soil moisture, and litter quality have an effect on bacterial community distribution (Nicol *et al*., 2008; Zinger *et al*., 2009; Rousk *et al*., 2010). Factors accountable for alteration in bacterial communities can be associated with plant species and land management practices. Recent studies further report that soil properties are vital in shaping the structure and composition of bacterial communities as well as affecting their ecosystem functions (Guong *et al*., 2012; Ferrenberg *et al*., 2013; Paula *et al*., 2014). Although effects of environmental factors such as climate, soil material and slope position on the dispersion of plant communities and soils are well-known, our knowledge of the

biogeographic distribution of soil microbial communities is only developing because of the newly introduced approaches for analyzingg the structure and metabolic activities of microbial communities.

### 2.1.1. Soil microbial properties

Diverse microbial communities are considered to promote ecosystem constancy, productivity and sustainability. Microbial communities vary tremendously with a great number of microbial species in a single gram of soil (Torsvik *et al*., 2002). Soils are regarded to be the most different microbial habitat on earth with reference to species diversity and community size. In a recent pyrosequencing survey (Lin *et al*., 2017), bacterial diversity of forest soil was taxonomically richer than agricultural soils. The most copius groups detected were members belonging to the Actinobacteria, Proteobacteria, and Bacteroidetes phyla while, members belonging to the Firmicutes, Acidobacteria, Gemmatimonadetes and Cyanobacteria phyla were observed in lower proportions (Fierer *et al*., 2009).

Bacteroidetes have been reported to be more predominant in agricultural setups than in the similar soils occurring under non-cultivated conditions, however the reverse is expected for Actinobacteria (Schellenberger *et al*., 2010). Bacteria of the genera *Rhizobium* are copious in soil, where they form symbiotic associations with legume roots. Land use modification changes the below-ground ecosystem, frequently resulting in loss of biodiversity and reduction of soil carbon. In most parts of the world, soils are well-known to be devalued in a certain way due to transition from forests and/or grasslands to agriculture consequently impacting on soil physical and chemical properties (Briar *et al*., 2012). Eroding processes such as losses in soil carbon, nutrient reduction and reduction in water holding capacity can happen rapidly and eventually become challenging to deal with. It is without doubt that land

use and management factors are important regulators of microbial communities in tropical soils than in many temperate systems, where friendly climatic conditions combined with access to fertilizers and other soil alterations help reduce variation in the soil environment (Bossio *et al*., 2005). In the past, soil microbial communities were examined using approaches based on culturing and isolating the microorganisms. Notwithstanding the perception that culture-dependent methods are not perfect for studies of the structure of natural microbial communities when used alone, they furnish in one of the more practicable entails of realizing the development habit, growth and possible purpose of microorganisms from soil habitats (He *et al*., 2008).

**2.2. Chemical properties of soil**

Soil chemical properties that are frequently affected by tillage systems are pH, soil total nitrogen, CEC and exchangeable cations. Ismail *et al*., (1994) and Rahman *et al*., (2008) stated that exchangeable Ca, Mg, and K, were importantly higher in the surface soil under non tilled (NT) compared to the ploughed soil. According to Ali *et al*, (2006), the lowest values of soil OM, N, P, K and Ca recorded in conventional till plots possibly due to the overturn of uppermost soil during cultivation shifts less fertile subsoil to the surface which results in possible leaching.

Soil organic matter (SOM) has long been reflected as the important quality factor affecting the physical, chemical and biological properties of soil. Organic matter in soil is described as a series of sections with dissimilar rates of decomposition (Rosell *et al.*, 2001; Lai, 2004). It has been extensively reported that physical properties, most notably structure, are largely governed by SOM content and quality (Khaleel *et al*., 1981). Management of organic carbon

has been recommended as a primary practice to enhance and conserve soil structure in short- and long term agricultural operations (Reeves, 1997).

### 2.2.1. Soil biological properties

Soil organic carbon (SOC) content is a biological property that is mostly affected by tillage (Doran, 1980). The soil organic matter content plays a major role in influencing the activities of soil organism which as a result influences the SOC dynamics. Cookson *et al*., (2008) discovered a diminished fungal biomass and enhanced bacterial biomass with extreme tillage usage. SOC has an impact on nutrient recycling and soil fertility status. Degradation of soil organic matter discharges nutrients, including nitrogen (N), into the soil (Murphy, 2015). Improved SOC content have been associated with a possible rise in food production; while this depend on management practice, soil type and environmental conditions (Barzegar *et al*., 2002; Zhang *et al*., 2012).

### 2.2.2. Physical properties of soil

Among the physical fractions, the clay content is the utmost vital constituent of soil. The clay fraction is defined as the fragment of soil particles with actual diameters of less than 2 μm. Clay is a colloid portion which plays a critical role in the binding larger particles of soil and producing a more stable compound. In dissolved solution, clay particles can flocculate or disperse. Distributed clay is portable in the surroundings and with moving water it can be moved across soil surface or infiltrates with water infiltrating water downwards inside the soil profile, which can be of concern in agriculture and the environment (Czyż and Dexter, 2008, Czyz and Dexter, 2009).

### 2.2.3. Saline soil

A saline soil is commonly described as a soil in which the electrical conductivity of the intensity extract in the root zone exceeds 4 dS $m^{-1}$ and has convertible sodium of 15 %. The growth of most crop plants is cut down at this EC, although many crops reveal low production at lower EC (Munns, 2005; Jamil *et al*., 2011). Soil salinity can slow down crop growth, horticulture and forage production in arid and semiarid regions. Salt may ascend naturally in the top soil or be brought in by irragation with salty water. Salinity is becoming widespread hazard due to land clearing, unjustifiable irrigation practices and through masses of turning marginal land into production (Rahnama *et al*., 2011).

Despite developing mechanisms for pressure tolerance, microorganisms can also contribute to some degree of tolerance to plants towards abiotic pressures like drought, terrifying injury, salinity, metal poisoning and high temperatures. In the past bacteria from different genera including *Rhizobium*, *Bacillus*, *Pseudomonas*, *Pantoea*, *Paenibacillus*, *Burkholderia*, *Achromobacter*, *Azospirillum*, *Microbacterium*, *Methylobacterium*, *Variovorax* and *Enterobacter* have been described to render tolerance to host plants under different abiotic stress environments (Grover *et al*., 2011).

### 2.2.4. Sewage sludge

The addition of sewage sludge changes the physical and chemical properties of soils due to organic matter input. This accumulation may importantly amend soil structure, increase soil moisture and porosity; increase humus content and cation exchange capacity (Barzegar *et al*., 2002). Furthermore, the addition of sludge to soil results in reduced soil pH and a rise in

electrical conductivity (Smith, 2009). Generally the use of organic modifications has been linked with an enhancement of microbial growth and activity (Bailey and Lazarovits, 2003), together with alteration in microbial community composition (Marschner *et al.*, 2003). These alterations are linked with modifications in operational abilities of soil microbial communities (Fierer et al., 2012). Agricultural land management is one of most important anthropogenic actions that greatly changes soil characteristics, including physical, chemical, and biological properties (Jangid *et al*., 2008). This practice is predominantly relevant in semi-arid environments, where inappropriate land management together with climatic restraints (scarce rainfall and reoccuring drought periods) can intensify rates of erosion and other degradation processes of agricultural land (Caravaca *et al.*,2002). It is highly suggested to use organic fertilizers in agricultural production to provide soils with plant nutrients and recover soil chemical, physical, and biological properties (Brady and Weil, 2001). Conventional fertilizers such as kraal manure and compost are commonly used for these purposes (Ferreras *et al*., 2006; Herencia *et al*., 2007). Land use types had a critical result on soil quality properties and also interrelationship between land use type and soil properties (Oyetola and Philip, 2014). Different land use systems such as agriculture (irrigated and rainfed), horticulture, forestry, pastures and wasteland systems leads to the change in physicochemical properties and also change in nutrient content (Ally-Said *et al.*, 2015). Studies have shown that variation in soil properties were found among different land uses (Abbasi *et al*., 2010; Yang *et al.*, 2013) and cultivation significantly affects the physical, chemical, and microbial properties of soils.

# CHAPTER THREE

# MATERIALS AND METHODS

## 3.1. Site characteristics

### 3.1.1. Geographical setting of the study area

The soils samples used for this study were collected from six ecosystems along the Dikabeya horticultural farm that is 8 km from Palapye waste water treatment (PWWT) plant. The study area (Fig. 3.1) lies within Eastern Palapye ($22^0$ 33"0'S and $27^0$ 8' 0"E) Botswana. This area consists of both commercial and subsistence arable farms.

### 3.1.2. Climate

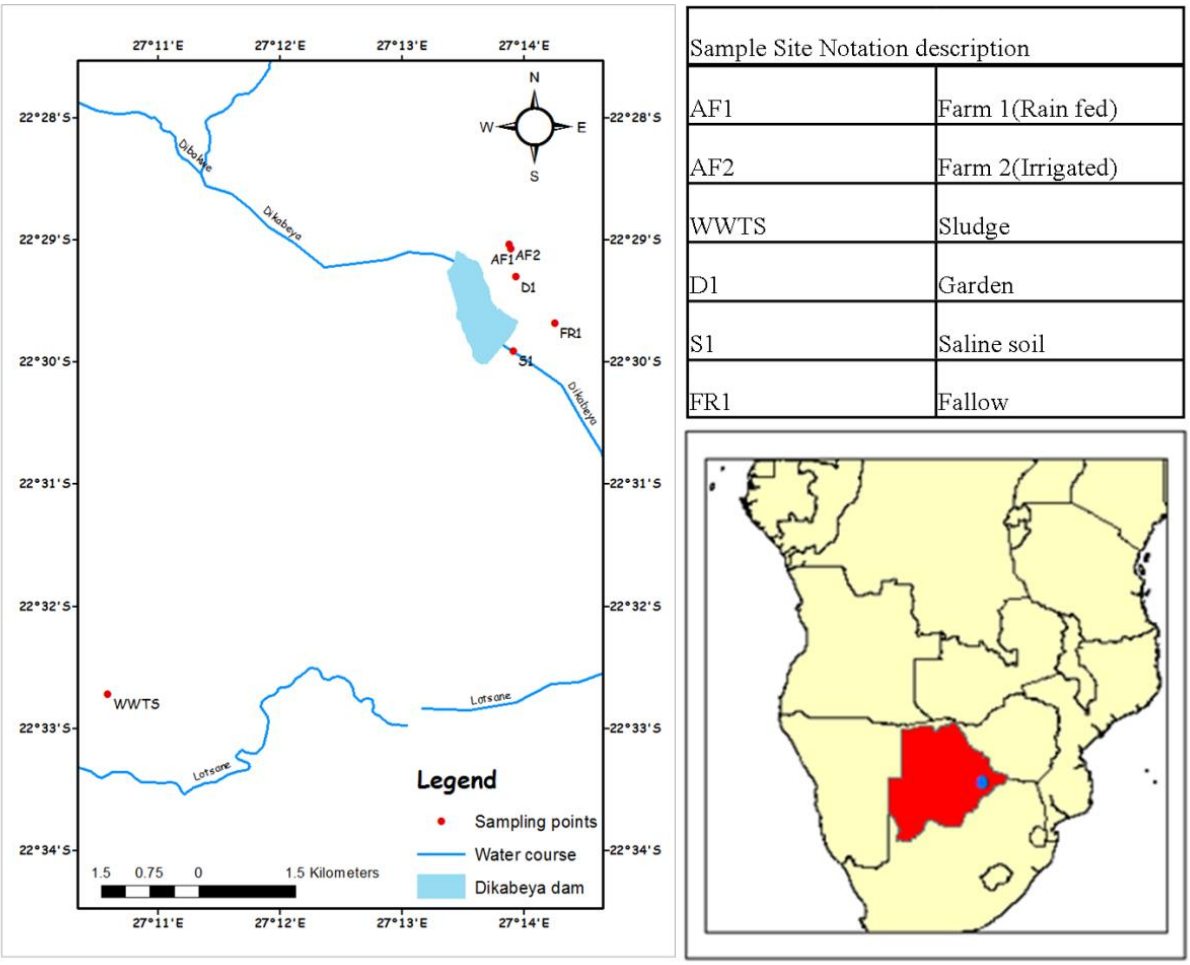
Palapye experiences semi-arid climate with average annual rainfall ranging from 250mm in the south-west to 650 mm in the northwest. Moreover, rainfall is seasonal, unreliable and changes yearly. Maximum temperatures usually occur during the October-March months, with June and July month's receiving the lowest temperatures. Only slight inter-annual changes in temperature ranges occur. The average annual maximum temperature is ranges between 28 °C and 30 °C and the average minimum temperature is between 14 °C and 16 °C (Kenabatho *et al*., 2012).

### 3.1.3. Vegetation

The study area lies within the hard veld and the vegetation is described by the prepotency of savannah vegetation including *Colophospermum mopane* (Mopane) and *Acacia* family. Trees around the study area source firewood, act as barriers from wind and provide fencing poles for locals.

### 3.1.4. Soils and geology

The soils of the study area have poorly developed horizons and are predominantly sandy silt loam in texture, with aeolian deposition and are classified as Ferralic Arenosols (FAO-WRB) or Ustic Quartzipsamments (Soil Taxonomy) (Kebonye *et al*., 2017).




| Sample Site Notation description | |
|---|---|
| AF1 | Farm 1(Rain fed) |
| AF2 | Farm 2(Irrigated) |
| WWTS | Sludge |
| D1 | Garden |
| S1 | Saline soil |
| FR1 | Fallow |

Fig.3.1: Geographical map of the study area showing the sample sites

The six land use sites where soil samples were obtained were approximately 2-3 km apart. Samples were collected in the mornings. Sampling sites were chosen based on land use and management practices. Samples were collected in triplicates from each ecosystems site using soil auger at a depth of 15 cm. All the instruments were disinfected with 70 % ethanol or

washed with distilled water before going into the next location for soil sample collection. Samples from the different locations were placed into separate labeled sterilized zip lock containers and taken to the laboratory for analysis using the method as described by Perez and Victor, (2014). About 1 kg of soil was collected from each site. Upon collection, the samples were placed in a cooler box with ice packs and then transported to the laboratory to be analyzed within 12 hours to reduce changes in soil properties due to storage.

