Microbial Diversity, Community Structure and Functional Characteristics of Sunflower Rhizospheric Soils

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DECLARATION

I declare that this thesis titled "Microbial Diversity, Community Structure and Functional Characteristics of Sunflower Rhizospheric Soil" is an original output of the research conducted by me under the supervision of Professor Olubukola Oluranti Babalola. Therefore, I, the undersigned, declare that this thesis submitted to North-West University for the award of Doctor of Philosophy in Science, Biology in the Faculty of Natural and Agricultural Sciences has not been previously submitted by me or anyone else at any University in part or entirety for the award of any degree and that all the information obtained from published literature have been acknowledged accordingly.

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Date: **……**

DEDICATION

This work is dedicated to my Heavenly Father, the Giver of strength, wisdom and power.

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LIST OF ABBREVIATIONS

ABA	Abscisic acid
ACC	1-aminocyclopropane-1-carboxylate
AM	Arbuscular mycorrhiza
AMF	Arbuscular mycorrhizal fungi
ANOVA	Analysis of Variance
ARA	Arabinose
ARC	Agricultural Research Council
ATP	Adenosine triphosphate
Bp	Base pair
BLAST	Basic Local Alignment Search Tool
BLASTn	Basic Local Alignment Search Tool n
°C	Degree centigrade
Ca	Calcium
CCA	Canonical correspondence analysis
Cl	Chlorine
CLPP	Community level physiological profiles
Cm	Centimeter
CO_2	Carbon (IV) Oxide
Cu	Copper
DNA	Deoxyribonucleic acid

DGGE	Denaturing Gradient Gel Electrophoresis
EPS	Exopolysaccharide
F	Forward
FAO	Food Agriculture Organization
Fe	Iron
Fe^{3+}	Iron (III)
G	Gram
g/l	Gram per liter
GLU	Glucose
H^{+}	Hydrogen ion
H	Hour
hr.	Hour
HCN	Hydrogen cyanide
IAA	Indole-3-acetic acid
ISR	Induced systemic resistance
ITB	Itsoseng bulk soil
ITR	Itsoseng rhizospheric soil
K	Potassium
Kb	Kilo-base
KEGG	Kyoto Encyclopedia of Genes and Genomes

MEGA	Molecular Evolutionary Genetics Analysis
MF	Mycorrhizal fungi
Mg	Milligram
Mg	Magnesium
Min.	Minute
Ml	Milliliter
Mm	Millimeter
Mn	Manganese
N	Nitrogen
Na	Sodium
NaCl	Sodium chloride
NCBI	National Center for Biotechnology Information
NGS	Next-Generation Sequencing
NH_3	Ammonia
N-NH4	Ammonium ion
$N\text{-}NO_3$	Nitrate
Nif	Nitrogen fixing
No	Number

NS	Not statistically significant
OD	Optical density
OM	Organic matter
OTU	Operation taxonomic unit
P	Probability
P	Phosphorus
PAN	Pannar
PAST	Paleontological statistics software package
PCoA	Principal coordinate analysis
PGP	Plant growth-promoting
PGPB	Plant growth-promoting bacteria
PGPR	Plant growth-promoting rhizobacteria
QIIME	Quantitative Insights into Microbial Ecology
qPCR	Quantitative polymerase chain reaction
RAST	Rapid Annotation using Subsystem Technology
RDPII	Ribosomal Database Project
RES	Resistivity
ROS	Reactive Oxygen Species
RNA	Ribonucleic acid

rRNA	Ribosomal ribonucleic acid
S	South
S	Sulphur
SA	South Africa
SE	Standard error
SHB	Sheila bulk soil
SHR	Sheila rhizosphere soil
Sp.	Species
Spp.	Species
SPSS	Statistical Package for the Social Sciences
SRA	Sequence read archive
Temp.	Temperature
tRNA	Transfer ribonucleic acid
TX	Texas
USA	United State of America
W	Weight
Wt	Weight
w/v	Weight per volume
Zn	Zinc
%	Percentage
µl	Microliter
<	Less than

≤	Less than or equal to
>	Greater than
≥	Greater than or equal to
µg/ml	Microgram per millilitre

GENERAL ABSTRACT

Rhizosphere zones are 'hubs' for microbial diversity comprising of diverse microbiomes compared with the nearby bulk soils. Rhizospheric microorganisms play major roles in plant health, development and yield. The exploitation of the soil microbiome is a promising means to boost the production of economically viable and important agricultural crops, like sunflower (*Helianthus annuus*). The sustainable production of sunflower plants is important and one key way to achieve this feat is to have a better understanding of the connection between bacterial diversity, ecosystem functioning and their response to environmental variables. Likewise, the respiratory responses of these bacterial communities need to be studied to comprehend their roles in the ecosystem. The impact of agricultural practices in farmland ecosystems on the structure of soil bacterial communities and the effects on soil functioning was evaluated using 16S amplicon sequencing. Rhizospheric soil and corresponding bulk soil samples were collected from four farms namely Sheila/Ditsobottla, Itsoseng, Lichtenburg and Kraaipan/Krayburg. Employing the MicroResp™ method, community-level physiological profiles (CLPP) was used to explore the activities and functional diversity of the entire microbial communities in these sunflower soils. The taxonomic structures, distribution and diversity of the bacterial communities and significant relationships between the bacterial communities and soil properties were investigated. The effects of soil properties, agricultural practices and cropping system on bacterial community diversity of the soil samples from the farms were observed. Furthermore, effects of carbon substrate utilization on bacterial community diversity of sunflower rhizospheric soils obtained from Sheila (SHR) and Itsoseng (ITR), as well as Ditsobottla and Kraaipan were also investigated. The results obtained from sunflower rhizosphere soils grown in SHR and ITR showed that sodium (Na^+) and clay

content positively influenced *Firmicutes*, *Deinococcus-Thermus*, *Cyanobacteria* and *Fibrobacteres*, while soil resistivity (Res) and Magnesium (II) ion (Mg^{2+}) positively influenced *Actinobacteria*, *Bacteroidetes*, *Thaumarchaeota*, *Aquificae Planctomycetes* and *Chloroflexi*. The CLPP analysis revealed that the microbial communities in SHR and ITR used the amino acids, including tryptophan and malic acid effectively. The metabolic rate of these carbon substrates may be due to the prevailing nature of some of the organisms, including *Actinobacteria* in the soils. Furthermore, the sunflower rhizospheric soils from Ditsobottla (R1) and Kraaipan (R2) were evaluated, it was observed that organic matter (OM) positively influenced *Lactobacillales*, *Myxococcales*, *Rhizobiales*, *Bacillales*, *Burkholderiales*, *Enterobacteriales*, *Sphingomonadales*, *Flavobacteriales* and *Nitrosomonadales* obtained from R1, while total N and pH positively influenced *Cytophagales*, *Planctomycetales*, *Gemmatimonadales*, *Caulobacteriales* and *Nitrospirales* from R2. The bacterial communities of the soil samples used the various carbon substrates (three amino acids, three carboxylic acids and six carbohydrates) as an energy source. Significant differences ($P < 0.05$) were only detected in tryptophan and methionine-amended soils. The effect of soil properties examined was observed to influence bacterial dispersal across the sites. *Planctomycetes* and *Proteobacteria* predominated the rhizosphere soils from Lichtenburg and Kraaipan sites. Significant differences ($P < 0.05$) in bacterial structure at phyla and family levels and predicted functional categories between soils across the sites were revealed. The effect of the analyzed soil parameters was observed to influence the bacterial spatial distribution across the sites. This study provides evidence of the dominant bacterial community structure in sunflower soils. The CLPP measurements of the rhizospheric soils were different from those of the bulk soil and the degree of the dissimilarities was based on the type of carbon substrates and the soil microbial composition. Further, various unclassified microbial groups were discovered across the

study sites, it is therefore recommended that efforts should further be made to isolate, characterize and identify these unclassified microbial species, as it might be plausible to discover novel microbial species that can further be harnessed for biotechnological purpose and sustainable production of sunflower and other agricultural crops. Moreover, the predictive functional traits at growing stage are important for their future study and exploration as bioinoculants for enhanced crop production and management and for improved crop yields.

Keywords: Plant growth-promoting microorganisms (PGPM), sustainable agriculture, amplicon sequencing, genomics, crop yield

DISSEMINATION OF RESEARCH RESULTS AND LIST OF PUBLICATIONS

List of Conference Presentations

A. Submitted and accepted abstract for the 18th International Symposium on Microbial Ecology. To be held in Lausanne, Switzerland, from 14-19 August 2022.

Title: Amplicon profiling of rhizosphere bacterial community structure and diversity associated with sunflower plant as influenced by cropping system

Authors: Blessing Chidinma Nwachukwu, Ayansina Segun Ayangbenro and Olubukola Oluranti Babalola

Candidate's Contributions: Managed the literature searches, wrote the protocol, did the laboratory work, performed all analyses, interpreted the results and wrote the first draft of the abstract and corrected the final draft for submission. Also, prepared the poster for presentation.

B. Presentation at the Biodiversity Genomics 2021 Conference (BG 2021), Online Everywhere, 27 September to 1 October 2021.

Title: Structural and respiration profiles of microbial communities from sunflower plant soils

Authors: Blessing Chidinma Nwachukwu, Ayansina Segun Ayangbenro and Olubukola Oluranti Babalola

Candidate's Contributions: Managed the literature searches, wrote the protocol, carried out the laboratory work, performed all analyses, interpreted the results, wrote the first draft of the abstract

corrected and the final draft of the abstract. Also, prepared the power point slides for presentation and presented the findings at the conference.

C. Presentation at the Annual Conference on Food Science and Technology (FOODCONF-21), Melbourne, Australia, July 12-14, 2021.

Title: Beneficial microbial structure of sunflower rhizosphere grown at Sheila and Kraaipan

Authors Blessing Chidinma Nwachukwu, Ayansina Segun Ayangbenro and Olubukola Oluranti Babalola

Candidate's Contributions: Managed the literature searches, wrote the protocol, carried out the laboratory work, performed all analyses, interpreted the results, wrote the first draft of the abstract and corrected the final draft. Also, prepared the power point slides for presentation and presented the findings at the conference.

D. Presentation at the 3RD Euro-Mediterranean Conference for Environmental Integration, Sousse, Tunisia, June 10-13, 2021.

Title: Comparative study of microbial profile and functional structures of sunflower rhizosphere grown in Sheila and Itsoseng

Authors: Blessing Chidinma Nwachukwu, Ayansina Segun Ayangbenro and Olubukola Oluranti Babalola

Candidate's Contributions: Managed the literature searches, wrote the protocol, carried out the laboratory work, performed all analyses, interpreted the results, wrote the first draft of the abstract

and corrected the final draft. Also, prepared the power point slides for presentation and presented the findings at the conference.

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Chapter 5: Rhizosphere competence of plant growth-promoting rhizobacteria (PGPR) and their application in sustainable food production

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Chapter 6: High- throughput sequencing survey of sunflower soil

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Chapter 7: Amplicon sequencing data profiling of bacterial community connected with the rhizospheric soil from sunflower plants

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Chapter 8: Comparative study of microbial structure and functional profile of sunflower rhizosphere grown in two fields

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Chapter 10: Structural diversity of bacterial communities in two divergent sunflower rhizosphere soils

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Authors: Blessing Chidinma Nwachukwu, Ayansina Segun Ayangbenro and Olubukola Oluranti

Candidate's Contributions: Managed the literature searches, performed the experiments, did the bioinformatics analyses of the genomic data and wrote the first draft of the manuscript.

CHAPTER ONE

1.0 General introduction

1.1 Introduction

One in every four persons, or approximately three hundred million people in sub-Saharan Africa suffers from food insecurity and lack of a healthy lifestyle as a result of high food prices and environmental factors such as drought, which has pushed many into poverty and hunger. Currently, the world's population is approximately 8 billion and projected to 9.7 billion in 2050 and 11 billion in 2100. Hence, to feed the ever increasing human population, the world will need novel approaches for sustainable food production (Adedeji et al., 2020). In order to achieve sustainable food production, sustainable agriculture must develop strategies and farming productive systems which are eco-friendly, profitable and can conserve natural resources and energy, consequently ensure food security, quality and safety (Igiehon et al., 2019).

Therefore, an immediate plausible strategy is to reduce chemical pesticides and fertilizers and supplement with microbe-based environmentally sustainable materials (Adesemoye et al., 2017). In the long-term, it would be important to replace harmful agrochemicals such as pesticides and chemical fertilizers with healthy and environmental friendly materials of agricultural beneficial bacterial capable of improving plant nutrition and conferring protection against abiotic (drought and salinity) and biotic (pests and pathogens) stress, which in turn enhance plant health and crop yields (Deng et al., 2019).

Specifically, increasing the abundance of soil bacterial species has shown to help improve plant health and yield probably through the inoculation of nonresident bacteria into the soil (Deng et al., 2019). In addition, the application of individual bacteria, identification of healthy and functionally variety of the bacterial population and their application for improving crop productivity is another major challenge. This is because the total soil bacterial population is an important portion in the rhizosphere, which represents the most abundant genome of the host plant (Igiehon et al., 2019).

Furthermore, the rhizosphere is a narrow region of the soil that is adjacent to the plant root, which is directly controlled by the root systems and their products, making it a nutrient-rich environment. The associated soil microbes inhabiting the region are called root microbiome (Nwachukwu and Babalola, 2021). Microorganisms coexisting and performing diverse interactions in a specific habitat are known as microbiota. The rhizosphere consists of various microbial populations including bacteria, fungi, algae, viruses, protozoa and archaea (Ali et al., 2017). The rhizosphere is a hotspot for bacterial communities performing unique functions that impact the plant and soil environment directly or indirectly such as improving soil quality, soil health, regulation of soil fertility, biogeochemical cycle and plant performance as a result of increased productivity of the crop (Sabale et al., 2019).

The host plant interactions with the bacterial community enable the plant to take up nutrients, improve their tolerance to different stresses (biotic or abiotic), enhance disease suppression, improve resistance to pathogens, release metabolites and phytohormones. The interactions of plant and bacterial communities control the plant root environment, resulting in nitrogen fixation and the secretion of up to 11% of organic carbon (Dennis et al., 2010). Different studies have shown that the soil bacterial community and the plant are important factors that affect the soil structure

(Babalola, 2010). According to Wang et al. (2011), several environmental conditions (salinity and drought) and interferences influence the bacterial community structure and functions, thus altering the soil characteristics and may have negative consequences on plant health and crop yield.

Sunflower (*Helianthus annuus* L.) is a tall annual plant of height between 50- 390 cm, with ovate leaves and the flowers are bright yellow or maroon inside. The stem is hairy and rough also made up of strong tap-root that is round in the early season, woody or angular later in the season (Fernández-Luqueño et al., 2014). Sunflowers are primarily cultivated for human consumption. The cooking oil from the seeds is predominantly used worldwide as a source of protein with high nutritional value (Oberholster et al., 2018).

Interestingly, in South Africa, sunflower (*Helianthus annuus* L.) is currently the third-largest grain crop cultivated after maize and wheat. The plant survives in warm dry climates found in many African regions and the Middle East. Sunflower plants strive and grow in temperate zones. However, it can perform well under various environmental conditions (Adeleke and Babalola, 2020b). They can survive in extreme heat because of their hardiness and some farmers refer to them as summer crops. Other uses of sunflowers include industrial purposes such as cosmetics, paints, lubricants, biofuel production and resins. In some countries, the seed cake left after oil extraction is used in livestock feed (Purwati and Herwati, 2016). Sunflower plants further have antimicrobial and anti-inflammatory properties used for treating some diseases (Muller et al., 2011). Also, sunflower plants are ornamental plants used in ancient religious ceremonies (Bozic et al., 2015).

A bacterial population inhabiting the sunflower rhizosphere is a metabolic network carrying out various metabolic and physical processes that control the biochemical and physiological characteristics of a living cell (Majeed et al., 2018c). Several chemical reactions occur in these processes, including the conversion of energy available in the environment into useful forms required by the organisms. Also, molecules are synthesized from the available nutrients in the environment, these nutrients are needed for cell growth (Lange et al., 2015). According to Muller et al. (2018), the metabolic network consists of reconstruction, analysis and modeling giving an awareness of the microbial ecology (Muller et al., 2018,Mwajita et al., 2013).

A specific group of bacterial communities influence plant performance and increase crop yields, the rhizosphere. These groups of the bacterial community are called plant growth-promoting bacteria (PGPR); they competitively colonize the host plant root region, promote plant growth and increase crop productivity (Schlemper et al., 2017). They are also indicators of rhizosphere competence. Presently, researchers have recorded that PGPR are a potential alternative to chemical- fertilizers used in agriculture. Studies have shown that plants attract a certain group of bacteria to the roots and as a result produce exudates (substance secretes by plants) (Nwachukwu et al., 2021).

PGPR use these compounds through diverse mechanisms (direct or indirect) to promote plant growth and water absorption as well as phytopathogenic suppression, decrease herbivory damage and increase nutrient bioavailability (Pii et al., 2015). The tripartite interactions between bacterial species, other indigenous microorganisms and sunflower plants are mutualistic symbioses of significant economic importance for increasing agricultural productivity (Igiehon and Babalola, 2018). These plant growth promoters are exploited for other agricultural benefits such as

biofertilizers and biocontrol agents, allowing notable reduction of chemical fertilizer use (Souza et al., 2015). Also, there is an increasing number of amendments to these bacterial communities that are used for profit-making.

Therefore, to understand the composition and role of the entire bacterial population in the rhizosphere, it is important to use more advanced approaches. Nonetheless, technical constraints have historically hindered the analysis of complex plant-associated bacteria. Amplicon (or marker genome sequencing as next generation sequencing technique) targets a specific region in a genome using PCR to create DNA sequences. This is referred to as amplicons and gives broader knowledge of the structure of the entire genomes in an environmental sample (Lindgreen et al., 2016). The amplicon genome sequencing gives a more detailed description of the genomic compositions in the samples than phylogenetic sequencing that targets the 16S rRNA genes (Salem et al., 2020). Recently, the high speed of performing next generation sequencing and reduced costs have expanded the study of sequence-based next generation sequencing approach (Razzaq et al., 2019). Recently, the amplicon genome sequencing was used to identify bacterial species in sorghum and sunflower rhizosphere soil (Oberholster et al., 2018).

Functional diversity is a major parameter to study root-associated bacterial population functioning, which is essential because bacterial species are key actors in the rhizosphere soil. A higher level of multifunctional roles has been associated with increased bacterial and environmental diversity, thus influencing the entire soil functioning (Maillard et al., 2019). Communitylevel physiological profiles (CLPPs) are fast screening methods used to determine the metabolic and physiological profile of bacterial communities based on their ability to use various carbon substrates (carbohydrates, amino acids and polymers) and to adapt their metabolism to various environmental

conditions, thus allowing the classification of certain functional traits of a community (Perujo et al., 2020). In recent studies, CLPP has been used to determine different bacterial community functional traits from soil samples (Lladó and Baldrian, 2017a, Jones et al., 2018**).**

Despite these foundational experiments, little is known about the root bacterial community of commercially produced crops. Further research on the root and rhizospheric microbiome of commercial agricultural crops and non-model plants is thus required using more advanced next-generation techniques (high throughput sequencing techniques) (Salem et al., 2020, Ambrosini et al., 2016). For instance, no research to date has extensively explored the root and rhizospheric soil microbiome of sunflower using high throughput sequencing techniques (Babalola et al., 2021c).

1.1 **Aim of the study**

To provide insight into the microbial diversity, community structure and functional characteristics of sunflower rhizospheric soils

1.1.2 **Objectives of the study**

The following specific objectives helped achieve this goal:

(i) To determine the physical and chemical properties of sunflower rhizosphere and comparable bulk soils.

(ii) To determine the bacterial diversity and taxonomic abundance of all bacterial species from the sunflower plant rhizosphere and comparable bulk soils

(iii) To determine the relationship between the bacterial communities and physical/chemical properties of the sunflower plant rhizosphere and comparable bulk soils.

(iv) To determine the functional traits of bacterial communities of the sunflower plant rhizosphere and comparable bulk soils using Community Level Physiological Profiles (CLPPs)

(v) To determine the metabolic profile of plant growth-promoting bacteria in the rhizosphere and corresponding bulk soils

(vi) To model species interactions in the sunflower plant rhizosphere and comparable bulk soils.

CHAPTER TWO

Perspectives for sustainable agriculture from the microbiome in plant rhizosphere

Overview

In the past 50 years, modern agricultural practices have significantly increased crop yields, primarily as a result of the use of fertilizers, chemical pest control, irrigation, and the production of hybrids. Current agricultural techniques heavily rely on synthetic chemical fertilizers and pesticides, which have led to a number of environmental issues such as groundwater contamination, deteriorating soil quality, and loss of biodiversity. A growing threat to present agriculture is posed by environmental conditions like drought, high temperatures, and CO2 brought on by global climate change.

Global crop production is becoming more and more necessary to meet the demands of our expanding population, which is expected to reach nearly 9 billion people by 2050. These demands include those for food, biofuel, and industrial processes. Worldwide agriculture is transitioning to a more sustainable and environmentally friendly strategy, spurred by rising demand and knowledge of the detrimental effects existing agricultural practices have on the environment and human health.

As part of the soil ecosystem, soil microbes are crucial for sustaining plant diversity, controlling soil fertility, and cycling nutrients. Rhizobacteria that colonize the rhizosphere of roots are referred to as plant growth-promoting bacteria (PGPB). Due to its favorable impacts on soil and crop productivity, the utilization of naturally occurring PGPB in sustainable agriculture has gained

relevance during the past ten years. Along with promoting plant growth, PGPB also gives plants the ability to withstand biotic and abiotic stressors. However, the rhizobiome found in the rhizosphere is made up of more than only rhizobacteria and AMF. There are known instances of other microorganisms living together in the rhizosphere. It has been established that the rhizosphere is home to a lot of the archaea groups classified as ammonium oxidizers.

Genes for metal and antibiotic resistance are frequently found in PGPB. Antibiotic resistance can either be a genetic trait or an acquired trait. The existence of particular traits, such as the presence of multidrug efflux pumps, which are involved in carrying out metabolic processes in bacteria, can be used to explain intrinsic resistance. A long evolutionary history that predates the "antibiotic era" is suggested by phylogenetic analysis of several genes involved in antibiotic resistance, which supports this. Gene mutations or the horizontal gene transfer of antibiotic resistance genes from other organisms can also lead to acquired antibiotic resistance. The biological implications of PGPB's antibiotic resistance is mostly unknown.

Also, Rhizobacteria communities that coexist in the rhizosphere act as plant growth promoters by fixing nitrogen, increasing the amount of nutrients available, suppressing phytopathogens, and inhibiting disease. Once more, it increases plants' ability to endure environmental stressors like high salinity and water scarcity (Olanrewaju et al., 2019, Babalola, 2010).

Furthermore, to identify and characterize the rhizobacteria of agricultural importance for increased food production. Presently, meta-omics techniques for identifying rhizobiomes of agricultural sustainable importance have been reported**.** Microorganisms were isolated and identified using the conventional or culture-based method for classifying and identifying microorganisms prior to the

development of the meta-omics techniques. Approximately 99% of the microorganisms in a sample were unknown because the conventional method of culturing could not identify all of the microorganisms in a sample. The non-culturable microorganisms and their functions are captured by the meta-omic methods. An emerging method for characterizing the structural and functional genomes in a given environmental sample is the meta-omics technique. It has enabled researchers to comprehend the variety of structures and capabilities exhibited by microbial communities in plant rhizospheres.

Chapter 3, gave an insight into understanding rhizobacteria that are involved in the stability of the environment, especially the agricultural soils. The evaluation of rhizosphere soil and the related bacteria for biotechnological applications is the main focus. Therefore, healthy and sustainable environments can benefit from the efficient use of rhizosphere soil and its bacteria in an environmentally friendly manner.

Abstract

The ever-growing human population globally has resulted in the quest for solutions to the problem of hunger by providing food security. The importance of plant-root associated microorganisms cannot be overlooked— plants rely on them. These root colonizers dominate the rhizosphere due to the abundance of available nutrients, relying on their host plant for nutrients and other essential requirements. The relationships between microbial communities and plants are controlled by the type of plant and microorganism involved. Advances in modern molecular techniques have led to the evolution of omic technology using nucleic acid molecules to study plant-microorganism associations capable of stimulating plant growth, improve yield and induce disease suppression.

This review elucidates the activities of microbial communities, especially nitrogen-fixing rhizobacteria associated with plant roots, nitrogen fixation as a mechanism of promoting plant growth, their importance and the challenges employing bioinoculants. Prospecting plant growth promoters using omic technology will advance sustainable agriculture globally.

Keywords: Nitrogen-fixing genes, Omic technology, Plant growth-promoting rhizobacteria, Root nodulation process, Soil fertility

2.1 Introduction

Life dwelling on earth depends on the celestial environment and is controlled by the soil beneath our feet. Soil is a critical part of the ecosystem with valuable resources important to us, as the topmost layer of the earth; it holds plants at the root and supports animal life. Above all, the soil is composed of numerous microorganisms interacting with each other. Soil ecosystem is a source of minerals, organic matters, liquids and gases. Moreover, soil acts as a water filter and a medium that encourages plant growth (Beckers et al., 2017a). All soil houses millions of organisms that contribute to the biodiversity and distribution of most of the antibiotics for fighting plant borne diseases (Zhou et al., 2020b).

In the context of this review, the soil region around the plant root that connects the network of plant root-associated microorganisms, especially bacteria, are found in the plant root system called the rhizosphere. Microbial communities in the rhizosphere perform dynamic beneficial interrelated functions in the plant root zone (Olanrewaju et al., 2019). The entire set of microbial populations inhabiting the plant root-associated region is referred to as the rhizosphere microbiome or rhizobiome. Some of these microorganisms may be pathogenic (harmful to plant growth) or non-

pathogenic, having symbiotic relationships with other rhizospheric microorganisms (Ali et al., 2017).

Lorenz Hiltner in 1904 introduced the term 'rhizosphere' due to Beijerinck's discovery of bacterial community inhabiting the soil with the ability to fix nitrogen. According to the Hiltner, rhizosphere was described as root-associated soil compartment influenced by root activity. Furthermore, rhizosphere is the ecological niche where microorganisms dwell, serving as a connection to other plant root zones. Microorganisms in the compartment perform significant activities that contribute to plant health, mostly nitrogen fixation (Olanrewaju et al., 2019). The rhizosphere inhabits myriads of microorganisms and is considered a well-advanced external functional plant habitat, which is the plant's second genome because plants are referred to as metaorganisms. Therefore, understanding the exact contributions of rhizobiome (mainly nitrogen [N] fixing bacteria concerning plant health and productivity) are important (Ali et al., 2017).

Bacterial species usually do not work in isolation but in association with other microbial species that have diverse beneficial impacts on plants, hence promoting plant growth or are non-beneficial with damaging effects on the host plants (Murphy et al., 2015, Bagyaraj and Ashwin, 2017). In the rhizosphere, the association between plant and the microbial communities occurs within the soil matrix (Sabale et al., 2019, Pii et al., 2015). Rhizosphere is a diverse habitat with a high abundance of microbial community called rhizobiome. The bacterial species are the most dominant microorganisms found in the rhizosphere, colonizing the microenvironment with mutual benefits (Bagyaraj and Ashwin, 2017).

The beneficial rhizobiome in the regions have close interaction with host plants, primarily contributing to the soil health for various agricultural purposes (Alawiye and Babalola, 2019). Among other benefits of rhizobiome is their interaction with other microorganisms and plants. Understanding the interactions in the rhizosphere is not solely for knowing their involvement in plant growth and development. Also, to understand the different metabolic activities that occur in the rhizosphere (Liu et al., 2015, Preece and Penuelas, 2016). As well as exploiting their connotation in phytoremediation approaches that can lead to the sustainable production of metabolites and crops (Schlemper et al., 2017).

More so, the microbial community control plant rhizosphere has led to competitors cohabiting in the environment in a mutual relationship (Liu et al., 2015). The positive relationship is a symbiotic relationship with the host plants or negative with damaging impacts that are from predators or pathogens (Igiehon et al., 2019). The microbial community associations in the rhizosphere influence plant growth and tolerance to stressors, (Rasmann and Turlings, 2016).

Studies have shown that the rhizosphere is the hotspot for diverse abundance of microbial populations conducting several taxonomical operations. The root region (rhizosphere) is a complex soil environment that attracts much microbial mainly bacterial genera from the bulk soil. The bacterial community attracted to the plant root region interacts with the plant and other microbial communities. As a result, metabolites are released for promoting plant growth. The plant roots and soil environment control the associations between microorganisms and plants as well as plants and microorganisms (Alawiye and Babalola, 2019).

The rhizobiome play an essential role in sustaining and stabilizing the ecosystem. These roles influence the plant through gaseous and nutrient exchanges, as well as plant litter, water filter and rhizodeposits. The plant root region is a path for nutrient and mineral absorption, which releases several organic and inorganic compounds known as rhizodeposits. The presence of rhizodeposits forms an exclusive environment in the rhizosphere, which serve as a pointer that diverse rhizobiomes are bound to be in abundance in the rhizosphere with high nutrients (Preece and Penuelas, 2016).

Moreover, the rhizosphere contains a pool of microbial diversity conducting different interactions and functions, such as growth promotion and pathogen resistance. The rhizosphere is controlled by root exudates (low molecular weight organic compounds secreted by plant roots, which are released into the rhizosphere, capable of influencing the rhizosphere, inhibit harmful microorganisms and promote plant growth), mucilages (polysaccharide substances extracted as gelatinous or viscous solution produced by most plants and some microorganisms) and border cells (those cells that separate from plant root tips and disperse into soil environment) released into the rhizospheric soil by plant roots.

Plants secrete border cells, exudates, rhizodeposit nutrients and mucilages that attract microorganisms to the region, which serve as food for microorganisms inhabiting in the rhizosphere. The rhizosphere is structured by groups of microorganism's mostly bacterial species that are mesmerized by the rhizodeposits disseminating from the plant roots (Orlikowska et al., 2017). Ultimately, contributing to making the soil the most complex and diverse habitat in the ecosystem to a point that many microbial species colonizing the rhizosphere release regulatory substances that can influence plant ability to survive in challenging soil environmental conditions,

including waterlogged areas, extreme cold or in deserts, that are a threat to sustainable agriculture (Alawiye and Babalola, 2019, Sabale et al., 2019).

Sustainable agriculture is an integrated farming system having a site-specific approach that would over time satisfy the human need for food, improve environmental quality and enhance natural resources. Generally, sustainable agriculture will improve the economy and result in enhancing the quality of life of farmers and society. Sustainable agriculture relies on fertile soil, but rapid desertification and land degradation have damaged the soil, resulting in an approximate loss of about 24 billion tons of fertile arid land world-wide (Pii et al., 2015, Zhou et al., 2016).

Plant microbial communities participate in transformations that impact on the composition of litter, which could lead to some changes in the turnover of nutrients, soil structure, physiology and characteristics. These might cause changes in the structure and composition of plant microbial communities (Xu et al., 2019). In addition, biotic and abiotic factors control the microbial activities in the rhizosphere region. The rhizobiome coexisting in the rhizosphere plays an essential role in improving soil quality, plant health and crop production (Zhang et al., 2017).

The inoculation of diverse bacterial species into the soil helps in improving plant-microorganism interactions, biogeochemical cycles and biological engineering. Therefore, it could encourage agriculturists from the application of agrochemicals, substituting the functions of these toxic chemical-based products for suitable beneficial bacteria (Li et al., 2019a). The overall understanding of the biodiversity and structure of functional and active root and free-living microorganisms in the rhizosphere (such as nitrogen-fixing bacteria, capable of fixing nitrogen for

plant use, which is paramount in enhancing crop performance and growth) in turn, increases agricultural crop production (Lagos et al., 2015).

To gain a complete understanding of the microbial network in the rhizosphere, it is necessary to integrate the interactions between the most abundant soil and plant root microorganisms (Zhou et al., 2020b). A detailed review of the interactions of the diverse nitrogen-fixing microbial community in the rhizosphere was therefore offered as well as a comprehensive review of the mechanism of nitrogen fixation, contributions and challenges of applying PGPR in sustainable agriculture was discussed. Omics techniques for identifying and characterizing microbial communities were highlighted.

2.2 Rhizobiomes of agricultural plants: Plant growth promoters

Rhizobiome referred to as rhizobacteria is a class of bacterial species found in the rhizosphere with the ability to enhance host plant growth. Plant growth is stimulated by diverse microbial communities present in the rhizosphere. Several plant growth-promoting microorganisms are cohabiting in the rhizosphere. Plant root assembles many plant growth-promoting rhizobacteria communities such as *Azospirillium*, *Rhizobium*, *Arthrobacter* and *Pseudomonas* into the internal structures and surface of plant roots. As well as, surrounding the plant environment (Lagos et al., 2015).

The rhizobiome play critical beneficial roles in the host plant, including plant nutrient cycling, plant productivity and immunity against diseases coupled with the specific capacity to further improves soil fertility (Lee et al., 2019). In this instance, the plant microbial community has been described to accouter plant hosts with new pools of genes attributed as extended genome or plant

genome (Vandenkoornhuyse et al., 2015, Igiehon et al., 2019). Meanwhile, some scientists have reported the richness of rhizobacteria in plant root regions and rhizospheric soils. Hence, any shift in the balance or changes in the edaphic conditions will certainly impact on the soil microbiota and crop yield (Lee et al., 2019). Various agricultural beneficial microorganisms and their functions in the rhizosphere of different plants are outlined in Table 2.1.

Recently, the attention of some scientists has been drawn to the critical effects of plant growth-promoting rhizobacteria (PGPR) to plant. The impacts have unveiled the different mechanisms of interactions between plants and bacterial communities in the rhizosphere. Acknowledging PGPR as a tool to enhance sustainable food production in the future is important (Babalola, 2010, Finzi et al., 2015, Igiehon et al., 2019). Various mechanisms are employed by PGPR to either promote plant growth or impact and generally on the plants, for example, the production of inhibitory activities against plant pathogens (antifungal and antibiotics metabolites) (Vandenkoornhuyse et al., 2015, Igiehon et al., 2019).

These mechanisms are mainly through biogeochemical cycles, significantly in the nitrogen fixation process by nitrogen-fixing bacterial species, specifically producing nitrogen-fixing genes (*nif*) that provide nitrogen for plant usage (Chen et al., 2019a, Igiehon et al., 2019). Another essential plant growth-promoting mechanism is the control of phytopathogens. An example is *Pseudomonas* sp. AF-54 isolated from sunflower rhizosphere with antagonistic effects on *Fusarium* sp. The bacterium can be a promising biocontrol agent against sunflower pathogens (Majeed et al., 2018b).

Chen et al. (2019b) studied PGPR communities of wheat plants and their beneficial effects on plant development. The results provided insights that proved a shift in root rhizobiome can affect plants and the microbiome. There are shreds of evidence that has implicated the activities in the rhizosphere that trigger the release of rhizodeposits that are of benefits to plant. So to traditionally observe the effects of various cultivated plants on the composition of PGPR found in the rhizosphere, there is need to understand the abundance and activities of the PGPR in rhizospheric soil (Hu et al., 2018, Chen et al., 2019a, Babalola, 2010).

The activities of the PGPR determine the number of organic compounds excreted by the roots. Thus, these compounds are collectively known as exudates (Babalola, 2010, Hu et al., 2018). The exudates and other rhizodeposits influence the physical and chemical structures of the rhizobacteria (Cregger et al., 2018). PGPR assimilate and release exudates deposits. The exudates are then discharged into the rhizospheric soil after modification (Crawford and Knight, 2017). It is important for the soil quality that increases when these deposits are absorbed by the PGPR that can finally boost plant growth (Orlikowska et al., 2017). For example, *Bacillus subtilis* secretes root polysaccharide that enhances the production of biofilm matrix and is generally advantageous to plants (Olanrewaju et al., 2019). Figure 2.1 outline the different mechanisms of action PGPR uses in improving plant growth.

Table 2.1: Some plant root-associated microorganisms and their influence on plants.

Microorganisms	Effects (Positive and negative)	Reference
Burkholderia sp., *Achromobacter* sp., *Azospirillum* sp., *Chryseobacterium* sp.	Stimulatory effects on plant growth.	Ambrosini et al. (2015)
Rhizobium spp. YAS34	Promote plant growth under stressed conditions and resistance to water. Make use of fertilizer more effectively by increasing nitrogen uptake.	Ali et al. (2017)
Proteobacteria, Acidobacteria	Indicate soil nutrient.	Beckers et al. (2017a)
Azotobacter vinelandii; Azotobacter Chroococcum	Promote plant growth.	Ali et al. (2017)

Frankia	Nitrogen-fixing, and P- solubilization.	Beckers et al. (2017a)
Candidatus Saccharibacteria	High indicator: To biotic and abiotic stresses; strict nutrient requirements; plant innate immune responses; Interact with host plant genotype and microorganism-to-microorganism interaction.	Starr et al. (2018)
Azospirillum lipoferum	Inhabits plant root niche and crude oil contaminated soil.	Singh et al. (2018)
Enterobacter	Produces siderophore, Indole Acetic Acid (IAA), and HCN; Fixes nitrogen, and P- solubilization.	Starr et al. (2018)

Pyrococcus furiosus, Flavobacterium	Biocontrol activity; P and K solubilization.	Singh et al. (2018)
Bacillus ceres	Causes rootlet rot.	Singh et al. (2018)
Curtobacterium,	Phosphorus solubilization and IAA production.	Ali et al. (2017)
Pseudomonas putida GR12-2	Ethylene, and ACC-deaminase inhibitor.	Alawiye and Babalola, (2019)
Pseudomonas fluorescens angstrom 313	Causes shunted growth in plant.	Mosimann et al. (2017)
Bacillus substilis	Causes sour skin and soft-rotting disease of onion.	Murphy et al. (2015)

Pseudomonas fluorescens; *Pantoea agglomerans*	Antifungal activity.	Alawiye and Babalola, (2019)
Azospirillum brasilense	Promotes the uptake of NO3, K+ and H_2PO_4.	Ali et al. (2017)
Burkholderia ambifaria MCI7	Antifungal activity, siderophore production, Increase shipped weight and plant performance.	Mosimann et al. (2017)
Burkholderia pseudomallei	Causes melioidosis.	Mora-Ruiz et al. (2018, Manivanh et al. (2017)
Halobacillus sp.	Biocontrol activity, IAA production and P-solubilization.	Mosimann et al. (2017)

Pseudorhodoplanes, Paenibacillus, ocuria	Indole Acetic Acid production, P- solubilization and nitrogen-fixing.	Şeker et al. (2017)
Arthrobacter	P-solubilization, production of IAA and biocontrol Agent.	Singh et al. (2018)
Pseudomonas fluorescens and *P. fluorescens* biotype F	ACC deaminase activity, Chitinase activity; increased grain yield and weight.	Şeker et al. (2017)

PGPR perceives different signals secreted from plant roots. Ultimately, it releases different signalling molecules that control various stress responses that are caused by edaphic conditions. This usually equips the plant with the ability to resist or tolerate the different stresses that would lead to root development, plant growth and increase in crop productivity (Crawford and Knight, 2017, Lyu et al., 2019). PGPR coexisting in the rhizosphere respond differently to root metabolic activities by stimulating biomass and activities (Lyu et al., 2019).

The growing pieces of evidence in the study of plant growth promotion have proved that PGPR improve soil health and quality, for this reason, promote plant development and growth. For example, PGPR speed up nutrients cycling, influence and increase available nutrient and nitrogen-fixation (Lyu et al., 2019, Finzi et al., 2015). Adding further, the PGPR do not perform activities in isolation; there is an association between the plant and microbiota in the micro- environment. This coexistence influences seedling survival that results in the advancement of plant growth performance and productivity (Crawford and Knight, 2017).

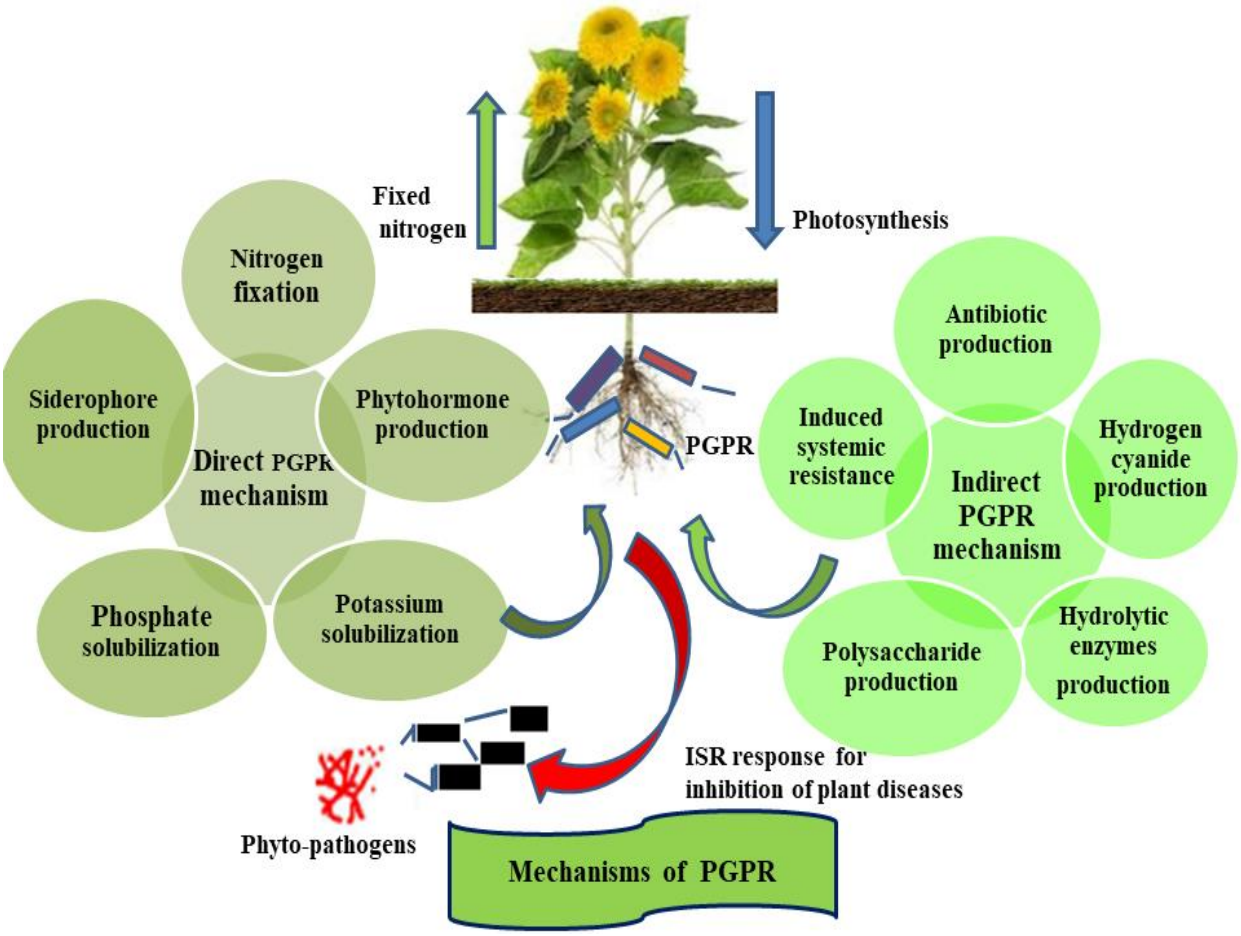

Figure 2.1: Mechanisms of plant growth promotion. PGPR promote plant growth by direct and/or indirect mechanisms. The direct PGPR mechanism includes nitrogen fixation, siderophore production, phosphate solubilization, potassium solubilization and phytohormone production, while the indirect PGPR mechanism includes antibiotic production, hydrogen cyanide production, hydrolytic enzyme production, polysaccharide production and induced systemic resistance. The PGPR also helps the plant to fight against phyto-pathogens. PGPR stands for plant growth-promoting rhizobacteria, while ISR stands for induced systemic resistance.

There are shreds of evidence that backs up the competence of PGPR in sustainable agriculture. Nevertheless, PGPR were first explored only for improving crop production (Lyu et al., 2019). Recently, it has been proposed that it plays a critical role in the suitable functioning of the agroecosystem (Oak, 2019). Scientists have shown that PGPR are beneficial for the improvement of soil quality, combating pathogens and the effects of climate change. Identically, it can help in the restoration of degraded land and decrease the effects of environmental soil pollutants (Lyu et al., 2019). Evidently, Majeed et al. (2018c) recorded different strains of *Pseudomonas fluorescence* and *Pseudomonas* sp. strains, for example *Pseudomonas* sp. AF-54 from sunflower rhizosphere as promising microorganisms with plant growth-promoting traits.

Other agricultural beneficial microorganisms present in the rhizosphere are mycorrhizal fungi (MF), have pesticidal genes that could destroy the negative effects of chemical-based pesticides applied on soil and indirectly enhance plant biomass (Bulgarelli et al., 2015). Few of the agricultural important fungal strains that provide mineral nutrients for plants are genera frequently isolated from the root region in high abundances, such as *Fusarium*, *Xylaria*, *Cladosporium*, *Dactylonectria* and *Trichoderma* (Muller et al., 2018). There are, however, reports on some fungi genera in low abundance, for example, *Aspergillus*, *Alternaria*, *Mucor*, *Leptoxyphium* and *Chloridium* (Pelagio-Flores et al., 2017, Lee et al., 2019, Crawford and Knight, 2017).

However, not only the rhizobacteria and AMF are members of the rhizobiome present in the rhizosphere. Other microorganisms cohabiting in the rhizosphere have also been documented. Lee et al. (2019) reported abundance of archaea groups commonly known as ammonium oxidizers in the rhizosphere. Some phyla of archaeal from the rhizosphere of the tomatoes plant recorded are

Aenigmarchaeota, *Crenarchaeota*, *Diapherotrites*, *Euryarchaeota*, *Pacearchaeota*, *Woesearchaeota* and *Thaumarchaeota.*

2.3. Plant root-bacterial interactions in the rhizosphere

Plant root plays different essential roles, including anchoring and supporting of the plant to the ground, absorption of water, inorganic nutrients and food. Additional, roles are nutrient storage, translocation of minerals and water to the stem, secretion and accumulation of various potential compounds, such as the secondary and primary metabolites peptides, sugars, carboxylic acids and proteins (Kuzyakov and Blagodatskaya, 2015). The underground world of plants is a network of interactions facilitated by the microbial community and plant roots. The plant root regions and soil are colonized by arrays of bacteria that promote the symbiotic relationship, resulting in the enhancement of nutrients' bioavailability and the release of organic substances into the habitat (Beckers et al., 2017a).

The relationship between plant root and bacteria was first recorded by Foster and Rovira in 1976, who considered different ultrathin sections of the wheat bacterial community with the aid of a transmission electron microscope (Kuzyakov and Blagodatskaya, 2015). The result showed that bacteria sparsely colonized immature roots in the root regions. Conversely, in the cortical cells and cell walls, rhizobiome were more abundant. Correspondingly, in an experiment it was reported that bacterial species in the rhizosphere were significantly different to the bacteria in the bulk soil, including numbers, types and sizes (Muller et al., 2018). Contrary to what was reported, abundant bacterial species were dominant in the tomato plant endosphere than other niches studied (Zhou et al., 2016).

In a study bacterial genera of more beneficial functional impact were found to be abundant in the rhizosphere. The abundant bacterial genera were *Alphaproteobacteria*, *Firmicutes* and *Actinobacteria.* Outside the rhizosphere, *Verrucomicrobia* were higher in the bulk soil compared to other niches (Lyu et al., 2019). However, there was a disparity in the results of a related study on soybean plant rhizosphere. A group of bacterial communities with a particular nutritional function was abundant in the root surface or rhizoplane while the least was in the surrounding bulk soil (Zhou et al., 2020b, Lee et al., 2019).

Besides, the metabolism of nitrogen, potassium and phosphorus, the bacterial community is involved in iron metabolism and uptake as well as the transportation of membranes (Fonseca-García et al., 2016). The diversity of bacterial communities that colonize the different below ground zones has been comprehensively studied to interpret the mechanisms that allow various interactions between the internal tissues, root surface, rhizosphere and bulk soil. Varieties of substrates are released through organic nutrients and growth-promoting materials that bacterial species act on, resulting in the promotion of plant growth (Tassi et al., 2017). Plants dispense peculiar organic substrates that are utilized by bacteria. Hence, it provides inorganic nutrients and growth-promoting substances that stimulate plant growth.

Accordingly, Singh et al. (2019b) isolated different species of rhizobacteria possessing multiple plant growth- promoting traits from sunflower rhizosphere soil. The interaction between PGPR and other resident microorganisms produced secondary metabolites with plant growth-promoting properties. Some of the rhizobacteria were member *Bacillus subtilis* strain Rhizo SF48, *Bacillus thuringiesis* strain Rhizo SF 23 and *Acinetobacter* sp. strain N15254. Also, various strains of

Pseudomonas and *Enterbacteria* spp were reported. Some of the identified PGPR inhibited the growth of *Fusarium oxysporum*.

Several factors, such as soil properties and type, plant genotype, different plant developmental stages and rhizodeposits, influence the level of interactions between bacterial communities and plant root systems (Beckers et al., 2017a). The different interactions that occur in the rhizosphere produce various available rhizodeposits, including those that depend on different abiotic and biotic factors as well as plant variants and developmental stages (Beckers et al., 2017a, Lagos et al., 2015). Although the upper part of the plant is held by soil at the root, plants need a variety of macro and micronutrients that play beneficial roles in their growth and development. Some nutrients are required in major quantity and are called macronutrient, namely nitrogen, carbon, sulfur, calcium and phosphorus. However, some nutrients are needed in smaller portions which are referred to as micronutrients, for example, zinc, iron, magnesium and nickel (Finzi et al., 2015, Fonseca-García et al., 2016).

Moreover, toxic elements, such as heavy metals in the soil have been reported to accumulat in plant tissues over time, when present in extreme conditions and above the permissible limits, for example, barium (Finzi et al., 2015). The negative impact of the presence of toxic elements in the soil is that it becomes toxic to plants, limiting plant growth. Ordinarily, a high amount of toxic elements within plant tissues have a direct or indirect impact on the plant (Beckers et al., 2017a). The direct impact generates oxidative stress that increases the inhibition of cytoplasmic enzymes and damages the cell structures (Vandenkoornhuyse et al., 2015). The indirect impact substitutes nutrients that are essential to plant cation exchange sites. In that case, the ions control the roles of different enzymes and proteins. Thereby, it stops metabolism and reveals the extent of toxicity in

the plant, for example ions (boron) which have been reported to be toxic to plant even at low concentrations (Tassi et al., 2017).

2.4. Soil fertility: Role of PGPR

The soil is a habitat for various macro-organisms, such as ants, earthworms, insects and termites as well as a substantial number of microorganisms that participate in nutrient cycling, mineralization of organic compounds, synthesis of phytohormones and other important processes that advance plants productivity. Furthermore, they help in improving and the maintenance of soil fertility (Susilowati et al., 2015).

PGPR colonize plant roots and have been implicated in all important activities that occur in the soil such as the promotion of plant health by the aiding plant take-up in many nutrients. In addition, control the pathogens that could affect the host plant negatively. The moisture content in the soil determines the colonization of PGPR in the rhizosphere and long term soil fertility. PGPR are responsible for synthesizing many biomolecules that enhance soil health (Vandenkoornhuyse et al., 2015).

Several organic compounds that operate as plant residues undergo decomposition and mineralization. These minerals increase soil health and fertility (Figure 2.2). The high amount of phosphate available to plants is as a result of P solubilization, an activity performed by PGPR. Additionally, other chemicals in charge of plant growth are formed and they influence the plant root morphology and enhance soil health and fertility. The soil environments are improved mainly by bacteria species (Suyal et al., 2015).

The presence of nitrogen (N) in plants is critical for protein and amino acid synthesis. Nitrogen is acquired from the atmosphere, rhizosphere and soil through the fixing of nitrogen. However, volatile compounds and metabolites are released by PGPR to improve plant and soil health (Igiehon et al., 2019). Likewise, different enzymes are released to control the increase and effect of pathogens, hence contribute to the biocontrol of phytopathogens and improvement of plant growth. These compounds boost crop yield and enhance soil health. The overall mechanisms are essential to achieve a more fertile soil (Jiang et al., 2016).

In contrast to conventional chemical-based fertilizers, which have been linked to the contamination of farmland following application, PGPR inoculant as a biofertilizer could boost resident soil microbial diversity in addition to promoting plant development. For instance, nitrate from chemical fertilizer can contaminate below-ground water, which can then contaminate surface water when runoff from the soil occurs after rainfall. After extended exposure to or intake, this may raise the risk of stomach cancer in adults and result in blue baby syndrome in infants. (Igiehon et al., 2019).

Interestingly, some PGPR act as both biopesticides and biofertilizers, for example, *Burkholderia cepacia*. A study on maize showed that these microorganisms stopped the negative impact of

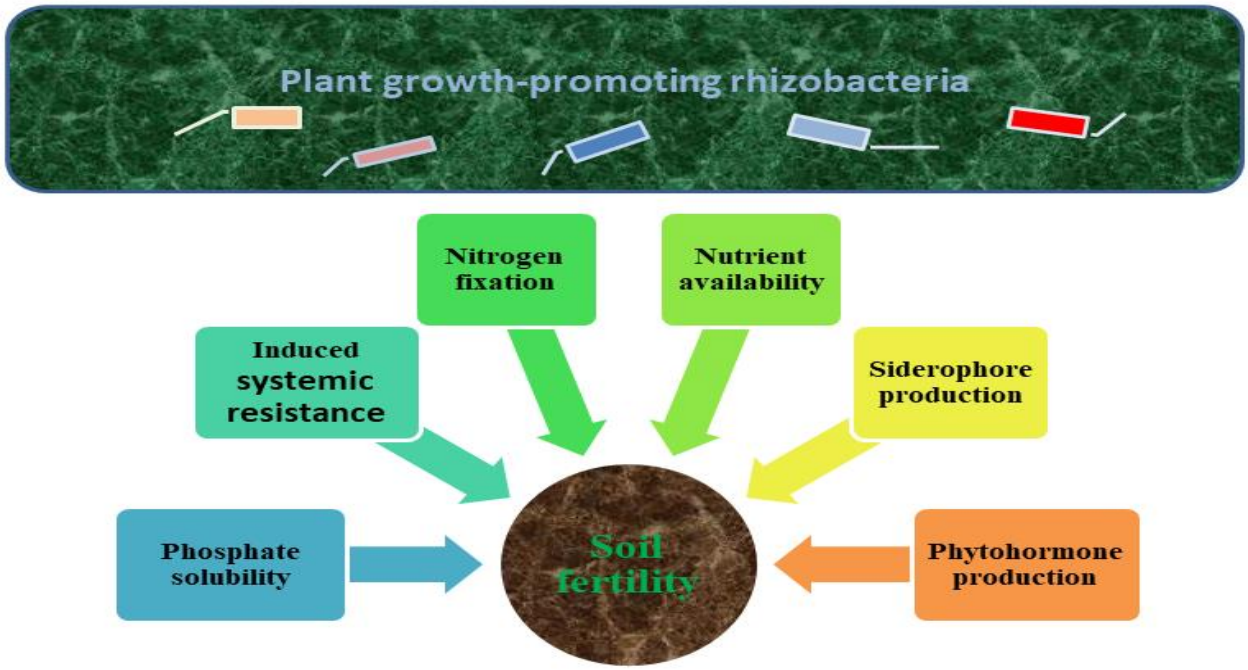

Figure 2.2: Contributions of plant growth-promoting rhizobacteria to enhance soil fertility. Plant growth promoting rhizobacteria contribute to soil fertility by fixing atmospheric nitrogen (nitrogen fixation), enhancing iron availability through siderophores production, enhancing nutrient availability through phytohormone production and phosphate solubility, as well as priming the defense mechanisms of host plants to improve resistance against wide range of phyto-pathogens.

Fusarium species and promoted maize growth in iron-deficient soil by producing siderophore. Some PGPR species produce siderophore and antibiotics with antagonizing properties that could stimulate systemic resistance, which may suppress the impacts of many pathogens which can be used for many agricultural purposes, including improving soil fertility (Manivanh et al., 2017).

Additionally, arbuscular mycorrhizal fungi with plant growth-promoting and pesticidal traits have been recorded as a beneficial rhizospheric microorganism with the potential of destroying the damaging effects of chemical fertilizers. The result increased plant biomass and improved soil fertility significantly proves that AMF is a promising microorganism for sustainable agriculture. Jadhav and Anil (2020) reported the influence of AMF on soil fertility and sunflower plant growth. Therefore, it is important to harness agricultural beneficial rhizobiome with the potential of enriching agricultural soil for sustainable agricultural development.

2.5. Biological nitrogen fixation: PGPR as nitrogen fixers

The rhizosphere is made up of a unique and enormous variety of bacterial species. Some are cultivable and known (1%), while 99% are yet uncultured, which makes it challenging for researchers to assess them. The variations of bacterial communities, mainly PGPR, shape the integral structure of the rhizosphere (Alawiye and Babalola, 2019, Igiehon and Babalola, 2018b). According to Olanrewaju et al. (2019), a large proportion of bacteria colonize different land plant roots and niches. These bacteria are assembled to make up the rhizobiome.

Among the plant root microenvironment the rhizosphere is a thin soil layer that surrounds plant roots, hence acts as crossroads for microbial interactions, which makes this zone an exceptionally special, active and important zone with the potential for diverse root activities and unique

metabolic processes (Zhou et al., 2020b). Rhizobacteria communities co-existing in the rhizosphere are plant growth promoters that play several roles, including nitrogen fixation, increasing available nutrients, phytopathogens suppressions and disease inhibitions. Again, it improves plant persistence and tolerance to environmental stress, such as high salinity and water shortage (Olanrewaju et al., 2019, Babalola, 2010).

Rhizosphere is a haven for diverse bacterial species with a unique structure and composition that is impacted by plant regulated mechanisms (Mukhtar et al., 2019). Variations in the rhizosphere are modified by most bacterial populations broadly advantageous to plants (Bulgarelli et al., 2015, Lee et al., 2019). Recently, there are detailed reports on bacterial components found in the rhizosphere and their influence on plants (Mukhtar et al., 2019, Kuan et al., 2016). It has been recorded that in the rhizosphere, nitrogen-fixing is the fundamental mechanism that enhances plant growth (Lee et al., 2019, Susilowati et al., 2015).

Nitrogen (N) is a major macronutrient important for the growth and productivity of all living organisms, bacteria and plants inclusive. Nitrogen is found in membrane lipids and nucleotide (Mukhtar et al., 2019, Zhang et al., 2017). It is among the major essential component of plant dry mass because it is the main constituent of chlorophyll (compound plants use from sunlight to produce sugars from water and carbon dioxide) and amino acids. When plants do not get enough nitrogen, they are unable to produce amino acids, and without amino acids plants cannot make the unique protein that plant cells require for growth (Babalola, 2010).

The biological fixing of atmospheric nitrogen is an essential microbial activity that supports life on earth. This process takes place when atmospheric nitrogen is transformed to ammonia (NH_3)

by a highly complex oxygen-unstable enzyme system found in free-living symbiotic diazotrophs, called nitrogenase (Igiehon et al., 2019). Nitrogen fixation is an energy-demanding process that requires the hydrolysis of a minimum of 16 moles of ATP per mole of reduced nitrogen (Souza et al., 2015).

Gram-negative rhizobacteria species possessing plant growth-promoting traits capable of fixing nitrogen dominated the rhizosphere, can be referred to as nitrogen fixers (Alawiye and Babalola, 2019, Igiehon and Babalola, 2018b). The common range of nitrogen-fixing rhizobacteria genera with the potential of improving plant growth and development, which consequently lead to increase in crop productivity, include *Azospirillum*, *Azotobacter*, *Acetobacter*, *Arthrobacter*, *Bacillus*, *Burkholderia*, *Serratia*, *Pseudomonas* and *Mitsuaria* (Bagyaraj and Ashwin, 2017, Babalola, 2010, Manivanh et al., 2017). Other important rhizobacteria strains such as *Pseudomonas fluorescens* Pf153 and *Pseudomonas* sp. DSMZ 13134 from a particular locality having different plant growth-promoting traits with influence on maize crop yield was reported by Mosimann and teammates (2017). Correspondingly, *Vibrio fluvialis* NWU37, *Pseudomonas fluorescens* NWU65 and *Ewingella Americana* NWU59 were identified, likewise *Streptomyces* species and lots of *Proteobacteria* (Alawiye and Babalola, 2019).

The common broad range of nitrogen-fixing bacteria present in the rhizosphere, which symbiotically fix nitrogen with a particular plant, are *Pseudomonas, Rhizobium, Azotobacter Paenibacillus, Allorhizobium, Klebsiella, Salinibacter, Agrobacterium, Azorhizobium, Bradyrhizobium, Sinorhizobium, Ochrobactrum, Phyllobacterium, Mesorhizobium* and *Devosia* are in synergistic association with leguminous, while *Frankia,* are in association with non-leguminous plants and trees that employ their functions (Mukhtar et al., 2019, Kuan et al., 2016).

Distinct features are used to differentiate members of nitrogen-fixing bacterial communities in various plant rhizospheres. Some plants inhabit different nitrogen-fixing bacterial communities, such as sunflower (Majeed et al., 2015a), rice (Miller, 2018), poplar (Mukhtar et al., 2019), halophytes (Mora-Ruiz et al., 2018), *Arabidopsis* (Ali et al., 2017) and tomato (Lee et al., 2019).

A study of different plant varieties reported that the genus *Pleomorphomonas* known for their nitrogen-fixing capacity was abundant in the rhizosphere (Kang et al., 2019). Similarly, Ambrosini et al. (2018) recorded nitrogen-fixing *Bacilli* belonging to the genus *Paenibacillus* spp from the sunflower rhizosphere. Many processes trigger bacterial-plant interactions, which have led to the development of beneficial relationships and have enhanced adjustments to different environmental challenges (Pii et al., 2015). Another distinct nitrogen-fixing bacterial species from the rhizosphere plant root system of mature *Populus deltoides* trees have been reported (Salas et al., 2017).

Notably, non-symbiotic bacteria fix the fewer amount of nitrogen, sufficient to the associated host plants compared to bacteria found in the root nodule (rhizobia) (Mosimann et al., 2017). Despite the slower fixing capability of non-symbiotic bacteria, few PGPR have proved to be very efficient in increasing this process by making the nitrogen nutrient that is short in supply accessible to plants. During a non-symbiotic nitrogen-fixing activity, free-living diazotrophs excite the growth of non-leguminous plants.

The common genera of this group are *Anabaena*, *Diazotrophicus*, *Azotobacter*, *Azocarus*, *Cyanobacteria* and *Gluconoacetobacter diazotrophicus* (Zhang et al., 2017, Zarraonaindia et al., 2015). The application of cultures with non-symbiotic nitrogen-fixing PGPR, mainly *Azospirillum*

and *Azotobacter* enhanced the productivity of crops (Pii et al., 2015, Souza et al., 2015) just as *Rhizobium* nitrogen fixation is important in the cultivation of various plants (Jadhav and Anil, 2020, Ambrosini et al., 2012). Ambrosini et al. (2018) reported that novel nitrogen-fixing *Paenibacillus helianthi* sp. nov. encouraged high growth and yield of sunflower plants.

The majority of rhizospheric nitrogen-fixing bacterial species populate the host plant root, some may emanate from other tissues, such as internal tissues, leaf, flowers and bulk soil (Salas et al., 2017). A high abundance of members from the phyla Proteobacteria and Spirochaetes were identified from the rhizosphere and bulk soils of rice farm, whereas Gemmatimonadetes, Acidobacteria and Planctomycetes were in depletion (Miller, 2018).

Among the nitrogen-fixing bacterial communities in the rhizosphere are bacteria that colonize the host plant root by establishing themselves in the root surface, while a fraction finds their way into the plant's internal tissues and is controlled by the innate immunity of the plant (Jiang et al., 2016). Some phyla recorded within the root surface and internal tissues are Alpha, Beta and Deltaproteobacteria. Few indicated classes are Chloroflexi and Bacteroidetes (Miller, 2018, Li et al., 2019a).

Bacterial communities in the rhizosphere use of a wide variety of substances that increase the higher colonization and persistence rates serving as sources of the nutrient (Crawford and Knight, 2017, Cregger et al., 2018). The rhizoplane, which is the plant root surface is one of the plant root systems, where bacteria inhabit and attach themselves to the root surface structures with the aid of either the cell surface polysaccharides, fimbriae or flagella (Olanrewaju et al., 2019, Miller, 2018).

Similarly, some reports stated that there is a fragile boundary between the rhizoplane and rhizosphere (Beckers et al., 2017a, Mukhtar et al., 2019, Olanrewaju et al., 2019). Before now, researchers have recorded that some nitrogen-fixing bacterial species, such as *Caulobacter* sp. and *Zoogloea ramigera,* identified from rhizoplane of some plants (Beckers et al., 2017a, Edwards et al., 2015). These bacterial species are capable of controlling the nutrient status of the surrounding soils and other factors, such as pH (Elmagzob et al., 2019).

Most scientists have overlooked the activities occurring in the internal tissues (endosphere), which makes this region the least studied. Endosphere is a plant microenvironment that harbours a lesser concentration of bacterial communities called endophytic bacteria (Gomes et al., 2018, Zhou et al., 2016). The interior root region being an exclusive niche has a fewer number of bacterial density compared to the root surface (rhizoplane) while rhizosphere has more abundant bacterial diversity (Elmagzob et al., 2019). Table 2.2 describes nitrogen-fixing genes and their functions.

The beginning of molecular communication between soil bacterial and host plants occurs through the discharge of signals as communication chemicals, for example flavonoid, capable of improving plants-microorganism relationship (Ali et al., 2017, Alawiye and Babalola, 2019, Carrell and Frank, 2015). Majeed et al. (2018c) recorded that this chemical helped in the choice of the greater befitting partners for their development and the destruction of the pathogenic once. The communication signal is recognized by NodD (bacteria receptor), which acts as a transcriptional activator of nodulation genes (NodA, NodB, NodC and NodFE). Evidently, Nod factors stimulate agent of root nodules inhabiting in the rhizobia.

Table 2.2: Rhizobial nitrogen-fixing genes and their predicted functions.