### 3.2. Field examination and soil sampling

The six land use systems were selected based on their management practices. Saline (S1) was basically a bare land with high soluble salt content that inhibited crop growth. The horticultural garden was a farm specializing in vegetable production (cabbage, beetroots, tomatoes and green pepper) under irrigation and fertilizer application (NPK 2:3:2). Arable farm 1 (AF1) was managed under rainfed subsistence agriculture devoid of fertiliser use. Arable farm 2 (AF2) was a commercial farm; producing field crops (such as maize, sorghum, watermelon and sweet potato) irrigated with water from Dikabeya Dam, and subjected to inorganic fertilizer application. Sewage sludge (WWTS) was a dried precipitate from Palapye sewage treatment. Fallow (FR1) was an undisturbed land usually used for grazing, it was abundantly vegetated and crop cultivation was never carried out in this land.

### 3.3. Laboratory analyses

The collected soil samples were air-dried, gently ground and passed through 2 mm sieve before physical and chemical analyses. Chemical analyses were performed at the laboratory of the Agricultural Research Centre in Sebele.

### 3.3.1. Physical properties

#### 3.3.1.1. Particle size distribution

Particle size distribution was determined by the method of Bouyoucos (1926). To 40 g air-dried soil (<2 mm) was added 40 mL of 5 % sodium hexametaphosphate (NaHMP) solution and 150 mL deionized water in a beaker. The suspension was enclosed with a watch glass and kept overnight. The content was afterwards stirred for five minutes with a high speed electrical stirrer, poured into a 1000 mL capacity calibrated cylinder and water added to make up to 1 litre mark. The suspension was mixed thoroughly in the cylinder with the plunger and a hydrometer (ASTM 152 H) inserted immediately after the plunger was withdrawn. Froth at the surface of the suspension in the hydrometer jar produced due to vigorous stirring with the plunger, was dispersed by adding one drop of amyl alcohol. The first reading for silt plus clay was taken after 40 seconds of plunger withdrawal from suspension. Afterwards, the suspension was mixed with the plunger as before and left undisturbed. After 4 hours, the hydrometer was inserted and the reading was recorded for clay. Finally, the percent values of sand, silt and clay fractions were incorporated into the ISSS triangle and soil textural class was established.

#### 3.3.1.1 Chemical properties

#### 3.3.2.1. Soil pH

The pH in $H_2O$ was determined using the electrode of pH meter (InoLab WTW series, pH 720 glass electrometer) in a 1:1 (soil to water) ratio (Hanlon & Bartos, 1993). Twenty grams of soil was weighed into a 50 mL beaker and twenty milliliters of deionized water was added. The soil-water suspension was poured into a bottle and stirred vigorously for 30 minutes. The

suspension was allowed to stand for 30 minutes at room temperature for equilibration. The pH electrometer was then standardized using buffer solutions of pH 4.0 and 7.0. Then, pH of the supernatant liquid was recorded (Hanlon & Bartos, 1993).

### 3.3.2.2. Electrical conductivity (EC 1:2.5)

A 1:2:5 soil: water suspension was prepared by adding 50 mL deionized water to 20 g soil in a beaker. After shaking the contents well, the suspension was kept still for one hour. After that, the EC of the supernatant liquid was measured at $25^0$ C using EC meter model EDT instruments, BA380 (Rhoades, 1993).

### 3.2.2.3. Organic carbon

Two grammes (2 g) of the air-dried soil sample was mixed with 10 mL of 2 N potassium dichromate ($K_2Cr_2O_7$) in a wide-mouthed 1000 mL volumetric flask. The flask was swirled gently to disperse the soil in the solution. Twenty millilitres of concentrated sulphuric acid ($H_2SO_4$) was added and the flask was gently swirled to mix the contents of the flask. It was left to stand for 30 minutes after which 200 mL of distilled water was added, swirled and allowed to cool. 10 mL of 85 % phosphoric acid ($H_3PO_4$) was added and the mixture in the flask was left for one hour to allow particles to settle. Both samples and standards were read at 620 nm wavelength and a 2 cm path length in Ultra violet (UV) spectrophotometer (Walkley and Black, 1934).

### 3.2.2.4. Phosphorus

Bray II method was used to determine extractable phosphorus. Soil sample (3g) was weighed into 50 mL extraction cups and stirred for 40 seconds before filtering with (Whatman filter paper 40) into dry clean plastic cups. The semi-automatic dispenser was used to pipette 5 mL

of extractant plus 20 mL of working solution (sulphuric- molybdate, distilled water, ammonium molybdate, potassium tartrate, 10 N sulphuric acid, ascorbic acid and boric acid) into a 50 mL plastic cup. The same procedure was followed in making the standard solution. The samples were left for 40 minutes to develop colour. The absorbance was determined at 670 nm using spectrophotometer, and the calibration graph of absorbance versus concentration was read off as the concentration of extractable phosphorus (P) in the soil (Bray and Kurtz, 1945).

**3.2.2.5 Extraction of basic cations**

Extraction vessel/syringe was prepared by tightly compressing 1 g ball of a filter pulp into the bottom of a syringe barrel with a modified plunger. Soil of 2.5 g was weighed in the extraction vessel. A squeeze bottle was used to add 1 M ammonium acetate at pH 7 to 5-7.5 mm above the soil. The extraction vessel was then placed in the mechanical vacuum extractor and allowed to run for 2 hours. The leachate was collected into 100 mL volumetric flask and filled to the mark with ammonium acetate.

**3.2.2.6 Exchangeable cations**

Dilution of 100 mL of a 1000 mg $L^{-1}$ primary standard solution (Magnesium standard solution) with distilled water was done in a 1 litre volumetric flask and it was made up to the mark. For the preparation of mixed standard solution 50 mg $L^{-1}$ of magnesium and 500 mg $L^{-1}$ of calcium measured using a pipette and taken into 100 mL volumetric flask. 50 mL of the 1000 ppm calcium were also added and mixed well. Pippetes of 2.0 mL, 4.0 mL, 6.0 mL, 8.0 mL, and 10.0 mL were used to prepare a working standard from the mixed standard solution into 100 mL volumetric flasks and it was made to the mark with 1 M ammonium acetate solution. The standards were containing 1.0, 2.0, 3.0, 4.0, 5.0 mg $L^{-1}$ magnesium and 10, 20,

30, 40, 50 mg $L^{-1}$ calcium. Ammonium acetate sample leachate and standards were taken into vials or test tubes and 9 mL of strontium chloride solution was added and mixed. The absorbance was measured using an atomic absorption spectrophotometer. Magnesium was measured at 285.2 nm and calcium at 422.7 nm wavelengths and the plot of absorbance versus concentration curves was plotted and reading of samples concentration was done (Kramer and Tisdall, 1921).

#### 3.2.2.7. Potassium and Sodium

Standard solutions for potassium and sodium were prepared using potassium and sodium stock solutions at 78 mg $L^{-1}$ and 46 mg $L^{-1}$ respectively Potassium standard solution series was taken into 100 mL volumetric flask by a pipette with 0.5, 10, 15, 20, 25 mL of the 78 mg $L^{-1}$ and made to the mark. The standard then was containing 0.0, 0.4, 0.8, 1.2, 1.6 and 2.0 cmol $kg^{-1}$ potassium in soil. The same protocol was followed in making sodium standard series with 0.5,10, 15, 20 and 25 mL of 46 mg $L^{-1}$ sodium taken into volumetric flask and made to the mark with ammonium acetate. The standards were containing 0.0, 0.4, 0.8, 1.2, 1.6, and 2.0 cmol $kg^{-1}$ in soil. AAS was used to measure the emission for standards and samples (Simard, 1993).

### 3.3.3. Bacterial analysis

#### 3.3.3.1. Bacterial isolation and quantification

The culture-based approach for bacteria identification was adopted to complement the metagenomics approach by taking into consideration the relative abundance of the viable selected bacteria. Gram negative (*E. coli*) and Gram positive (*S. aureus*) bacteria were selected represent the viable species within the Proteobacteria and Firmicutes respectively. *E. coli* medium and Mannitol salt agar (MSA) selective media were used for isolation and

quantification of *E. coli* and *S. aureus*. The media were prepared according to the manufacturer's instructions (Harlequin™). Fresh soil samples were collected, one gram of soil was diluted tenfold, and serial dilutions were spread plated on the respective selective agar media. The plates were incubated at 37$^{o}$C for 24 hours using Incotherm economy incubators (Labotec, South Africa). Only plates with 30 to 300 colonies were considered quantifiable and were counted using a colony counter (BioCote, Stuart, UK). Isolates growing in respective selective media were randomly picked and confirmed by gram stain procedure.

#### 3.3.3.2. DNA extraction

DNA was extracted from each of the soil samples collected from the six land use systems. One gram triplicate of each soil sample was subjected to DNA extraction using a Power Soil DNA Extraction Kit (MO BIO Laboratories, Carlsbad, CA, USA according to manufacturers instructions). DNA samples were checked for quality and purity using a Nanodrop spectrophotometer (GENOVA NANO). Metagenomic sequencing of DNA was performed at the University of Pretoria. Only samples with DNA concentrations above 10 ng uL$^{-1}$ were sent for next-generation metagenomic sequencing.

#### 3.3.3.3. 16S rRNA metagenomic sequencing

The 16S rRNA metagenomic sequencing was done using the 16S rRNA sequencing workflow for the Ion PGM™ System (Salipante et al., 2013). Briefly on the procedure, the two primer pools (V2-4-8, V3-6-7-9) were simultaneously used to amplify 7 of the hyper variable regions of the 16S rDNA gene in bacteria. After generating the amplicons, the Ion Plus™ Fragment Library Kit was used to ligate barcoded adapters and synthesize libraries. Barcoded libraries from the sample were pooled and templated on the OneTouch2™ system followed by 400 bp sequencing on the Ion PGM (Fig.3.2). Automated analysis, annotation and taxonomic

assignment carried using the Ion Reporter Software pipeline. Classification of base pair sequences was through alignment to either the curated MicroSEQ ID or curated Green genes databases (Salipante et al., 2013).

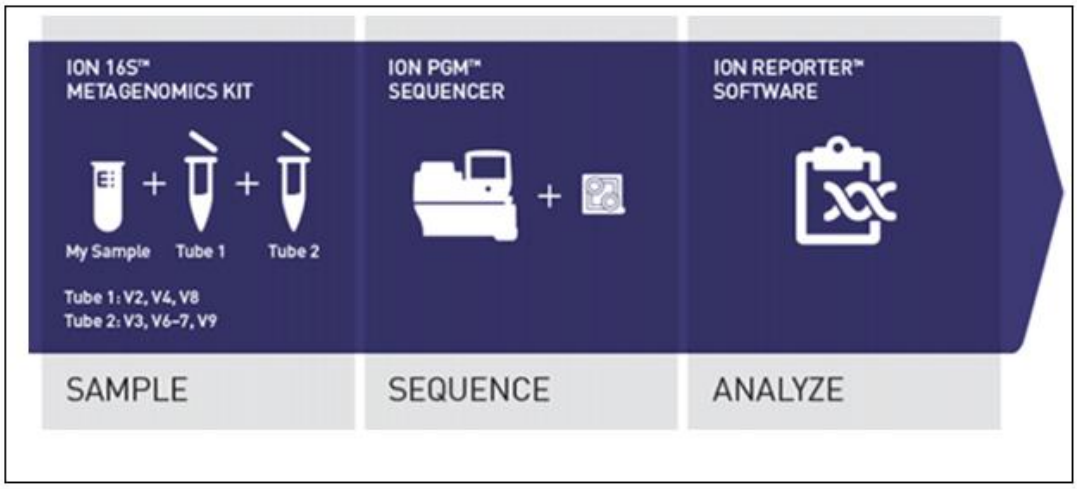



Fig. 3.2: Schematic diagram of Ion 16S Metagenomics workflow (Salipante et al., 2013). Two sets of primer pools were simultaneously used to amplify 7 of the 9 hypervariable regions of the 16S rDNA gene in bacteria: Pool 1: Primer set to amplify V2-V4-V8, Pool 2: Primer set to amplify V3-V6-V7-V9.

### 3.4. Statistical analysis

Average counts from triplicate cultured plates were used to obtain the mean and standard deviation. Statistical analyses for bacterial quantification were performed using Microsoft Excel 2010. Metagenomics results were analysed through the ion torrent reporter bioinformatics software. Classification of reads was through alignment to either the curated MicroSEQ ID or curated Green genes databases (Salipante et al., 2013).

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# CHAPTER FOUR

# RESULTS

## 4.1. Physicochemical properties

Selected physical and chemical properties of the soils are summarized in Table 4.1 with the details shown in Appendix A. Variations in physical and chemical characteristics were observed in all of the land uses but with no clearly defined trends. The pH ($H_2O$) of the soils ranged from 5.18 to 7.62 with the highest in the saline soil. Soil electrical conductivity (EC) values ranged from 0.02 to 0.57 dS $m^{-1}$ with little variations across the soils. On average the EC at the sludge site was higher than the other land uses (0.57 dS $m^{-1}$). Organic carbon was contrasting with values ranging from 0.20 to 57.50 % and was highest in the sewage sludge. Phosphorus showed the widest span with values ranging from 5.74 to 2641 ppm. The soils showed an increased amount of phosphorus in sludge (Table 4.1). Calcium concentration ranged from 1.15 to 6.73 cmol $kg^{-1}$. Magnesium concentration in the soil varied from 0.49 to 1.23 cmol $kg^{-1}$. Potassium was present in all samples and its concentration ranged from 0.14 to 1.26 cmol $kg^{-1}$. Sodium ranged from 0.06 to 0.39 cmol $kg^{-1}$. Cation exchange capacity (CEC) ranged from 1.98 to 8.62 cmol $kg^{-1}$ and averagely higher in sewage sludge. The sand fraction of the soils ranged from 79.35 to 92.03 % . The amount of clay fraction ranged from 5.80 to 17.83 % and silt was between 2.10 to 3.38 %.

Table.4.1. Selected physico-chemical properties of the soils in the studied ecosystems

| Sample ID | Ecosystem | Location | pH | EC | OC | P | Ca | Mg | K | Na | CEC | Clay | Sand | Silt | TC |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | $H_2O$ | $dS.m^{-1}$ | % | ppm | ----------cmol.$kg^{-1}$---------- | | | | | ---------%--------- | | | |
| FR1 | Fallow | 22º29'18.46"S 27º14'4.15"E | 5.18 | 0.02 | 0.36 | 5.74 | 1.19 | 0.49 | 0.25 | 0.06 | 1.98 | 12.35 | 84.27 | 3.38 | LS |
| S1 | Saline soil | 22º29'33.83S 27º13'41.76"E | 7.62 | 0.44 | 0.50 | 24.90 | 1.23 | 1.23 | 0.41 | 0.39 | 4.05 | 13.07 | 82.77 | 4.16 | SL |
| WWTS | Sludge | 27º10'22.02"E 22º32'22.59"S | 5.84 | 0.57 | 57.50 | 2641.3 | 6.31 | 0.87 | 1.26 | 0.18 | 8.62 | 17.83 | 79.35 | 2.82 | SL |
| D1 | Garden | 22º28'52.38"S 27º13'45.93"E | 5.75 | 0.03 | 0.66 | 132.1 | 1.56 | 0.66 | 0.14 | 0.14 | 2.40 | 8.39 | 88.96 | 2.65 | LS |
| AF1 | Arable Farm1 | 22º28'37.62"S 27º13'14.34"E | 5.50 | 0.03 | 0.65 | 9.11 | 3.49 | 0.76 | 0.50 | 0.66 | 4.81 | 5.80 | 92.03 | 2.17 | S |
| AF2 | Arable Farm2 | 22º29'33.83"S 27º13'41.76"E | 6.44 | 0.06 | 0.47 | 284.9 | 2.93 | 0.73 | 0.26 | 0.14 | 4.05 | 8.65 | 89.25 | 2.10 | S |

LS: Loamy sand; S: Sand; SL: Sandy loam; WWTS: Sludge, A1F1: Arable farm1, AF2: Arable farm2, D1: Garden, S1: Saline soil, FR1: Fallow, pH: soil pH, Ec: Electrical conductivity, Oc: Organic carbon, P: Phosphorus, Ca: Calcium, Mg: Magnesium, K: Potassium, Na: Sodium, CEC: Cation exchange capacity, TC: Textural class.

## 4.2. Molecular and Metagenomics analysis of soils

### 4.2.1 DNA concentrations

The purity of the DNA ranged from 1.5 ng $\mu L^{-1}$ to 2.0 ng $\mu L^{-1}$ with the highest in arable farm 2 which did not qualify for the 16s rRNA Metagenomics sequencing (Fig.4.1). The DNA concentrations varied from less than 1 ng $\mu L^{-1}$ to 83.6 ng $\mu L^{-1}$. The sludge had the highest average DNA concentration (83.6 ng $\mu L^{-1}$), followed by saline (25.8 ng $\mu L^{-1}$), garden (19.1 ng $\mu L^{-1}$) and arable Farm 1 (6.38 ng $\mu L^{-1}$). Soil DNA samples above 10 ng $\mu L^{-1}$ were selected for the 16S rRNA Metagenomics sequencing (Fig.4.2).

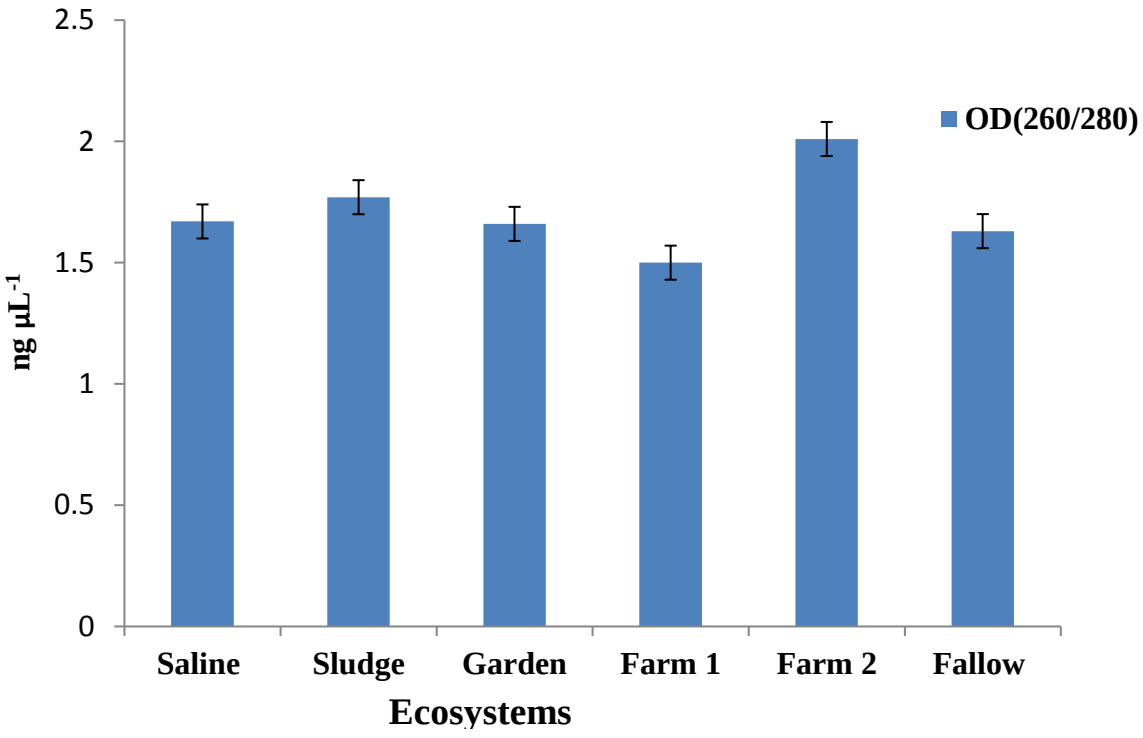


Fig.4.1: The purity of DNA measured by NanoDrop spectrophotometer across all the samples. The ratio of absorbance at 260/280 between 1.80 and 2.0 indicate pure DNA free of RNA/protein contamination.

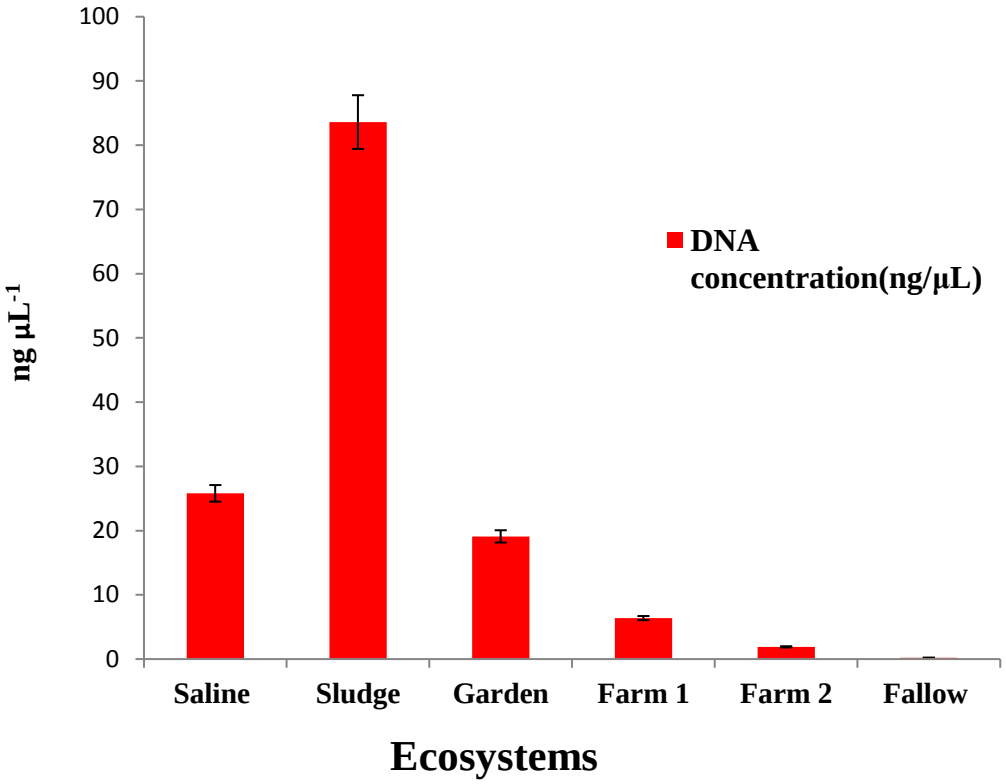


Fig.4.2: The quantity of DNA measured by NanoDrop spectrophotometer across all the samples. The DNA concentrations of the samples were calculated from UV absorbance at 260 nm.

### 4.2.2 Phylum and class level analysis

From the selected samples, quality sequences from sludge (33008 reads), garden (20251 reads) and saline (11043 reads) were obtained following high throughput DNA sequencing. Over 96 % of the sequence reads were mapped and classified at phylum and class level, only 1 % was unmapped for sludge, 3 % and 4 % were unmapped for garden and saline respectively. Averaged across the selected samples, the most abundant bacterial phyla were Proteobacteria which ranged from 63 to 84 % and these phyla consistently had the highest relative abundances across all samples (Fig. 4.3A-C). Actinobacteria were present in garden

and saline soils with relative abundance ranging from 18 to 23 % and only 2 % represented in sludge. Firmicutes phyla were well represented across all sites with values ranging from 4 to 12 %. Bacteroidetes were found in a higher percentage in the sludge sample (6 %) compared to the garden (1 %) and saline samples (0.7 %). Sludge showed higher phylum diversity that was not present in the saline and garden although the difference in abundance is less that 1 %. The most abundant bacterial classes were Betaproteobacteria ranging from 12 to 21 %. Alphaproteobacteria percentages ranged from 22 to 32 % and were represented in all samples. Gammaproteobacteria also ranged from 17 to 29 % across the three samples. Deltaproteobacteria was present in sludge (5 %) and saline soil (2 %). Epsilonproteobacteria (2 %), Flavobacteria (2 %), Clostridia (2 %) and Cytophagia(2 %) were only present in the sludge (Fig.4.4D-F). Bacilli were consistently present in both garden and saline soils at 11 % respectively.

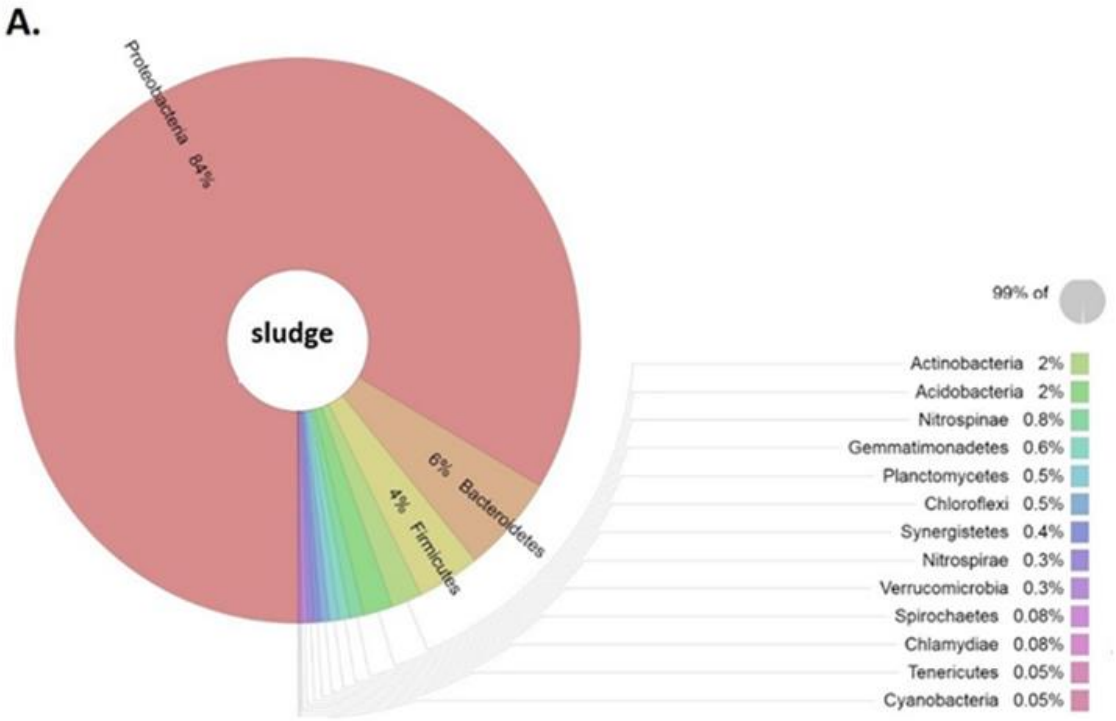


Fig.4.3(A): Krona charts showing the A. phylum-level diversity and distribution of bacterial populations in sludge.