Nitrogen-fixing genes	Functions	References
*nif*A	Particular nitrogen-fixing regulatory protein and transcriptional stimulator; Regulates the expression of genes encoding accessory functions by the aid of their RpoN-dependent -24/-12 promoters and the nitrogenase structural genes	Brink et al. (2017)
*nif*B	Protein that controls biosynthesis iron-molybdenum cofactor	Lemaire et al.(2018)
nifD	Nitrogenase molybdenum-iron protein and forms alpha chain	Backer et al. (2018)
nif H	Nitrogen-fixation enzyme protein	Chauhan et al. (2015)
*nif*K	Nitrogen-fixating enzyme (nitrogenase) molybdenum to iron protein; forms beta chain	Kuan et al. (2016)
*nif*Q	Protein that aid in nitrogen fixation and binding of molybdenum to iron	Lemaire and Gastal, (2016)
*nif*W	Stabilizes nitrogen-fixing enzyme, nitrogenase	Srivastavia, (2017)
nif X	Molybdenum-iron cluster binding protein	
nif Z	Nitrogen-fixing protein	Srivastavia, (2017)

*fix*A	Nitrogen-fixing gene, electron transfer flavor protein and beta chain	
*fix*B	Nitrogen-fixing gene, proteins aid electron transfer flavor and alpha chain	Brink et al. (2017)
*fix*C	Nitrogen-fixing protein and oxidoredutase enzyme	Kumari et al. (2019)
*fix*H	Nitrogen-fixing and cation transport gene	Gastal et al. (2015)
*fix*J	Double component nitrogen-fixing regulatory protein and a direct positive regulator of *nif*A	Lemaire and Gastal, (2016)
*fix*K	Expression of *nif*A, fixGHIS and fixNOQP Ferrodoxin and FNR/CRP transcriptional activator and regulatory gene; Ferrodoxin, such as protein, control the gene for nitrate respiration and one of two stigma 54 proteins.	Backer et al. (2018)

2.6. Contributions of plant growth-promoting rhizobacteria to sustainable agriculture and food security

Generally, farmers worldwide need new innovative ideas for farming to increase farm production that can take care of the human population, which is estimated to be approximately 7 billion globally. For food security to be actualized, it requires a process of producing adequate food and improving its quality. This process, referred to as the global green revolution, must maintain the ever-increasing human population without sabotaging environmental protection and impacting the environment negatively (Igiehon and Babalola, 2018b). However, sustainable agricultural developement is needed to ease these issues.

According to National Research Council, the advancement of recent eco-friendly agricultural practices, energy conservation strategies and natural resources that are promising to food security are the crucial goals of sustainable food production (Igiehon et al., 2019, Grafton et al., 2015). Researchers have indicated that the most promising approaches to accomplish these objectives are to substitute harmful pesticides and inorganic fertilizers with environmental friendly symbiotic microbial formulations, for example *Bradyrhizobium* species with the possible attribute of improving plant health and crop yields, while protecting plants from biotic stressors, for example, pests, phytopathogens and abiotic stressors, such as drought, climate change and different environmental pollutants (Lang et al., 2019, Lugtenberg, 2015). There are reports on the identification and application of PGPR as a substitute to chemical-based fertilizers. The usage of PGPR as an alternative approach has enhanced the abundance and richness of soil microorganisms and have influenced plants health and yield positively (Babalola et al., 2007).

Bacterial (microbial) cultures are combined with chemically advanced carriers with the help of liquid or solid fermentation approaches. The bacterial isolates are alternatively integrated into plants in mixed or pure culture through seed or soil application, biological priming and seedling dip. Again, it is the application of individual bacterial and identification of functionally appropriate rhizobiome. Their importance in promoting crop productivity is another basic and critical task since the rhizobiome is essential, thus it is a description of the plant host second genome (Babalola, 2010, Beckers et al., 2017b, Beckers et al., 2017a). Therefore, to attain food security, there is a need to depend on the advancement of plant diversity, growth and seed yield. The major way this can be accomplished is by inoculation of crops with rhizobacteria, such as *Rhizobium* species (Igiehon et al., 2019, Igiehon and Babalola, 2018b), as discussed below.

2.6.1. The effects of rhizobial inoculant as a plant growth promoter

Rhizobium species are bacteria capable of reducing atmospheric nitrogen to ammonia through nodule formulation in the stems or roots of host plants. Due to the agricultural and environmental significance of *Rhizobium* species, many researches have been conducted on the bacteria (Ambrosini et al., 2018). The inoculation of seeds with *Rhizobium* species was recorded to improve the formation of the nodule, nitrogen absorption and seed protein. Some seeds, such as sunflower (*Helianthus annuus* L), soybean (*Glycine max* L.), lentil and pea inoculated with *Rhizobium* species enhanced root nodulation, root and shoot microbial diversity, seedling height and shoot biomass, as a result, increased plant growth and crop yield significantly (Ambrosini et al., 2018). Other yield constituents included the number of seeds per pod, total number of pods per crop and sum of branches bearing pod per plant was as well improved (Enebe and Babalola, 2018, Brader et al., 2017).

In a field and greenhouse study the researchers reported that *Vicia sativa* L. plants were modified with *Azospirillium*, the result showed that there was a notable increase in nitrogen-fixing activities, N content and percentage (Igiehon and Babalola, 2018b). A similar experiment on peanut plant showed that there was an improvement in the number of root nodules, root dry weight and plant growth. Chicken pea treated with *Rhizobium* species both in greenhouse and field experiments compared to the controls improved plant growth (Lawson et al., 2017). *Rhizobium* species were more effective on the plant growth when co-inoculated with other plant growth-promoting microorganisms. *Rhizobium* species co-inoculated with few bacterial species will not only improve the effectiveness of *Rhizobium* species, but will advance crop production (Kuan et al., 2016, Lang et al., 2019).

Some beneficial bacterial species that have favorably improved crop productivity by co-inoculation with *Rhizobium* species are *Azotobacter*, *Bacillus*, *Enterobacter*, *Serratia* and *Pseudomonas* (Ambrosini et al., 2016). The co- inoculation of *Rhizobium leguminosarum bv. trifoli* and *Azospirillium lipoferum* enhanced white clover root nodulation (Igiehon and Babalola, 2018). Furthermore, there are reports on *Rhizobium* and *Azospirillium* co-inoculation on the bean with increased nitrogen-fixing activity in the crop (Lang et al., 2019, Lugtenberg, 2015, Igiehon and Babalola, 2018b).

The co-inoculation of *Rhizobium* and *Bacillus* species on cowpea resulted in the bacterial species stimulating plant growth by increasing the nutrient uptake and root weight (Lang et al., 2019, Lugtenberg, 2015, Igiehon and Babalola, 2018b). The combination of *Bacillus, Rhizobium* and *Azospirillium* species advanced nitrogen fixation and root nodulation in pigeon pea. The co-inoculation of *Streptomyces lylius* WYEC108 and *Rhizobium* sp. promoted the growth of pea by

increasing the number of nodules. Again, it enhanced the colonization of *Streptomyces* at the root and root nodules (Li et al., 2019a). The combinations of various inoculants have been explored in different crop experiments but there is still a need for further research.

Furthermore, the incorporation of *Rhizobium* sp. in crops stimulates crop growth and serves as a substitute to expensive chemical-based fertilizers. Using relevant species as an inoculant in environments with nitrogen gas depletion can be a better avenue to improve the growth and development of crops (Lyu et al., 2019, Igiehon and Babalola, 2018b). Taking in mind the inexpensive rate of inoculation and many agricultural benefits, farmers are encouraged to take advantage of the numerous benefits of these bioinoculants and biofertilizers on crops.

2.6.2. The effects of rhizobial inoculant on plants photosynthesis and chlorophyll concentration

Nitrogen is an essential plant regulating element for growth because it is limited in the environment. Plants require an abundance of nitrogen than other mineral nutrients for their growth. Nitrogen is a major component of many organic compounds, including proteins, chlorophylls and plant growth regulators (Lyu et al., 2019). The visible appearance of the crop with nitrogen deficiency is stunted growth, reduced branching and yellow colouration of the leaves. Nitrogen as a monomer of protein is essentially required for the total plant cell enzymatic processes. It is present in proteins and vitamins. Also, act as a key player in photosynthetic processes in photosynthetic plants (Zhou et al., 2016).

Plants inoculated with rhizobial strains in field and greenhouse experiments indicated a considerable increase in the chlorophyll levels of the leaves. As well, there was 140% notable

increase in photosynthetic activities in plants modified with rhizobial strains compared to the controls (Xiao et al., 2017). Again, soybean inoculated with *B. japonicum* resulted in the buildup of chlorophyll content. There was an increase in diverse growth indices, such as height, number of leaves, the width of the stem, number of flowers, pod development and root extension, which distinguished the treated plant from the control (Bulgarelli et al., 2015, Igiehon and Babalola, 2018b). The impact of P and *B. japonicum* was studied in a field experiment on the growth of cowpea. Consequently, there was a drastic increase in the chlorophyll level of the leaves. However, there is still a study gap on the application of K, P and *Rhizobium* as well as the formation of chlorophyll in *Phaseolus vulgaris* (Deng et al., 2019).

2.6.3. The effects of rhizobial inoculants on the mineral nutrient assimilation by plants

The availability and assimilation of mineral nutrients are important for plant growth. Mineral nutrients, such as nitrogen (N), magnesium (Mg), calcium (Ca) and potassium (K) are required by various plant varieties for their growth and increase crop productivity. The mutualistic association between *Rhizobium* species and plants can be the main N source in majority of the cropping systems, where approximately 80% of the N originating is from biological fixing of N. In a study, amendment of crop seeds with Rhizobial cultures improved N uptake and root nodulation (Deng et al., 2019, Finzi et al., 2015).

Similarly, seeds modified with *Rhizobium* spp. showed an increase in plant N from 19 to 42 mg per plant (Lange et al., 2015, Lugtenberg, 2015). In a study in Brazil *Phaseolus vulgaris* amended with *Rhizobium* sp. cultures significantly increased N from about 20 to 60 kg per of N per hectare (Garrido-Oter et al., 2018, Igiehon et al., 2019). Potassium is another essential mineral nutrient

conserved in the soil, mostly in a non-soluble form which cannot be easily absorbed by plants and as a result limits plant growth. Assimilation of potassium is essential for plant function and development. For the non-soluble P form to be solubilized by PGPR, it should pass through acidification, chelation and different enzymatic activities (Resende et al., 2014).

Mineral nutrient uptake depends on different factors, such as the interactions at the rhizosphere, the concentration of the minerals and the ability to replace them in the soil. This challenge can be resolved by the introduction of *Rhizobium* spp or other phosphates solubilizing bacterial species, such as *Burkholderia*, *Bacillus*, *Erwinia* and other important mineral nutrients into the rhizosphere soil (Souza et al., 2015, Manivanh et al., 2017). Apart from the inadequacies of the main mineral nutrients required by plants, micronutrients can also become limited in the rhizosphere, causing low crop production. Some of the essential micronutrients needed by plants are iron (Fe), molybdenum (Mo), zinc (Zn) and boron (B). It was recorded in a study that the amendment of Pigeon pea (*Cajanus canjan* L. Millsp) with *Rhizobium* strains inoculant relatively increased the number of available mineral nutrients in the rhizosphere and as a result boosted the capacity of plant nutrient uptake (Lyu et al., 2019).

The impact of *Rhizobium* strains inoculants on the increase of minerals nutrients uptake in the rhizosphere and plant growth have been reported by various authors (Pii et al., 2015, Majeed et al., 2015b, Igiehon and Babalola, 2018b). A study showed the influence of *Rhizobium* strains on mineral nutrients assimilation by *Phaseolus vulgaris*, which resulted in a notable increase in Ca, P, K, S and Mg uptake in all plant parts. The introduction of *Rhizobium* strains has improved the significant uptake of different micronutrients, including Cu, Fe, Mn, Zn, Fe, B and Mo in various plant part as recorded in some studies (Igiehon et al., 2019, Kuan et al., 2016).

2.7. The relationship between of PGPR, iron acquisition, crop productivity and phytopathogens extermination for sustainable agriculture

Important mineral nutrient, such as inorganic N, Fe and P required by plants, can be acquired through symbiotic associations with rhizobacteria. Plants often absorb Fe from reduced ferrous ion (Fe^{2+}), although the ferric ion (Fe^{3+}) is readily available in the soil. Most plants have been recorded to release chelators and siderophore that attach to Fe^{3+} and aid in retaining it in the soil solution. Chelators significantly release Fe^{3+} to the root surface, where it is transformed to Fe^{2+} that is then absorbed rapidly. Grasses secrete the siderophores that are absorbed alongside Fe^{3+} across the plasma membrane (Kuan et al., 2016, Lange et al., 2015, Lugtenberg, 2015, Igiehon and Babalola, 2018b).

Nevertheless, few PGPR can separate insoluble Fe from the soil with the help of bacterial siderophores, therefore makes it accessible for the host plant, which is evidence that some plants can take in bacterial Fe^{3+} siderophore complexes require for their growth (Lange et al., 2015, Lugtenberg, 2015). However, there is controversy on the benefits of bacterial Fe^{3+} siderophore complex absorption to plants iron nutrition. Some scientists recorded that the contributions of Fe from the aforementioned sources to plants and stated that the total iron requirement is little. Although, few accepted their essential benefit contributions in calcareous soils (Igiehon et al., 2019, Lugtenberg, 2015). A study showed that bacterial siderophore, such as pseudobactin and ferrioxamine B, did not provide a sufficient amount of iron for plants (Igiehon et al., 2019, Igiehon and Babalola, 2018b).

The bioavailability of Fe in the rhizosphere soil is reduced by obtaining Fe through PGPR siderophores. As a result, it impacts on the proliferation of fungi, which may be harmful to plants in soils deficient of Fe. Plants are relatively more productive in soil rich in microorganisms than in the soil with a low amount of microorganisms. This acknowledges the fact that PGPR help host plants by obtaining mineral nutrients (Beckers et al., 2017b, Deng et al., 2019).

2.8. Challenges applying PGPR in the field for sustainable agriculture

The technology of bioinoculation holds the potential for the future. However, several challenges are encountered in the field application of PGPR in sustainable agriculture. Few main bottlenecks need to be addressed to elevate the efficacy of PGPR inoculation in field applications. The development of recent inocula depends on laboratory screening assays that mainly depend on PGPR mechanisms, such as nitrogen fixation, calcium phosphate solubilization, the activity of ACC deaminase and auxin synthesis (Sessitsch and Mitter, 2015, Hristeva and Denev, 2017). Screening of pure culture in the laboratory with plant growth promotion potential does not result from microorganisms that can promote plant growth in the field (Mukhtar et al., 2019, Murphy et al., 2015, Prasad et al., 2019a).

Unfortunately, some of these organisms with the potential of improving plant growth in the field are discarded during in vitro screening in the laboratory because their mechanisms are not fully understood. The most critical challenge is the development of PGPR based products that can be applied commercially, coupled with the process of screening and efficient selection of the most potential organisms (Sessitsch and Mitter, 2015). Some factors are being considered when isolating, screening and selecting PGPR. Some criteria that are considered include the competence

of the host plant to adapt to a specific environment, pathogens and climate change (Salas et al., 2017, Itelima et al., 2018).

Moreover, other means of selection and isolation are the spermosphere model. This approach is an enrichment method that employs different seed exudates that are used as a source of nutrients, for instance the one recorded as potential nitrogen-fixing bacteria from the rice rhizosphere. Isolating promising microorganisms with the capacity to inhibit soil-borne pathogens is another essential method of selection. Therefore, the potential microorganisms should be collected particularly from soils that have suppressive effects on the specific pathogen (Purwati and Herwati, 2016). Other methods include selection based on characteristics associated with PGPR, such as antibiotics and siderophore production as well as activity of ACC deaminase. To solve the problem of selecting the excellent and promising strains of PGPR, there is a need for upgrading to advanced assessment systems and efficient bioassays (Sessitsch and Mitter, 2015).

Another is the application of PGPR as a biological control agent against phytopathogens, such as fungal pathogens. In an experiment performed in greenhouse systems, the result showed that PGPR have the potential in controlling different phytopathogens (Hristeva and Denev, 2017). This was achieved because of the controlled and consistent environmental conditions in the greenhouse throughout the planting season. Nevertheless, it is impossible to achieve a constant environmental condition in the field where instability in the biotic and abiotic factors are very high and the rivalry between indigenous micro and macrofauna is a challenge too (Schlemper et al., 2017, Itelima et al., 2018).

However, the understanding of the previously mentioned factors can be useful in deciding the appropriate type and concentration of PGPR strains and time of inoculation. Likewise, soil types, cropping strategies and farm management practices ome factors that could encourage PGPR survival and increase (Itelima et al., 2018). Hristeva and Denev (2017) reported that sunflower rhizosphere soil inoculated with PGPR inocula showed that there was an impact of soil type on sunflower rhizobiome population density and structure. For those reasons, the idea of engineering the rhizosphere to improve the function of PGPR are becoming more popular (Schlaeppi and Bulgarelli, 2015, Chaudhary et al., 2020, Parnell et al., 2016).

Other areas of concern are to identify the appropriate carrier and to establish more excellent composite formulations that can guarantee the survival, proliferation and actions of PGPR in field applications. Also, concentrate on compatibility with chemical-based seed treatments. To overcome the challenges of the field application of PGPR, approaches such as optimizing the conditions for growth before the formulation, the improvement of carriers and application technology can be helpful (Şeker et al., 2017, Chaudhary et al., 2020).

Some farms cultivated with high-value crops are frequently fumigated with broad-spectrum biocidal fumigants that can change the structure of the biological community in the soil (Majeed et al., 2015b, Macouzet, 2016). However, long-term exposure to these fumigants affects the microbial community interactions, which can be helpful in nutrients acquisition and mobilization. The biocidal fumigants are damaging to soil health and pose a challenge to PGPR inocula colonization in the rhizosphere. While plant breeding has helped achieve the green revolution, still few of the frameworks of bioinoculants have been made to combine the microbiome-based form

of plant breeding to accomplish a heritable PGPR population that can improve crop productivity and food security (Macouzet, 2016, Hristeva and Denev, 2017).

The green revolution has revealed the negative impact of inorganic fertilizers, herbicides and pesticides application in the soil environments. The applications of chemical-based products have led to soil damage, especially as soil contaminants. The combination of plant growth promotion and bioremediation would be an advantageous approach in solving agricultural problems globally. Besides, plotting microbiota consortia to mitigate different forms of plant growth promotion and bioremediation is an important form of this approach (Barquero et al., 2019). The production of bioinoculant for a particular soil condition, defeating environmental restrictions and training of farmers and farmworkers on how to efficiently incorporate the bioinoculants to crops are essential in the development and deployment of many important bioinocula (Prasad et al., 2019a).

The different challenges discussed must be surmounted before registering commercialized PGPR products for public use such as the scaling up of fermentation conditions, large scale production of PGPR and maintaining product quality, efficacy and stability (Bashan et al., 2016). Factors that should be taken into consideration before commercialization are cost, duration of shelf-life, ease of application, rate of colonization and compatibility. Before deciding the commercialization of PGPR products, cost capitalization and possible markets must be considered and studied (Itelima et al., 2018). Also, knowledge of the levels of pathogenicity, toxigenicity and allergenicity is of utmost importance. Studies on potential gene transfer and persistence in the environment are needed to devise means to enhance safety measures of commercial products. Categorizing the products under the proper category, either as a biocontrol agent or biofertilizer, is important as well (Orlikowska et al., 2017, Barquero et al., 2019, Parnell et al., 2016).

2.9. Meta-omics techniques for identifying rhizobiomes of agricultural sustainable importance

Before the emergence of the meta-omics techniques, microorganisms were isolated and identified using the traditional or culture-based method for isolating and classifying microorganisms. This conventional method for culturing could not identify the entire microorganisms in a sample, which made approximately 99% of the microorganisms unknown. Meta-omic method captures the non-culturable microorganisms and their functions (Fadiji and Babalola, 2020). The meta-omics technique as an emerging approach is used for characterizing the functional and structural genomes in a given environmental sample. It has unlocked the understanding of diverse structures and functions exhibited by microbial communities in the plant rhizosphere (Alawiye and Babalola, 2019). (Table 3 highlights the different methods of identifying agricultural beneficial microbial species.)

The meta-omic techniques have aided in studying microbial populations at different levels and functions. Meta-omics techniques include metatranscriptomics, used to differentiate the transcriptome profile of the microbial community and their interactions, utilizing mRNA sequencing and microarray applications (Vargas-Albores et al., 2019). Another is metaproteomics an approach used to classify the totality of complementary protein at a particular time in an environmental sample. This results in understanding the interactions among microorganisms and microorganisms, and between plants and microorganisms (Yu et al., 2019).

Metaproteomics assists in understanding microbial protein profiles and complex metabolic pathway, and identifies the multi-functional genes of microorganisms (Vargas-Albores et al., 2019,

Adegboye and Babalola, 2013). Furthermore, metagenomics with the application of shotgun metagenomic analysis (Illumina MiSeq, NextSeq and HiSeq) predicts at the gene level the entire taxonomic and molecular functions of the microbial community in an environment. Metabolomics specifically evaluates small molecules of low-molecular-weight in biological systems. It is a quantitative and qualitative analytical approach used for metabolite profiling of a huge portion of metabolomes (Jansson and Hofmockel, 2018, Oulas et al., 2015).

Meta-omic applications are classified as next-generation sequencing (NGS) analyzing tools. The NGS approaches build on the strength of omics tools to study plant-associated microorganisms in an agricultural environment. These meta-omics techniques have facilitated the understanding of the variations, structures, abundance, richness and distributions of plant rhizosphere microbial communities (Adegboye and Babalola, 2013, Fadiji and Babalola, 2020). Aside from comprehending the properties mentioned earlier, it is as well used in identifying microbial community members with exceptional qualities and functions in the rhizosphere. Microbial communities of different plants studied using the omics approaches are sunflower, wheat, maize, sorghum, oat, cowpea and soybean (Maropola et al., 2015, Donovan et al., 2018).

Recently, the attention of scientists is shifting from the previous Illumina system or shotgun metagenomics to the latest third-generation sequencing techniques. The most recent third-generation techniques, for example, MinION (Nanopore) and PacBio completes its reads within 4-6 hours and can take up ultra-long reads of hundreds of thousand bases, unlike the Illumina system that takes days, which is the major advantage over the Illumina system. Other advantages of the third-generation techniques are that they are relatively inexpensive, portable and user-friendly. Furthermore, no special training or equipment is required for data analysis. It can be

connected to a laptop or computer system, and can easily generate a microbial profile from full-length 16S or 18S rRNA gene sequence at a significant high taxonomic resolution (Pii et al., 2015, Brader et al., 2017). However, some limitations are encountered when using the MinION (Nanopore) and PacBio, for instance error rate and low accuracy level, which may be due to the restriction to a specific application and continuous long reads.

However, these limitations can be corrected with further improvements (Fadiji and Babalola, 2020). For better knowledge of the microbial variations and structures in the rhizobiome, the combination of more than one of the omics tools is to be employed; this significantly makes the meta-omics technique a fascinating technique in system biology.

Table 2.3: Methods of identifying agriculturally beneficial microorganisms.

Methods	Applications used	Functions	Limitations	Reference
Culture-based	Plating on general or selective culture media view with the aid of a different microscope, biosensor imaging	Isolate and identify agricultural important microorganisms, identified mechanisms of a cell to cell interactions, the pattern of colonization and detection phyto-stimulatory genes	Limited information; Loss of unculturable microorganisms	Fadiji and Babalola, (2020)
Amplicon sequencing/ targeted metagenomics	16S and 18S rRNA amplification using PCR machine, cloning or DGGE, high throughput sequencing,	Extraction of specific genomes of interest from environmental and legumes samples, identifying microbial population and functional genes performing specific metabolic activity;	Limited information about the genome; Damaging sampling	Jünemann et al. (2017), Oulas et al. (2015)

	RFLP, FISH, confocal laser scanning microscopy (CLSM), secondary ion mass spectrometry (SIMS), biosensor or stable isotope labelling, oligonucleotide probes, bioinformatics tools and database soft wares.	analyze microbial structure, identification of microorganisms involves in phyto-biocontrol and macergens genes, characterizing nutrient availability, analyze AMF genome		
Whole-genome metagenomics	Shotgun metagenomics-Illumina Hiseq or Miseq,	Extraction of entire genomes in an environmental sample, Identify both	Number of replicates is limited; require	Martellacci et al. (2019), Donovan et al. (2018)

	bioinformatics tools and database soft wares	known and novel genes and microorganisms responsible for plant-microorganisms interactions, microorganism-microorganism interactions, Identify metagenomes with potential functional and structural genes.	computational power	
Metaproteomics	LC/FT-ICR, 2-dimensional electrophoresis	Profiling of proteins using protein expression profile analysis	Needs computational power; comparison to transcriptomic data	Hu et al. (2018), Alawiye and Babalola, (2019)
Metabolomics	LC-MS, GC-MS, NMR, FTIR, ESI-Micro-T of MS	For identifying and characterization of both novel and known; targeted and	Requires a few numbers of replicate and computational	Sabale et al. (2019), Lagos et al. (2015)

		untargeted metabolites and their specific functions, Profiling of metabolites with biological control ability against phytopathogens; assesses metagenomic pathways	power; large amount of metabolites	
Metatranscriptomics	mRNA sequencing and microarray applications	Screening of untargeted genome; Assessment of metabolic pathways and gene expression	Few numbers of replicates; requires computational power and comparison to metagenomics data	Delmont et al. (2015), Alawiye and Babalola, (2019)

2.10. Conclusion and future prospects

This review has provided detailed insights into various microbial communities and significant impact of PGPR with more focus on the nitrogen-fixing rhizobacteria with specific roles in sustainable agriculture. The nitrogen-fixing genes of rhizobacteria can promote plant growth even when host plants are exposed to biotic and abiotic stressors, such as a prolonged shortage of water and high salinity content. The PGPR can as well act as biofertilizers that can improve soil fertility and increase crop yields, which would reduce the world-wide dependence on chemical-based fertilizers and pesticides known to have a damaging impact on the environment. This is particularly important as these biological fertilizers are inexpensive and ultimately eco-friendly.

The understanding of PGPR mainly the nitrogen-fixing rhizobacteria are required because they are significant microbial groups that propel the activities in the plant microenvironment. The knowledge of the microbial community is advancing into evolution involving integrating different disciplines and applications that are needed. Coupled with the interrelationship between multiple disciplines in science, in-depth knowledge of the rhizosphere and harnessing metabolic products. Advancements in studies are required when more advanced technologies and data analysis software is employed. At this present time, studying the rhizosphere is an emerging field of microbiome research that is significantly progressing in the use of novel techniques. These include fingerprinting and third next-generation sequencing MinION (Nanopore) and PacBio applications in understanding the activities of the microbial communities in the rhizosphere regions. Scientists have the challenge of quantifying the 16S rRNA, 18S rRNA or internal transcribed spacer (ITS) sequences, a Polymerase Chain Reaction (PCR) based method and the sequencing technique that

targets a single genome, either 16S rRNA, 18S rRNA or ITS genes that do not provide comprehensive information on the microorganisms' metagenome diversity and functions.

Omic techniques have bridged the shortfalls of the conventional techniques and the phylogenetic surveys in identifying and characterizing the entire microbial genomes and the genetic features and traits that enable microorganisms to survive in the soil environments. The data obtained using the meta-omic approaches will give a broader view (the description of the microbial components) and assists in improving the methods of combating plant diseases and improving plants growth. For the most part, promote functions worthwhile in agriculture and have a favourable impact on food production globally, and increase food security.

CHAPTER THREE

Elucidating rhizosphere associated bacteria for environmental sustainability

Overview

One of the biggest issues facing the world population right now is feeding them nutritious food that contains a balanced diet of nutrients. The agroecosystems are harmed by the careless application of chemical fertilizers to increase agricultural yield. Similar to this, chemical pesticides used to control plant pathogens and insect pests have had negative impacts on insect predators, including bee populations worldwide, soil biota, and both terrestrial and aquatic flora and fauna. Agrochemicals undoubtedly have a variety of deleterious consequences on human health. As a result, agricultural techniques that involve a greater use of chemical pesticides lead to a reduction, alteration, or even a total loss of native and advantageous microbiodiversity.

Environmental sustainability is a key concern on the global stage. It is well recognized that human activity contributes to environmental degradation, affects the health of the soil, obstructs plant growth and development, and depletes nonrenewable resources. We urgently require the development, adoption, and promotion of environmentally sustainable ways in order to reduce the excessive demand for and usage of synthetic chemicals while also taking into account their long-term adverse impacts on global agricultural ecosystems. Therefore, agricultural sustainability is crucial in this time because it offers the possibility to meet our future agricultural and human needs, something which can be done by using toxic, conventional and unsustainable farming techniques.

Similar to this, the extensive application of chemical fertilizers is causing rich soils to turn acidic. Farming practices that are new and appropriate must be made clear to farmers in order for them to comprehend and embrace them. This is required to address the issue of enhancing productivity while preserving environmental stability.

Plant growth-promoting rhizobacteria, also known as PGPR, are useful as stress-management agents in plants because of their ability to promote plant development, they have been receiving more and more attention in recent years. The natural home of microbes that are advantageous for plants is soil. They participate in several soil activities that affect the general health of plants, crop yields and productivity. Numerous attempts have been made to investigate the diversity of native soil microbes, their distribution and behaviour in soil habitats. In order to ensure that microbial inoculants function effectively and have a positive effect on soil health.

Growth-stimulating bacteria, like PGPR, have the ability to enhance soil health through a variety of mechanisms, including phosphate solubilization, nitrogen fixation, heavy metal sequestration, production of phytohormone (such as gibberellins, indole acetic acid, or cytokinins), decomposition of crop residues matter, suppression of phytopathogens and mineralization of soil organic, etc. As a result, they can help plants grow even in low-nutrient soils.

The biotechnology sector also benefits from PGPR since they offer a vast number of novel genes and biochemical pathways to search for antibiotics, enzymes and a variety of other important substances. As a result, PGPR are widely used in agriculture. Understanding the mechanisms through which PGPR effect soil health and therefore assure sustainable agriculture is crucial for appropriate use of PGPR. Therefore, the main goal of this study is to emphasize the mechanisms

that PGPR uses, their function in enhancing soil health, and their contribution to raising agricultural productivity on a sustainable basis.In the next chapter (chapter 4), a detailed understanding of these microorganisms and their interactions should facilitate an improvement of plant growth and sustainable crop production in soil and soilless agriculture. Hence, the goal of this review is to provide light on metagenomic approaches used to investigate the microbial ecology and related microbiota of plant roots. Additionally, the various DNA-based methods and how they contribute to the development of plant microbiomes are discussed. Metagenomics is allowing us to gain a better understanding of these microbes and their biotechnological potentials, which can then be utilised to create novel, useful, and eco-friendly bio-fertilizers and bio-pesticides.

Abstract

The abundance of nutrient accumulation in rhizosphere soils has placed the rhizosphere as an "epicenter" of bacterial concentrations. Nonetheless, over the years, little attention has been given to bacterial inoculants and soil-like substrates. The reason is that many farmers and experiments have focused on chemical fertilizers as an approach to improve plant growth and yield. Therefore, in this research the focus is on assessing the application of rhizosphere soil and its associated bacteria for biotechnological applications. This review has been structured into major subunits: rhizosphere soil as a treasure trove for bacterial community concentration, biodegradation of lignocellulose for biofuel production, rhizosphere soil and its bacteria as soil amendments and the role of rhizosphere soil and its bacteria for bioremediation and biofiltration. Hence, the efficient use of rhizosphere soil and its bacteria in an environmentally friendly way can contribute to healthy and sustainable environments.

Keywords: Bioadsorbent, Biofuel, Bioremediation, Plant-microorganism interactions, Soil amendment, Sustainable development goals, Soil health

3.1. Introduction

The United Nations (UN) have released blueprints for seventeen sustainable development goals (SDGs) to achieve a better and more sustainable life for people globally by 2030. The second goal (zero hunger) was adopted to end hunger and achieve food security by 2030, ensuring that everyone has sufficient food. This goal seeks sustainable solutions to end hunger in all its forms and to achieve food security. The aim is to ensure that everyone has enough good-quality food to lead a healthy life (Backer et al., 2018). Therefore, to achieve this goal, there is a need for better access to food and widespread sustainable agriculture promotion. This entails improving the productivity and income of small-scale farmers by promoting equal access to land, technology and markets, sustainable food production systems and resilient agricultural practices. It also requires increased investments through international cooperation to bolster the productive capacity of agriculture, especially in developing countries (Backer et al., 2018, Adedeji et al., 2020).

Over a decade, the fight against hunger globally has progressively improved and the proportion of the undernourished population decreased from 15% (2000–2002) to 11% (2014–2016) (Zhu, 2016). Nonetheless, approximately 800 million people do not have constant access to quality and safe food. If prevailing conditions persist, the zero hunger objectives will not be achieved by 2030 (Igiehon and Babalola, 2018b). The incessant hunger is no longer the problem of food availability.

Still, many countries failed to attain the millennium development goals (MDGs) against hunger and human-induced environmental degradation due to advanced food insecurity, especially in sub-

Saharan Africa and southern Asia (Adedeji et al., 2020). Hence, there is a need for novel biotechnological applications of beneficial plant growth-promoting bacteria to improve soil fertility and plant health and, as a result, produce sufficient healthy quality food without having any negative impacts on the environment (Backer et al., 2018).

In agro-based industry, plant root-associated bacteria have a beneficial effect on the growth and yield of crops and forest trees (Enebe and Babalola, 2018, Baghaie and Aghilizefreei, 2019). Plant growth-promoting rhizobacteria (PGPB) are consortia of bacterial species that colonize the plant root region (rhizosphere), impacting plant growth and health advantageously. The PGPB are agricultural bioresources that stimulate plant growth and productivity. They also incite plants' resistance to different phytopathogens in a wide variety of crops, including vegetables, fruits and some trees (Babalola et al., 2020c).

The diversity of bacterial species in the rhizosphere has been used as a biological indicator to estimate soil quality and fertility because they play a critical role in nitrogen fixation, hormone production and nutrient distribution (Yan et al., 2017a). Similarly, they have contributed to the production and oxidation of methane and acetone, and have resulted in the enhancement of the soil pH, water composition, organic carbon content and porosity (Keswani et al., 2019, Ju et al., 2020).