**B.**

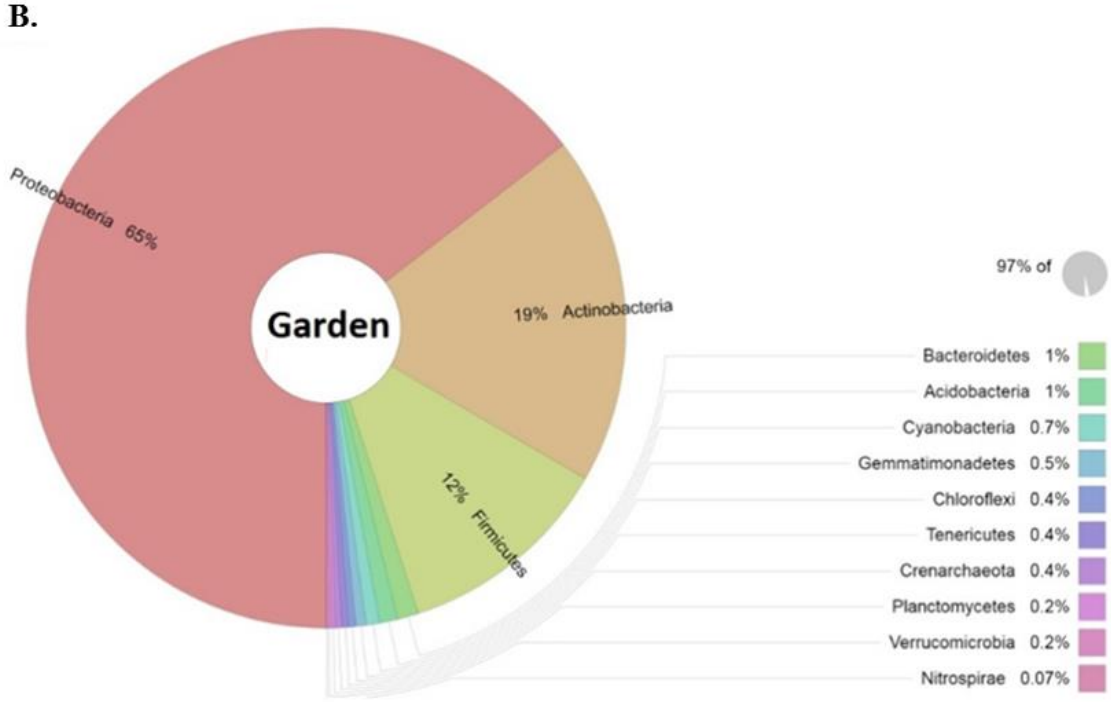


Fig.4.3(B): Krona charts showing the phylum-level diversity and distribution of bacterial populations in garden.

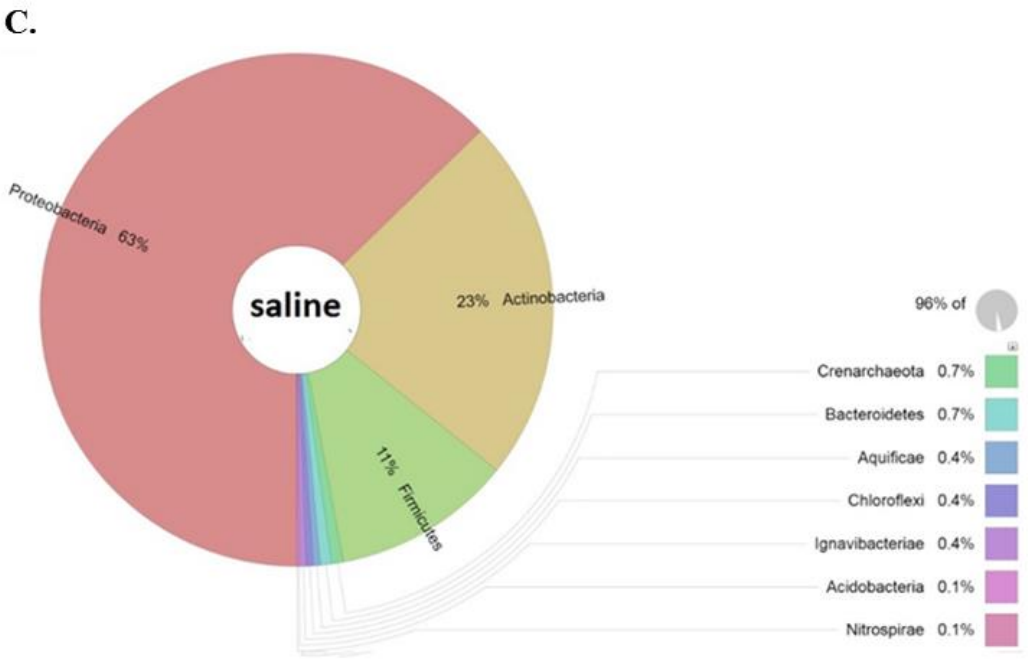


Fig.4.3(C): Krona charts showing the phylum-level diversity and distribution of bacterial populations in saline.

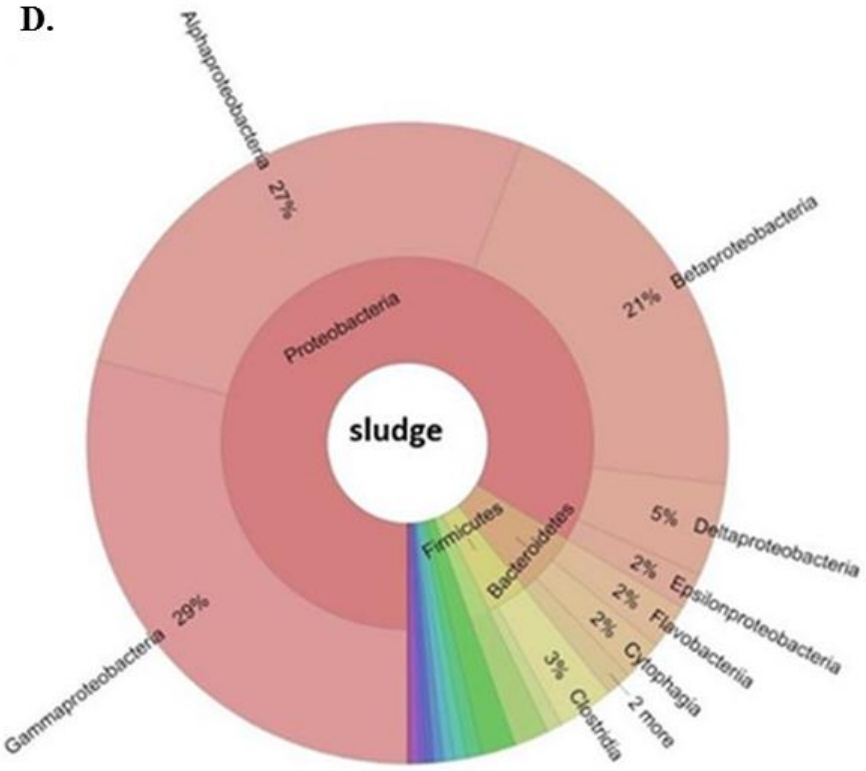


Fig.4.4(D): Krona charts showing the class-level diversity and distribution of bacterial populations in sludge.

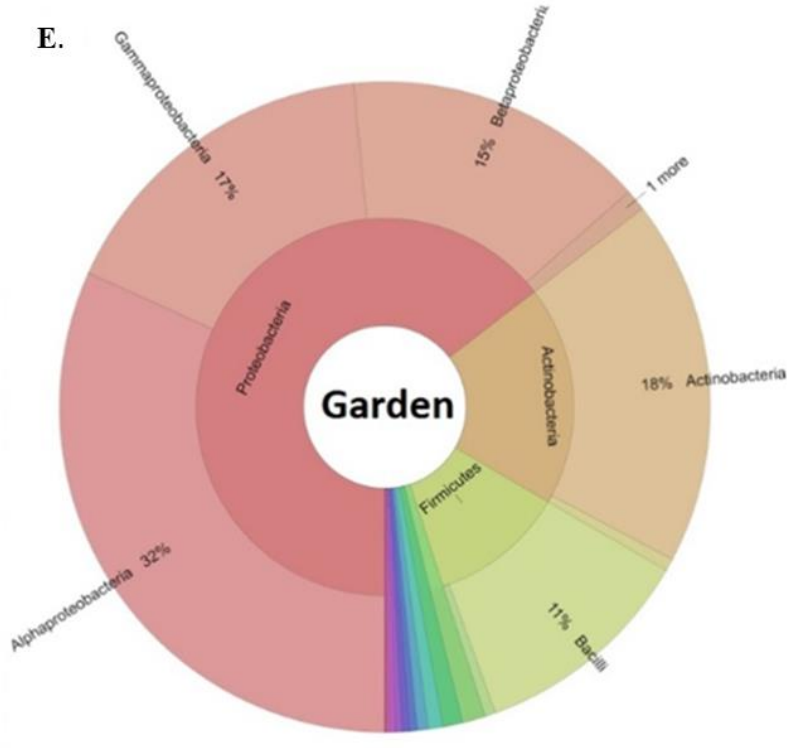


Fig.4.4(E)**:** Krona charts showing the class-level diversity and distribution of bacterial populations in garden.

**F.**

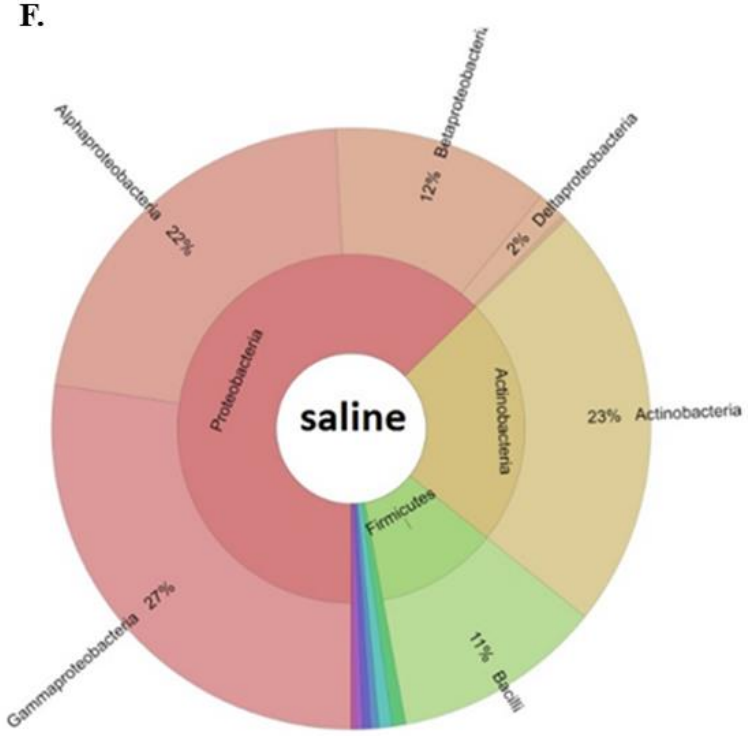


Fig.4.4(F). Krona charts showing the class-level diversity and distribution of bacterial populations in saline.

### 4.2.3 Order and family level analysis

Results at order level show that within the class alphaproteobacteria; Sphingomonadales was the most abundant order in the garden soil (27 %) and saline soil (14 %) compared to sludge (4 %). Both Rhizobales and Rhodospirilales ranged from 2 to 9 % across all the samples. In the gammaproteobacteria, order Xanthomonadales values ranged from 12 to 19 % and it was present in all samples. Pseudomonadales ranged from 3 to 5 % in sludge and saline respectively. Sludge showed more diversity consisting of orders not present in garden and saline soils; Oceanospirrales (4 %), Chromatiales (3 %), Orbales (3 %) and Altermonadales (2 %). Within the betaproteobacteria the order Burkhodriales was found in all the samples with values ranged from 6 to 12 %. Nitrosomonadales were at 3 % and 5 % respectively in saline and garden soils (Fig.4.5A-C). More order diversity was found in the sludge consisting of Myxococcales, Desulfovibrionales, Flavobacteriales and Camphylobacterales all at 2 % abundance. With a relatively higher abundance of the order Actinomycetales at 12 % and 13 % respectively, there is more consistency observed between garden and saline soils in comparison to sludge (1 %).

Garden and saline soil also revealed a higher abundance of Bacillales both at 11 %. Acidomicrobiales were present only in garden and saline soils 5 % and 2 % respectively. Orders Clostriales and Cytophagales were present only in sludge although at a lower abundance of 3 % and 2 % respectively.

The family with the highest relative abundance is Sphingomondaceae at 27 % in garden and 14 % in saline soils. Xanthomondaceae was also relatively high across all the samples; saline (19 %), garden (14 %) and sludge (10 %). Saline and garden soils also revealed a higher abundance of Bacillaceae both at 10 %. Rhodospirillaceae (9 %) and Rhodobiaceae (6 %) were present in the

sludge soil, whereas Oxabacteraceae (8 %) showed up as most abundant in the garden soil compared to the saline soil (5 %). Rubrobacteraceae (5 %) is another family in saline soil (Fig. 4.6D-F).