The rhizosphere soils serve as an exclusive natural niche, which houses myriads of bacterial species and their compositions differ with plant species. The most predominant root-associated bacterial community found in the rhizosphere soil are Betaproteobacteria (e.g., *Burkholderia*), Bacteroidetes, Alphaproteobacteria (such as *Rhizobia*), Gammaproteobacteria (like *Pseudomonas*) and Firmicutes (e.g., *Bacillus*) (Nuccio et al., 2020). The rhizosphere soil is composed of a high

abundance of bacterial population compared to the bulk soil. These bacteria from the rhizosphere soil can be harnessed and used in an ecofriendly approach as promising biotechnology for the production of antimicrobials, and can serve as biocontrol, bioremediation and biofertilization agents, thereby improving soil health, soil fertility and crop yield and ensuring environmental sustainability (Yurgel et al., 2019).

Environmental sustainability acknowledges the importance of advancing and controlling the biological and physical systems that bolster both the short- and long-term value of all forms of life on earth without jeopardizing the diversity and well-being of natural ecosystems (Kamutando et al., 2019). By virtue of the ecological services rendered by rhizobacteria, Ambrosini et al. (2018) have recommended further research on the factors that aid in the maintenance of the rhizosphere bacterial community and promote practices that advance rhizosphere conservation and protection. Despite the critical role played by rhizobacteria in redressing soil fertility and environmental sustainability, there still remains the need for further understanding of the mechanisms through which rhizobacteria perform their ecological roles and how such roles can be exploited for environmental sustainability. Therefore, critical discussion on the diversity of bacteria in the rhizosphere soils and their role in lignocellulose degradation, biofuel production, biofiltration and bioremediation, as well as the possibility of achieving soil amendment, was provided.

3.2. The rhizosphere soil as a treasure trove for bacterial community concentration

The rhizosphere is known as the region of the soil that surrounds the root where biological, physical and chemical properties of the soil are modulated by plant processes. The rhizosphere is a hotspot of plant-bacteria interplay within the soil environments (Huang et al., 2014). It is

colonized by diverse bacterial communities, which are functionally and structurally controlled by soil type and texture, environmental factors and plants (Yurgel et al., 2019). Studies have revealed that the plant root exudates and other rhizodeposits lure beneficial bacteria to the rhizosphere, although uninvited ones are also enticed (Backer et al., 2018, Lindström and Mousavi, 2019). The host plant induces selection pressure on the development of the rhizosphere microbiome, which favors and attracts a specific plant microbiota due to variations in the composition of the root exudate (Bukhat et al., 2020).

Odelade and Babalola (2019) stated that there is a higher bacterial biomass in the rhizosphere soil compared to the bulk (rootless) soil, which is as a result of increased availability of substrates for bacterial growth through root exudates, resulting in greater population density and community structure in the root region that may be different from those in the bulk soil. This was supported by a study by De Luna et al. (2005), who stated that the bacterial cell population in 1g of rhizosphere soils is typically 108–1012, surpassing that of the bulk soil, due to various root exudates and rhizodepositions in the root region. This high bacterial density in the rhizosphere soils has been ascribed to the high level of available substrate and humidity (Long et al., 2018).

Reports have suggested that root exudates and various rhizodeposits perform key roles in the richness and diversity of bacterial communities in the rhizosphere. Root exudates have the most diversified nutritional composition compared to other rhizodeposits. They are also versatile in composition and influenced by the plant host and environmental factors (Tian et al., 2020). The root exudates attract beneficial bacterial species to the rhizosphere of plants, while some unwanted bacterial species are also lured (Babalola et al., 2020c). The constituents of root exudates vary between plant species and cultivars, which leads to variation in the rhizosphere bacterial

community. These variations can be manipulated to create specific selective effects on the rhizosphere microbiome (Bakker et al., 2012).

Dennis et al. (2010) stated that root exudates carry out a limited role in controlling the bacterial communities in the rhizosphere compared to the other rhizodeposits (volatile compounds, mucilages, slough-off root cells and lysates) due to large variations in exudate composition and dynamics shown by various studies. Collective evidence indicates that bacterial communities are oftentimes distinct from similar plant cultivars and from bulk soil, but not always. The authors classified these plants as having a delicate rhizospheric effect (Sasse et al., 2018, Lettice, 2018). The mechanisms by which hosts winnow the ambient community to form their microbial communities are not fully understood, although plant functional traits, such as the cuticle composition, may be responsible (Huang et al., 2014). However, shaping and establishment of the rhizosphere microbiome is a selective and dynamic process that involves several mechanisms such as signal recognition, chemotaxis, biofilm formation and antibiosis (Tian et al., 2020).

Root exudation includes the secretion of enzymes, oxygen and water, ion, mucilage and diverse carbon-containing metabolites. The plant root system produces various metabolites, while the root tips secrete most of the root exudates, which are low molecular weight organic substances (such as amino acids, amides, organic acids, sugars, enzymes, phenolic acids and coumarin), high-molecular-weight compounds (such as proteins and mucilages) and other substances, including sterols that attract bacteria to the rhizosphere (Lu et al., 2018, Kari et al., 2019). However, the components of the exudates vary in the amount released, molecular weight and biochemical functions. These exudates act as attraction signals that influence the ability of bacteria to colonize the roots. To proliferate and be established in the rhizosphere, the organisms must be able to use

root exudates, colonize the root or rhizosphere effectively and be able to compete with other organisms (Bukhat et al., 2020). Rhizobacteria locate plant roots through cues exuded from the roots and root exudates, which stimulate plant growth-promoting rhizobacteria (PGPR) chemotaxis on root surfaces. Root exudates can also stimulate flagella motility in some rhizobacteria (Sarhan et al., 2018, Palansooriya et al., 2020). These traits are essential for the colonization of the rhizosphere.

The impact of plant roots was examined on rhizosphere and bacterial communities, and it was deduced that root length, biomass, density, volume and surface area create distinct ecological niches for some bacterial species to improve advantageous interactions in the rhizosphere (Phour et al., 2020). It has been established that since the root tips make the initial connection with the bulk soil, the bacterial communities and rhizodeposits are notable in maintaining the rhizosphere (Naylor and Coleman-Derr, 2018, Igiehon and Babalola, 2018b).

Despite variations in the dynamics and composition of root exudates, a subset of the bacterial population is designated as the core rhizosphere microbiome, which are ubiquitous across plant species and environment (Bukhat et al., 2020). The microbiota uses the root exudates as a source of energy, and the common genera in the rhizosphere include *Burkholderia, Bacillus, Microbacterium, Azospirillum, Serratia, Pseudomonas, Erwinia, Aeromonas, Mesorhizobium, Rahnella, Acinetobacter, Enterobacter* and *Acinetobacter* (Sarhan et al., 2018, Palansooriya et al., 2020). Conventionally, bacterial species in the rhizosphere were isolated and identified using the traditional or culture-based method for isolating and classifying microorganisms. This method's main inadequacy is that it cannot identify the entire microorganism in a sample, making approximately 99% of the microorganism unknown (Baghaie and Aghilizefreei, 2019). Thus, only

a few bacterial populations have been identified from the rhizosphere soils using conventional techniques, but not until the advent of next-generation sequencing techniques (dos Santos and Maranho, 2018). High-throughput sequencing has made the identification of most rhizobacteria possible and also lends credence to their functional role in the rhizosphere (Table 3.1).

Bacterial communities from the rhizosphere have been implicated in synthesizing extracellular hydrolytic enzymes responsible for biodegradation into the soil. Therefore, they are viewed as the leading force manipulating the terrestrial ecosystems. The abundance of nutrients in the rhizosphere not only contributes to plant growth and development but also maintains the beneficial soil bacterial community inhabiting the rhizosphere soil (Babalola, 2010, Backer et al., 2018). Some studies conducted on rhizosphere soils have reported the presence of beneficial bacterial communities essential for biotechnological applications (Table 3.1). Methanotrophic bacteria capable of producing methane from NH4+ were identified from rice paddy rhizosphere soil (Zhu et al., 2018).

3.3. Biodegradation of lignocellulose for biofuel production by rhizospheric bacteria

Agricultural and wood residues produced after harvesting and processing of plants are abundant biomass on earth, and these biomass resources have remarkable energy capacity (Chen et al., 2020). Several tons (estimated to be around 10-50 billion produced annually worldwide (Sharma et al., 2019) of these wastes are produced annually from corn, wheat, soybean, timbers etc. and mainly composed of lignin, cellulose and hemicellulose. The lignocellulose biomass, recognized as the most abundant biopolymer on earth is a predominant component of the cell wall of plants. It is an inexhaustible raw material for biofuel production. The plant cell wall is a heterogeneous

complex of carbohydrate polymers (cellulose and hemicellulose) and an aromatic polymer (lignin) (Enagbonma and Babalola, 2019, Mathews et al., 2015).

Lignin gives the plant a rigid structure that provides protection against the hydrolysis of cellulose and hemicellulose. In spite of the plethora of lignocellulose in nature, the high cost of hydrolyzing them into smaller monosaccharides have made using them unappealing. Therefore, this has led to the search for cheaper means of hydrolyzing lignocellulose biologically (Zwetsloot et al., 2020). Lignocellulose is used in many industrial processes for the production of chemicals, fuels, polymer precursors, paper and pulp, food and flavor compounds. Presently, the search and production of a renewable form of energy resources are of increased interest, which is the outcome of insubstantial areas for petroleum-based fuel production that is depleting and harming the environment (Harun et al., 2010).

For so many decades, plant materials and animal feed composed of carbohydrates have been utilized as biomass energy resources for substituting fuel production. Hence, the fuel produced from this form of resource is referred to as biofuels (Sharma et al., 2019). Several countries and the environment will gain from the profitable use of renewable fuel from biomass rather than petroleum-based automotive fuels (Morgan Jr et al., 2017). The use of biofuel decreases the dependence on petroleum-based oil and the impacts of greenhouse gases, ameliorates air quality and generates new employment opportunities (Harun et al., 2010). The biofuel from lignocellulosic waste streams is ecofriendly, cost effective and thus a priority all over the world. The main challenges of using this waste stream are: the amount of polymerization, the protective lignin is recalcitrant to degradation thus providing a less surface area for enzymatic hydrolysis, biomass particle size and the crystalline nature of cellulose sheathed by hemicellulose (Sharma et al., 2019).

Due to the recalcitrant nature of lignin towards degradation, the conversion of lignocellulose to biofuels and other renewable energy resources involves pretreatment processing, such as biological, chemical, mechanical and thermal processes. The biological pretreatment process involve the use of microorganism for conversion and degradation of lignocellulose streams into sugars to produce biofuel (Mathews et al., 2015). Biological processing is gaining popularity because it requires less energy, involves no chemicals and creates less pollution.

Naturally occurring bacterial species from different niches have developed cellular mechanisms to acquire energy from plant biomass through the production and release of carbohydrate-active enzymes (Harun et al., 2010). The plant cell wall is degraded by these enzymes, which synthesize monosaccharides that can be utilized as biofuel and other value added products. The use of plant biomass by bacteria is crucial for life on Earth because of their involvement in carbon flux in the environment (Velvizhi et al., 2019). The enzymes capable of degrading plant cell wall are widely used in industrial applications, including fuels and chemicals production, and also in the food and feed industry (Harun et al., 2010). Enzymatic degradation of lignocellulose biopolymer is important for sustainable agricultural residues. These residues can be degraded to glucose by cellulases, such as cellobiohydrolase and endoglucanase (Chen et al., 2020).

The two main mechanisms used for biofuel production are direct and indirect fermentation processes (Dou et al., 2020b). The direct fermentation process is a microbial process that breaks down the starting plant materials into sugars capable of being fermented, and later transformed into alcohol (Liu et al., 2018). Bacterial species, for example *Clostridium, Lactobacillus, Microbacterium* and *Leuconostoc,* catalyze the production of fermentation products directly from the substrate (Zwetsloot et al., 2020, Liu et al., 2018).

Table 3.1: Bacteria present in rhizosphere soil and the techniques used in identifying them.

Technique used	Bacteria reported	Plant	Reference
Denaturing Gradient Gel Electrophoresis (DGGE)	*Sphingobacteriales, Flavobacteriaceae, Xanthomonadaceae, Cyanobacteria*	lettuce, soybean, potato, maize	Gómez Expósito et al. (2017b), Chaudhary and Shukla, (2019)
Quantitative PCR (qPCR) analysis	*Bacillus velezensis NJAU-Z9*	pepper	Zhang et al. (2018)
G3 PhyloChip microarray analyses	*Atribacteria, Dependentiae, TM6, Latescibacteria WS3 Marinimicrobia, SAR406, Omnitrophica, OP3, BRC1. Acidobacteria, Gemmatimonadetes* and *Tenericutes*	wheat, barley	Sarhan et al. (2018)
Restriction Fragment Length Polymorphism (RFLP)	*Azospirillum, Pseudomonas chlororaphis, P. frederiksbergensis, Bacillus aryabhattai* and *Paenibacillus peoriae*	maize	Kari et al. (2019)
DNA-Stable Isotope Probing (DNA-SIP)	*Nostocales, Stigonematales, Streptomyces, Bacillus, Alicyclobacillus,*	rice	Wang et al. (2020a), Long et al. (2018)

	Clostridium. *Rhizobiales*, *Rhodospirillales*, Myxococcales and Actinomycetales		
16S amplicon sequencing	*Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Acidobacteria*, *Firmicutes*, *Verrucomicrobia*, *Planctomycetes*, *Actinobacteria*, *Cyanobacteria* and *Gemmatimonadetes*	wheat, maize, potato, soybean	Matthews et al. (2019), Zhong et al. (2019)
Shotgun sequencing	*Stenotrophomonas*, *Rahnella*, *Sphingomonas*, *Janthinobacterium* *Luteibacter*, *Arthrobacter*, *Streptomyces*, *Bradyrhizobium*, *Methylobacterium*, *Ramlibacter*, *Nitrospira*, *Nocardioides*, *Geodermatophilus* and *Burkholderia*	soybean, sunflower, sugar beet	Lu et al. (2018)
Culture-based	*Bacillus*, *Pseudomonas*, *Ochrobactrium*, *Providencia*, *Achromobacter*, *Burkholderia* and *Enterobacter*	wheat	Kumar et al. (2018a)

This process does not require the step of converting the starting plant materials to gas. Comparatively, the indirect fermentation uses the pyrolysis of the starting plant materials to generate a mixture of different gases, such as hydrogen, carbon dioxide and carbon monoxide. Then, acetogenic microorganism are used to transform the gases generated into ethanol (Umadevi et al., 2018).

The production of biofuel from lignocellulose requires different biological processes, such as delignification or pretreatment method (the release of free hemicellulose and cellulose from the lignocellulosic material), depolymerization (hydrolysis) of the carbohydrates polymers from the hemicellulose and cellulose to produce free sugars, and finally, fermentation of mixed hexose and pentose sugars to produce ethanol (Morgan Jr et al., 2017). Various pretreatment processes have been used to alter the plant cell wall; these treatments are necessary to ensure the quality of products made from polysaccharides (Mood et al., 2013). For example, in biofuel production, pretreatment is necessary because lignin prevents access of carbohydrate-degrading enzymes from binding, which limits product yield (Mathews et al., 2015).

Biomass pretreatment methods are divided into physicochemical (steam pretreatment/auto hydrolysis, wet oxidation and hydrothermolysis), physical (milling and grinding), chemical (oxidizing agents, dilute acid, organic solvents and alkali) and, lastly, biological or a combination of all the methods (Alvira et al., 2010). Thereafter, the cellulose and hemicellulose are hydrolyzed into monomers by enzymatic or acid hydrolysis. Furthermore, for the fermentation process, bacteria are used to break down these monomeric sugars into alcohols (Morgan Jr et al., 2017). The integration of these process configurations for biofuel production is efficient, cost-effective

and economical. (The schematic process of biofuel production from lignocellulose biomass is depicted in Figure 3.1.)

Lately, there is increased interest in rhizosphere soils because they house diverse bacterial populations that synthesizes uncommon cellulolytic enzymes important in biofuel industries (Xin et al., 2017). Cellulolytic enzyme such as monospecific endo-β-1, 4-glucanase was obtained from rhizosphere soil through metagenomics-based strategy for bioethanol production (Wierzbicka-Woś et al., 2019). Cellulolytic bacteria such as *Erwinia*, *Sporocytophaga*, *Ruminococcus*, *Clostridium*, *Fibrobacter* and *Cellulomonas* from the rhizosphere degrade cellulolytic materials containing small amounts of lignin (Rai et al., 2019).

Significantly, the degrading mechanism of lignocellulose by aerobic and anaerobic bacteria species differ due to its macromolecular arrangement (Valliammai et al., 2020). Anaerobic bacteria often arrange the cellulase or hemicellulase apparatus into cellulosomes (multi-enzyme complex) composed of enzymes with different activities, including carbohydrate esterases, polysaccharide lysases and glycoside hydrolases (Artzi et al., 2017). The catalytic constituents of cellulosomes are primarily made up of dockerins (a non-catalytic module's structure that coheres to cohesion modules) situated in the large non-catalytic protein acting as a stage. The association between cohesins and dockerins allows the incorporation of the hydrolytic enzymes in the complex (Hyeon et al., 2014). It has been reported that scaffoldins are in charge of binding the whole complex via a non-catalytic carbohydrate-binding module (CBM) to crystalline cellulose (Artzi et al., 2017, Hyeon et al., 2014).

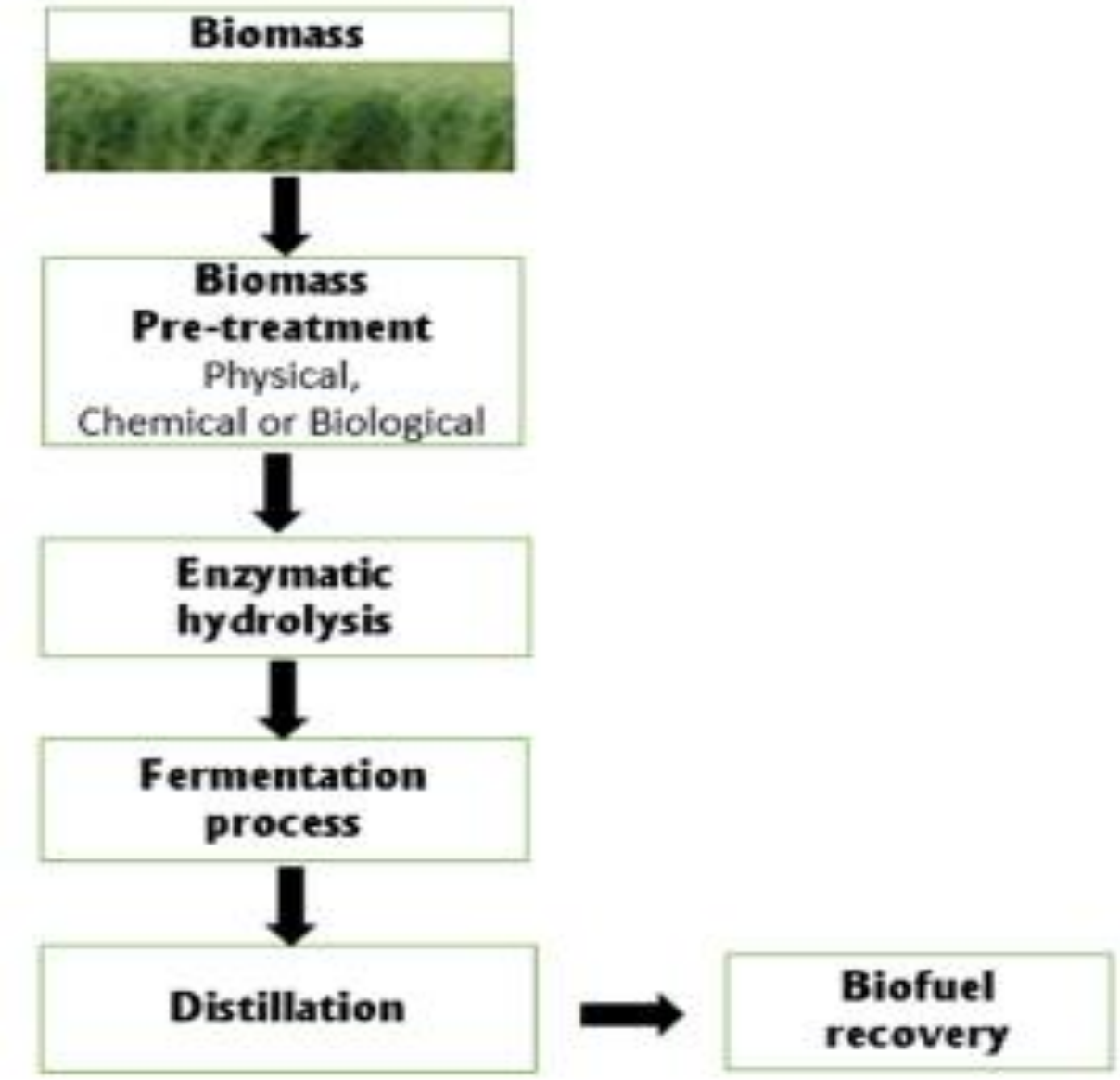

Figure 3.1: Schematic representation of the steps involved in biofuel production from lignocellulose biomass.

Many studies regarding cellulosomes are mainly focused on anaerobic bacteria such as *Clostridium* species (Xin et al., 2017). Others include *Bacteroides cellulosolvens*, *Ruminococcus albus*, *Acetivibrio cellulolyticus* and *Ruminococcus flavefaciens* (Artzi et al., 2017, Prasad et al., 2019b). The potential approach of advancing hydrolytic activity systems is the design and construction of cellulosome-based complexes (Artzi et al., 2017). Likewise, cellulosomes capable of incorporating bacterial enzymes from non-aggregating systems could be produced to augment hydrolytic activities and, accordingly, biomass saccharification (Silva et al., 2018).

Moreover, genetic manipulations are used to introduce genes capable of synthesizing cellulosome into bacteria able to degrade simple sugars (Behera et al., 2017). However, they do not have functional machinery able to degrade plant cell walls. Alternatively, bacteria naturally synthesizing cellulosomes could be manipulated to increase their capacity to produce ethanol from lignocellulose (Valliammai et al., 2020). Xylanosomes, which are self-organizing protein complexes, were designed and constructed using cellulosomes as template (Dou et al., 2020a). They were designed particularly for hemicellulose hydrolysis, but showed alliance with cellulases, and potential use of these nanostructures in cellulose hydrolysis has recommended (Artzi et al., 2017).

Rhizosphere soils are hotspots for bacterial phyla capable of degrading cellulose, such as *Acidobacteria*, *Firmicutes*, *Fibrobacteres*, *Alpha-proteobacteria*, *Actinobacteria* and *Verrucomicrobia* (Dobrovolskaya et al., 2020, Bao et al., 2019). Most strains of these phyla are degraders of plant biomass polysaccharides (Taubert et al., 2019) and can also decompose lignin and phenolic compounds (Zwetsloot et al., 2020). Examples of organisms isolated from the rhizosphere of various plants that produce cellulose and may be used in biofuel production (Table

3.2). Many bacterial populations can transform complex lignocellulose polymers into monosaccharides with the aid of lignocellulolytic enzymes, which are essential for various far-reaching industrial processes, have been isolated from rhizosphere soils (Costa et al., 2020). In a recent study, cellulose degrading bacteria (*Bacillus,* C*hryseobacterium* and *Pseudomonas*) were isolated from the rhizospheric soil and endosphere of forage grass timothy (*Phleum pratense* L.) (Saleh et al., 2019). Similarly, bacterial genes capable of degrading cellulose and xylan have been identified from bacteria such as *Acidobacteriaceae bacterium* from rhizosphere soil (Umadevi et al., 2018). Table 3.2 shows some rhizobacteria isolated from the rhizosphere of different plant species that can be used in biofuel production.

Mukhtar et al. (2019) reported thirty-eight and forty-five bacteria from the rhizosphere soil of *Atriplex amnicola* and *Salsola stocksii,* respectively. These organisms possessed xylanase, cellulase, and many other enzyme activities at 1.0–1.5 M NaCl concentration. The fermentation of simple sugars from lignocellulose degradation by these enzymes is an exceptional potential in biofuel production. Consequently, the knowledge of bacterial activities and environmental conditions influencing the alteration of vast quantities of carbon materials in rhizosphere soils could contribute to new opportunities that can benefit the environment (Table 3.3) (Umadevi et al., 2018, Taubert et al., 2019, Saleh et al., 2019, Zwetsloot et al., 2020).

3.4. Biofertilization: The use of rhizosphere bacteria as a soil amendment for plant growth promotion

Plant growth-promoting bacteria, such as *Azospirillum*, *Rhizobium* and *Azotobacter* enhance the growth of different plants and, thus, are used for biofertilization of many crop plants (Antar et al.,

2020). The effects of plant growth stimulation have been attributed to atmospheric nitrogen fixation, potassium and phosphate solubilization, production of plant growth hormones (auxins, gibberellins, ethylene and cytokinins), polyamines and diverse amino acids produced by PGPR, which improve the nutritional availability of plants directly (Tian et al., 2020). There are several factors to be considered in the formulation of biofertilizer. These factors include the choice of appropriate microorganisms with the potential to colonize plant rhizosphere, the growth profile of the bacteria, appropriate carrier and types and optimum conditions of organisms. The success of the products also depends on the method of application and shortage of the formulation (Itelima et al., 2018).

Table 3.2: Rhizobacteria that produces enzyme that can be used in lignocellulose degradation for biofuel production.

Rhizobacteria	Plant	Country	Reference
Arthrobacter, Brevibacterium, Bacillus, Chrysobacterium, Stenotrophomonas, Streptomyces, Pseudomonas, Xanthomonas Paenibacillus	*Phleum pretense* L.	Canada	Saleh et al. (2019)
Bacillus, Pseudomonas, Kocuria	*Salsola stocksii* and *Atriplex*	Pakistan	Mukhtar et al. (2019)
Streptomyces	*Zea mays*	South Africa	Adegboye et al. (2018)
Arthrobacter, Pseudomonas	Quercus sp.	Spain	Lasa et al. (2019)

Any material added to a soil to improve the physical and chemical properties of the soil, such as structure, water infiltration, water permeability, aeration and drainage, which, as a result, provides a more suitable environment for plant roots and health, is referred to as soil amendment (Palansooriya et al., 2020). Owning to the distinct physical and chemical properties of rhizosphere soils due to the microbial activities, they are the epicenter for nutrient accumulation. However, reports on soil and soil-like substrate amendments are limited. Currently, other methods widely employed as soil amendment include the excessive application of growth enhancers, chemical fertilizers, pesticides and soil sterilization approaches, such as fumigation with methyl bromide (MeBr). Although these treatment methods can be efficient for controlling environmental stresses, they have harmful impacts on human health and the environment and long-term adverse effects on soil health and quality (Hamid et al., 2019).

Table 3.3: Biotechnological products manufactured from some rhizospheric bacterial phyla and its industrial applications.

Bacterial phylum	Plant rhizosphere	Biotechnological product and application	Industrial application	Reference
Actinobacteria	*Helianthus annuus*, *Zea mays*, *Triticum aestivum*, *Glycine max*	Kanamycin enhanced shoot growth.	Actinobacteria secrete cellulases suitable for cellulosic biofuel production.	Yadav et al. (2018), Patel et al. (2016)
Proteobacteria	*Zea mays*, *Oryza sativa*, *Saccharum officinarum* and *Glycine max*	Bioinoculants- significantly increased crop yield, biomass dry weight, nodulation, phosphorus and nitrogen uptake. Bioprotectants protect plant from phytopathogens.	Bioremediation strategies for the degradation of oil spill contamination (edible such as fats and lipids; and crude oil) as well as carbamate and organophosphate insecticides.	Kour et al. (2019)

			Z*ymomonas* - produces abundance of alcohol for industrial use Acetic acid bacteria can be employed for the production of acetic acid, vinegar, ascorbic (vitamin c), glucoronic, galactonic, arabonic acids and sorbose.	
Firmicutes	*Triticum aestivum* and *Vitis vinifera*	Nitrogen-fixing ability, enhance soil porosity and produce compound similar in activity to indole-3-acetic acid with the capacity to stimulate plant growth. Biocontrol activity effective against a wide range of phytopathogens, such as Fusaricidin identified as a potential	*Paenibacillus polymyxa* produce 2, 3-butanediol (BDL) forms methyl ethyl ketone used as a liquid fuel additive by dehydration. Produce cell wall degrading enzymes (proteases, β-1,3-glucanases, xylanase, chitinases and cellulases) available in	Song et al.(2019)

		antifungal agent, has been identified from *P. polymyxa* E68. In addition, control *Fusarium oxysporum.* Flocculants production.	detergent formulations, leather processing, food industry (starter culture for yogurt production, additives and beer production), waste management and chemical synthesis. *Lactobacillus pentosus* has been applied in sulfite waste liquor fermentation. Flocculating or flocking agents used for water treatment.	
Bacteroidetes	*Brassica napus*	Use alternative enzymatic mechanisms to solubilize biopolymers apart from	Produce enzymes exhibiting activities such as degrading cellulose, lignin or chitin. In addition, various lipids,	Wieczorek et al. (2019), Gkarmiri et al. (2017b)

		glycosidic hydrolases, the so-called "polysaccharide-utilizing''. Degrade complex polysaccharides in soils and contributes to synergistic breakdown of solubilized chitin oligosaccharides.	polysaccharides, or proteins used in industries such as leather processing, detergent, paper and shoe production. Used for biofuel production. Used in phytoextraction of heavy metals from polluted soil.	
Acidobacteria	*Castanea crenata*, *Saccharum officinarum*, *Vigna mungo* and *Solanum lycopersicum* L	Produce exopolysaccharide (EPS), which provide protection against environmental stress and enable bacterial survival under unfavourable soil conditions. Form soil matrix, serve to sequester water and nutrition, and be involved in bacterial cell-surface adherence and	EPS possess physical and chemical properties such as thickening, gelling, stabilizing, suspending, emulsifying, texture-enhancing and coagulating. Although some of these bacterial products (e.g., gellan gum, dextran, alginate and xanthan) have been	Kielak et al.,(2017)

	soil aggregate formation. In addition, they produce plant growth-promoting traits and phytohormones. Produce biofilms, which enhance rhizobacterial root colonization by holding moisture and protect plant roots from phytopathogens.	commercialized successfully. In the food and fodder production industry, EPSs are used as gellings, thickening and suspending agents. For instance, xanthan (from *Xanthomonas campestris*) is used as food additive. EPSs are bioemulsifiers that are used in the cosmetic and chemical (e.g., pesticide) industries. Use in environmental technologies, such as phytoremediation and bioremediation in soil and water by enhancing oil and heavy metal

			recovery. In addition, use in human health and chemical industry.	
Nitrospirae	*Panax ginseng* Meyer	Plant growth promoter: Nitrite-oxidizing bacteria (NOB) are involved in nitrification, including the oxidation processes of ammonia and nitrite. In addition, the are known to convert nitrite to nitrate, improve shoot/ root biomass, improve nutrient uptake, alleviate cold stress in plants and serve as biocontrol agent.	Potential in the petroleum industry for the exploration of petroleum, clean-up of oil spills both in in-situ and ex-situ conditions and enhance microbial oil recovery. Bioconversion of food waste and activated sewage sludge into useful products. Biohydrometallurgy (microbial recovery of minerals from ores), used for fuel production and for clean-up of oil spills and	Wei et al. (2020)

	deterioration of petroleum products.

Promising alternatives such as using materials like bacterial soil amenders (PGPR) integrated with soil for enhancing the physical and chemical qualities of the amended soil, consequently improve plant health, performance and growth. Plant growth-promoting rhizobacteria can improve crop growth through various mechanisms (direct or indirect), including improving water acquisition, increasing soil nutrient bioavailability, suppressing plant diseases and decreasing herbivore damage (Ghori et al., 2016). Cui et al. (2019) inoculated maize with *Bacillus amyloliquefaciens* B9601-Y2, which consequently controlled the impacts of *Biopolaris maydis* (southern corn leaf blight) by colonizing the main roots and root hairs and later migrating to the stems and leaves. Likewise, *Bacillus amyloliquefaciens* B9601-Y2 notably improved maize seedling growth (height, number of leaves) and chlorophyll content.

The physical and chemical parameters of rhizosphere and bulk soils are relatively variable, which shows that the rhizosphere soil has an abundance of physical and chemical properties (Fan et al., 2018). This is due to different bacterial interactions and high nutrient concentrations. Several studies have revealed that the rhizosphere soils have significant levels of organic carbon, nitrogen, ammonium nitrate and clay content as well as soil mineral nutrients such as phosphorus, iron, zinc, magnesium, copper, manganese, sodium, calcium and potassium compared to the bulk soils. This is due to symbiotic nitrogen fixation, root exudation of organic acids, cluster root formation, plant secretion of phosphatases and rhizosphere pH modification (Angst et al., 2018, Fan et al., 2018). An experiment conducted by Maseko et al. (2017) showed that the rhizosphere soils had approximately two-to-three times more phosphorus, copper, sodium and potassium, and six times more manganese than the bulk soils. The clay content in rhizosphere soils increases their

waterlogging and soil porosity. The high concentrations of these minerals naturally increase bacterial and phosphatase activities, which could be due to root exudation and, as a result, improve plant growth and yields (Angst et al., 2018).

Considering the exclusivity of the bacterial population in the rhizosphere soils, many authors have suggested their use as soil amendments in low-input farming systems (Deng et al., 2019) (Table 3.4). A major challenge with applying and using biofertilizers on the field is their inconsistency and thus unreliability, which calls for innovative solutions. This could be as a result of the diverse growth habitat and community structure of plant roots. The can create a stressful environment for the proliferation of the product (Timmusk et al., 2017). Another limiting factor for their use is their selectively, resulting in variable quality and efficiency on the field. Innovative solutions that direct the product to the desired location and target crop and an understanding of the root microbiome dynamics and flux in plant metabolic networks can increase the chances of product effectiveness and reproducibility (Singh et al., 2019c).

3.5. The role of rhizosphere soil and its bacteria for bioremediation and biofiltration

Recently, researchers have given in-depth attention to bioremediation, which is a process that mainly stimulates and uses microorganism such as bacteria, plant enzymes or plants to degrade and treat target pollutants in soil and other environments by manipulating the environmental conditions (Singh et al., 2019c). Phytoremediation, a combination strategy used by plants and microorganisms to remove pollutants from the environment, has proven to be effective in

Table 3.4: The rhizosphere soil and rhizobacteria as a soil amendment.

Plant	Impact of rhizobacteria on the plant	Reference
Capsicum annuum L.	The soil amended with *Bacillus velezensis* improved pepper seedling plant height and stem diameter of the plant trees and yields more than those pepper plants grown on un-amended soil	Zhang et al. (2018)
Arabidopsis thaliana	Combined mixture of rhizosphere soil or soil-like substrates and *Bacillus* mixtures resulted in a significant increase in plant root fresh, shoot fresh weight, nutrient uptake, chlorophyll content and plant diameter. Also, increased the transcript levels of ammonium and nitrate uptake genes in the plant	Calvo et al. (2019)
Helianthus annuus	*Pseudomonas fluorescens* A506, *P. gessardii* strain BLP141 and *P. fluorescens* strain LMG 2189 improved plant growth, yield, physiology, proline and antioxidant activities, and reduced the malanodialdehyde content in inoculated soil	Saleem et al. (2018)
Ocimum basilicum L.	Rhizobacteria consortium (*Bacillus lentus*, *Pseudomonas* sp. and *Azospirillum brasilens*) had positive effects on the antioxidant activity and	Enebe and Babalola, (2018)

	chlorophyll pigment content under water-induced and salinity stress	
Festuca rubra	Bacterial consortium immobilized in the mixture of perlite and sawdust (ratio 1:1:1 v/v) (diameter in 3–10 mm) led to a substantial improvement of plant roots, stem length and stem biomass, as well as influenced the elongation of the plants in all soil treated. Soil additives (phosphate fertilizer and sewage sludge) and an immobilized consortium of microorganism had a positive effect on plant growth (longer root, stem length and stem biomass) in comparison to the control	Grobelak et al. (2018)
Eucalyptus globulus	Co-application of biochar (20 t hm^{-2}) and PGPB (5 × 10^{10} CFU mL^{-1}) amendments significantly decreased the concentrations of soil total P and NH_4^+-N, whereas they advanced total K, NO_3-N, and soil water content, hence maintained soil sustainability in eucalyptus plantation	Ren et al. (2020)
Curcuma longa	*The Curcuma longa soil amended with B. subtilis* MML2490 and *P. aeruginosa* MML2424 enhanced plant growth promotion and management of turmeric rhizome rot disease, thus appeared promising for commercialization	Chenniappan et al. (2019)

decontaminating soil polluted with heavy metals and hydrocarbons. Some bacterial species have synergistic interactions (direct or indirect) with environmental factors capable of removing hydrocarbon and heavy metal pollutants from soils, which consequently successfully increases the movement of these pollutants to the above-ground plants' biomass. Rhizoremediation, a phytoremediation approach, is plant-microbe cooperation with the potential to remove soil pollutants through the action of microorganisms and plant enzymes in the rhizosphere (Correa-García et al., 2020).

Many PGPR strains capable of removing pollutants from environmental media are found in the rhizosphere soils (Kalaimurugan et al., 2020). dos Santos and Maranho (2018) reported the efficiency of *Bacillus*, *Alcaligenes*, *Microbacterium* and *Curtobacterium* isolated from the rhizosphere soil in heavy metal transformation. For example, *Pseudomonas fluorescens* isolated from rhizosphere soil was inoculated into maize soil polluted with cadmium in research conducted by Asilian et al. (2019). Their discovery revealed that maize soil amended with 2 mmol kg^{-1} Tween 80 had a better Cd uptake than the control (soil uninoculated). Moreover, they reported that soil samples inoculated with 4 mmol kg^{-1} Tween 80 increased translocation efficiencies and phytoextraction (a subprocess of phytoremediation in which plants remove heavy metals from soil or water even at relatively low concentrations) than soil uninoculated with *P. fluorescens.* Therefore, they proposed that phytoextraction of heavy metals can be achieved by *P. fluorescens.* The properties and mineral compositions of rhizosphere soil place them as an excellent accumulator (adsorbent) of heavy metals and a promising technique for soil remediation capable of purifying soil from contaminants (Ghori et al., 2016).

This is evident in research where Mourato et al. (2015) used lead (Pb) metal phytoextraction to reveal that *Brassica nigra* and *Brassica juncea* rhizosphere soils have high heavy metal-bioaccumulating ability than those uninoculated. Other salient findings showed that cultivar of *B. juncea* rhizosphere soil was the most adsorbent of Pb (up to 3.5% on a dry weight basis) compared to the uninoculated soil (Tangahu et al., 2011). Hence, the tight binding of Pb to plant and soil materials explains the low movements in soil and plants. In another experiment, the researchers reported using of grass species (*Cenchrus ciliaris*) rhizosphere soil as a tool for removing Pb and Cu from aqueous solutions (Kumar and Fulekar, 2018).

The result from this investigation revealed that the rhizosphere soil absorbed Pb up to 97.31 ppm and 188.3 ppm of Cu. They concluded that rhizosphere remediation using *C. ciliaris* rhizosphere is a compelling and effective green innovation for remediation of heavy metals from soil. Xu et al. (2020a) used cadmium (Cd^{2+}) resistant *Pseudomonas* sp. 375 from heavy metal polluted rhizosphere soil as an adsorbent to remediate water body polluted with Cd^{2+}. *Pseudomonas* sp. was used as an inexpensive and potential bioadsorbent for bioremediation of Cd^{2+} from wastewater. Other rhizobacteria and pollutants they removed are listed in table 3.5.

Additionally, Canizo et al. (2019) stated that bacterial byproduct such as Extracellular polysaccharides or Exopolysaccharides (EPS) can be employed for the bioremediation of heavy metals from soil and water. The review gave an extensive assessment of the biosorption of crystal violet (CV) dye from effluents and natural water using *Rhodococcus erythropolis* AW3 biomass as a biosorbent. The results revealed that Langmuir isotherm

model had the highest biosorption capacity (289.8 mg g^{-1}) in removing CV dye from effluents and natural water using *R, erythropolis*.

Through the process of decomposition, bacterial species can decompose plant materials to produce a remarkable number of greenhouse gases. Yuan et al. (2018) reported that paddy soil adds 10% of total global atmospheric methane (CH_4) emissions. However, this depends on the bacterial species involved. Nevertheless, rhizosphere soils harbor many methane oxidizing bacteria such as acetoclastic *Methanosaeta* and hydrogenotrophic *Methanocella* that function as biofilters. These bacteria can adsorb the methane produced in the soil by bacteria and reduce the quantity of methane released into the surroundings.

In a study to ascertain if bacteria in the rhizosphere soil can mitigate the emission of methane, Aimen et al. (2018) reported that methanotrophic bacteria colonizing the rhizosphere soil were capable of oxidizing most of the methane formed in the rhizosphere before it was discharged. This is because *pmoA* and *mcrA* genes, which encode methane monooxygenase subunit, possess a strong affinity for methane; even at a low concentration of 6.25 kg ha^{-1} methanotrophs can minimize the emission of methane by 60% (Davamani et al., 2020).

Table 3.5: Rhizobacteria and pollutants removed from the environment.

Rhizobacteria	Isolation source	Pollutant	Reference
Bacillus thurigiensis, B. pumilus and *Rhodococcus hoagie*.	*Panicum aquaticum*	Petroluem	Viesser et al. (2020)
Lysinbacilus fusiformis L8, *Bacillus weihenstephanensis* UT11, *Paenibacillus* sp. M10-6,	*Hosta undulata*	Alkylphenol	Chang et al. (2020)
Ensifer, Novosphingobium, Norcardioides, Streptomyces, Rhizobium	*Coronilla varia, Vigna unguiculata*	Phenantrene	Jiao et al. (2019)
Mycobacterium gilvum	*Phragmites australis*	Pyrene, benzo[a]pyrene	Toyama et al. (2011)
Bacillus subtilis, Bacillus amyloliquefaciens	*Lactuca sativa* L.	Cadmium	Wang et al. (2020b)
Microbacterium hydrocarbonoxydans,	*Phragmites australis*	Colored distillery	Chaturvedi et al. (2006)

Achromobacter xylosoxidans,		effluent		
Bacillus subtili, B. megaterium,				
Alcaligens faecalis,				
Pseudomonas migulae				
Alcaligenes, Bacillus,	*Prosopis laevigata,*	As(V), Pb(II),	Roman-Ponce	et
Curtobacterium,	*Spharealce*	Cu(II), Zn(II)	al.(2017)	
Microbacterium	*aangustifolia*			
Bacillus sp. CIK-512	Zea mays	Pb	Ahmad et al. (2018)	

3.6. Concluding remarks

Biotechnology is an important and rapidly developing field of technology worldwide for its significant contribution to food, health and environmental sustainability. In the quest of employing naturally occurring materials for biotechnological purposes, rhizosphere soil bacterial materials were reviewed as a prospective essential tool. This is because they house important beneficial bacteria useful in the production of biofuel, bioremediation of heavy metals and biofilteration of gases, and can also be used as soil amendment. To maximize the complete benefits of rhizosphere soil for biotechnological purposes, there is a need for further critical research to use the metabolic potentiality of bacteria found in the rhizosphere soils, subsequently unveiling their complete potential. Moreover, to certify a sustainable application of rhizosphere bacterial materials in the future, experiments should be performed to ameliorate factors that stimulate and improve rhizosphere soil restoration by bacteria.

CHAPTER FOUR

Metagenomics: A tool for exploring microbiome for sustainable agriculture

Overview

The development of omics approaches, which have lately acquired popularity in the study of microbial diversity and abundance, was facilitated by developments in molecular biology. In 1998, the term "metagenomics" was first used to describe the analysis of the whole genetic components extracted straight from the environmental samples. More significantly, it is a key technique used to investigate the intricate community of bacteria that inhabit environmental materials by examining the nucleotide sequences.

Targeted or shotgun sequencing strategies may be used in metagenomic research; the choice will mostly rely on the nature of the environmental investigations to be carried out. Recent years have seen a significant increase in the use of environmental samples' genomes as an important tool for comprehending of the ecological biodiversity, functional, structural and evolutionary history. It fully negates the necessity for specimen isolation and culture in the lab. Soils, sediments, water, plants, occasionally acquired from marine sources, benthic specimens, gut contents and feaces are only a few examples of the environmental samples used for the study. When processing complicated environmental samples, the information from the traditional Sanger DNA-sequencing approach will be irrelevant because it can only be used for single DNA sequencing.

These kinds of samples do contain a lot of DNA, from hundreds to millions of different people. Although conventional/traditional sequencing has made it possible to amass sizable barcode DNA reference libraries, the amount of DNA in an ambient sample is greater than what can be constrained by conventional sequencing. With the use of next-generation sequencing (NGS), it is now possible to directly recover DNA sequence information from environmental samples. These data are said to have a wide range of uses, some of which include comparing the microbiota in healthy and unwell people; studying the ecosystem's biodiversity; studying DNA evolution; and analyzing feces or intestinal DNA fragments.

To confidently present the many taxa found in the environmental samples, the genetic data will be compared with an increasing standard reference library of identifiable organisms. Through the application of annotation and DNA clustering using alignment and phylogenetic techniques, recent advances in computer approaches have improved the research of biodiversity across time and geography. Ecological study is increasingly focused on exploiting huge amounts of sequence data as a result of the commensurate increases documented in number of data and both breadth using NGS platforms. This method has reduced the inaccuracy and bias in PCR-related data, and it also greatly reduces run time.

Recently, a variety of platforms have been found, each with benefits and drawbacks. These platforms have been recognized as using unique templates for preparation and various chemicals for sequencing signal detection. The section used the agricultural plants as a case study to go into great detail about the metagenomics approach for community analysis of microorganisms

inhabiting the rhizosphere beneficial for food production. Additionally, a thorough review of the computational techniques and tools used for the study of the communities of rhizospheric microbes, including taxonomic composition, similarity and differences, alpha and beta statistical analyses, and functional analysis of microbial genes, were covered. The next section (chapter 5), focuses on trends in PGPR use and implementation, it promotes PGPR as one of the alternative methods for sustainable agro-ecological systems, where intensive agricultural practices, including the use of external chemical inputs have decreased crop productivity.

Abstract

Microorganisms are immense in nature and exist in every imaginable ecological niche, performing a wide range of metabolic processes. Unfortunately, using traditional microbiological methods, most microorganisms remain unculturable. The emergence of metagenomics has resolved the challenge of capturing the entire microbial community in an environmental sample by enabling the analysis of whole genomes without requiring culturing. Metagenomics as a non-culture approach encompasses a greater amount of genetic information than traditional approaches. The plant root-associated microbial community is essential for plant growth and development, hence the interactions between microorganisms, soil and plants is essential to understand and improve crop yields in rural and urban agriculture. Although some of these microorganisms are currently unculturable in the laboratory, metagenomic techniques may nevertheless be used to identify the microorganisms and their functional traits. A detailed understanding of these organisms and their interactions should facilitate an improvement of plant growth and sustainable crop production in

soil and soilless agriculture. Therefore, the objective of this review is to provide insights into metagenomic techniques to study plant root-associated microbiota and microbial ecology. In addition, the different DNA-based techniques and their role in elaborating plant microbiomes are discussed. As an understanding of these microorganisms and their biotechnological potentials are unlocked through metagenomics, they can be used to develop new, useful and unique bio-fertilizers and bio-pesticides that are not harmful to the environment.

Keywords: Bioinformatics, Micro-ecological niche, Omic techniques, Rhizosphere, Shotgun metagenomics, Sustainable food production

4.1. Introduction

The soil environment is a highly complex terrestrial environment and one of the main reservoirs for microbial diversity, harboring a wide range of different genomes. The soil is the hotspot of a myriad of agriculturally beneficial microorganisms. These microorganisms interact both with one another and with plants to improve soil quality and enhance crop productivity. The microorganisms therefore have positive influences on agricultural sustainability. Sustainable and innovative agriculture are among the solutions to the global challenges regarding the degeneration of the quality and quantity of natural resources for growing crops and identifying key agriculturally beneficial microorganisms and their function.

The relationships between agriculture, environmental sustainability and social systems suggest that the developments in agriculture are dependent upon many complex interactions, including

green technology and innovations, biodiversity conservation, plant and animal health, cultural acceptance, pollution management, economy and political stability (Goel et al., 2017) as well as urban agriculture. Moreover, some estimates suggest that the world population will reach 10 billion by 2050, and with such dramatic population growth there is a need to improve crop production for sustainable agriculture and food security (Levy and Lubell, 2018).

In 2015, the United Nations established seventeen sustainable development goals (SDGs) to improve the livelihood of human populations worldwide. The SDGs second goal, termed zero hunger was proposed to solve the problem of hunger by advancing sustainable agriculture globally, hence, accomplishing global food security and increased global nutrition. The goal of this agenda is to seek sustainable solutions to end all kinds of hunger, thereby achieving food security in the world by 2030 (Grafton et al., 2015). The SDGs target is to ensure that everyone globally has good quality food and a healthy life.

To realize this goal, there should be demand for better access to quality food, widespread awareness of modern agriculture and the use of advanced technologies (Agler et al., 2016). To improve agricultural productivity governments and agricultural stakeholders can provide assistance to farmers, including financial incentives for small scale farmers, promotion of equal access to land, trainings on the use of innovative technologies, creation of markets that are suitable food promotion systems, and good agricultural practices. Also, there is a need to increase investment and research through international bodies to boost agricultural production capacity in developing countries (Singh et al., 2019d). Enabling environment for sustainable production of

foods in bioponic systems, which is a type of urban agriculture, should be encouraged. Urban agriculture is an emerging technology that aims to tackle the different aspects of 'urban sustainability' which include, but is not limited to food security, waste management and equitable food supply (Lovell and Taylor, 2013, Ackerman et al., 2014, Russo et al., 2017). In developed nations, urban planning captured agriculture as a transient activity for empty geographical areas that can later be converted into more lucrative commercial, industrial and residential land uses (Van Veenhuizen and Danso, 2007). Nevertheless, urban agriculture has become a durable initiative by improving profits via mass production of perishable vegetables that are costly to transport from rural settlements, specific marketing approaches, including plant growth using organic manure, crop selection diversification and integrating non-agricultural services e.g. tourism, establishment of decent and lively dwelling environment, cooking services, nature rehabilitation and environmental enlightenment (Plakias, 2016, Pölling et al., 2016, Pölling et al., 2017).

For almost two decades, the fight against world hunger has experienced little progress due to the gradual integration of advanced technologies into farming practices in the developing nations. Despite a global decrease in the number of undernourished persons in the past 15-20 years, over 790 million people still experience irregular access to an adequate food supply (Grafton et al., 2015). Thus, if this current trend continues the zero hunger mandates will not be achieved by 2030. Therefore, the need to increase production strategies and overcome various challenges is critical. It is necessary to make the availability of nutrients a priority, and to give precedence to the

cultivation of plants that are more tolerant of marginal lands, biotic factors and environmental changes (Brader et al., 2017, Chadwick et al., 2015).

Studies have shown that improving soil health can increase crop productivity by 10-50 %, and when it is combined with the use of microorganisms with plant growth-promoting traits there can be a 50 - 60% productivity increase (Abram, 2015). This approach can reduce the use and dependence on chemical fertilizers. To reach sustainable agriculture goals many agricultural microorganisms are present as an alternative to chemicals. Unfortunately, it is estimated that 99% of microorganisms found in the natural environment are non-culturable using conventional techniques (Brader et al., 2017, Chadwick et al., 2015).

Microbial communities perform essential functions in the soil ecosystem despite the fact that the functions of the unculturable microorganisms are largely unknown. Microorganisms differ in the ways that they forage for food, harness energy, interact, compete and associate with each other. However, the diversities that we are aware of are just a small part of the microbial population. To better understand the total soil microbial community, it is possible to employ metagenomics, the sequencing and analysis of DNA extracted from environmental samples. This approach may be referred to as ecogenomics, environmental genomics or community genomics (Abram, 2015).

Metagenomics can contribute significantly to the study of agroecosystems. Metagenomics can facilitate an understanding of the huge microbial diversities available in the soil environment, for the production of novel molecules of therapeutic importance, biotechnological advancements and for sustainable agriculture (Garrido-Oter et al., 2018). Until now, most previous research has

focused on biological features of cultivable microorganisms and how these organisms can decrease environmental impacts. While this approach has met with some measure of success, it is intrinsically limited due to the fact that it ignores the role of the vast majority of soil microorganisms (Bevivino and Dalmastri, 2017).

In the soil environment, the interactions between microbial community members help in maintaining the soil ecosystem integrity, nutrient recycling, conservation of natural habitats, balance of the food chains, and important physiological activities in the plants and animals. A highly diverse microbial taxa are also linked with plants in a singular entity that is known as holobiont (Suárez and Stencel, 2020, O'Brien et al., 2019); these plants associated microbial taxa interactions could be neutral, pathogenic or beneficial (Igiehon and Babalola, 2018a). Thus, all agricultural production depends on closely related biological organisms that co-inhabit and interact with microorganisms and their internal and external environments (Martínez-Porchas and Vargas-Albores, 2017).

Microbial communities associated with agricultural practices have not yet been well studied (Bevivino and Dalmastri, 2017). However, the emergence of metagenomics has offered exceptional opportunities to understand the microbial community structure in a specific microniche of interest. This approach is important, since microbial community structure provide insights into the characteristics of microbes (e.g. bacteria, archaea and eukaryotes) as determined by functional gene composition, including the microbial composition (that is, the identity and relative abundances of all taxa) in the community (Philippot et al., 2013a). Therefore,

understanding the structural profile of bacterial, archaeal and fungal communities and their compositions is now possible (Martínez-Porchas and Vargas-Albores, 2017) in soil and soilless agriculture.

4.2. The problematics of urban agriculture: a motivation for the use of omics tools to ensure sustainable agriculture

Urban agriculture involves food production and consumption within cities and is practiced by different individuals motivated and driven by different socio-economic conditions (Simon Rojo et al., 2014). It can range from 'urban gardening' to 'large scale urban farming' (Duží et al., 2017).

It is sacrosanct to mention some of the adverse effects of urban agriculture irrespective of its positive benefits (Duží et al., 2017), as such knowledge will help to decide on the way to go to ensure environmental and agricultural sustainability. Some urban areas are presently not conducive to live in since they are characterized by a particular amount of air pollution cum soil contamination from activities such as urban agriculture. Also, urban soils especially soils situated close to industrial clime or heavy traffic roadways may habour organic wastes that may pose some health and environmental hazards (Nehls et al., 2015, Schwarz et al., 2016). Urban agriculture can also result in the degradation of urban milieu by contributing to an increase in carbon footprint if it is not practiced in an eco-friendly manner (Mok et al., 2014, Duží et al., 2014). Therefore, despite the stern motivation to encourage urban agriculture, the environmental and health implications of this practice should not be underestimate (Duží et al., 2017). Agricultural practices in the rural areas have also come with their shortcomings. Some of these challenges can be addressed by

harnessing the microbiome of such environments as it is believed that some microbes are endowed with the capacity to remove and/or detoxify pollutants emanating from agricultural and industrial sites (Igiehon and Babalola, 2019a, Igiehon, 2015). Such microbes with novel ability to create a pollutant-free environment can be detected by exploring omics tools (Ruiz et al., 2021, Guerra et al., 2018, Ma et al., 2020).

4.3. Omics approaches used for soil and soilless microbiome analyses in the context of sustainable agriculture

In the mid-1980s, scientists began to sequence the 16S rRNA genes from isolated bacterial samples (Brader et al., 2017, Head et al., 1998, Handelsman, 2004, Fadiji and Babalola, 2020). In 1998, the term metagenome came into general use when Handelsman et al. (1998) described soil microorganisms as essential sources of novel natural compounds. In general, studies conducted using a metagenomic approach are conducted by collecting environmental samples, DNA extraction of the total DNA, which can be done using DNA extraction kits (Table 4.1). The DNA samples are enzymatically fragmented with the use of commercially available library preparation kits such as Nextera Tagmentation (Illumina) kits and Fragmentase (New England Biolabs) which uses either endonucleases or transposases (Sabale et al., 2019). Other DNA fragmentation methods that generates DNA fragments of many kb in length include the use of acoustic shearing, centrifugal shearing, sonication, needle shearing and point-sink shearing. Furthermore, the concentration and purity level of the DNA libraries are determined by using the Qubit and Nanodrop spectrophotometer. Also, the integrity of the sampled DNA are evaluated by loading

DNA sample on agarose gel and differentiating size distribution to a suitable marker, such as lambda DNA (Farouk et al., 2020). On the other hand, DNA samples can be run on an Agilent TapeStation® using a Genomic DNA ScreenTape.

Further, the extracted DNA fragments can then be cloned into a suitable bacterial plasmid that acts as a vector. The ideal plasmid vectors are characterized by their small size, origin of replication (Ori site), unique restriction site, selective marker (antibiotics resistance) and multiple cloning sites (Granjou and Phillips, 2019). Finally, the fragments are analyzed using fragment analyzer system (a parallel capillary electrophoresis machine that automates the analysis of the quantity and quality of library preparations) and normalized. Sequencing is performed using different sequencing platforms such as Illumina, Pyrosequencing, ABI SOLiD sequencing system, as well as Nanopore sequencing (Zhang et al., 2021, Martin, 2011). Then the short reads are de-multiplexed and data are analyzed (Mahmoud et al., 2019).

The advancement of next-generation sequencing techniques (e.g. amplicon sequencing and shotgun metagenomics) has great potential in alleviating the challenges of capturing the whole profile of the microbial communities (Jansson and Hofmockel, 2018). Shotgun metagenomics is used extensively to study the microbial diversity and functional genes in various environmental samples (Table 4.2). This sequencing method is a recent replacement of the phylogenetic marker (taxonomic) or targeted genes sequencing (Zhang et al., 2021). For instance, recent studies have shown that Illumina Hiseq and MiSeq sequencing have been used to study microorganisms from

Table 4.1: Widely used DNA extraction kit for identifying rhizospheric microorganisms.

DNA extraction kit	Application	Company	Reference
DNA Qiagen plant mini kit	Isolation of pure total DNA. Purification of DNA from fungal and plant tissues.	Qiagen, USA	Goodwin et al. (2016)
Genejet Bacteria Genomic DNA Kit	Isolation of microbial DNA void of contaminants from wide range of gram negative and gram positive bacteria based on special silica membrane technology.	Thermofisher Scientific, USA	Goodwin et al.(2016)
Modified Mericon extraction	It is specific to easily, and rapidly purify DNA in a user-friendly spin-column format. Isolation of high-quality microbial DNA from plant tissues.	-	Oulas et al. (2015)
Plant DNAzol Reagent	For isolating microbial DNA of plant tissues.	Invitrogen, USA	Zhao et al. (2019)
Fast DNA SPIN Kit for soil	Aids in microbial DNA isolation from soil, plant and living tissues.	MP Biomedicals, USA	Zhao et al. (2019)
EZNA Plant DS mini kit	Recovery of microbial DNA from fresh, dried or frozen plant tissue having a high	Omega Bio-Tek, USA	Zhao et al. (2019)

	concentration of polysaccharide, polyphenols, or lower DNA quantity.		
ZR Soil Microbe DNA Kit MiniPrep	Rapidly isolates microbial DNA from humic free PCR-quality genomic DNA from microorganisms in the soil. Isolate DNA from tough-to-lyse bacteria, fungi, algae and protozoa inhabiting in a wide range of soil types.	Zymo Research, Irvine, CA, USA	Zhao et al. (2019)
Genejet soil DNA kit	Fast and efficient isolation of high-quality microbial genomic DNA from soil samples and other environmental samples. It uses silica-based membrane technology in the form of a convenient spin column.	Thermofisher Scientific, USA	Zhao et al. (2019)
EZNA SP plant DNA kit	Fast isolation extraction of high-quality microbial DNA from the different variety of plant tissues (root/ leaf), and species.	Omega Bio-Tek, USA	Mahmoud et al. (2019)
Nucleospin Plant II-Lysis Buffer (PL1 and PL2)	Isolation of microbial DNA from materials from plants, fungi species and other biological samples.	Macherey Nagel, Germany	Mahmoud et al. (2019)
Quick-DNA Faecal/ Soil Microbe kit	Simple and rapid isolation of inhibitor-free, PCR quality microbial DNA and host cell from		Mahmoud et al. (2019)

	a variety of sample sources (biological and soil samples).	Zymo Research, Irvine, CA, USA

farmland soil. Babalola et al. (2020a) studied the diversity of microbial species from sunflower rhizosphere soil from Bloemhof, South Africa. These researchers found that the microbial community consist of mainly six genera *Conexibacter, Streptomyces, Methylobacterium, Burkholderia, Geodermatophilu* and *Nocardioides.* Wang et al. (2019a) identified Chloroflexi, Actinobacteria, Acidobacteria, Gemmatimonadetes and Proteobacteria using Illumina HiSeq from farmland soils in Jilin province, China.

Also, Wu et al. (2018b) identified different bacterial phyla from *Rehmannia glutinosa* rhizosphere and bulk soil samples using 454 pyrosequencing. The major bacterial phyla reported were Proteobacteria, Bacteiodetes, Actinobacteria and Firmicutes. *Bacillus subtilis, Alternaria alternata* and other microbial species have also been identified in plant rhizosphere. The limitation in this method is that it requires up-to-date databases for accurate microbial identification (Wu et al., 2018a).

Illumina MiSeq has also been used to sequence microbiome of plant roots and organic matter obtained from bioponic system. In the study, it was observed that the plant roots and organic matter contain different microbial taxa (Wongkiew et al., 2022). Management practices, plant type and substrate qualities of another type of urban agriculture initiative e.g. rooftop farming have been reported to significantly impact the abundance and composition of microbial community and other living organisms of the rooftop farming environment, stressing the need of experimental and/or practical studies unique to rooftop agriculture (McGuire et al., 2015, Williams et al., 2014, MacIvor and Ksiazek, 2015, Bretzel et al., 2017, Ksiazek et al., 2014, Aloisio et al., 2019).

Metagenomics has been used to detect microbiome genomic characteristics that are involved in plant-microbial interactions (Bulgarelli et al., 2015; J. Xu et al., 2018). These microbiome genomic characteristics include abiotic and biotic stress response, production and use of different metabolites, chemotaxis, ability to produce toxin and cellular mobility. Beneficial characteristics that are also useful to plant growth such as phosphate solubilization, siderophore production, nitrogen fixation and mobilization and indole-acetic acid production capacities are embedded in some microorganisms associated with plants in soil (Sessitsch et al., 2012, Xu et al., 2018, Carrión et al., 2019, Li et al., 2019b), bioponic and hydroponic environments. These microbial functional traits needed for plant growth and sustainable agriculture have been studied using NGS technique (Igiehon et al., 2019).

Although, metagenomic analyses provide in-depth understanding of the functions of plant microbial community in the soil, evaluation of the associations between plant and microorganisms will only be comprehended when these functional characteristics or traits are studied in-situ. This can, however, be accomplished when metagenomics techniques are combined with other techniques such as transcriptomics and proteomics (Trivedi et al., 2021).

Even though the most commonly used next-generation sequencing technique is the Illumina system, recently the attention of researchers is shifting towards the application of MinION (Nanopore) and PacBio (Oulas et al., 2015). The new sequencing approaches are the latest third-generation sequencing technology. The main advantage of the third generation sequencing approach is that the reads are completed in between 4-6 hours rather than in days.

Table 4.2: Some advantages and disadvantages of amplicon and shotgun metagenomic sequencing.

Sequencing techniques	Advantages	Disadvantages	Reference
Amplicon sequencing	i. Short preparation time and little DNA input number; Allows flexibility of different experimental designs, inexpensive and reduced turnaround time.	i. Lower sensitivity; primers used for PCR amplification can introduce bias by binding to regions that are not conserved across all targeted microbe.	Benitez et al. (2017), Sabale et al. (2019)
	ii. Highly targeted approach to discover and screen genetic variants; widely used for phylogeny and taxonomy profiling.	ii. Limited resolution, short read lengths and high sequencing errors. Lower reproducibility and poor quantification due to deficient random sampling, sequencing error and amplicon biases.	Abram (2015) Marchesi and Ravel (2015), Wu et al. (2018b)
	iii. Uses oligonucleotide probes to capture the regions of interest and delivers	iii. Difficulty in assessing operational taxonomic units (OTUs).	Lucas et al. (2018) Goodwin et al. (2016)

highly targeted resequencing, including in different regions, like GC rich regions.	iv. Single marker gene due to constant horizontal gene transfer and difficulty in defining the specie.
iv. Supports multiplexing of hundreds to thousands of amplicons per reaction to achieve high coverage.	v. Identify amplicon only genus level
v. Efficient variant identification and characterization; discovery of rare somatic mutations, such as tumor combined with germline DNA.	

Shotgun metagenomic sequencing	i. No bias with PCR amplification and more efficient for taxonomic composition classification.	i. DNA preparation methods, sample complexity and sequencing platforms could lead to bias.	Ngara and Zhang (2018), Li (2018)
	ii. Detailed microbial diversity and composition; Better resolution and increased accuracy.		Garrido-Oter et al. (2018) Sabale et al. (2019)
	iii. Do not target and amplify a specific gene.		Vargas-Albores et al. (2019).
	iv. Metagenomic data can be used for additional analysis, such as assembling, binning, antibiotics and metabolic function profiling		

Further, this technique can obtain ultra-long reads up to hundreds of thousand bases (Goodwin et al., 2016). This approach is less expensive, can be connected to a laptop or computer, and does not require any special computer training or equipment for data analysis (Kerkhof et al., 2017). The third-generation technology generates a bacterial and archaeal population profile derived from the full length of the 16S rRNA gene sequence to provide high taxonomic resolution (e.g. at the genus level) (Johnson et al., 2019, Winand et al., 2019).

After sequencing and obtaining the metagenome data, it is essential to preprocess the raw data in order to have high quality reads for further analysis, and this can be achieved by using UCHIME, MG-RAST and RDP tools (Bolger et al., 2014), as well as Trim Galore and Trimmomatic software (Sun, 2020). The platforms frequently used for deionizing metagenome data are MOTHUR and QIIME 2, while UCHIME is employed for cross-checking and expulsion of chimeras from the raw sequenced metagenome datasets (Santamaria et al., 2018). At the end of pretreatment and processing, the reads are grouped depending on their discrete barcodes, and then the primers are removed (Sabale et al., 2019, Kumar Awasthi et al., 2020). A number of computer software programmes for categorizing reads into taxonomic and functional groups are available, including daSILVAta (Dröge et al., 2015) and MEGAHIT (Liu et al., 2015).

Also, *De Novo assemblers*, including Meta-IDBA, metaSPAdes, Genovo, Ray Meta and Contig Extender, have emerged recently and they are employed in sequencing novel microbial genomes where there is no reference metagenome sequence available for alignment (Table 4.3) (Kumar et

al., 2018b). Metagenome sequence reads are assembled as contigs, and the coverage quality of the sequence data depend on the size and continuity of the number of gaps in the data.