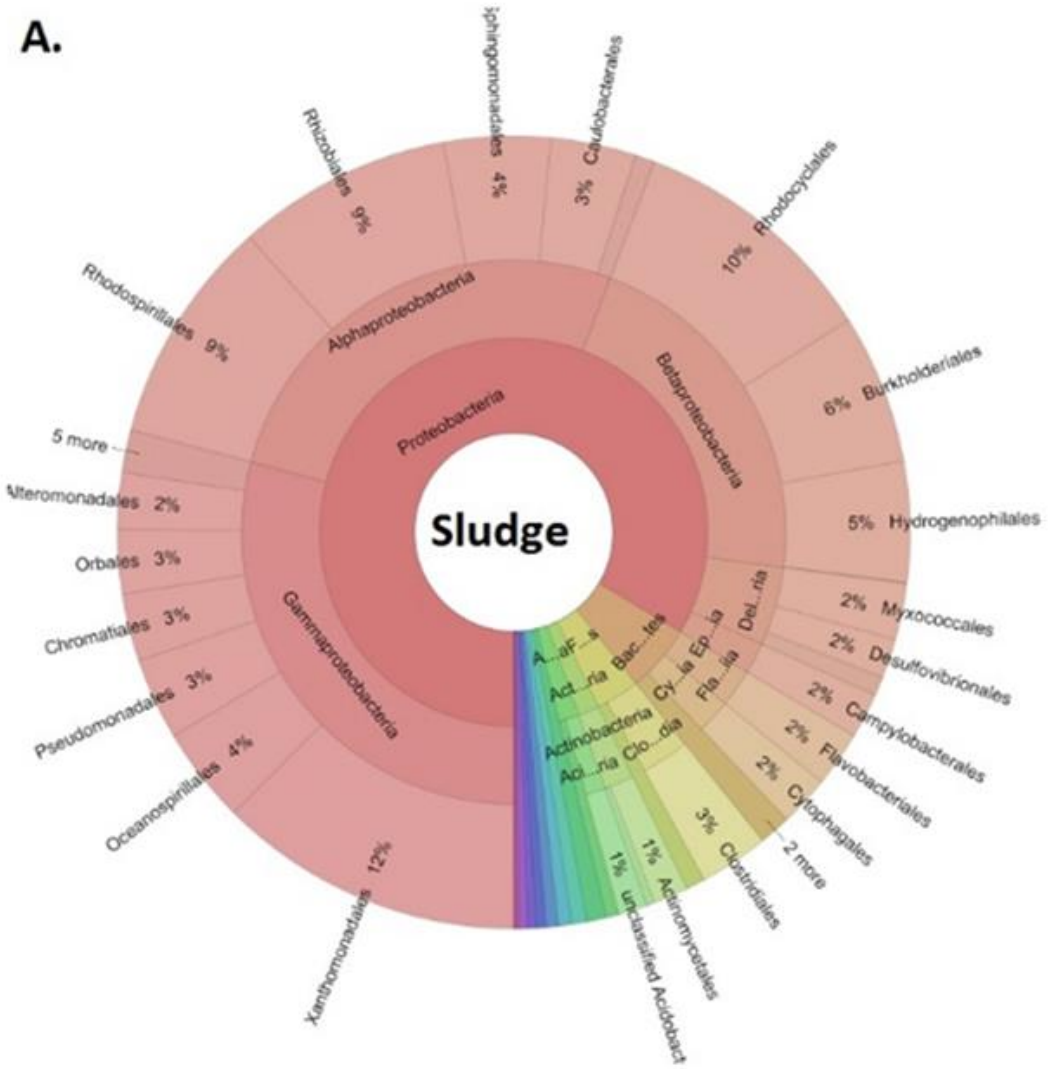


Fig. 4.5(A): Krona charts showing the order-level diversity and distribution of bacterial populations in sludge.

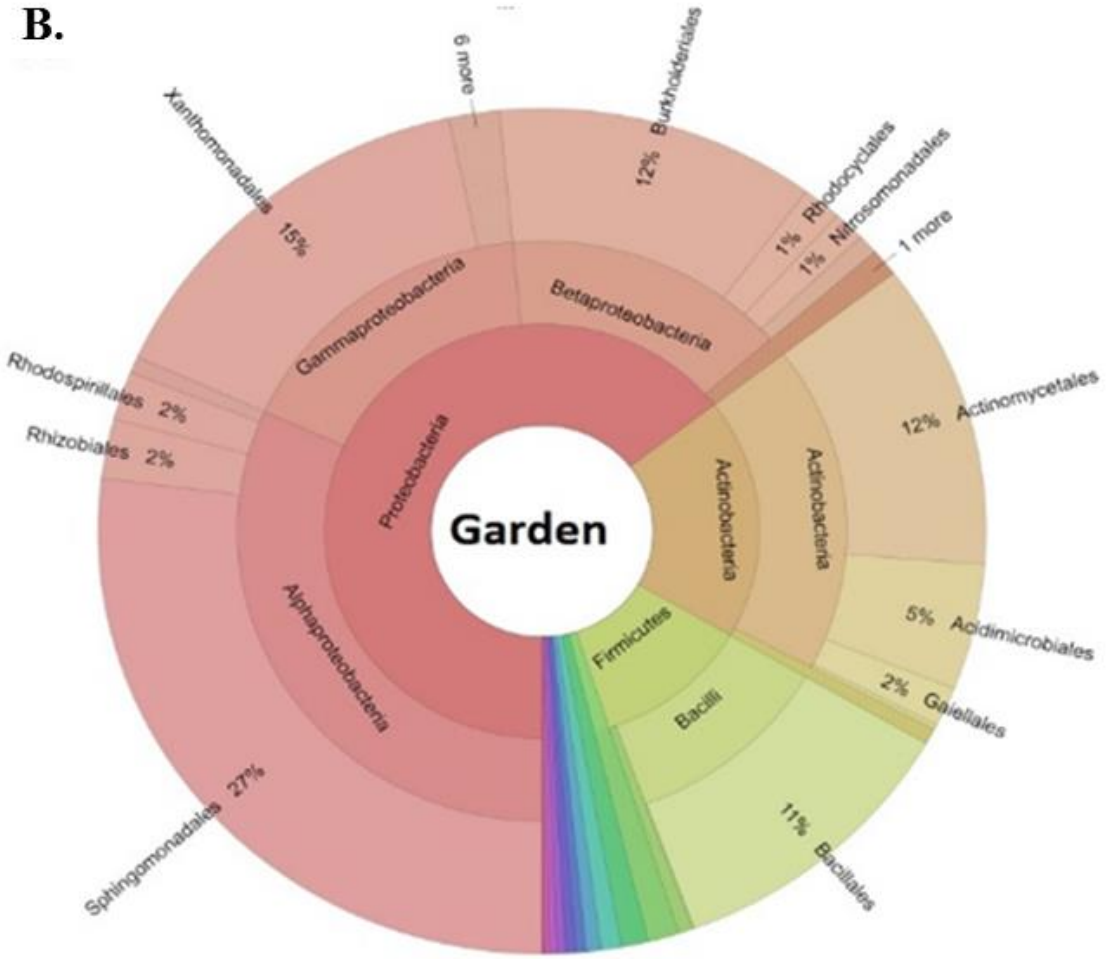


Fig. 4.5 (B): Krona charts showing the order-level diversity and distribution of bacterial populations in garden.

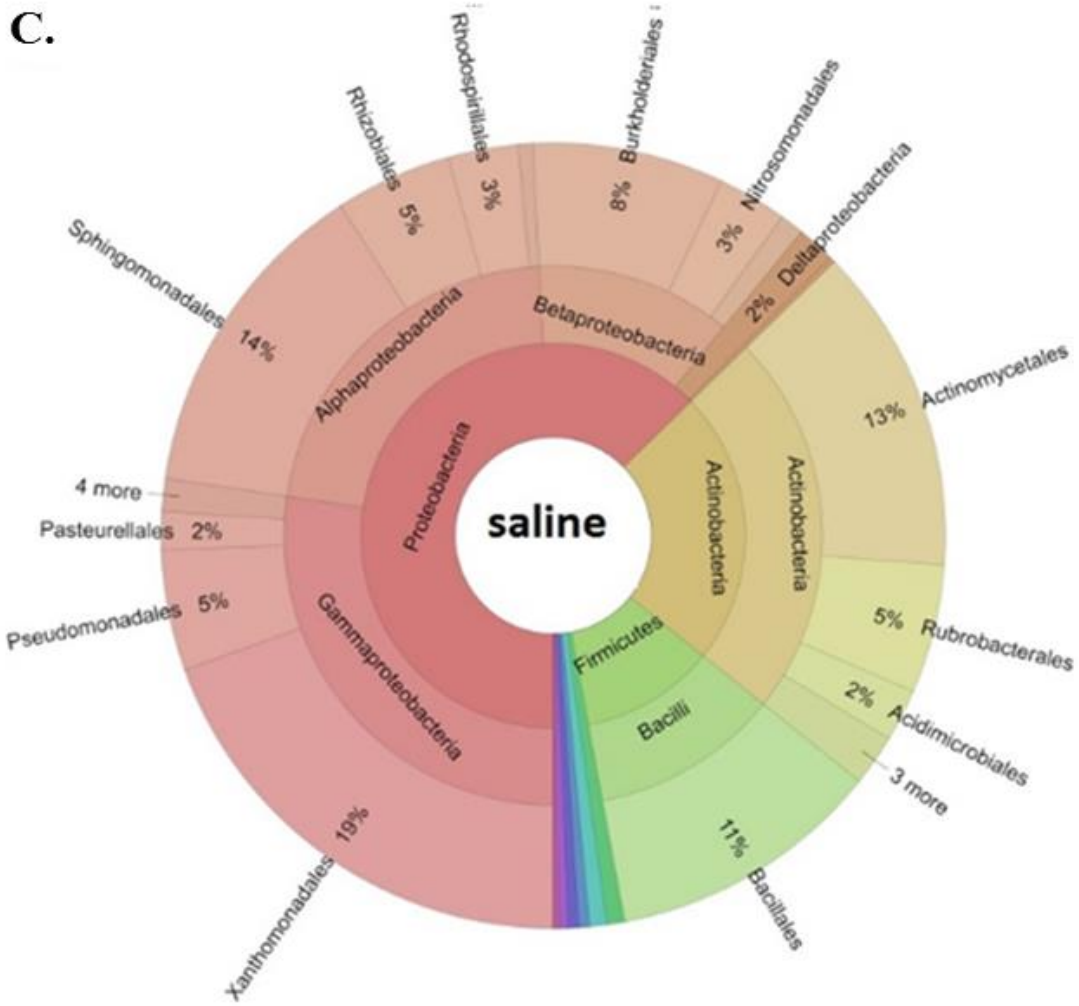


Fig. 4.5(C): Krona charts showing the order-level diversity and distribution of bacterial populations in saline.

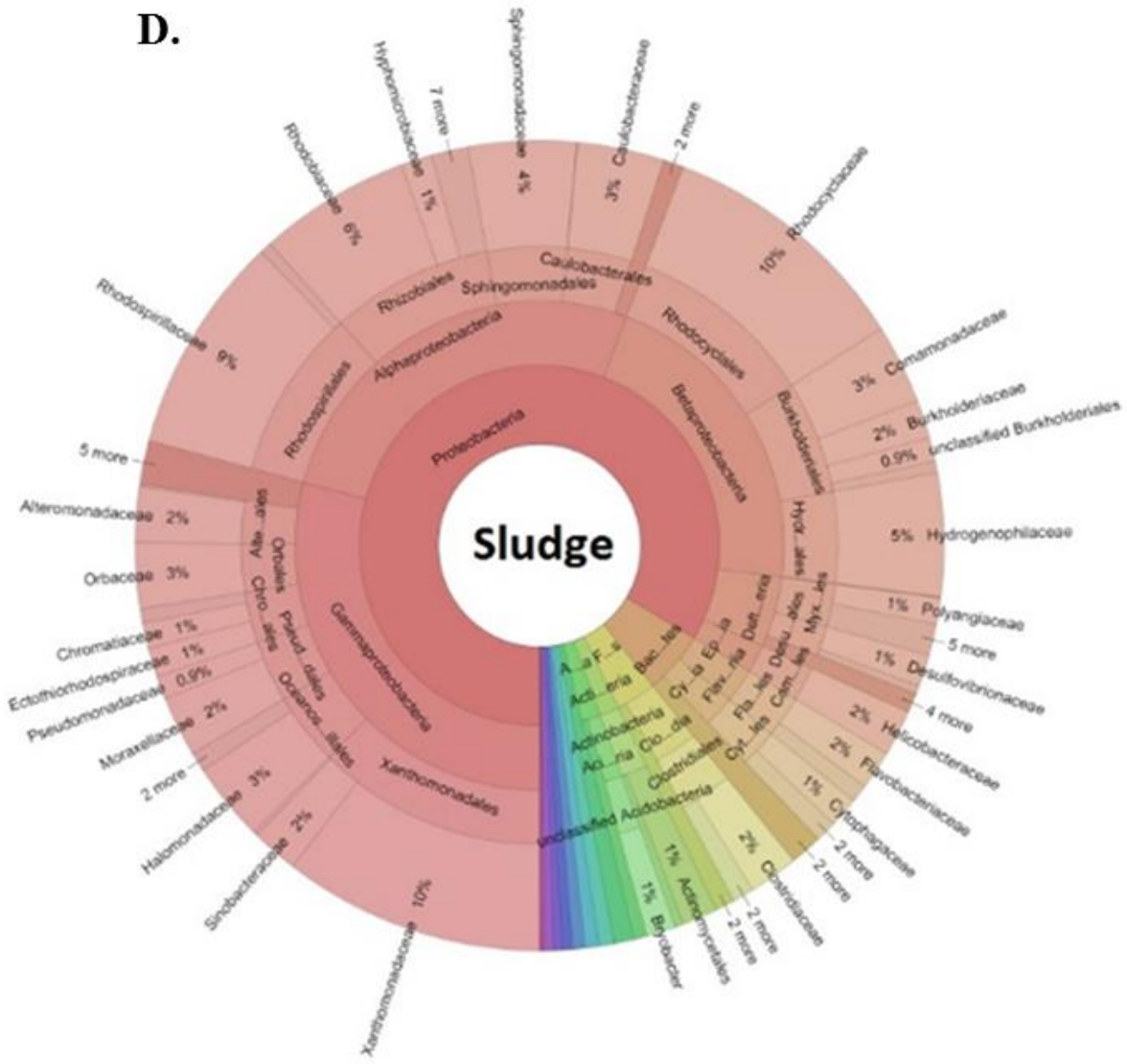


Fig. 4.6(D): Krona charts showing the family-level diversity and distribution of bacterial populations in saline.