Generally, the problem of *de novo* assembly is confirmed to be NP-Hard and as a result this problem cannot be resolved effectively (Medvedev et al., 2007). Some challenges of employing the *de novo* assemblers are memory usage, higher assembling time and CPU on paired-end or joined data sets are higher than single-end data sets, for example ABySS (Khan et al., 2018a). Contigs can be separated using SqueezeMeta. SqueezeMeta employs different procedures to separate the contigs. Essentially, binning algorithms classify contigs emanating from the same genomes because their nucleotide structure is similar (Tamames and Puente-Sánchez, 2019). Also, SqeezeMeta uses DAS Tool to combine the numerous binning results to form a single set (Sieber et al., 2018).

Table 4.3: Some popular phylogenomic analytical tools for phylogenetic and taxonomic classification.

Popular phylogenomic tools	Use	Reference
Accurate Species TRee ALgorithm (ASTRAL)	Provides a statistically reliable valuation of the true species tree from unrooted gene trees, under the multi-species coalescent model.	Rabiee et al. (2019)
Unifrac metric	Places communities in a phylogenetic context by building a large phylogenetic tree, and then estimating the quantity of the tree that is shared between more than one communities. Furthermore, calculates the amount of phylogenetic diversity within a single sample.	Hollister et al. (2015)

Molecular Evolutionary Genetics Analysis (MEGAX)	Helps in comparative analysis of homologous gene sequences either from different species or from multigene families with a distinct emphasis on inferring patterns of DNA and protein evolution as well as evolutionary relationships.	Kumar et al. (2018b)
EDGAR (Efficient Database framework for comparative Genome Analysis using BLAST score Ratios)	Calculates genomic subsets, compares gene content, and use for some visual result representation such as Venn diagrams or pairwise synteny plots. Furthermore, used for various sophisticated analyses with emphasis on phylogenetic and statistical analyses.	Yu et al.(2017)

IMG/M (Integrated Microbial Genomes with Microbiome Samples)	Estimates pairwise Alignment fraction and ANI values to indicate the phylogenetic distance between genomes. It also provides a phylogenetic tree browser, synteny plots and phylogenetic profiles like gene distributions. In addition, contains annotated archaeal, bacterial and metagenomic sequence datasets.	Markowitz et al.(2012)

4.4. Phylogenomic analysis of environmental microorganisms that are of importance in sustainable agriculture

Phylogenomics involves evolutionary reconstructions which gives insights into the relationships that exist between organisms by comparing the entire genomes and their functions. The result from a phylogeny (species tree) is primarily required for many analyses with the purpose of gaining more insights into biological process by reorganizing the evolutionary history (Figure 4.1) (Emms and Kelly, 2019).

Although, this strategy is among the best and commonly accepted computational techniques in analyzing sequenced metagenomic data, but there are still many limitations affecting its usage, for instance the species tree. The main areas that fall under phylogenomics are establishment and clarification of evolutionary relationships, prediction of gene functions, and prediction and retracing of lateral gene transfer (Raymann, 2014).

Phylogenomic analyses usually involve different steps, including comparing the pairwise sequence among the entire genomes, that is, resolution of homologous relationships among gene-coding genes, like the gene families inference or orthologous groups (Rabiee et al., 2019). This places restrictions on the number of data set through which de novo inference of similar relationship can use (Emms and Kelly, 2019). Actually, the step prefers culture-independent and model organisms, as well as clinical isolates which bring about bias in sampling, resulting in unbalanced data sets, and also without reflecting the correct microbial diversity in the sampled community. This could give misleading information on the phylogenetic inferences. Lastly, the widely accepted clonal

approach for microbial replication stops gene mutations in numerous genetic backgrounds, hence makes it extremely difficult to identify and differentiate the impact of mutation and homogenous genetic background (Hollister et al., 2015).

The challenges of phylogenomic analyses of genes of interest in large microbial community include failure to recognize the actual original genetic determinants, partial assembly, assembly errors, redundancy and inaccurate positive results (Yu et al., 2017, Young and Gillung, 2020). To overcome the challenge of relatedness, it is better to focus more on the observable changes that occur during evolutionary histories rather than only the genomes genetic features (Earle et al., 2016).

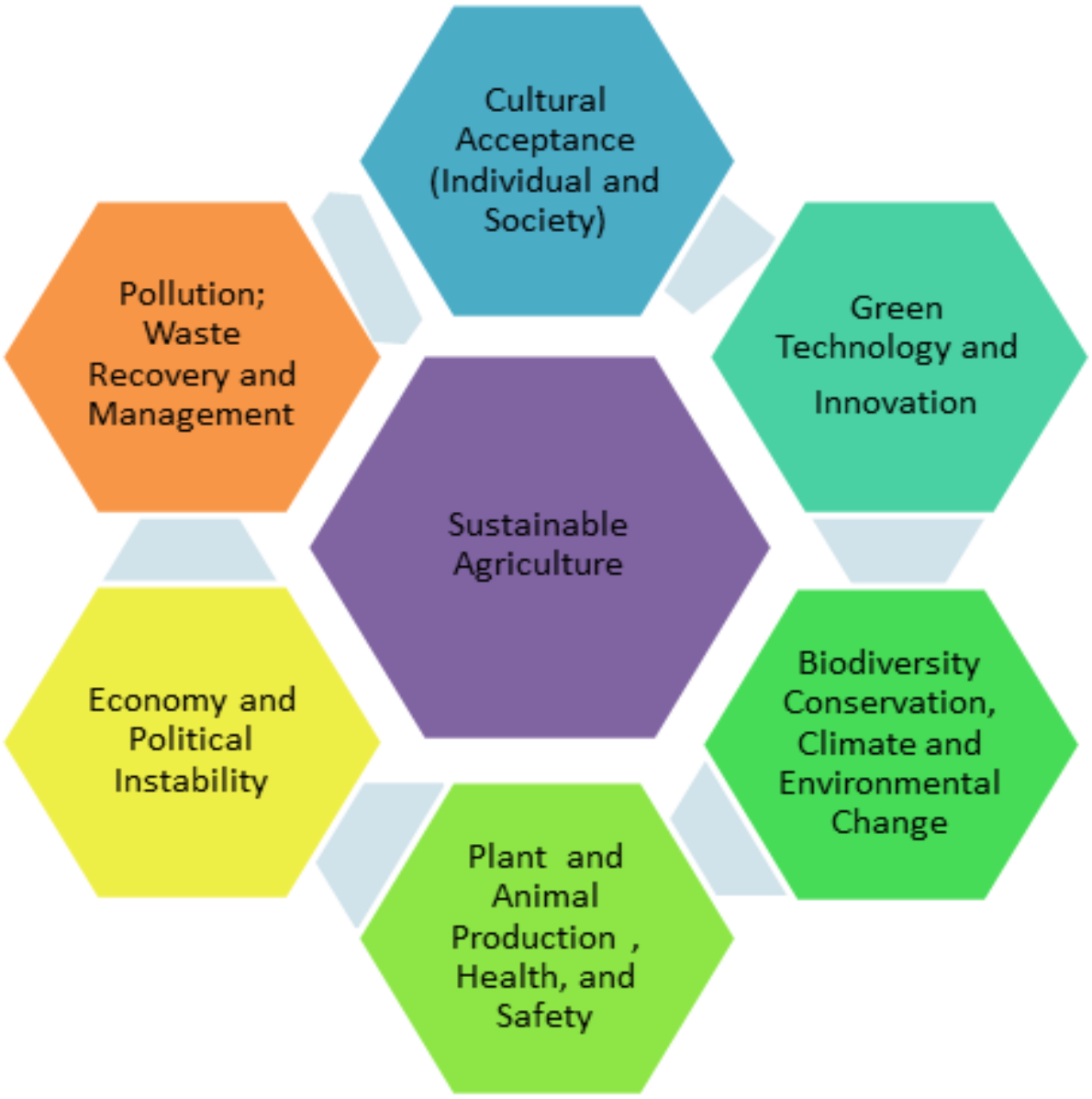

Figure 4.1: Integration of sustainable agriculture into the society.

For most experiments involving microbial community analyses, it is essential to focus on evolutionary history of the microbial community of study against the host phylogency background (Kumar et al., 2018b). Unfortunately, proving the hypothesis is not feasible when studying microbial taxonomic diversity and genomes between different host species because the significant differences are not reasonable between niche adaptation and true co-evolution. In spite that the correlation between host species phylogenic tree and microbial community member phylogenies can be employed as evidence of co-diversification. However, it does not provide convincing knowledge of the basis for genetic interrelatedness (Earle et al., 2016, Markowitz et al., 2012).

4.5. What can metagenomics do?

4.5.1. Metagenomic applications for sustainable sustainable agriculture

Meta-omic approaches as next-generation sequencing methods are used to explore metagenomes from the environmental samples and are used to identify essential genes of special functions and/or bioactive compounds. Meta-omics supports different scientific research areas in agricultural sciences and provides an understanding of the functional potential of genomes in different species that are of agricultural benefits (Handelsman, 2004).

Currently, many molecular information arising from proteome (proteins) and transcriptome (all sets of RNA) sequencing is important for better comprehension of gene content, specific functions and metabolic networks that are then translated to livestock or crop breeding, disease resistance, health and yields. Consequently, they have led to several advancements in agriculture and

biological sciences (Prosser, 2015). Also, different pieces of evidence reported have shown the contributions of genomic studies in agriculture that have span beyond identifying or manipulating genes from samples associated with a particular trait and genomic breeding (Goel et al., 2017, Pinu et al., 2017).

Agricultural genomics is focused on finding novel solutions through studying livestock or crop genomes. For this reason, researchers have obtained information that would deal with issues of protecting the crops or livestock and the increase of food products in the food industry, so that food security is achieved (Makonde et al., 2015). Microbial communities from the soil and plants are key players in agriculture, determining plant health, soil features and biogeochemical characteristics, as well as influence crop yields and ultimately quality traits. The knowledge of the soil environment is sparsely available. For example, researchers have recorded that on earth, soil is the largest reservoir of carbon, while the prokaryotic microorganisms are richly abundant in the soil environment. The traditional techniques over the years have only identified a few of these microorganisms (Pinu et al., 2017).

The application of metagenomics in agriculture has not only profiled plant growth-promoting rhizobacteria (PGPR) but also has described their functional characteristics. The approach has proved to be an appropriate technique in understanding the different interactions occurring in the soil environment, rhizosphere and plant tissues (Gupta et al., 2018). The metagenomic approach has successfully identified diazotrophs from the rhizosphere soil of red kidney beans by targeting nitrogen fixing gene (nifH) (Sabale et al., 2019).

Correspondingly, metagenomics is revealed as a useful tool in tracing the shift in taxonomic component and functional redundancy of rhizobiomes. Soil microorganisms are unlimited and critical members of the ecosystem playing integral roles in triggering plant growth, development, defense and stress responses in plants (Gupta et al., 2018). Therefore, for progressive sustainable agriculture production, the knowledge of the relationship between plants and soil microbial community using metagenomics will be helpful in designing crop systems. Applying metagenomics techniques in the study of soils inoculated with organic manures would be useful in formulating strategies for fertilization and reduce farmers' dependence on chemical-based fertilizers (Manjula and Narsimha, 2015).

At this present time, the toxic impacts of chemical-based fertilizers and pesticides on the agricultural ecosystem, plant health and development are having a noticeable damaging influence on the environment. Among the abundance of microorganisms are beneficial agricultural microorganisms that can act as a critical option to attaining agricultural sustainability production. On the contrary, the full potentials of these microbial communities are still untapped (Goel et al., 2017).

Another key point is that considering the intriguing resources that could be unravelled from the microorganisms, hence the need for developing novel genes with plant growth-promoting traits that serve as bioinoculants which can contribute to agricultural sustainability (Gupta et al., 2018). Ordinarily, identifying and characterizing the rich microbial community becomes unavoidable. According to Goel et al. (2017), metagenomics can be used for sustainable agriculture

advancement programs that can regularly assess the plant growth-promoting microorganisms. For this reason, it requires a comprehensive, correct analysis and characterization of essential beneficial plant growth-promoting microorganisms and their functionalities rather than just cataloguing.

Similarly, functional metagenomics can be employed for the reshaping and readdressing of microbial activities in the rhizosphere, otherwise termed rhizosphere engineering (Goel et al., 2017, Louca et al., 2018). In recent whole-genome studies, metagenomic applications have unlocked the diverse metabolic, genomic and phylogenetic resources stored in the metagenome of soil microbial communities (Garrido-Oter et al., 2018). Agricultural soil is reported as the most challenging and diversified terrestrial environment that is richly embedded with unlimited resources. While, enabling many soil microbiologists to overcome the complex challenges of culturing microorganisms in a pure state, and capturing the uncultured and unculturable percentage. The metagenomic approaches for screening libraries from soil genomes are sequence-

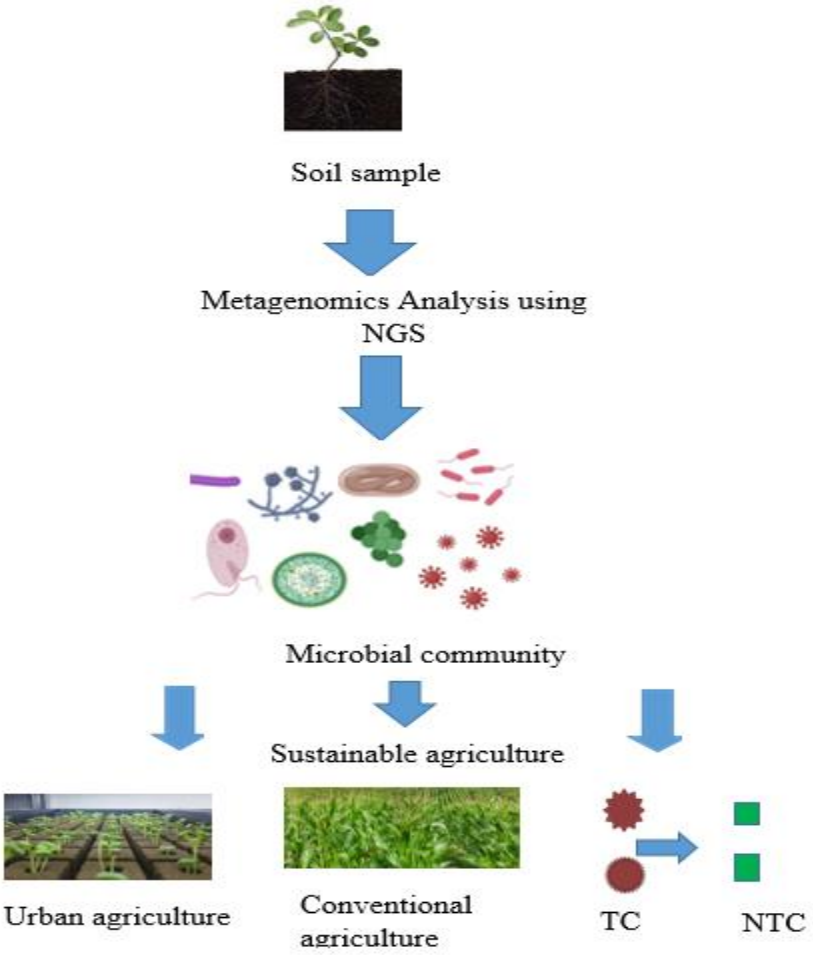

Figure 4.2: Soil microbial genetic diversity and their applications in sustainable agriculture. Microbes obtained and identified from rhizosphere soil can be harnessed to enhance plant growth and productivity in both urban and conventional agriculture. Some of such microbes may also play a role in converting toxic contaminants to their respective non-toxic forms. NGS-Next generation sequencing, TC-Toxic contaminants, NTC-Non-toxic contaminants.

driven and function-driven techniques that are suitable, flexible and feasible. Employing one or both of the methods helps researchers to evaluate a larger number of new products important for agricultural purposes (Garrido-Oter et al., 2018) (figure 4.2).

Altogether, metagenomics provides information on culture-independent soil microbial communities that are influenced by environmental conditions, agricultural management, and land-use practices, like the use of pesticides, fertilizers and tillage systems. Different biomolecules are synthesized due to the response of diverse microorganisms to edaphic changes, including other essential factors. To sum it up, the concentration of metabolites that are released differs owing to different factors, namely nutrient concentration, pH and temperature affecting the uptake and secretion of metabolites in the soil environment (Gupta et al., 2018), and even in bioponic systems.

Microorganisms present in bioponic systems aid in the breakdown of organic matter to the forms that can be utilized by plants (Cáceres et al., 2018, Sharma et al., 2017). Knowing that bioponics is driven and facilitated by microbial activities, understanding microbial interactions and functions are key to the assessment and enhancement of the efficiency of bioponic environments. Metagenomic studies involving microbial compositions and functions, co-occurrence microbiome network as well as ‘discriminant analysis’ have provided insights into the microbial community of bioponic systems (Hu et al., 2017, Langille et al., 2013, Thomas et al., 2011). For instance, symbiotic interconnectivity and keystone taxa can be detected using co-occurrence microbiome networks, while discriminant analysis can unveil microbial biomarkers that are significantly present under certain conditions (Thomas et al., 2011, Tan et al., 2020). Metagenomics

investigations, when coupled with efficiency assessment may, therefore, help to enhance bioponic systems (Bao et al., 2021) and sustainably improve urban agriculture.

4.5.2. Plant-microorganism interactions: soil health

Soil agro-ecosystem is a well-known hotspot of network systems with different biodiversity because it is a special environment. Soil hosts plant and diverse microbial communities performing many diverse interactions. The soil at the plant root region (rhizosphere) is colonized by numerous microbial populations (rhizobiomes) coexisting and performing a variety of beneficial functions and, therefore, make the rhizosphere soil a habitat where there are exchanges of energy among microbial communities. The rhizobiomes are the notable driving force that influences the soil structure and physical-chemical compositions that guide the ecosystem and for that reason contribute to the organization.

Rhizosphere soil has a special capability to conform to environmental changes due to the microbial communities inhabiting the soil that is sensitive to climate changes, land use and management. The microbial ecologist has documented that the natural ecosystem, including soil environment, has the capacity of self-healing — that is, returning to its initial state after disturbance. Over the years, soil has been used for the cultivation of crops and food production. The soil as the most biologically active terrestrial niche with a large number of cultivable, un-cultivable, unknown and known microbial communities is challenging to study (Jansson and Hofmockel, 2018). Soni and Goel (2011) employed the metagenomic approach to evaluate the microbial population from different rhizosphere soil samples. They reported metagenome from rhizosphere soil with ni*f*H

genes having nitrogen-fixing ability. Metagenomic studies on soil biochemistry have revealed many functions that are not known to be of importance to life sustenance (Goel et al., 2017).

Along with the use of various intense agricultural management practices that affected the soil microbiome without positive influence, many practices, including the use of agrochemicals, excessive use of chemical fertilizers, erosions, saline run-off on soil environments, economical use of land for housing and multiple cropping, are the leading cause of soil degradation over a long period that has led to the challenges to sustainable agriculture (Maropola et al., 2015). Add to this the use and overuse of machinery that deteriorate the fertility, structure and vigour of soil. Consequently, microbial life cannot be sustained. To sustain soil health and improve soil characteristics, it is necessary for the agricultural ecosystem and the survival of humans.

Metagenomics has improved the knowledge of the microbial community that is essential to the plant environment and the interactions that occur in the soil environments that are negatively impacted by land preparation practices. In a report by Chávez-Romero et al. (2016) different microbial genera, including *Bacillus*, *Sinorhizobium*, *Agromyces*, *Lysobacter* and *Streptomyces* from soil impacted by tillage-residue management were documented. Similarly, metagenomics was employed to identify Firmicutes and Actinobacteria from wheat plant materials that were used in soil amendment resulting improved soil by increasing the abundance of microbial communities (Chávez-Romero et al., 2016).

Presently, different modern biotechnological strategies have been employed to identify the essential microbial community that can improve soil health, nutrient availability and increased

crop yields. Microbial diversity identified from soil using the shotgun metagenomic technique was incorporated into the soil through agricultural practices (soil amendment). The soil amendment increased soil fertility, improved plant growth and crop productivity. The frequently identified microorganisms from the soil with the potential of impacting soil health, include Spirochetes, Norcadia, Chloroflexi and many more microbial communities (Manjula and Narsimha, 2015).

Furthermore, the interactions that occur between plants and microorganisms in the rhizosphere is ever dynamic and complex (Philippot et al., 2013a, Turner et al., 2013). Some of these interactions have been shown to sustainably improve food production in agriculture, while others are not. Even though plant-microbial interactions can enhance aboveground crop yield, compounds generated in the aboveground region may have either positive or negative impacts on the rhizosphere microbial community structure and vice versa (Igiehon and Babalola, 2018a). For instance, there was a decline in the diversity of belowground endophytes owing to the action of salicylic acid, which triggered the defense mechanism of *Arabidopsis thaliana* in the phyllosphere region, whereas, plants devoid of jasmonate-elicited defense mechanism showed greater epiphytic diversity. This further showed that specific plant immune system differentially affect belowground microbial communities (Philippot et al., 2013a). Also, symbiotic interactions have been reported to occur between plants and microorganisms. A typical example of such is the interaction between plant and mycorrhizal fungi, in which the fungi aid in the supply of nutrient to the plant hosts, while the plants supply carbon substrates to the fungi for survival (Zarik et al., 2016). For example, a consortium of arbuscular mycorrhizal fungi (AMF) co-inoculated with Rhizobium species was

also observed to improve the yield of soybean plants in the North West Province of South Africa (Igiehon et al., 2020).

Symbiotic interactions do not only arise between plant and microorganisms, but there are also microorganism-microorganism interactions. In particular, the interaction between some plant growth promoting bacteria and arbuscular mycorrhizal fungi (AMF) is symbiotic as the bacterial species help the AMF in establishing symbiotic interaction with their host plant while AMF enhance the invasion capacity and diversity of the bacteria. Nevertheless, the bacteria and AMF benefit in other ways from the symbiosis. Symbiosis has also been observed between *Burkholderia* species (a bacterium) and the fungus *Rhizopus*, which depends on metabolites produced by the bacterium to produce spores and survive (Braga et al., 2016).

During plant growth, there may be modification of the surrounding soil, nutrient content and soil microbial community (Bennett et al., 2017, Fujii et al., 2018). Such modifications might feed-back on plant survival and growth (that is, plant-soil feedback), thereby changing plant population (Bennett et al., 2017). The impacts of mutualists on plant-soil feedback are mainly positive since they pacify the negative plant-soil feedback caused by nutrient depletion or presence of natural enemies. Positive plant-soil feedback is said to occur when, for instance, there is an increase in the amount of nutrient and population of mutualists in the environment, while negative plant-soil feedback might occur as a result of nutrient depletion, release of autotoxic chemicals and increase in the population of natural enemies (Bennett et al., 2017, Kulmatiski et al., 2017, Smith-Ramesh and Reynolds, 2017). AMF can facilitate positive plant-soil feedback and this might be influenced

by the relative quantities of various natural enemies and nutrients since AMF helps more in the absorption of phosphorus than nitrogen and protect host plants against pathogens, but not root herbivores (Teste et al., 2017, Delavaux et al., 2017). Thus, AMF can drive positive plant-soil feedback when there is a limited amount of phosphorous or when plant pathogens are more than root herbivores. Rhizobacteria can cause positive plant-soil feedback in symbiotic relationships with leguminous plants by enhancing the growth of the legumes and feedback as free living bacteria (Revillini et al., 2016).

4.5.3. Rhizosphere soil microbial genetic diversity assessment in relation to sustainable agriculture

Soil is considered as one of the most diverse environments containing billions of microorganisms. The soil environment is mainly composed of bacterial and fungal species that are made of thousands of taxa. Again, large organisms, like nematodes, ants and moles cohabit in the soil (Sergaki et al., 2018). Soil microbial ecosystems are composed of microflora and microfauna that co-exists while playing different essential roles in improving plant growth and all-round developments. This is mostly dependent on soil diversity and integrated nutrient management systems (Priya et al., 2018).

A gram of soil from the rhizosphere could contain numerous microbial units (10^9) and distinct taxa upto $4x10^6$ CFU/ ml (Bulgarelli et al., 2015, Lagos et al., 2015). The common traditional cultivation of rhizospheric microorganisms has hampered microbiological studies. In effect, a large number of bacteria and other groups of microorganisms are yet to be cultivated in the

laboratory. Microorganisms identified using the common traditional culture methods are less abundant in the soil micro-niches. Besides, the well-known microbial species isolated from soil habitat are mostly from Proteobacteria, Bacteroidetes, Firmicutes and Actinobacteria, the few major phyla that can be cultivated easily in the laboratory under growth conditions (Babalola, 2010).

Previous studies have shown that metagenomic approaches have given a more comprehensive picture of the rhizospheric microbiome, and many new rhizosphere microorganisms will be discovered in the shortest space of time (Figure 4.3). Most of these microorganisms have been implicated to be useful for the enhancement of crops in urban agriculture, while others have the potential to remove contaminates from the environment. Moreover, other researchers identified Proteobacteria, Acidobacteria, Bacteroidetes, BRCI, Chloroflexi, Actinobacteria, Firmicutes, Cyanobacteria, Planctomycetes, Chlorobi and Nitrospira from rhizosphere soil samples with the aid of metagenomic applications.

Plants have relied on the beneficial symbiotic cohabitation of microorganisms widely used in sustainable agriculture. Metagenomic applications have explored and unlocked the access to rhizosphere microbial communities of many plants by targeting beneficial functional genes present in the microorganisms. Some of such genes are associated with nitrogen fixation (*nif*H) and Cold Shock Protein (CSP) (Goel et al., 2017).

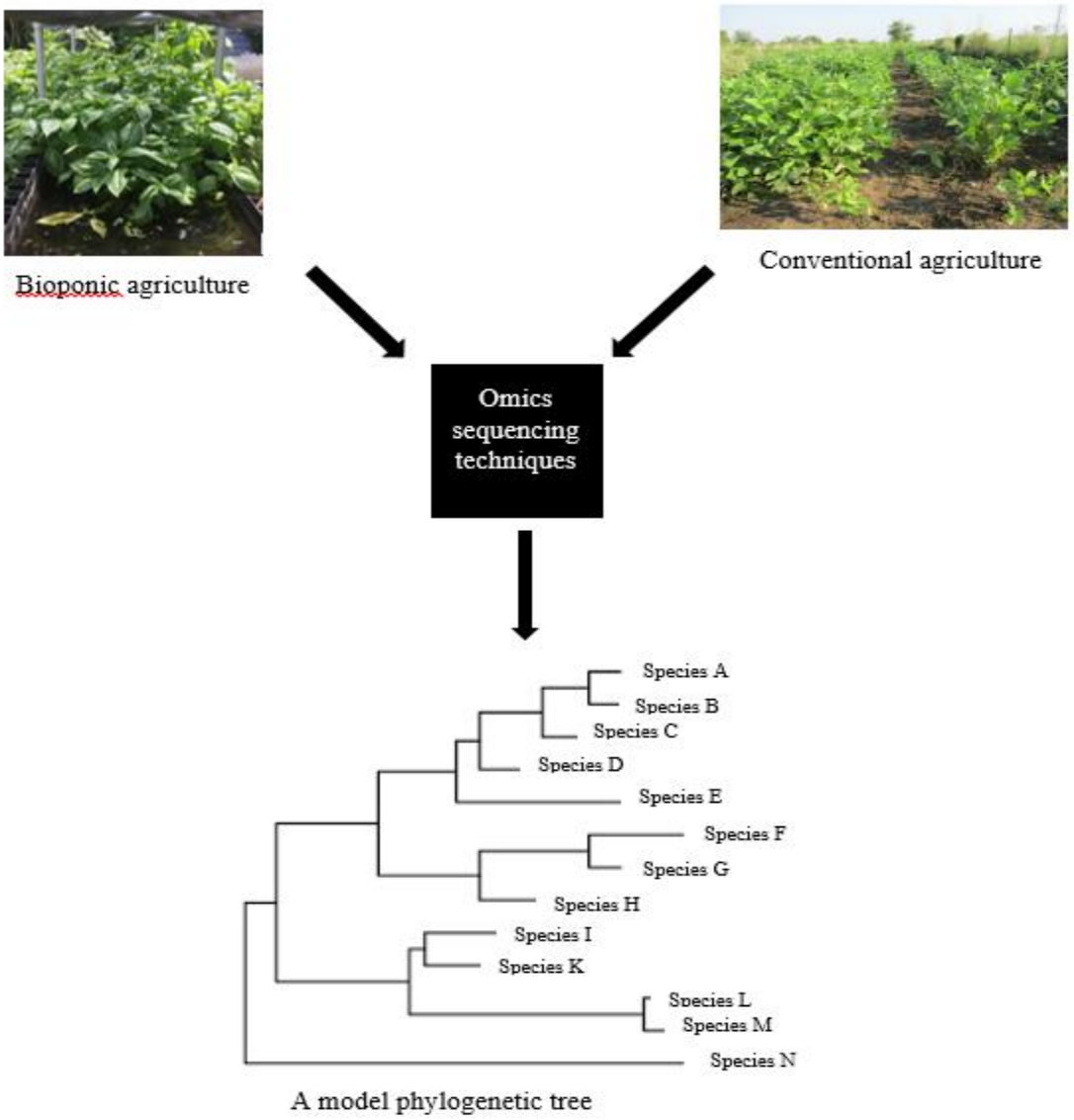

Figure 4.3: A phylogenetic tree of microbial species obtained from omics analyses of urban and conventional agriculture. Root associated samples of bioponic plants and rhizosphere soil samples can be obtained and sequenced using omics techniques, followed by construction of a phylogenetic tree of the microbial species that are of importance in achieving sustainable agriculture.

4.5.4. Exploring beneficial microorganisms of biogeochemical cycles

Agricultural sustainability relies on the biogeochemical cycle and diverse microbial communities play important functional roles in biogeochemical cycles in soils. Biogeochemical cycles often rely on the type of biodiversity accessible in a specific soil environment and the variety of crops planted. Application of metagenomics approaches has explored and identified novel genomes involved in biogeochemical cycles. The genomes of *Kuenenia stuttgartiens* a bacterium involved in anaerobic ammonium fixing have been documented (van Niftrik et al., 2008).

Similarly, novel protein cytochrome, an important component for iron oxidation involved in the formation of acid mine drainage, was identified using the combined shotgun and MS-based proteomics with genomic analysis of soil microbial community (Bevivino and Dalmastri, 2017). In brief, *nif*H genes involved in nitrogen cycling were identified using the metagenomic approach. In another event, a new, complete genome sequence of *Ferroplasma* type II and *Leptospirillum* group II was identified. Correspondingly, the partial genome sequence of *Ferroplasma* type I, *Leptospirillum* group III and G- plasma linked to biogeochemical cycles were identified with the aid of metagenomics (Goel et al., 2017).

4.5.5. Identifying novel biocatalysts from the soil environment

Living or biological systems (e.g. biocatalysts or enzymes) are classified as essential proteins that speed up metabolic reactions. In that case, they play significant roles in all biological organisms.

Metagenomic approaches are adopted in identifying entirely novel genes that encode new classes, types of enzymes as well as identifying enzyme substrate specificity. Indeed, metagenomics enables researchers to understand the cells participating in metabolic processes and identification of new bioactive compounds (Murphy et al., 2015). Specifically, new enzymes of agricultural importance are being identified and developed with metagenomic applications. Its application in agricultural soils reduces the dependence on chemical-based fertilizers and promotes environmental sustainability.

Functional metagenomics with compelling evidence is an effective tool to discover new enzymes from the soil, and to understand the enzyme activities and functions. The demand for production of enzymes and biocatalysts in commercial scale for agricultural purposes are rapidly growing. Presently, metagenomics is the technology to provide the needed enzyme molecules on a massive scale (Ghosh et al., 2018). Few of the main enzymes used to improve soil fertility, crop growth and yield are sulfatases, dehydrogenase and phosphatases. They are also known to reduce the use of pesticides for agricultural productions (Peng et al., 2018). Extremophilic esterases, with a contrasting active site compared to the one formed by culturable microorganisms capable of increasing the crop performance, have been identified from soil using metagenomics (Murphy et al., 2015).

Metagenomic applications have unlocked novel halotolerant enzymes from the soil which have great potentials in tolerating high salinity in the soil. It is equally important in industrial applications for bioethanol production and lignocellulosic deconstruction (Salem et al., 2020). Soil

microbial sources with attention to bacteria, fungi and yeast are the reservoir of many enzymes. Eventually, it is attracting special attention for their tremendous agricultural benefits (Ghosh et al., 2018). Enzymes employed in the food and agrochemicals productions were reported by (Ahmed et al., 2018). Above all, agricultural applications of enzymes are advantageous in enriching and fertilizing the soil, as well as ensuring continuous crop production (Ahmed et al., 2018)

Furthermore, some biotechnologically important enzymes, including amylases, xylanases, lipases, cellulases, proteases, have been unlocked from genetically untapped soil resources through metagenomic approaches. Lipases were discovered from novel soil microorganisms (Salem et al., 2020). Currently, there is a record of lipases identified from species of plants and rhizosphere metagenomes. Equivalently, a new alkaline stable lipase was identified from a metagenomic library constructed from agricultural soil (Peng et al., 2018). Cellulase a naturally occurring and the most abundant biopolymer on earth is recorded as the third largest used industrial enzyme globally for catalyzing the hydrolysis of cellulose (Salem et al., 2020).

Metagenomic approaches have captured new cellulases from compost soils, soils from regions and other natural environments by creating the libraries and screening all active clones (Ahmed et al., 2018). New cellulase was reported from the soil of a sugar cane farm by screening functional genes of the metagenomic libraries (Peng et al., 2018). Cellulase has the potential for the biological conversion of crops, like sugar cane, sugar beet, corn and wheat biomass into renewable liquid biofuels. Significant successes have been met by several bioenergy research centers (López-López

et al., 2014). Again, in animal farming, cellulases are added to animal feeds to improve digestibility and promote agricultural sustainability through animal production (Ghosh et al., 2018).

Specifically, the metagenomic approach has identified novel xylanases from metagenome of compost soil that showed thermostability and alkali stability properties (Alves et al., 2018). Furthermore, two novel phytases enzymes with industrial benefits were identified from soil microbial communities with the aid of function-based metagenomic approaches. The two phytases belonging to the phytase family were identified for their phytases activities.

Interestingly, the first phytase enzyme has an uncommon histidine acid phosphatase as a conserved motif of the active site of PhyX possessing extra amino acid residue, while the second, member of a new type phytase is encoded by multiple open reading frames (ORFs) differentiating it from all known phytases that are encoded by a single ORF (Tan et al., 2015). Generally, the enzymes identified from soil environment as mentioned above have different potentials for increasing soil fertility, influencing crop productivity and promoting sustainable agriculture.

4.5.6. Paleogenomic survey

Among the many emerging applications of metagenomics is paleogenomics, alternatively called genome-wide ancient DNA analysis. This is a new field in science that is based on the analyzing, and reconstruction of genomes in extinct plant and animal species (Tan et al., 2015). As it is known, that soil is a habitat with unlimited species of organisms, including plants. The soil habitat have a variety of genetic materials and functions. The metagenomic approach has provided insights

to the means traits evolve and the relationship between extinct organisms and living species. It has been established that DNA from samples is not limited to one species of a gene (Kim et al., 2014).

The ancient DNA of plants is fragmented, destroyed and mixed with genomes of the abundant microbial variations (Novak, 2018). Gupta et al. (2018) stated that palegenomic applications with high-throughput sequencing have provided access and understanding of the nuclear genomes of the extinct organisms. These approaches could revolutionize the field of paleobiology and assist in deciphering genome researches focusing on extinct species

4.6. Conclusion and future prospects

The relevance of microorganisms in plant health and the development of the plant environment are well established, but the majority of the rhizosphere microbial communities is yet to be characterized and is not explored. The combination of the conventional method with metagenomic approaches to assess the function and structure of the microbial community has provided insights into soil microbiome. Meanwhile, to identify plant signals, a wealth of resources and fundamental factors in the rhizosphere will provide microbial and chemical markers to describe how plants attract and stimulate advantageous microorganisms in soil and soilless agriculture.

Metagenomics has contributed to sustainable agricultural development by bringing to light many untapped soil microorganisms, their functionalities, genes for various applications, improving crop productivity, phytopathogen resistance and nutrient cycling. In addition, shotgun metagenomics and bioinformatics tools have facilitated data interpretation of metagenomic data and resolved the

challenges of pooling metagenomic data to establish the entire metagenomes of individual microbial community members, then correlating the diversity of different environments and analyzing the challenges in diversity.

CHAPTER FIVE

Rhizosphere competence of plant growth-promoting rhizobacteria and their applications in sustainable food production

Overview

The various bacterial genera are essential parts of soils. The bacteria genera participate in a variety of biotic activities that keep the soil ecosystem active for nutrient cycling and resilient for the production of crop. They increase soil structure, produce multiple plant growth regulators, protect plants from plant diseases by their regulation or suppression, and bioremediate polluted soils by sequesteration of harmful heavy metals and breaking down xenobiotics. They all serve to stimulate plant growth (like pesticides). Rhizobacteria, which live around and inside plant roots, are in fact more capable of converting, mobilizing, and solubilizing nutrients than bacteria found in bulk soils.

Rhizobacteria are the main factors in the recycling of the nutrients in the soil, and as a result, they are essential for soil fertility. Following the integrated plant nutrition management system, biological approaches to boosting crop output are currently gaining significant traction among agronomists and environmentalists. In this context, rigorous research is being conducted all over the world with the motivation to investigate a diverse variety of rhizobacteria containing unique qualities like salinity tolerance, pesticide degradation/tolerance, heavy metal detoxifying potentials, biological control of plant pathogens and insects, along with the typical plant growth

promoting properties like phytohormone, 1-aminocyclopropane-1-carboxylate, siderophore, nitrogenase activity, ammonia production, hydrogen cyanate (HCN) and phosphate solubilization, etc.

As a result, a variety of symbiotic (Bradyrhizobium, Rhizobium and Mesorhizobium) and non-symbiotic (Bacillus, Pseudomonas, Klebsiella, Azospirillum, Azotobacter and Azomonas) rhizobacteria are now emloyed globally as bio-inoculants to enhance plant growth and development under many stress conditions like fungicides, heavy metals, insecticides, herbicides, salinity, etc.

Despite the fact that the methods by which rhizobacteria improve plant growth are not completely understood, plant growth-promoting rhizobacteria have been reported to demonstrate the aforementioned abilities to hasten the development and growth of plants. The goal of the current review is to clarify the idea of rhizobacteria in the contemporary context and their fundamental processes for promoting plant growth and developement with recent studies. In order to gain a broad understanding of how these helpful rhizobacteria function and are applicable, the most recent paradigms for a variety of applications of these rhizobacteria in various agro-ecosystems have been explicitly described. Furthermore, research was performed (chapter 6) and published to announce the structure of sunflower rhizospheric soil microbial communities at Sheila, Itsoseng and Kraaipan towns located in the North West Province of South Africa.

Abstract

Sustainable food production, among other non-intensive production systems, involves the application and use of myriads of plant growth-promoting microorganism capable of enhancing soil health, plant growth and protection that lead to increased crop productivity. Plant growth-promoting rhizobacteria (PGPR) are free-living rhizobacteria that enhance plant growth due to several modes of action that start with their ability to colonize both the intracellular and extracellular rhizo niche in their search for a carbon source. In the past few decades, the focus of developing biosafety agro-products has been shifted from agrochemical based application to a more sustainable system without posing negative impacts on the soil microflora or fauna. Application of PGPR inoculants on soils and seeds improves biological nitrogen fixation, solubilization of phosphate and secretion of phytohormones required for growth, especially application in a stressed environment. While soil-borne pathogens continually opposing the functions of these microorganisms, PGPR has advanced diverse strategies in the form of nutrient, iron and space competition, systemic resistance, antibiotics synthesis, lytic acid, hydrogen cyanide and siderophore production for the food production.

Keywords: Biocontrol, Biofertilization, Biosafety agro-products, Biostimulation, Chemical inputs

5.1. Introduction

Intensive agricultural practices that include the prolonged use of inorganic fertilizers and chemical pesticides have led to huge agricultural and ecological damage. The excessive use of chemical

fertilizers exerts damaging impacts on soil microorganisms, altering the fertility status of soil and pollutes the environment (Nwachukwu et al., 2021). The progressive increase in human's population is projected at more than 9 billion by 2050 world-wide. According to the United Nations, Africa's share of global population is predicted to grow from 17% in 2020 to 26% by 2050 and 39% by 2100, while the population of Africa's most populous nation, Nigeria is expected to surpass that of United States by 2050 (Adeleke and Babalola, 2020a, Alori and Babalola, 2018).

Sustainable food production has become the main factor in improving food security globally, particularly in developing nations. This has resulted in an impending negative effect on the human race, which needs to be addressed without delay (Oberholster et al., 2018). One of the problems in addressing food security is that the ecosystem is already under stress and more people are undernourished. This makes it very difficult to conquer the challenge of food security in many developing nations (Igiehon and Babalola, 2018b).

The majority of the farmers especially in developing nations practice subsistence farming, but nowadays the rise in mechanized farming practice and the use of agrochemicals as external inputs are out of control, which adversely affect the soil environment and biodiversity (Şeker et al., 2017, Busby et al., 2017). Crop yield in the majority of the developing nations are low compared to the developed nations of the world. Many of these developing countries lose crops worth billions of US dollar yearly; the intensive agricultural practices destroy the ecosystem, result in land degradation and an increase in diseases and pest infestation. Still, some countries in developing countries such as Nigeria, South Africa, Zambia, Ethiopia, Mozambique and Liberia rely on

agriculture and have experienced an increase in their gross domestic product (GDP) growth in agriculture (Igiehon et al., 2019).

Although the advantageous outcomes of chemical-based fertilizers and pesticides are instantaneous, the indiscriminate and prolonged usage of these harmful chemicals in agriculture is dangerous. Artificial chemical inputs in the form of chemical fertilizers and pesticides destroy the soil structure, resulting in the depletion of available nutrients, contaminate water resources that alter the soil biological population and result in the degradation of the environment. They ultimately pose negative impacts on crop yield and increased susceptibility towards biotic and abiotic stresses. Furthermore, humans are exposed to these harmful chemicals through the possible entrance from the food chain as heavy metals (Sergaki et al., 2018).

The progressive increase in food production is essential for the development and continuity of developing countries and poor economies. Recently, many new agricultural technological innovations have evolved and should be implemented by policymakers because the growth and development of nations would rely on advanced and environmentally friendly technologies such as hybrid disease-resistant species, modern management practices and climatic modified seeds (Babalola, 2010). The acceptance of these technologies by farmers is quite slow, due to some negative consequences over time, high cost and dependability. With respect to these concerns, the use of novel agricultural beneficial microorganisms for sustainable agriculture will be an alternative strategy to increase crop yield without having any long term negative effects on the ecosystem (Gomes et al., 2018, Singh et al., 2018).

Most indigenous soil microorganisms, in particular rhizobacteria residing in the vicinity of plant roots, perform different beneficial interactions with plants and play critical roles in sustainable agriculture by improving soil quality and health and making nutrients readily available to plants (Keswani et al., 2019). These special groups of microorganisms possess potential characteristics to promote plant growth due to their interactions in the region of the soil in close proximity to the roots (rhizosphere) are called plant growth-promoting rhizobacteria (PGPR) (Kloepper and Schroth, 1981). PGPR interact and carefully selects their mutual partners through natural selection, creation of host specificity and sensitive to environment where there is lesser microbial diversity (Lyu et al., 2019). PGPR improve plant growth through different mechanisms such as increasing the availability of nutrients to plants and producing essential metabolites without influencing the environment negatively (Nwachukwu et al. 2021).

Many PGPR strains have been reported to survive adverse environmental conditions such as drought, salinity stress, unavailability of nutrients and high heavy metal contamination. PGPR trigger major biological functions in the soil but also have been engineered to improve crop productivity through healthy competition in the soil and breakdown of nutrients (Lang et al., 2019). In this review, the various mutualistic interactions of PGPR with plant roots in the rhizosphere and the major plant growth-promoting traits, including their mechanism of actions, are highlighted. The commercialization of PGPR products and challenges in developing countries are also highlighted. Insight is given into the use of PGPR as an effective microbial consortium for advanced agrobiology and livelihood, especially in less developed countries (Adesemoye and Egamberdieva 2012). The review focuses on trends in the use and application of PGPR, where

intensive agricultural practices such as the use of external chemical inputs have led to a decrease in crop productivity and thus recommends PGPR as one of the alternative means for sustainable agro-ecological systems.

5.2. Rhizosphere: The 'epicenter' of microbial activities

The rhizosphere is the region that connects the plant roots and soil from which organic and inorganic substances and signaling molecules are transferred into other soil regions allowing lasting mineralization processes controlled by agricultural important microorganisms and their activities (Tirry et al., 2018, Urana et al., 2019). Rhizosphere houses numerous groups of organisms interacting with the plants and other microorganisms, such as archaea, bacteria, fungi and their environment (Nwachukwu et al., 2021). Most microorganisms found in the rhizosphere improve plant growth and health through different mechanisms.

Usually, plants secrete and discharge a significant amount of carbon fixed by photosynthesis; the soil microbial community and their roots use majority of these discharged materials known as root exudates, including primary metabolites (organic acids, sugars, inorganic ions, vitamins, amino acids, enzymes, purine and gaseous molecules) as a source of food. Collection of these root exudates have been performed using both qualitative and metabolomic approaches, including sampling root exudates from plants grown on soil and obtaining root exudates from in vitro cultures or hydroponic grown plants.

However, root exudates composition in soil will be instantly changed which is attributed to their adsorption to the soil particles as well as microbial degradation, actually, the exudation from soil microbial communities also modify plant exudation profile (Nwachukwu et al. 2021). Due to the challenges of the process of root exudation and low reproducibility from plants grown in the field have led to other approaches which excludes the extension of soil-culture. Many factors influence the exudation process, including biotic and abiotic stress conditions such as toxic ions (e.g. trace elements (heavy metal ions), extreme temperature, high salinity (generating both ion toxicity and osmotic stress), nutrient starvation (P deficiency) and water status (Igiehon et al.2019).

Crop cultivation in artificial field conditions can modify exudate composition. Factors such as aeration in vitro culture or hydroponic cultures in temporary immersion systems (TIS) have impacted on root exudates composition and plant development. The main advantage of these in vitro sterile systems is the reproducible exudation pattern which is important for the study of the effects of stress factors and nutrient deficiency (Regueira et al. 2018). Hence, some researchers highly recommend this method for exudate collection to prevent the presence of rhizosphere microorganisms with the ability to affect root exudate composition (Zandalinas et al., 2016). In some cases, root exudates are collected from in vitro or hydroponic cultures using plants grown in soil while in most studies root exudation are from in vitro or hydroponic cultures, where the liquid medium containing compounds is obtained.

In respect to this, root exudates are collected by various physicochemical processes, which includes through sorption filters buried in the ground (Vives-Peris et al., 2020) or extraction

processes with different solid or chemicals phase extraction (Naveed et al., 2017). The invitro methods allow the culture of excised organs such as shoots and roots or entire plants. However, this can influence root exudate composition because many studies have reported that root exudation is affected by the presence of shoots in the culture, as they control CO_2 absorption by photosynthesis in some plants such as watermelon (Ling et al. 2013), and combination of eggplant and tomato (Liu et al. 2009). The modification in the aerial tissues and as well as changing the scion of grafted plants were reported to affect root exudation processes.

Moreover, traditional stationary methodologies of culture are employed to obatain root exudates from invitro-cultured plant tissues. Recently, many automated TIS have evolved, some allow forced ventilation, CO_2 enrichment, culture hairy roots and cell suspensions. The first and one of the most commonly used systems is RITA (Recipient á Immersion Temporaire Automatique) which was originally developed for plant mass propagation. It has also been approved for metabolites from root culture capable of reducing hyperhydricity challenges in plant material grown in vitro. The major advantage is that TIS substantially increase metabolite and biomass production, hence their use for obtaining specific metabolites from root exudates. Some metabolites obtained from root culture using TIS are betalains from *Beta vulgaris* (beet) having colorant and antioxidant properties (Regueira et al. 2018).

These plants exudates have various available nutrients, playing a significant role in attracting different groups of microorganisms to the rhizosphere (Lang et al., 2019). Besides, the available nutrients in plants, they also depend on the interactions among microorganisms in the rhizosphere

using these exudates, thereby creating a connection between microorganisms and plants (Jeyanthi and Kanimozhi, 2018). The capability of microorganisms to colonize the host plant roots and improve soil health is to determine the efficiency and success of PGPR as an inoculant for crops (Mosimann et al., 2017). Examples of commercially available PGPR are listed in Table 5.1. Only microbial species that can strive and survive the competition in the rhizosphere are effective in expressing several genes and communication between cells through quorum sensing (a cell-to-cell microbial communication process that uniquely controls various physiological and metabolic activities) can emerge as promising PGPR (Lyu et al., 2019).

Beneficial plant-microbial interactions that exist in the rhizosphere between the plant roots and the microorganisms can be mutualistic (both organisms benefit from each other), commensalistic (one of the organisms benefits while the other obtain neither benefit nor harm), and amensalistic (one of the organisms is harmed while the other is unaffected) forming the bases for rhizobacteria to improve crop yield (Busby et al., 2017). However, for rhizobacteria to be classified as PGPR it must be able to colonize the root surface, have the capability to survive, proliferate and compete with other indigenous and nonresident microbiota (Babalola et al. 2021). Above all, it should promote plant growth (Odelade and Babalola, 2019).

In the rhizosphere, several bacterial species have been recorded to symbolically interact with host plants and as a result release organic plant growth-promoting substances in the form of exudates as important nutrients for soil bacterial growth and metabolic roles. This has been achieved by studying various microbial species, their functional roles through next-generation sequencing

techniques, the plant cultivars and the rhizosphere. PGPR demonstrate different mechanisms of action generally to promote plant growth either directly or indirectly, or both (Kumari et al., 2019).

PGPR improve plant growth directly by enhancing nutrient acquisition, mainly essential minerals such as phosphorus, vitamins and nitrogen from the environment. Furthermore, other growth factors such as enzymes, water, production of growth hormones, solubilization of phosphorus and iron sequestration by siderophore in the absence of pathogens (Arif et al., 2017, Backer et al., 2018). Indirect mechanisms reduce the effects of plant pathogens by protecting plants from phytopathogens. Indirect mechanisms such as competition, production of antibiotics, antifungal metabolites, production of hydrogen cyanide, siderophore and induced systemic resistance indirectly promote plant growth (Ju et al., 2020).

Kloepper and Schroth (1981) postulated how PGPR alter the rhizosphere niche; the authors buttressed the diverse effects of PGPR on plant growth and development upon inoculation (Kumari et al., 2019). The application of microbial inoculants, such as PGPR, are beneficial for stimulating plant root for growth, pest control, plant stress control and destruction of plant diseases leading to agricultural productivity (Arif et al., 2017, Backer et al., 2018). Plants naturally can fight against diseases, but the application of microbial inoculants as biocontrol agent helps advance these abilities.

Besides, several live microorganisms, such as *Rhizobium*, *Anabaen*a, *Pseudomonas* and *Azotobacter* species are used as microbial inoculants to improve nutrient uptake and nutrient pool. These microbial inoculants can be incorporated into soil, seeds and plants for enhancing

biofertilization and biostimulation serving as eco-friendly and substitute to chemical fertilizers (Ju et al., 2020, Zhang et al., 2017). The synergistic effect caused by microbial inoculants and resident microorganisms improve plant nutritional activities that involve breaking down, solubilization and mobilization of nutrients. Hence increasing crop yields have reduced the use of chemical fertilizers. The acceptance and application of microbial inoculant as plant improving agent is spreading widely in many developed countries where agriculture is the key driver of the economy (Adedeji et al., 2020).

5.3. Direct mechanisms of plant growth promotion by PGPR

5.3.1. Biofertilization

Biofertilization refers to the application of microbial inoculants on soil, plant surfaces, or seeds, which is important to enhance crop production. The microbial inoculants colonize the rhizosphere and supply essential nutrients required for plant use resulting in improved plant growth. The application of microbial inoculant improves the soil texture, such as soil porosity and soil aggregate structure. These biofertilizers with relatively low density have no negative impacts on the environment and are non-toxic in even high concentration. They have no adverse effects on crops and underground water because they do not contain heavy metals such as arsenic, mercury, lead, vanadium and cadmium. Studies have proved that these heavy metals could alter the composition and diversity of soil microbial population and nutrient cycling (Wan et al., 2020). Ultimately, these chemicals could find their way into the food chain causing diseases such as stomach cancer (Adeleke and Babalola, 2020b).

Table 5.1: Examples of commercially produced PGPR, mechanisms of action and plant responses.

PGPR	Product/ Country	Plant	Mode of action	Plant response	Reference
Paenibacillus helianthi sp. nov., *Streptomyces* sp., *B. subtillis*	BIOVED, AgBio, USA, Hungary, Italy	Sunflower	Nitrogenase activity; nitrogen fixation	Increased nutrient content and dry weight	Jeyanthi and Kanimozhi (2018), Tassi et al. (2017), Ambrosini et al. (2018)
P. thivervalensis, Azospirillum, A. brasilense, Paenibacillus polymyxa,	AtEze, Spot-Less, BlightBan, frostban, Bio-save- USA, India, Iran, Pakistan, Brazil	Maize (*Zea mays*)	The hydrolytic enzyme, phosphate solubilization, Siderophore and IAA production	Increase tolerance to salt; Increase plant leaf and height	Gomes et al. (2018) Kumari et al. (2019)

Lipoferum, *Pseudomonas putida*, *Serratia marcescen*, *P. fluorescens*,					
Pantoea agglomerans RK-92, *B. megaterium* TV-91C, *B. subtilis* TV-17C, *B. megaterium* TV-87A, *Bacillus megaterium* TV-3D, *B. megaterium* KBA-10	EcoGuard, Kodiak, Subtilex, Yeild-Shield, Companion, Bioyield, - USA, Turkey	Cauliflower (*Brassica oleracea L.*)	Nitrogen fixation, Phosphate solubilization, IAA production	Nitrogenase activity, phosphate solubilization, IAA production, Increased mineral nutrient uptake	Kumari et al. (2019)

Bacillus, B. amyloliquefaciens, Bacillus subtilis, B. pumilus, P. fluorescens, Serratia marcescens	BIOVED, HiStick N/T, Mepplus– USA, Brazil, India, Egypt, Hungary	Tomato (*Solanum lycopersicum*)	Siderophore and IAA production, phosphate solubilization Nitrogen fixation, antiviral activity	Increased crop yield and nutrient uptake, nutrient content, biologically controls viral pathogens	Kumari et al. (2019)
B.cereus, E. cloacae, B acillus drentensis, Providencia vermicola	Sonata, Serenade-USA, Saudi Arabia, India, Egypt, Pakistan,	Mung bean (*Vigna radiata)*	N-fixing and phosphate solubilization Siderophore and IAA production, Activities of	Increases nutrient uptake, Enhance gaseous exchange, photosynthetic pigments, and water retention.	

Ama-2, *Rhizobium, B. pumilus* Sol-1, *Brevundimonas* Kro13, *Alcaligenes* sp. Mal-4, *Kluyvera ascorbata* SUD165, *P. putida*, *Ochrobactrum*,	Bangladesh	nitrogenase, ACC-deaminase, and acetylene reducing	Improved growth and seed yield, Increases the stimulation of mung bean plant growth reduces Pb and Cd uptake decreases the toxic effect of chromium to seedlings by reducing Cr (VI) to Cr (III)

Bacillus spp., *B. amyloliquefaciens*	Germany, USA, Iran, China	Cotton (*Gossypium)*	Antibiotic activity; N-fixing and phosphate solubilization, Production of plant hormones (auxins, cytokinins, and gibberellins)	General improves growth and biocontrol of phytopathogens	Deng et al. (2019)
Bacillus simplex T7, *Pseudomonas putida* BA-8	Ecosoil-USA, Turkey	Grapes (*Vitaceae family*)	Antibiotic activity, IAA production, phosphate solubilization, N- fixation	Promote grafting capacity at nursery conditions	Jeyanthi and Kanimozhi 20 (2018)

B. polymyxa (LAB/BP/01) *B. subtilis*	Inomix ® biostimulant Spain	Cereals	Production of antimicrobial compounds and metabolites induced plant resistance to pathogens, uptake of nutrients	Improve plant growth and control of pathogens	Tassi et al. (2017)
Azospirillum sp.	Nitrofix ®Cuba	Wheat, barley, carrot, maize, cabbage	Secretion of hormones, nitrogen fixation	Enhance nutrient and water uptake, stimulate root developm ent, generally promote plant growth	Mosimann et al. (2017)

B. subtillis BR62, *Rhizobium* sp. base formulation *Peanibacillum durus* PD74	Micosat F® cereal Italy Mamezo®, Processing Seeds®, Hyper Coating Seeds ® Japan	Tomato, soybean, cereals, beet, legumes, sunflower	Nitrogen fixation, HCN production, produce enzymes that can destroy the cell wall of pathogenic fungi	Increase plant height, shoot dry weight and nodules	Mishra et al. (2017)
Azotobacter, P. fluorescens, phosphobacteria	Greenmax Agro Tech Life, Gmax, Biosink - India	A wide range of plants	Nitrogen fixation Solubilize phosphate, produce siderophore, alkaline phosphatase and IAA	Increase crop yield, nutrient uptake, resistant phytopathogens	Babalola et al. (2007)

Biofertilizers are formulated products of microbial inoculants in a liquid or powdered carrier containing PGPR as the active ingredients which, when applied to the seeds or into the furrows, enhance plant growth and increase yield, in a wide range of agricultural crops (Wan et al., 2020).

Several studies have documented the critical role biofertilizers play in maintaining soil health, promoting long term soil fertility, increasing organic farming and reducing the use and application of chemical fertilizers (Ju et al., 2020, Zhang et al., 2017). To use biofertilizers, there must be an efficient formulation of microbial inoculants, proper selection of suitable carrier and construction of adequate application methods.

In a study by Babalola and Akindolire (2011) which characterized PGPR from rhizospheres of eight food crops plants in South Africa, different PGPR strains belonging to *Burkholderia gladioli, Vibrio fluvialis, Serratia plymuthica, Pseudomonas fluorescens, B. cepacia, P. putida, S. ficaria, P. luteola, Erwinia* spp, *Aeromonas hydrophila, Rahnella aquatilis, Acinetobacter baumannii, A. calcoaceticus, Escherichia vulneris, Proteus penneri, Ewingella Americana* and *Shigella* spp. were used with productive effects as biofertilizers. All these strains produced hydrogen cyanide and ammonia to indicate their functions as biofertilizers in increasing crop productivity

5.3.2. Nitrogen fixation

Nitrogen (N) is one of the most essential nutrients for plant growth and development that ultimately increases crop productivity. Although atmospheric air is composed largely of N gas (N_2) at 78 %, N is one of the most limiting nutrients for crop production globally (Valentine et al., 2018). This

is because nitrogen (N_2) in the atmosphere is very stable due to the strong triple bond between the two N atoms in atmospheric nitrogen (N_2), which takes a lot of energy to break, and thus plants cannot convert it to its usable form (Hassan et al., 2016). Only a few prokaryotic organisms known as diazotrophs possess the enzymatic machinery required to break the strong bond that holds the two N atoms together. Atmospheric N_2 is biologically fixed through symbiotic means by the root nodulating Rhizobia group, or non-symbiotically by the free-living PGPR strains such as *Azospirillum* and *Azotobacter* spp. Endophytes such as Cyanobacteria (*Gluconoacetobacter diazotrophicus*, *Anabaena*, *Noctoc* and *Azocarus*) provides 7 - 80 kg N_2 per hectare annually (Hu et al., 2018). This process of converting atmospheric N_2 into a usable form of N by the enzymatic action of diazotrophs described above is called biological nitrogen fixation (BNF).

The BNF is an economically beneficial and eco-friendly alternative to chemical fertilizers and accounts for up to 290 million tonnes of N per year, providing N to plants (Mishra et al., 2017). For several years, rhizobia inoculants have been used for cultivating legumes as biofertilizers. Like the developed world, many sub-Saharan African countries, such as South Africa and Namibia use rhizobia inoculants for N uptake. Due to its economic benefits, rhizobia have been widely used as a substitute for chemical fertilizers and frequently used to boost the production of legumes (Igiehon et al., 2019).

The mechanism of N fixation requires a complex enzyme system such as nitrogenase, mainly encoded by the *nif* genes. The *nif* genes are structural genes involved in the Fe protein activation, donation of an electron, activation of iron-molybdenum and stimulation of plant regulatory genes

needed for the synthesis and function of enzymes (Etesami and Maheshwari, 2018). The *nif* genes are primarily found as a cluster of around 20–24 kb with about seven operons of 20 proteins variants. The advantageous impacts of *nif* genes have been well documented. Some studies have also proved that *nif* genes can improve N fixation through genetic engineering. However, others argued how N fixation traits of plants could be enhanced and harnessed (Babalola et al., 2007, Igiehon et al., 2019). Nevertheless, the focus of these methods is still on promoting plant growth and sustaining N levels in the soil.

Diazotroph is any microorganism that can grow without any source of external N. Diazotrophic microorganisms provide to the host plant fixed N that can be exchanged for fixed carbon which is then released as root exudates (Majeed et al., 2018b, Prasad et al., 2019a).

5.3.3. Mineral solubilization and nutrient uptake

For a plant to grow optimally there is a need for adequate mineral nutrients supply. However, even with the presence of mineral nutrients in the soil, plants may still show deficiencies because of the unavailability and insolubility of essential mineral nutrients to support plant growth. PGPR solubilized mineral nutrients such as potassium (K), iron (Fe) and phosphorus (P) and then makes it accessible for plants' use (Adeleke and Babalola, 2020a).

P is the second major important mineral nutrient after N required for plant growth (Majeed et al., 2018c, Zhang et al., 2019b). P is abundant in soil in both organic and inorganic forms, but is insoluble and the uptake of available P by plants is minimal (Zhang et al., 2017). PGPR trigger

plant growth by enhancing P solubilization. Plants absorb insoluble P forms such as monobasic ($H_2PO^-_4$) and diabasic ions (HPO_4^{2-}). Unavailable P in the soil are in insoluble forms such as phosphotriesters (organic), apatite (inorganic), phosphor monoesters and soil phytate or inositol phosphate (Zhang et al., 2017). Therefore, to eradicate the challenge of P deficiency in soils, phosphatic fertilizers are frequently applied to the soil by some farmers (Orlikowska et al., 2017). To solve the problem, phosphate solubilizing activity of PGPR are important. PGPR mineralizes and solubilize inorganic phosphorus by improving root system. Hence, the PGPR provide the available forms of P to the plants serving as potential biofertilizers.

PGPR solubilizes inorganic phosphate by secreting organic acids with low molecular weight, such as carboxylic acids, lactic acid, succinic acid, glycolytic acid, formic acid, fumaric acid and propionic acid, which lower the pH in the root area. Consequently it releases the confined forms of phosphate, such as Ca phosphate in calcareous soil (Xiao et al., 2017). Generally, organic phosphorus mineralization occurs by various enzymes such as phytase and C-P lyases phosphatases that catalyze the hydrolysis of phosphoric esters (Wan et al., 2020).

Additionally, other mineral nutrients such as iron and zinc are made available through the biosynthesis of compounds capable of promoting plant growth. Although the mineralization and phosphate solubilization can be impacted by environmental factors, they play a critical role in agricultural sustainability, especially in soils short of P (Fabiańska et al., 2019). Another essential macronutrient is K. Naturally, the accumulation of K in soils is minute and the majority of the K is found in silicate minerals or insoluble rocks. The short supply of K in soil results in poor

development of plant roots, tiny seeds and low crop yields. This generally presents as a concern to sustainable crop production. The search for solutions to alleviate the shortfall of K in soils has led to the innovation for environmentally friendly and economical options of K uptake by plants and to sustain the K in soils for improving crop production (Jeyanthi and Kanimozhi, 2018, Adedeji et al., 2020, Olanrewaju et al., 2019).

The solubilization of K occurs through the secretion of organic acids by PGPR. Interestingly, PGPR such as *Bacillus*, *Pseudomonas*, *Burholderia* and *Paenibacillus* species, which possess K solubilizing activity have been reported to provide the available forms of K to plants (Castelle and Banfield, 2018, Mosimann et al., 2017, Orlikowska et al., 2017). These microbial communities are referred to as potassium-solubilizing microorganisms (PSM) because they release unavailable K and make it accessible to plants. PGPR as potential biofertilizers can reduce the use of chemical fertilizers and eco-friendly presents an approach for crop production.

5.3.4. Biostimulation, production of plant growth regulators and bioactive compounds

The process of modifying the soil environment, during which PGPR are excited to produce substances capable of promoting plant growth, is called biostimulation (Olanrewaju et al., 2019). Biostimulants are biochemical substances that promote plant growth in the absence of pesticides, nutrients or soil improvers (Andrade et al., 2018). The chemical structure is closely related to that of natural plant hormones and the mode of action is being estimated as the production of plant hormones or phytohormones (Saleem et al., 2018). Recently, PGPR based stimulants are widely applied in agricultural practices globally, numerous Africa countries inclusive. PGPR based

stimulants directly or indirectly improve crop quality by enhancing nutrient uptake and tolerance to stresses such as salinity and drought (Enebe and Babalola, 2018, Etesami and Maheshwari, 2018). Across the global markets, different PGPR formulations have been registered and are commercially available. Some of the commercialized species are *Enterobacter, Pseudomonas, Variovarax, Serratia, Bacillus, Azobacter* and *Azospirillium* (Andrade et al., 2018). Although, their use in the cultivation of plants still presents a small fraction worldwide (Fabiańska et al., 2019).

Organic compounds required in minute concentrations capable of manipulating the physiological processes and regulate plant growth are called phytohormones or plant growth regulators (PGRs) (Itelima et al., 2018). Phytohormones are phytostimulants that are chemical compounds required for the physiological and biochemical development of plant growth. *Pseudomonas, Bacillus, Acetobacter, Xanthomonas, Bradyrhizobium, Penicillium* and *Aspergillus* are some genera of PGPR capable of producing phytohormones (Youseif et al., 2017, Kloepper and Schroth, 1981, Barquero et al., 2019).

Globally today, agricultural systems are frequently impacted by different stresses (biotic and abiotic) which often affect plant growth and crop yield. Yearly, farmers experience 30-50% losses due to stress (Youseif et al., 2017). These stresses can be human induced or inherent. The common abiotic stresses are drought, temperature, salinity and accumulation of heavy metals. Hence, these stresses stimulate plants and PGPR to produce the phytohormones. The phytohormones are found in minute amounts that exert control on the morphological, physiological and biochemical processes, even the structure and genetic expressions in plants and facilitate plant-microbial

interactions. Therefore, having notable effects on soil microbial composition, soil fertility and nutrient compositions, though the production of these organic chemicals is efficiently managed (Jeyanthi and Kanimozhi, 2018).