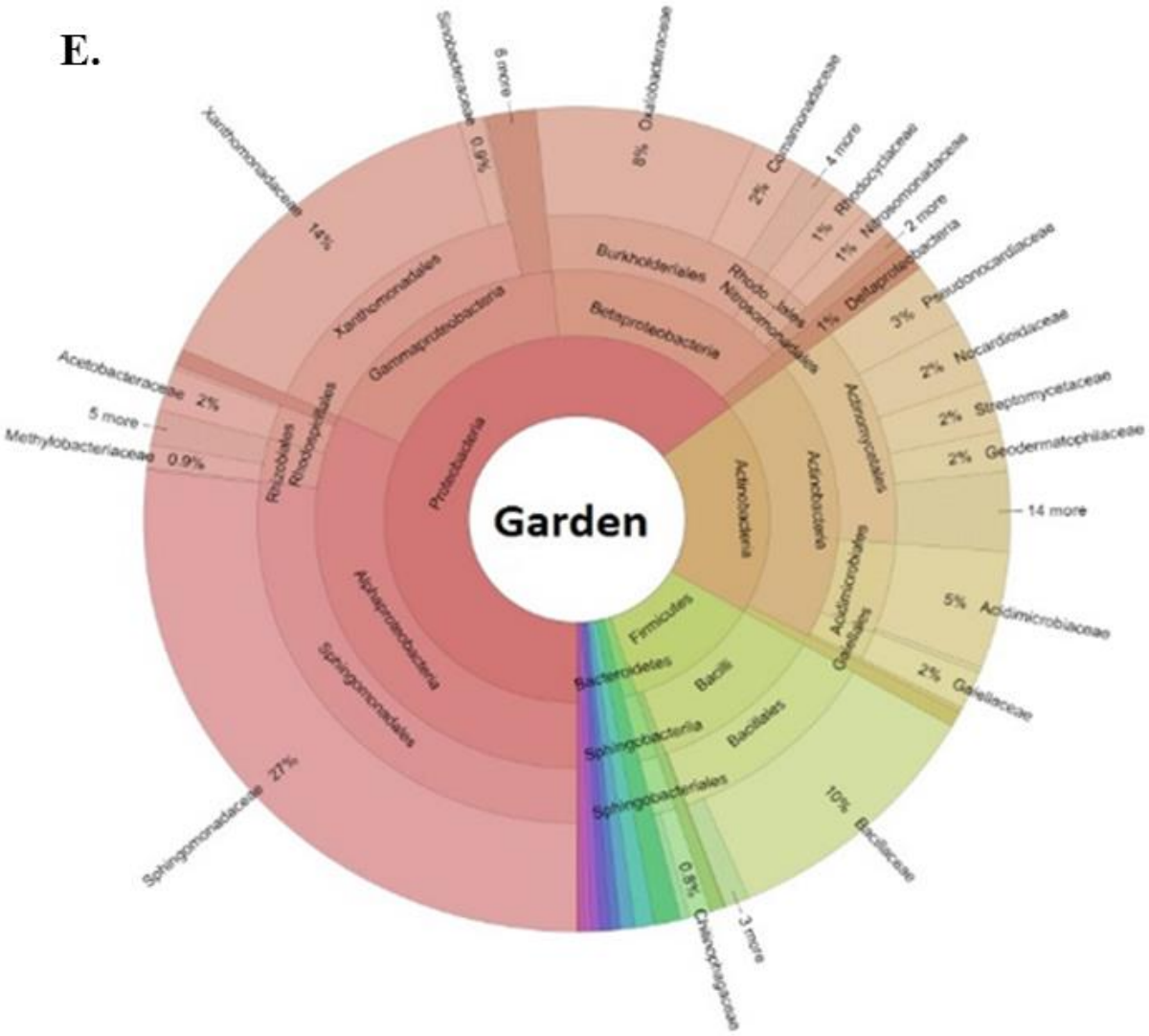


Fig. 4.6 (E)**:** Krona charts showing the family-level diversity and distribution of bacterial populations in garden

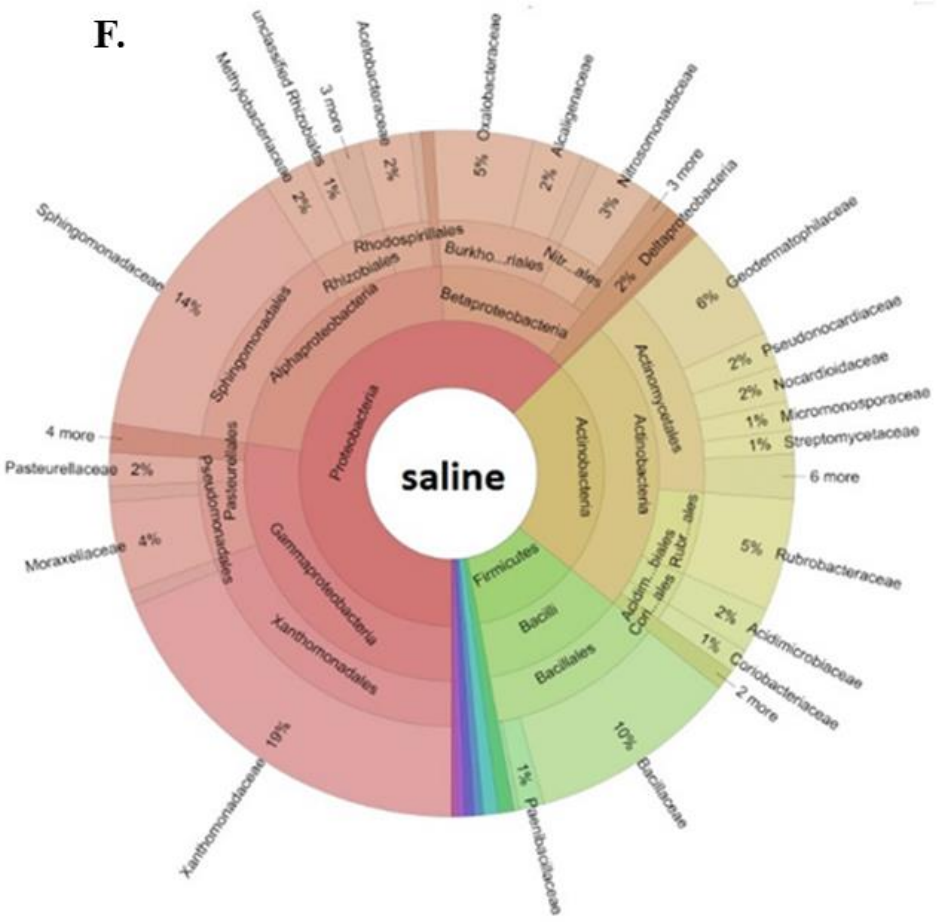


Fig. 4.6(F) continued**:** Krona charts showing the family-level diversity and distribution of bacterial populations in saline.

### 4.2.4 Genus level analysis

Metagenomics 16S rRNA sequencing also revealed some bacterial population at the genus level. At least twenty two different bacteria were revealed at genus level varying in relative abundance across all the sites (Fig.4.7a-c). Out of the twenty-two genus recorded, the sludge soils had the highest diversity comprising of thirteen bacteria genera; *Rhodovibrio* (1 %), *Dongio* (1 %), *Sphingomonas* (1 %), *Bryobacter* (1 %), *Pseudoxanthomonas* (1 %), *Frischella* (2 %), *Dechloromonas* (1 %), *Phenylobacterium* (2 %), *Clostridium* (2 %), *Lysobacter* (2 %), *Thermomonas* (3 %), *Thiobacillus* (4 %), and *Parvibaculum* (5 %). Garden and saline soils have eight and nine different bacteria genera respectively, with seven types common to both ecosystems. Garden soil had the highest abundance of *Sphingomonas* (20 %) compared to saline (8 %) and sludge (1 %). *Bacillus* was present in both garden and saline soils at a relative abundance of 6 % and 5 % respectively. *Oceanobacillus* was higher in saline (3 %) compared to garden (1 %). *Herbaspirillum* was detected at equal abundances (5 %), whereas *Pseudoxanthomonas* and *Geodermatophilus* were slightly higher in saline soil (5 %) compared to garden soil (4 %). *Pseudoxanthomonas* was also detected in the sludge at a relatively lower percentage (1 %). *Nitrospira* (2 %) and *Rosemonas* (1 %) were present only in saline soil.

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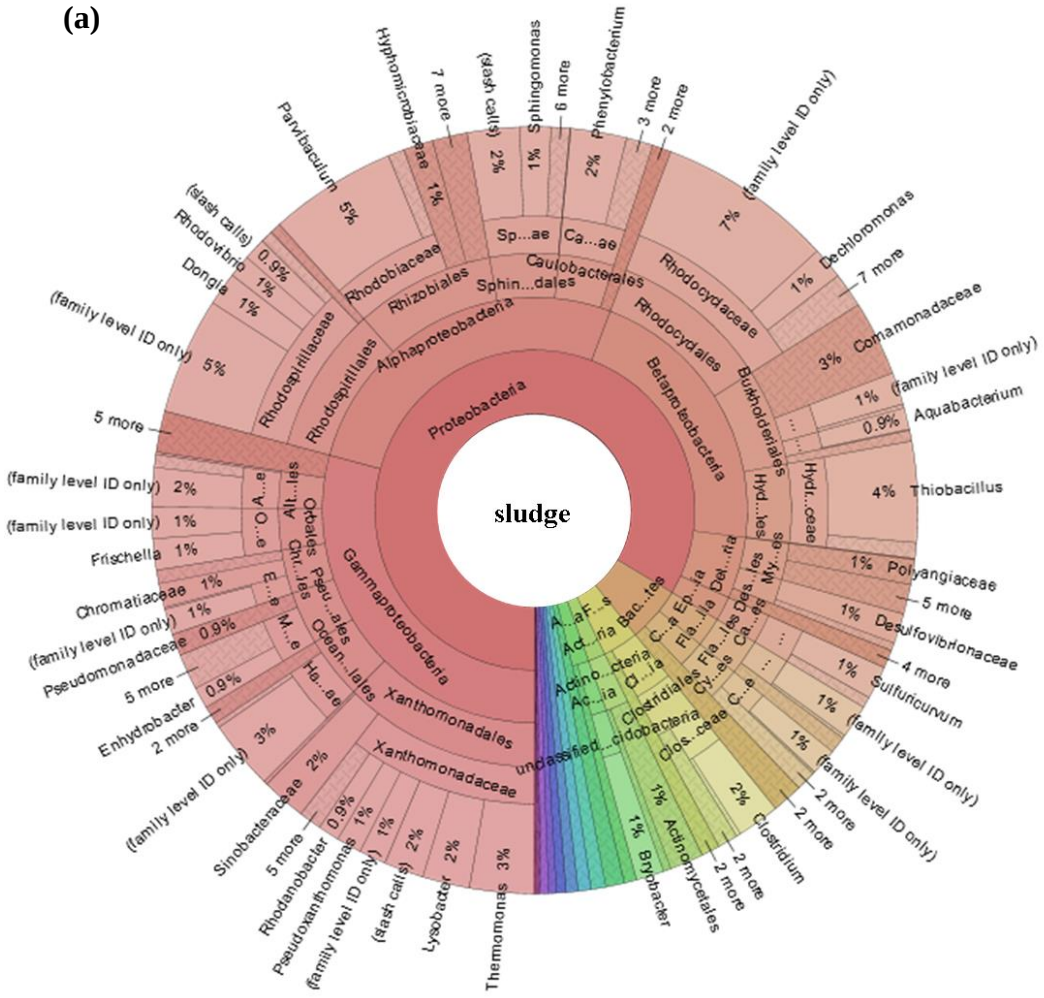


Fig. 4.7 (a): Krona charts showing genus-level abundance, diversity and distribution of bacterial population in sludge

**(b)**

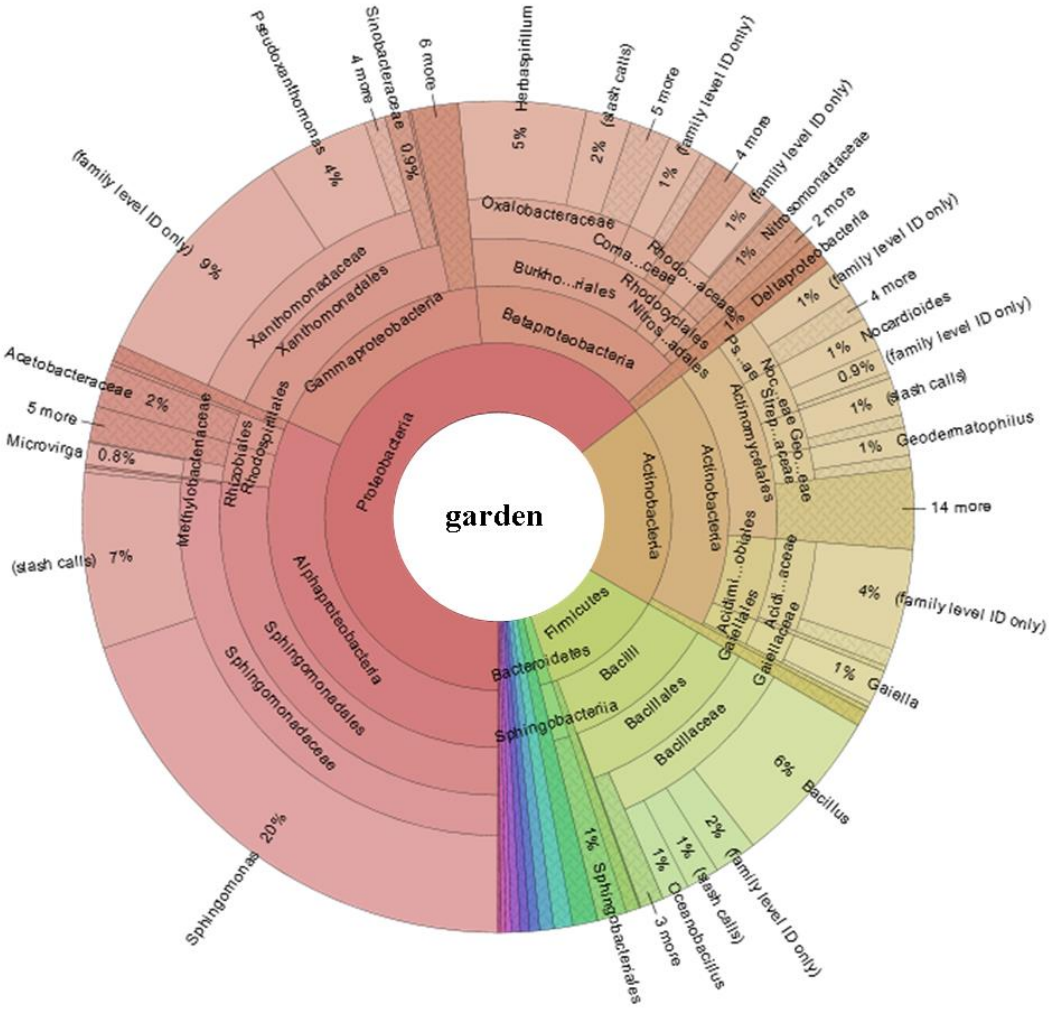


Fig 4.7(b): Krona charts showing genus-level abundance, diversity and distribution of bacterial population in garden

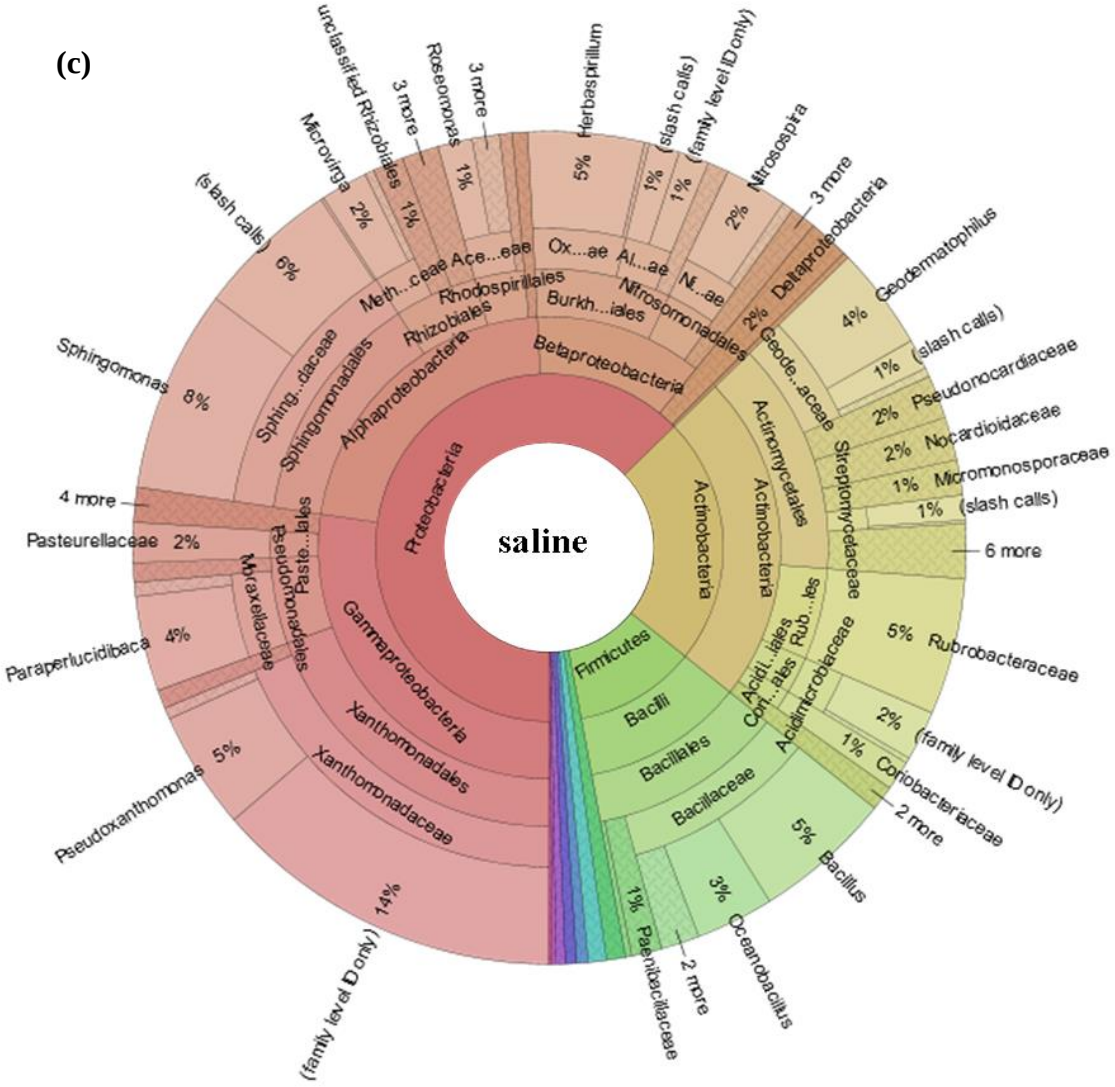


Fig 4.7(c): Krona charts showing genus-level abundance, diversity and distribution of bacterial population in saline

### 4.3. Bacterial isolation and quantification

The results obtained for the isolation and quantification of bacterial species in different ecosystems are presented in Fig.4.8. *S. aureus* (Gram positive) was quantifiable across all six ecosystems ranging from 1.7 x $10^7$ cfu $mL^{-1}$ in farm 2 soil to 0.5 x $10^7$cfu $mL^{-1}$ in garden soil. *E. coli* (Gram negative) were only quantifiable in sludge (6 x$10^7$ cfu $mL^{-1}$) and garden soil (0.3 x$10^7$cfu $mL^{-1}$).

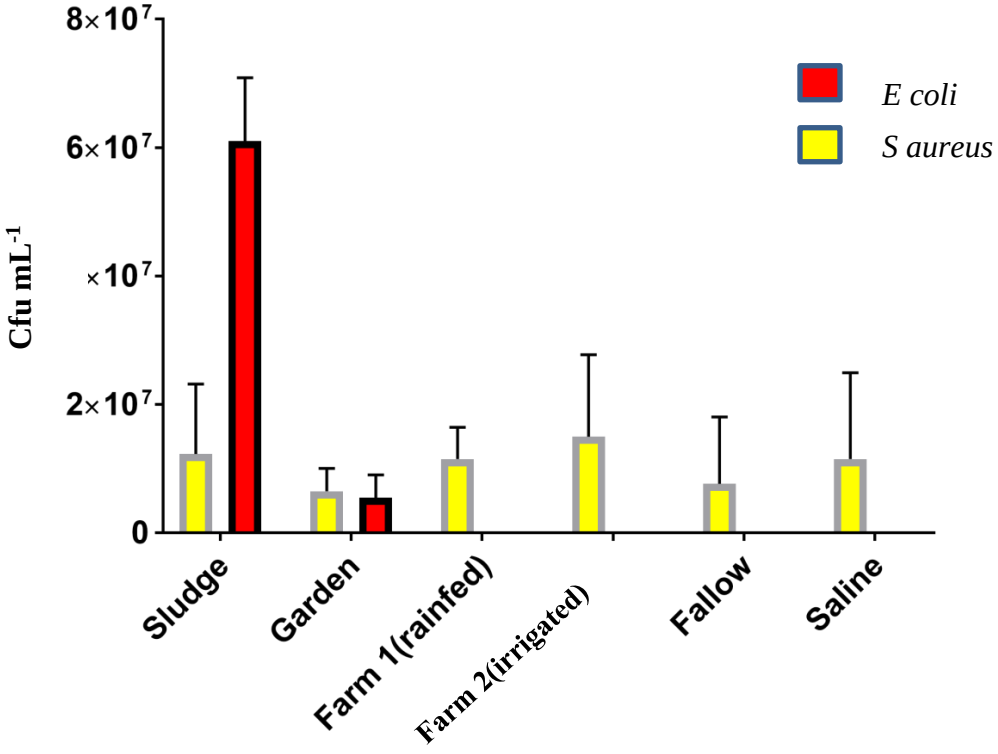


Fig.4.8: The total viable count of estimated number of bacterial diversity

# CHAPTER FIVE

# DISCUSSION

## 5.1. Influence of soil physicochemical properties on the bacteria community

Chemical properties are known to be appropriate indicators of soil quality and the growth of microorganisms (Fierer *et al*., 2007a). Organic matter content, clay content and cation exchange capacityare the three properties that mostly influenced bacterial abundance and diversity in the ecosystems. The sewage sludge has the highest organic matter, clay contentand cation exchange capacity and these, in turn, influenced soil bacteria the most as it recorded the highest bacterial occurrence and diversity. The bare fallow land has the least organic matter content and couldn't sustain enough bacteria community as indicated by failure to produce the required DNA quality for metagenomics (Fig.4.2). The saline soil with the highest electrical conductivity, an index of salinity, produced relatively high DNA yield. The presence of bacterial DNA in saline soil is an indication of salinity tolerance among the bacteria population. Salinity tolerant soil microbes respond to osmotic stress by synthesizing osmolytes which allow them to keep their cell turgor and metabolism (Yan *et al*., 2015).

Acidobacteria is the phyla most affected by high salinity (Naether *et al*., 2012), whereas poor water holding capacity resulting from low clay and organic matter content had a huge influence on Acidobacteria and Bacteriodetes (Xun *et al*., 2016). Water content and salinity have spatio-temporal variations in soils. Osmotic potential is a function of the amount of soluble salts present in soil solutions and is greatly affected by both soil water content and salinity, which in turn influences microbial activities in the soil environment (Yan *et al*., 2015).

The extremely low EC values in bare soils could be attributed to its dominantly sandy texture and high porosity which promotes the leaching of soluble salts from the soil (Eze and Meadows, 2014). EC has been found to influence the size and the activity of soil bacterial biomass, which in turn plays a key role in the biochemical cycle (Tripathi *et al*., 2006). While EC was very low in fallow, farm 1, farm 2 and garden respectively, it was relatively high in sludge and saline. This could be attributed to leaching of soluble substances from the nearby Dikabeya dam and water from the wastewater treatment plant. With pH values ranging from 5.18 to 7.62, the soils all fall within the suitable range for the optimum production of most plant species (Islam *et al*., 1980). Eventhough the overall phylogenetic diversity of bacterial community has been shown to have a positive correlation with pH peak diversity in soils near-neutral pH (Lauber *et al*., 2009). In this study the pH is commonly lower on the surface than deeper profiles within the soil, as a result of interaction with OM (Lozupone and Knight, 2007). Rousk *et al* (2010) previously showed that phylum composition is affected by changes in soil pH. The organic carbon contents of the soils were generally low except in sludge. This could be attributed to the high rate of organic matter mineralization in soils (Busby *et al*., 2007). The high amount of organic carbon in sludge, driving the high bacterial population, could be due to soil organic carbon being the basis of soil fertility. Soil organic carbon stimulates the structure, biological and physical health of soil, and acts a buffer against harmful substances.

Soils with large quantities of negative charge are more fertile because they retain more cations (McKenzie *et al*., 2004). There is an observed trend that long term applications of organic manure are beneficial for the build-up of soil organic matter and thus improve different features of soil fertility such as bacterial diversity (Liang *et al*., 2012) and in this study, high

negative charges was demonstrated by the higher bacterial diversity in the sewage sludge. Phosphorus was considerably high across all the samples. The highest amount of phosphorus was experienced in sludge. Normally the main source of phosphorus in sewage sludge is organic waste, mostly of human origin and from industries. The garden and farm 2 also produced high phosphorus values (132.1 and 284.9 ppm), which were associated with the application of phosphate fertilizers. Phosphorus level is consistently the second most important factor in all aspects of bacterial community structure (richness, evenness, composition, and phylogeny) in soils (Allison *et al*., 2007).

The observed differences in CEC could be attributed to clay and organic matter contents associated with different soils as it showed that it was high in soils with higher clay content. This was notable in this study because the CEC value was high (8.62 cmol $kg^{-1}$) in sludge hence high value of clay content (17.83 %). Knowledge of the CEC in soil is a great way to characterize the soil on the content of ionic elements, the concentration of clay, texture, the point of compression levels of porosity and permeability. Information about the possible need for fertilizer and correction of the soil acidity is also availed.

The exchangeable basic cations of the soils were mostly saturated with $Ca^{2+}$ followed by $Mg^{2+}$, $K^{2+}$, and $Na^{+}$. This order of abundance is in agreement with Thomas (1982) because of the soil parent material and agricultural treatments (Garden, farm 1 and farm 2). The particle size distributions represented a textural range of loam sandy in fallow, sandy loamy in saline and sludge and sand in arable farm and farm 2. The results showed that particle size affect the bacterial diversity of soils. The clay fraction has a more dissimilar bacterial community than silt or sand fractions (Sessitsch *et al.*, 2001) because clay is a colloid fraction which plays a significant role in binding together of larger particles of soil and produce more stable

compound particles. This is evident in sewage sludge because it yielded more bacterial diversity ascribed to high clay fraction. This study adds observed evidence that agricultural land management practices impact the bacterial population richness and diversity of soils, through alterations in soil physical and chemical properties, which is in line with Faoro *et al*., (2010) and Postma-Blaauw *et al*., (2010) where they state "Transformations of grassland to arable and harmfully affect both copiousness and functional diversity of soil biota. Further strengthening of the cropping system by enhanced fertilization and reduced crop diversity applied minor and differential effects on different soil biota groups."

**5.2 Abundance, diversity and distribution of bacteria in ecosystems**

The most dominant bacterial group belonged to the phylum Proteobacteria which is constant as it is described by several studies related to agricultural ecosystems (Borneman *et al*., 1996; Smit *et al*., 2001; Valinsky *et al*., 2002). Proteobacteria are a phylum of Gram negative bacteria, very common in soil environments and are linked to a wide range of purposes as they are involved in carbon, nitrogen, and sulfur cycling (Spain *et al*., 2009). The results of the present study are in agreement to those previously described for other soil types such as crops, forests, and grasslands (Janssen, 2006; Youssef *et al*., 2009). Members belonging to the Proteobacteria phylum occupied the highest richness in all soil samples. Proteobacteria members are prevalent in many soil ecosystems, including the rhizospheres, saline soils and semiarid soils (Spain *et al*., 2009) as observed in the study. Their relative richness which increases with high organic carbon availability in soils is in line with findings from previous studies such as Fierer *et al*., (2007b) and Eilers *et al*., (2010).

The Alphaproteobacteria was one of the most ample classes across all samples, comprising orders Sphingomonadales and Rhizobiales, which play roles in degradation of inorganic

compounds and nitrogen fixation respectively (Liu and Liu, 2013). The Betaproteobacteria consist of several groups of facultative anaerobic bacteria that are also versatile in their degrading capabilities. Examples include genus *Nitrosomonas*, which was detected in both saline and garden soil. This genus play an vital role in nitrogen fixation. The Rhodocyclales genus within the Betaproteobacteria is also important in degrading inorganic compounds, and its high abundance in sludge compared to the soil types is not surprising. Deltaproteobacteria, although detected at relatively low numbers compared to other classes of Proteobacteria, has an important ecosystem function. An example is the *Myxococcales*, comprising of soil dwelling organic degrading and sulfur reducing bacteria. Contributing in carbon cycling and producing secondary metabolites, Actinobacteria were dominant in saline and garden soils compared to sludge. This finding is also consistent with the findings in a study by Jenkins *et al*., (2010). Actinobacteria are a phylum that consists of many Gram- positive bacteria that play a vital function in the cycling of organic compounds and have been linked with soil organic matter production, as well as producing of the black pigments called melanin, which is associated with soil humic acid (Shivlata and Satyanarayana, 2015).

Members from Firmicutes phylum show a clear inconsistency among the samples, particularly in saline and garden soils, where their percentages are higher than in sludge where it was expected to be high. One would hypothesize that members of Firmicutes phylum particularly genus Bacillus are expected to be predominant in the sludge, however, their persistence and abundance is associated with their ability to resist desiccation (Edwards *et al.*, 2016). This is a rather unexpected finding when considering the general role of the members of the Firmicutes, most associated with diseases, particularly in humans.

The higher abundance of Bacteroidetes phylum in sludge relative to saline and garden soil is expected because of their common occurrence in the gut of animals (Drake and Horn, 2007). However, from the Bacteroidetes phylum have been established to be common in several different ecosystems, where their purpose is to degrade polymeric organic matter (Fierer *et al*., 2007b). They have been detected to be the major phyla living under anaerobic conditions existing in the rhizosphere and near surface soil ecosystems (Martinez-Alonso *et al*., 2010). Less copious phyla (Acidobacteria, Chloroflexi, Chlamydiae, Crenarchaeota, Planctomycetes, Verrucomicrobata, Spirochaetes, Cyanobacteria, Nitrospirae, Synergistetes, Tenericutes, Aquificae, and Ignavibacteriae) were also identified in this study. Nitrospirae which comprise the genus *Nitrospira* are recognized to contribute in soil nitrification (Baker *et al*., 2013). The presence of members originating from the Cyanobacteria phylum was unexpected considering their aquatic nature. However, a study found they can be present in soil crust in arid ecosystems and are documented as primary producers, along with their ability to fix nitrogen (Powell *et al*., 2015). Associates of the Gemmatimonadetes phylum were also determined by the metagenomics approach, and are important to note because they are difficult to cultivate using traditional culture approach (Lage and Bodonso, 2012). This group has been noted in different soil ecosystems, particularly dry soils, also at low abundance (DeBruyn *et al*., 2011).

Ratios between the number of Proteobacteria and Acidobacteria in an ecosystem might reflect the nutrient status of the soils examined (Smit *et al*., 2001) i.e high ratio of Proteobacteria to Acidobacteria indicate that the soil is more fertile because Acidobacteria are mostly affected by low clay content and organic matter. Members belonging to Acidobacteria phylum were also present at very low numbers and this shows that the soils in the study are less fertile. It is very important to highlight this phylum which is often affected by soil pH and available

phosphorus. At the phylum level, less diversity of the most dominant phylum such as Acidobacteria (0.1 %), Cyanobacteria (0.7 %) and Actinobacteria (2 %) is observed; therefore it does not appear to be sufficient for evaluating differences in soil bacterial communities. Consequently, differences in diversity across the samples are better appreciated when evaluating classifications at lower levels. At the genus level, bacterial communities of the selected ecosystems show an important variation in terms of diversity and abundance for example in saline soil there is less genus as compared to garden and sludge soil. The sludge soil had more diversity (13 genera) as many bacterial communities present in it were absent in the garden (8 genera) and saline (9 genera) soils. The following genera *Thiobacillus, Clostridium, Parvibaculum, Thermomonas, Lysobacter, Bryobacter, Phenylobacterium, Rhodovibrio, Dongia, Frischella,* and *Dechloromonas* were only found in varying abundance in the sludge soil and were not present in the garden and saline soils. The results at genus level also revealed a higher abundance of Sphingomonas (member of the phylum Proteobacteria) in the garden soil (20 %) when compared to the lower abundance in saline (8 %) and sludge (1 %) soils. In the previous studies of Lepleux *et al*., (2013), members of the Bacillus genera (showing higher abundance in garden and saline soils relative to sludge), were described as essential bio indicators of weathering and the presence of complex minerals (e.g. silicate). We can better appreciate the variations in diversity and abundance at the level of family genus and species (not indicated here) from each sampling site, which could also indicate that the differences are mainly due to different soil management factors at each site.

The culture method was used to complement the metagenomic approach in order to isolate and quantify specific viable bacteria in different samples. Although, culture approach appears to be an unachievable goal, looking into the fact that majority of bacteria are not regularly

cultured and identified under artificial laboratory conditions, since less than 1 % of the total bacterial population has proven to be cultivable on standard media. Some of the rare bacteria can be anticipated to be challenging to be discovered amongst the colonies that do grow in the in an agar (Curtis *et al*., 2002; Gans *et al*., 2005). The results from culture approach also suggest that the physicochemical properties of the soil may greatly affect bacterial species occurrence, diversity and abundance as clearly demonstrated by the unquantifiable numbers of *E. coli* in all ecosystems except sludge and garden.

# CHAPTER SIX

# CONCLUSION

Using a combined culture and metagenomics approaches, this study has discovered the array of bacteria found in soils of Palapye central Botswana. Phylogenetic classification and quantification of the bacterial communities obtained from garden and saline soils were found to be mainly from the major phyla, Proteobacteria, Actinobacteria, and Firmicutes. Less abundant phyla included; Bacteroidetes Acidobacteria, Gemmatimonadetes, and Cyanobacteria. However, there is still need for improvement in designing more sensitive primers for metagenomic approaches that could lead to more depth in identifying and quantifying more bacteria at genus and species levels. The presence of unmapped bacterial sequences identified in the ecosystems could suggest novel bacteria and goes further to support the need for a continued buildup of a comprehensive global soil biodiversity database through extensive research, particularly in relatively understudied regions.

Soil organic matter, cation exchange capacity and texture impacted the abundance and diversity of the bacterial community in the semi-arid soils. The soils from saline, garden, and sludge have promising potentials to contribute considerably to global C and N biogeochemical cycling due to the predominance of Proteobacteria. The genus level analysis suggests the influence of CEC, OC and clay content on bacterial communities because they are higher in sludge where these properties are abundant. The relationship between bacterial diversity and habitat disturbance is very multifaceted and can subjected to the level of disturbance; some disturbed habitats can even reveal higher diversity than forest systems depending on the management practices adopted. This study adds empirical evidence that soil bacterial communities are affected considerably by management practices of the ecosystems.

Dissimilarities in bacterial community structures in the six different ecosystems studied reflect soil management practices occurring at different time scales. Furthermore, this study opens up new frontiers in expanding the metagenomics studies in this region and in attempting to identify bacteria which are useful to humans and ecosystem functions.

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# APPENDIX A

Selected physico-chemical properties of the soils in the studied ecosystems.

| Sample ID | Agroecosystem | Location | pH | Ec | Oc | P | Ca | Mg | K | Na | CEC | Clay | Sand | Silt | TC* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | H2O | Dsm-1 | % | cmol.kg-1 | | | | | | g.kg-1 | | | |
| FR1 | Fallow | 22°29′18.46″S | 5.24 | 0.0157 | 0.41 | 4.48 | 1.23 | 0.5 | 0.27 | 0.06 | 2.06 | 18.01 | 80.2 | 1.79 | LS |
| | | 27°14′4.15″E | | | | | | | | | | | | | |
| FR2 | Fallow | 22°29′17.87″S | 5.16 | 0.0184 | 0.43 | 8.37 | 1.15 | 0.41 | 0.24 | 0.07 | 1.87 | 8.72 | 90.56 | 0.72 | S |
| | | 27°14′4.14″E | | | | | | | | | | | | | |
| FR3 | Fallow | 22°29′16.80″S | 5.15 | 0.0133 | 0.25 | 4.37 | 1.18 | 0.56 | 0.24 | 0.04 | 2.02 | 10.31 | 82.05 | 7.64 | LS |
| | | 27°14′5.44″E | | | | | | | | | | | | | |
| S1 | saline soil | 22°29′33.83S | 7.66 | 0.302 | 0.46 | 29.72 | 1.22 | 0.81 | 0.49 | 0.32 | 2.84 | 13.07 | 82.77 | 4.16 | SL |
| | | 27°13′41.76″E | | | | | | | | | | | | | |
| S2 | saline soil | 22°29′33.86S | 7.67 | 0.699 | 0.47 | 23.47 | 2.28 | 1.58 | 0.4 | 0.61 | 4.87 | 17.43 | 79.49 | 3.08 | SL |
| | | 27°13′43.60″E | | | | | | | | | | | | | |
| S3 | saline soil | 22°29′30.48″S | 7.53 | 0.324 | 0.56 | 21.58 | 2.83 | 0.99 | 0.33 | 0.29 | 4.44 | 12 | 86.57 | 1.44 | LS |
| | | 27°13′44.81″E | | | | | | | | | | | | | |
| SS1 | Sludge | 22°32′22.59″S | 5.43 | 0.716 | 59.9 | 2854 | 6.73 | 1.11 | 1.28 | 0.18 | 9.3 | 18.91 | 76.5 | 4.59 | SL |
| | | 27°10′22.02″E | | | | | | | | | | | | | |
| SS2 | Sludge | 22°32′26.86″S | 6.03 | 0.497 | 55.8 | 2524 | 5.9 | 0.6 | 1.25 | 0.17 | 7.92 | 17.83 | 80.56 | 1.61 | SL |
| | | 27°10′17.66″E | | | | | | | | | | | | | |
| SS3 | Sludge | 22°32′26.89″S | 6.07 | 0.504 | 56.7 | 2546.2 | 6.3 | 0.9 | 1.26 | 0.19 | 8.65 | 18.06 | 79.68 | 2.26 | SL |
| | | 27°10′19.98″E | | | | | | | | | | | | | |
| D1 | Garden | 22°28′49.91″S | 6.12 | 0.036 | 0.7 | 118 | 1.8 | 0.53 | 0.33 | 0.24 | 2.9 | 7.44 | 90.41 | 2.16 | S |
| | | 27°13′46.69″E | | | | | | | | | | | | | |
| D2 | Garden | 22°28′52.38″S | 6.04 | 0.0271 | 0.35 | 155.6 | 1.41 | 0.48 | 0.09 | 0.11 | 2.09 | 8.32 | 87.32 | 4.36 | LS |
| | | 27°13′45.93″E | | | | | | | | | | | | | |
| D3 | Garden | 22°28′52.38″S | 5.08 | 0.0201 | 0.92 | 122.6 | 1.46 | 0.48 | 0.2 | 0.07 | 2.21 | 9.42 | 89.14 | 1.44 | LS |
| | | 27°13′46.57″E | | | | | | | | | | | | | |
| AF1 | Arable farm1 | 22°28′37.62″S | 5.55 | 0.0174 | 0.69 | 9.29 | 4.25 | 0.78 | 0.63 | 0.06 | 5.72 | 6.72 | 91.84 | 1.44 | S |
| | | 27°13′14.34″E | | | | | | | | | | | | | |
| AF2 | Arable farm1 | 22°28′36.92″S | 5.36 | 0.0414 | 0.76 | 4.72 | 2.01 | 0.73 | 0.25 | 0.08 | 3.07 | 3.44 | 93.85 | 2.72 | S |
| | | 27°13′41.83″E | | | | | | | | | | | | | |
| AF3 | Arable farm1 | 22°28′37.32″S | 5.6 | 0.0191 | 0.49 | 13.33 | 4.21 | 0.78 | 0.61 | 0.05 | 5.65 | 7.24 | 90.4 | 2.36 | S |
| | | 27°13′42.95″E | | | | | | | | | | | | | |
| A1F1 | Arable farm2 | 22°29′33.83″S | 6.53 | 0.0474 | 0.53 | 180.2 | 2.73 | 0.98 | 0.32 | 0.23 | 4.26 | 5.08 | 92.76 | 2.16 | S |
| | | 27°13′41.76″E | | | | | | | | | | | | | |
| A1F2 | Arable farm2 | 22°29′33.76″S | 6.03 | 0.0869 | 0.47 | 540.2 | 2.69 | 0.86 | 0.22 | 0.13 | 3.9 | 7.88 | 89.22 | 2.9 | S |
| | | 27°13′41.14″E | | | | | | | | | | | | | |
| A1F2 | Arable farm2 | 22°32′22.59″S | 6.76 | 0.0408 | 0.42 | 134.4 | 3.36 | 0.35 | 0.23 | 0.05 | 3.99 | 13 | 85.77 | 1.23 | LS |
| | | 27°10′22.02″E | | | | | | | | | | | | | |