PGPR can enhance plant growth and development in stressed and natural conditions, therefore using competent microorganisms could advance agricultural sustainability and environmental stability. In stressed soil environment, PGPR produce specific low molecular compounds such as rhizobiotoxine around the rhizosphere capable of controlling phytopathogens by suppressing ethylene production. Moreover, to increase survival under stressed conditions, particular bacteria transcription proteins regulate gene expression in plants (Hu et al., 2018, Jeyanthi and Kanimozhi, 2018, Youseif et al., 2017). *Arthrobacter* sp. AF-163 produced phytochemicals capable of improving drought stress and crop growth in sunflower plants by altering membrane integrity and reactive oxygen species movement and also built up glycine, proline and betaine. Bacteria modulate the positive responses causing distinctive gene expression related to activation and expression of transcription factors, ethylene and salicylic biosynthesis in stressed condition (Majeed et al., 2018c).

Some PGPR such as *P. putida, Glucunobacter* sp.*, Agrobacterium* sp.*, Alcaligenes piechaudii, Micrococcus luteus* and *Streptoverticillum* sp. are known to synthesize high levels of ethylene, IAA and hydrogen cyanide to reduce salt stress and enhance sunflower plant root growth, as a result improved root structure development, N content and nodule biomass (Backer et al., 2018, Oak, 2019, Tassi et al., 2017).

PGPR producing auxin, cytokinins and gibberellins ameliorated antioxidant enzymes of different plants rhizosphere such as groundnut, lettuce and canola under gnotobiotic conditions. Hence, the mechanisms of action improved signaling molecules that affect gene expression, plant growth and yields (Chaudhary et al., 2020, Itelima et al., 2018). Various PGPR such as *Serratia*, *Rhizobium* and *Pseudomonas* species used as bioinoculants to cultivate common crops such as rice, sunflower and legumes, produced phytohormones involved in nodule formation and elongation of the root system (Garrido-Oter et al., 2018, Alori and Babalola, 2018, Mukhtar et al., 2019). *A. thaliana* seedlings treated with ACC tolerated drought better when compared to the uninoculated seedlings, and also increased in root hair length. Further investigation revealed that the mechanism is dependent on ethylene (Babalola et al., 2007).

Sunflower plants soil treated with *Costus* sp. triggered the production of phytohormones (auxins, cytokinins and gibberellins) as a result boosted the plants' growth compared with chemical fertilizers (Jadhav and Anil, 2020). Nonetheless, PGPR improve plant stress tolerance and growth by reducing the use and damaging effects of inorganic fertilizers on soil health leading to environmental and agricultural sustainability.

5.3.5. Siderophore production

Among the essential minerals abundant in the rhizosphere soil needed by plants is Fe, which is not readily available for plant and bacterial use. Generally, ferric iron (Fe3+) is the major form of Fe on earth but is insoluble. Plants and microorganisms require a sufficient amount of Fe, which is of concern in the rhizosphere where there is a high competition for Fe by plants and microorganisms.

To alleviate this problem and make Fe readily available in the root region, PGPR secrete low molecular weight and high-affinity iron-chelating compounds called siderophores (Zhang et al. 2019).

Ever since the report by Kloepper et al. (1981) for the occurrence of enhanced plant growth by siderophores produced by *P. fluorescence*, the role of siderophores produced by several PGPR strains in plant growth promotion has been reported in several studies (Ju et al. 2020; Singh et al. 2019b). PGPR strains *P. fluorescence* KBS6-17, *Serratia marcescens* KBS-6H and *Enterobacter sokazaki*-NAS6B are reported to produce siderophores confirmed by the change in the color of the blue CAS agar media into yellow and orange after inoculation and incubation for 5 hours at room temperature (Zhang et al. 2019).

Siderophores are grouped based on their functional capability as carboxylates and hydroxamates (Alori et al. 2017; Olanrewaju et al. 2019). PGPR produce different types of siderophores, such as ferroxamine and Pseudobactin that chelate in plant rhizosphere and as a pointer to show. Furthermore, PGPR species such as Streptomyces spp. and Trichoderma spp. reportedly produce siderophores comparable to those produced by the plants (Zhao et al. 2020). Siderophores are known to be synthesized by microorganisms under iron deficiency stress condition, hence to assure the growth and development of their cells. These microorganisms upsurge soluble iron in the soil, stimulate the chelation of iron and make it accessible for themselves and also for the plants (Colombo et al. 2020).

In a study, Zhao et al. (2020) reported that in *Arabidopsis thaliana*, *Trichoderma asperellum* Q1 synthesized siderophores in the soil by augmenting the transformation of poorly soluble Fe. Cucumber seedlings grown in soil inoculated with *Trichoderma asperellum* showed notable increase in the siderophores and the soluble Fe by improving the activities of ferric chelate reductase (FCR) in the roots (Zhao and Zhang, 2015). Sunflower plants inoculated with PGPR produced siderophores by stimulating plant cell division that enhanced root growth compared to uninoculated plants (Lindström and Mousavi 2019).

The ferric siderophore complex produced by *P. putida* and *P. fluorescens* is taken up by plants, resulting in the restriction of microorganisms from accessing the Fe in plant roots and other regions (Olanrewaju et al. 2019). The siderophores synthesized by several PGPR may be very useful for stimulating the absorption of iron in an iron-deficient environment by plants (Colombo et al. 2020). Also, the PGPR presence in soil induce the production of siderophores and help plants to reduce the toxicity of heavy metals in the stressed environment (Ayangbenro and Babalola 2017).

5.4. Indirect mechanisms of plant growth promotion by PGPR

5.4.1. Biocontrol of phytopathogens

Globally, diseases damage many crops and before harvest a large percentage is lost. Many farmers use chemicals to control pests and this is becoming a concern due to health implications, hence PGPR offer a potential solution. Presently, awareness and acceptance of biopesticides are on the rise globally. Biocontrol is the use of microorganisms to diminish the frequent occurrence of plant

diseases. Biopesticide selectively controls various organisms capable of causing diseases in plants without being toxic. PGPR are capable of controlling various phytopathogenic microorganisms and use at least one of the following mechanisms, including competition with pathogens for nutrients and specific ecological niche at the root region, the production of antibiotics and anti-fungicides, lytic enzyme, hydrogen cyanide production, induced systemic resistance and signal interference (Oak, 2019, Tassi et al., 2017). Some biocontrol agents are highlighted in Table 5.2.

PGPR as biocontrol agents exert these mechanisms of action to antagonize pathogenic bacteria, fungi and nematode depending on their severity. *Pseudomonas fluorescence* is the most studied biocontrol agent because of its antagonistic actions against many phytopathogens (Mukhtar et al., 2019). Besides, PGPR manipulate the involvement and application of important rhizobacteria and their metabolites in suppressing the impact of phytopathogens, and as a result, promote plant health and minimize loss during harvest (Andrade et al., 2018, Etesami and Maheshwari, 2018). Recently, farmers' interests and preferences have shifted to the application of integrated alternative pest control strategies that are viable, inexpensive and eco-friendly, including spore-forming *Bacillus* and other microorganisms with biocontrol ability (Olanrewaju et al., 2019). Likewise, Singh et al. (2019a) reported that all ACCd producing PGPR isolates identified from sunflower soil had an antagonistic effect on *Fusarium oxysporum* that causes *Fusarium* wilt in the sunflower. Furthermore, the ACCd producing PGPR exhibited a minimum of four plant growth-promoting traits, including the production of phytohormones (IAA) and provided mineral nutrients, such as P and N fixation that significantly improved seed and vegetative growth parameters of the sunflower plant compared to the control.

PGPR produce antimicrobial secondary metabolites and siderophores to chelate Fe and conduct a multifactorial process that stimulates induced systemic resistance which relies on various compounds, including volatiles and c-LP surfactin (Mukhtar et al., 2019). The combined impacts of the strategies mentioned earlier would increase crop yield through adequate bioformulation of spores from viable PGPR and concentrated culture supernatants with antimicrobial metabolites.

5.4.2. Induction of systemic resistance

Some PGPR trigger physical or chemical changes that are related to plant defence, a process referred to as induced systemic resistance (ISR) through the action of various phytohormones (Romera et al. 2019). It is the mechanisms by which PGPR protect plants from phytopathogenic infection by eliciting the production of different metabolites such as jasmonic acid, salicylic acid and ethylene, which is released in the form of stress response signaling within plants. The production of these chemicals within the plants stimulate their resistance ability against infection by various phytopathogens (Kumari et al., 2019). PGPR such as *Azospirillum*, *Bacillus*, *Enterobacter* and *Pseudomonas* species have been used as active ingredients in several biofertilizer products by farmers worldwide to activate induced systemic resistance.

Such inoculants containing this PGPR are often applied on the seed coat before planting to initiate ISR against pathogens such as *Pseudomonas syringae* and *Erwinia trachephila* that cause leaf spot and bacterial wilt, respectively (Majeed et al., 2018c). Common ISR elicitors are siderophore, MAMPs (when formed by pathogens are called pathogen-associated molecular patterns: PAMPs) are microbial molecules secreted by microorganisms, like chitin, flagellin and lipopolysaccharides

Table 5.2: Examples of PGPR used as a biocontrol agent against diseases and pathogens of different plants.

Plant	Disease/pathogen	PGPR	Reference
Sunflower	Sunflower wilt fungus	*Pseudomonas cepacia*	Tassi et al. (2017), Ambrosini et al. (2018)
Beans	*Sclerotium rolfsii*	*Pseudomonas cepacia*	Youseif et al. (2017)
Maize	Corn earworm; *Fusarium miniforms*	*P. maltophila* *Enterobacter agglomerans,* *P. cepacia* strain 406 and 526	Mosimann et al. (2017)
Rice	Rice sheath blight Rice root nematode *Rhizobium solani*	*Bacillus cereus*, *Streptomyces* spp., combined with *P. fluorescens* and *Burkholderia* sp., *P. fluorescens* Fp7 and Pf1	Andrade et al. (2018), Xu et al. (2019)
Tomato	Cucumber mosaic virus; Root knot nematode	*B. pumilus*, *B. amyloliquifacians* strain IN 937a;	Kumari et al. (2019), Lee et al. (2019)

		P. chitinolytica	
Wheat	Septoria tritici Other diseases	*P. aeruginosa* strain Leci; *P. putida* strain BK8661 *Rhodococcus, Pseudomonas, Bacillus, Penicillium* and *Beauveria* species	Fabiańska et al. (2019)
Barley	Powdery mildew	*B. subtilis*	
Sugar beet	*Fusarium oxysporum, Rhizopus stolonifer*, *Phona beta*, *Pythium ultimum,* Cyst nematode	*P. fluorescens* F113 *P. fluorescens*	Mosimann et al. (2017)
Mung bean	Root-knot and rot	*P. aeruginosa, B. subtilis*	Igiehon et al. (2019), Youseif et al. (2017)
Sugar cane	Red rot	*P. fluorescens other* PGPR	Fabiańska et al. (2019)

(Romera et al., 2019), cyclic lipopeptides, antibiotics (2,4-diacetylphoroglucinol) and some volatile compounds such as acetoin, ketones, 2,3-butanediol, alkanes, alcohols, sulfides, alkanes, esters and sesquiterpenes (Backer et al., 2018, Hu et al., 2018).

5.4.3. Antibiotics production

Soil-borne pathogens are known for their harmful effects on plant health and crop yield. Globally, many crops are destroyed due to the devastating impacts of pathogens, which reduce crop yield. The severity is more in Africa where crop yield is reduced to approximately 40% and harvest further to 20% (Adedeji et al., 2020, Lang et al., 2019). Recently, antibiotic production is among the research area extensively studied for biocontrol of phytopathogens and has been the most effective treatment to suppress phytopathogens globally (Olanrewaju et al., 2019).

Antibiotics are low molecular weight compound. Among its numerous functions are to inhibit the proliferation of pathogenic microorganisms and their metabolic activities (Deng et al., 2019). Antibiotics production is the major antagonistic activity to inhibit phytopathogens growth, thus playing significant roles in disease control and also can be used as biocontrol agents (Oak, 2019). In recent times, antibiotics produced by PGPR have been employed to suppress the negative impacts of pathogens in the plant root environment. Such antibiotics include phycocyanin, oomycin-A, pyrrolnitrin and 2,4-diacetyl phloroglucinol (Ju et al., 2020).

Evidently, studies have recorded that PGPR produce butyrolactones, zwittermycin A, xanthobaccin phenazine-1-carboxylic acids, pyrrolnitrin, kanosamine, iturins, fengycins

phenazine, circullin, colistin, polymyxin and 2,4-diacetylphloroglucinol (2,4-DAPG) with biocontrol effects on pytopathogens, such as *Fusarium oxysporum, Rhizoctonia solani, Podosphaera fusca* and *Streptomyces griseoviridis* that cause plant diseases, including leaf and seedling blight in rice, *Fusarium* root rot and tomato wilt (Backer et al., 2018, Garrido-Oter et al., 2018, Oberholster et al., 2018).

These biochemicals are basically produced by *Bacillus, Pseudomonas* strains, *Stenotrophomonas* sp. and *Streptomyces* spp. as active chemicals against phytopathogens (Lee et al., 2019, Tirry et al., 2018, Zhang et al., 2017). Some plant species naturally produce antibiotics that control plant diseases and are as well produced by soil microorganisms, for example Phloroglucinols (Backer et al., 2018, Ambrosini et al., 2018, Li et al., 2019a, Lindström and Mousavi, 2019). The biocontrol agents control plant diseases and have contributed to increasing crop productivity.

5.4.4. Competition for nutrients and binding sites

Additionally, poor soil health is a concern globally, PGPR capable of producing biochemical substances with inhibitory effects on phytopathogens, have been used to improve soil health in many African countries such as Nigeria, Benin and South Africa (Babalola and Akindolire, 2011, Alori and Babalola, 2018). The inoculated PGPR as biocontrol agent competes and in most cases out-compete the phytopathogens either for nutrients or binding sites in the host plant root region (Etesami and Maheshwari, 2018). Such competition acts by constraining phytopathogens from binding to the plants, therefore makes it relatively impossible to proliferate and infect the plant.

For example, nutrient competition is the biocontrol mechanism of *Pythium aphanidermatum* (Zhang et al., 2017).

Apart from inherent growth and availability of sufficient nutrients in the rhizosphere, some properties help PGPR competes favorably with phytopathogens and enhances their survival. These properties include the presence of flagella, chemotaxis and liposaccharides, and the use of synthesized exudates (Olanrewaju et al., 2019). In the ecological niche competition, PGPR dominate the niche and prevent colonization by phytopathogens until the available nutrients, such as Fe, is exhausted.

Recently, PGPR inoculants have been reported to have antifungal activity and compete against pathogenic fungi such as *Fusarium oxysporum* (Ambrosini et al., 2015). Similarly, the competitiveness of *Bacillus megaterium* in improving tomato plant growth has also been reported (Lindström and Mousavi, 2019). PGPR most times work in close association with other biocontrol mechanisms to halt the actions of phytopathogens (Hu et al., 2018, Singh et al., 2019b, Singh et al., 2019c).

5.4.5. Hydrogen cyanide production

Several PGPR produce hydrogen cyanide (HCN) in a process called cyanogenesis. Some of these PGPR can be inoculated into the soil to stimulate the production of HCN (Ambrosini et al., 2018). HCN is a volatile, broad-spectrum antimicrobial compound implicated in the biocontrol of phytopathogens associated with *Fluorescent pseudomonads* (Backer et al., 2018). The cyanide

ion obstructs the action of metalloenzymes, especially copper compose of cytochrome c oxidase. Secondary metabolite synthesized by gram negative bacteria is formed from glycine and catalyzed by the enzyme HCN synthase that is encrypted by biosynthetic genes such as *henA, henB* and *henC* (Castelle and Banfield, 2018).

Moreover, HCN stops the electron transport to the target cells and disrupt the energy supply leading to the death of organisms (Hu et al., 2018). HCN interrupts the proper activities of enzymes and inhibits natural receptor mechanisms. Some strains of Fluorescent *Pseudomonads* produce HCN and they have been implicated in the elimination of soil-borne pathogens (Lindström and Mousavi, 2019). PGPR such as *P. fluorescens* strain CHAO, *Bacillus, Stenotrophomonas, Brevibacterium* and *Pseudomonas* species isolated from rhizosphere soil produced HCN that inhibited the growth of pythium (Ambrosini et al., 2018). PGPR produce HCN in soil capable of altering the plant physiological activities by stimulating the formation of root hair (Deng et al., 2019).

5.4.6. PGPR mitigation of abiotic stresses in plants

In many agricultural fields globally, crop productivity and yield are affected by several toxicity, acidic soils and nutrient deficiency (fertility stress) limit the growth and productivity of various economically important crops in several agricultural fields globally. Apart from the intensive agricultural practices such as the prolonged use of external chemical inputs in many agricultural fields, the spreading impact of the global climate change causes several abiotic stresses on major crops worldwide. The predominant abiotic stresses such as drought, high soil salinity, nutrient

deficiency, acidic soils and metal toxicity significantly reduce the growth and yield of most cultivated crops (Daryanto et al., 2015, Naveed et al., 2014, Dimkpa et al., 2009b).

There are several mechanisms by which PGPR reduces the negative impacts of such abiotic stresses in plants and hence promotes growth indirectly. PGPR has been used as biofertilizers in the past few decades as an alternative strategy to reduce dependence on chemicals while improving plant growth and yield in a sustainable way (Rascovan et al., 2016). Many of these PGPR have abiotic stresses that adversely affect crop production. Abiotic stresses such as drought, salinity, metal developed various mechanisms to exert beneficial effects on abiotic stress in plants. One of the mechanisms PGPR uses to alleviate abiotic stress in soils is by producing the enzyme ACC deaminase. Upon inoculation of PGPR with this ability, the ACC deaminase reduces the plant ethylene levels, resulting in longer roots and relief from several abiotic stresses. Examples of PGPR that significantly alleviated different abiotic stresses in several agriculturally important crops include *Bacillus polymyxa* under nutrient deficient stress, *Azosprillum brasilense* on drought and salt stress, *P. fluorescence* on salt stress, *Pseudomonas fluorescence*, *P. putida* and *Azosprillum lipoferum* on drought (Dimkpa et al., 2009b).

Figure 5.1 illustrates the mechanisms by which certain plant growth-promoting rhizobacteria elicit induction of systemic tolerance (IST) in plants. In this interaction, various types of abiotic stresses such as drought, salinity and fertility stress could be mitigated by the production of certain bioactive compounds by the PGPR residing on the root surfaces. For instance, the secretion of cytokinins by the bacteria can result in the accumulation of abscisic acid (ABA) in the leaves,

which elicits stomatal closure under drought stress. Whereas the secretion of antioxidants and the enzyme, ACC-deaminase by PGPR results in the degradation of reactive oxygen species (ROS) and ACC precursor (Yang et al., 2009). These processes elicit induced systemic tolerance (IST) in the plants against drought and salt stress conditions and promote plant growth indirectly (Backer et al., 2018, Hu et al., 2018).

5.4.7. Lytic enzyme production

PGPR synthesize extracellular enzymes known as lytic enzymes. These enzymes hydrolyze polymeric compounds such as protein, cellulose, chitin and hemicellulose as an important form of biocontrol (Mukhtar et al., 2019). Lytic enzyme as a multifunctional naturally occurring protein protects plants from climatic factors, abiotic and biotic conditions (Olanrewaju et al., 2019). Lytic enzymes are secreted by PGPR to directly suppress the activities and growth of different phytopathogens, which are of concern. The presence of lytic enzymes in soil degrades a different range of compounds from the plant, and also destroys or digests fungal pathogen cell wall structure. The application of PGPR in soil activates the most critical eco-friendly mechanism (hydrolytic enzyme production) for the suppression of soil pathogens (Backer et al., 2018).

Similarly, PGPR produces and stimulates beneficial enzymes, such as lipases, chitinases, proteases and glucanases involved in the lysis of the cell wall. These enzymes degrade plant residues and nonliving organic matter to obtain carbon nutrition. In an experiment, *Myxobacteria* produces a lytic enzyme that has shown effectiveness against fungal phytopathogens (Kumari et al., 2019). Plant diseases such as bipolaris leaf spot induced by *Pythium* sp. and *Bipolaris* sp. are reportedly

suppressed by glucanase and beta-1, 3-glucanase produced by *Lysobacter and Lysobacter enzymogenes* strain C3, respectively (Şeker et al., 2017). Furthermore, *Serratia marcescens* produces chitinase that has an antagonistic effect on *Sclerotium rolfsii.* Nevertheless, hydrolytic enzymes directly control phytopathogens and save plants from different biotic and abiotic stresses affecting sustainable agriculture (Oak, 2019).

5.5. Role of PGPR in phytoremediation

The green technology is emerging eco-friendly and inexpensive strategies to improve agricultural soil contaminated with heavy metal which results in advanced soil health (Tirry et al., 2018). Phytoremediation involves the use of specific plant cultivars that are heavy metal tolerant, and as well as plant growth-promoting microorganisms (Urana et al., 2019). Phytostabilization is a major strategy that makes up phytoremediation that uses a particular plant cultivar to stabilize heavy metal contaminants, consequently reducing their bioavailability in soil (Ayangbenro and Babalola, 2017).

For soil remediation to be considered efficient, it requires plants to intercept, take-up, accumulate, sequestrate, stabilize and translocate heavy metal contaminants (Singh et al., 2019b). Despite the potential of cleaning up heavy metals in the soil, phytoremediation is faced with some factors that can slow down or stop the process (Singh et al., 2019b). For instance, such factors are nutrient availability, a type of pollutant, plant species, enzyme class, soil component and pH (Ayangbenro and Babalola, 2017). The plant microbial community determines the success of phytoremediation process. Likewise, microbial biomass, soil enzymes and microorganisms are major mediators of

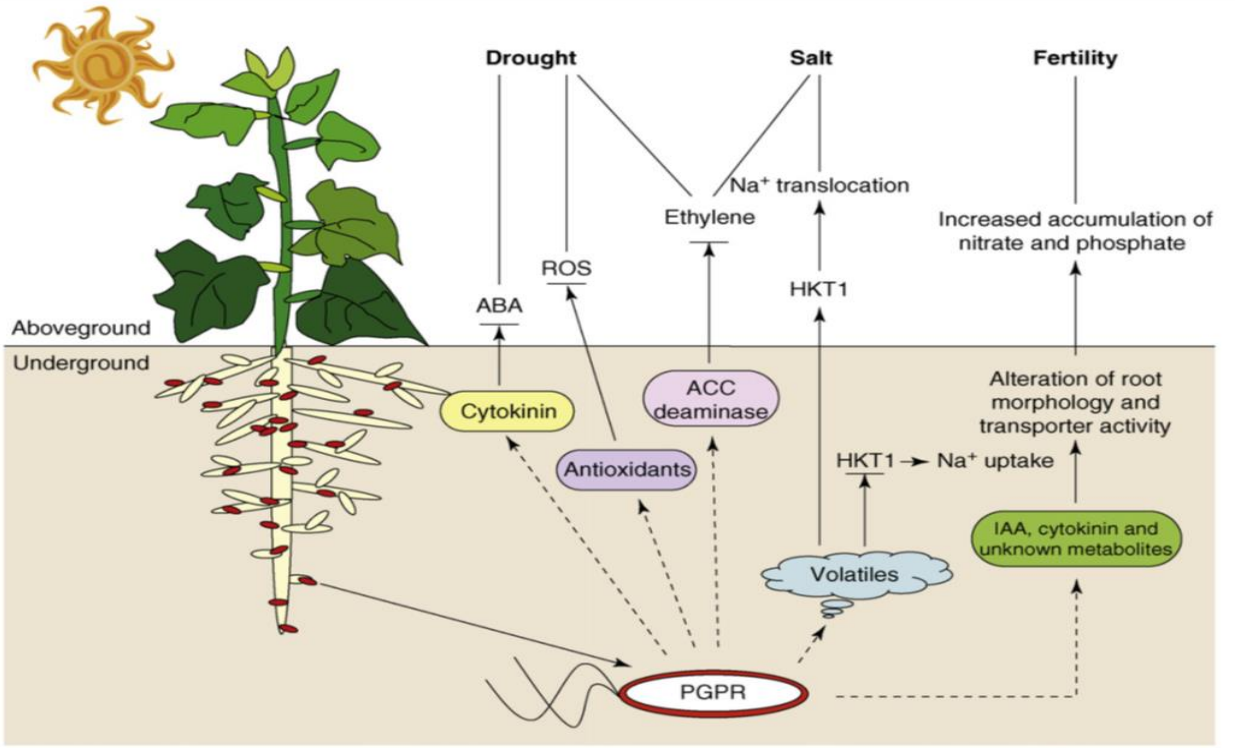

Figure 5.1: A schematic representation of how PGPR are involved in the mitigation of various abiotic stresses, including drought, salinity and nutrient deficiency (fertility) stress and elicit induced systemic tolerance (IST) in plants. PGPR stands for plant growth promoting rhizobacteria. Source: (Hassen, 2009).

biological processes even serve as an indicator of heavy metal stress (Tirry et al., 2018, Ju et al., 2020).

When heavy metal tolerant PGPR are inoculated into polluted soil, they stimulate plants to perform tasks such as heavy metal sequestration, recycling nutrients, control pathogens, mineralization, sustain soil structure and detoxify chemicals. As a result, the plants release exudates, including amino acids, phytohormones, vitamins and proteins for the microorganism (Keswani et al. 2019). The soil enzyme action responds quickly to metal stress, for example, catalase can degrade hydrogen peroxide (H_2O_2) and stops it from contaminating soil organisms (Arif et al., 2017, Peng et al., 2018).

Correspondingly, enzyme activities involved in soil organic carbon (C) (saccharase and β-glucosidase), P (acid phosphatase) and N (urease) cycles are considered important indices that present as the potential for soil enzyme synthesis by microbiomes (Arif et al., 2017, Urana et al., 2019). Microbial community diversity reduces with increasing levels of heavy metals such as zinc (Zn) and cadmium (Cd) in soil (Khan et al., 2018b, Tiwari and Lata, 2018). In a heavy metal polluted soil *Trigonella foenum-graceum* and *Brassica juncea* with PGPR inoculated into the soil showed rapid degradation of phenanthrene. This was as a result of high microbial population and exudates secreted by plants and root-associated microbial consortium (Urana et al., 2019).

PGPR such as *Pseudomonas* sp. GHD-4 improved soil enzyme activities and enriched microbial community diversity by degrading lead (Pb) and reducing its concentrations in soil (Xu et al., 2019). Heavy metal contaminated rhizosphere soil inoculated with PGPR has been reported to

have greater microbial diversity compared to uninoculated soil (Ju et al., 2020). PGPR involve in phytoremediation do not only benefits plant growth, but also improves soil quality (Peng et al., 2018).

5.6. Techniques of applying microbial inoculants to enhance plant growth

Microbial inoculants are made up of live microorganisms, mainly bacteria such as rhizobia or fungi capable of improving plant growth and development. Besides, it could be a pure or mixed culture. Similarly, they exist in solid or liquid form (Santos et al., 2019). A good carrier for preparing microbial inocula should provide a conducive environment for PGPR to live and grow. They also have long cellular viability at room temperature for two months and above. A suitable carrier is characterized by being ecologically friendly, easy to apply, stable, nontoxic to inoculant and plants. Also, they are easy to sterilize using gamma-irradiation or autoclave, readily available and are in scarce form. In addition, it (the carrier) has good pH buffering capacity, moisture absorption capacity, is easy to process and should be free from lump-forming materials.

The carrier type determines the application of microbial inoculant. Solid microbial inoculant materials such as peat are bead-like or granules are rich in organic matter and are used for microorganism immobilization (Moretti et al., 2018). In addition, liquid inoculants are microbial cultures in broth form rich in cell protectors and nutrients. Moreover, liquid inoculants can be suspensions in organic oils, or mineral or oil in water suspensions. In seed inoculation, liquid or powder microbial inoculants are used to coat the seeds. Likewise, other methods of liquid inoculant applications are in-furrow or foliar application, or spray on soil (Santos et al., 2019).

Again, other materials and techniques for carrying microorganisms are through lyophilization, industrial and agricultural residues and polymers for cell encapsulation (Moretti et al., 2020). Carriers from agricultural and industrial residues such as wheat bran, clay, sawdust, rice bran, lignite powder and peat can be augmented with inorganic or chitin containing media such as betonies, vermiculite, silicate and kaolin for soil or seed inoculation (Fukami et al., 2016). The use of different microbial inoculants around the world and in Africa over the past decades has increased, resulting in higher crop yields.

5.7. Application methods of PGPR in field

The applications of PGPR in field conditions have been studied extensively and reported. A variety of PGPR have been used as soil bioinoculants intended to enhance the supply of nutrients to plants. Diverse species of PGPR such as *Clostridia, Rhizobium, Bacillus, Azotobacter, Klebsiella* and *Azospirillum* sp. have successfully been inoculated in the fields to understand the mechanisms and effectiveness of PGPR to increase plant development and productivity. The beneficial effects of inoculations of PGPR (*Rhizobium* species, including *Sinorhizobium*, *Mesorhizobium*, *Allorhizobium*, *Bradyrhizobium* etc.) have significantly increased plant vegetative and yield by nitrogen-fixing symbiosis with leguminous plants (Basu et al. 2021). Additionally, Clostridia was reportedly isolated from rice soils and their interaction also increased after re-inoculating straw to fields which elevated the C to N ratio in the soil. *Azospirillum* has reportedly increased wheat yields in both field and greenhouse conditions. *Azospirillum lipoferum* increased rice yield to approximately 1.8 t ha−1 in a field experiment (Cassan and Diaz-Zorita, 2016). ^{15}N tracer

techniques in a field study showed that *Azospirillum lipoferum* and *Azospirillum brasilense* contributed 7–12% of wheat plant nitrogen (Ilyas et al. 2020).

Several field studies have confirmed that *Azospirillum diazotrophicus* isolated from rhizosphere soil increased sugarcane plant growth by improving N to over 200 kgN ha−1 year−1 (Fukami et al. 2016). *Acetobacter*-sugarcane system has now become an active experimental model and the diazotrophic character (nif+) is a significant module of this system (Anaya and Huber-Sannwald, 2015). *Azoarcus* sp. BH72 isolated from Kallar grass (*Leptochloa fusa* Kunth) colonized rice rhizosphere soils which reportedly improved microbial interactions and positively increased rice yield in both field and laboratory conditions (Chauhan et al. 2015). In field trials, many species of Enterobacteriaceae, including diazotrophs mainly those isolated from the rhizosphere of agricultural plants with plant growth-promoting potential colonize sugarcane, sorghum, rice, maize and other crops (Vegan et al. 2016). Also, improvement of soybean yield, seed size, fat content and drought stress by microbial inoculation correlated with enhancement in soil microbial assortment in the rhizosphere since it was previously reported by Igiehon et al. (2021) that microbial communities could be affected by *Rhizobium* and mycorrhizal fungi inoculation as well as fertilizer amendment.

5.8. Commercialization of PGPR products and challenges in developing countries

The green revolution has sadly introduced chemical pesticides, herbicides and inorganic fertilizers into agricultural soils that led to huge damage to the soil environment in the form of pollutants. To

resolve this problem, there is a need to design microbial consortia that can help to solve aspects of plant growth potential and bioremediation (Sergaki et al., 2018).

In many developed countries, including the United States of America, Australia, Europe and in some developing countries like Brazil, Argentina and India, there is a well-documented success in the commercialization of PGPR products as biofertilizers and biocontrol agents (Keswani et al., 2019, Prasad et al., 2019a, Urana et al., 2019). Despite the numerous benefits of PGPR inoculants, the development and their commercial application in sustainable crop production and protection in developing nations, particularly in Africa are still very scarce (Keswani et al., 2019).

Developing PGPR into new microbial inoculants depends on initial laboratory screening assays that are aimed at detecting particular PGPR traits such as phosphate solubilization, N fixation, auxin biosynthesis, siderophore production, ACC deaminase activity and antibiotic production (Backer et al., 2018, Prasad et al., 2019a). Screening of PGPR for these plant growth-promoting traits under *in-vitro* assays does not necessarily result in isolates that can improve plant growth under field conditions (Keswani et al., 2019, Singh et al., 2018, Moretti et al., 2020).

A major challenge in the commercialization of PGPR are developing consortia of highly efficient microorganisms with high rhizosphere colonization and persistence (Chaudhary et al., 2020). PGPR are frequently inoculated on plant materials but without a suitable carrier or quantities. They do not allow effective colonization of the rhizosphere under field condition, especially due to competition with indigenous soil macro and micro fauna (Sergaki et al., 2018). An additional challenge emanates from soil management practices such as fumigation of soil with broad-

spectrum biocidal fumigants that changes the biological community structure of the soil, including introduced microbial inoculants (Chaudhary et al., 2020, Gopalakrishnan et al., 2015).

The formulation of bioinoculants exceptionally for particular soil conditions reduces environmental challenges. Also, training of farmers and workers on how to expertly apply bioinoculants on crops is critical in the advancement and distribution of beneficial inocula (Urana et al., 2019). In fact, the need to educate the general population and farmers on the advantages of formulated products containing beneficial microorganisms in agriculture and to explore the full richness of fertile land for sustainable food production is crucial (Itelima et al., 2018).

5.9. Conclusion

Initially, chemical inputs improved plant growth and productivity, but later exercised their negative effects. These long-aged norms have not only adversely damaged the soil and its inhabitants, but also a threat to humans through the food chain and run-off from soils into water. Soil infertility and production areas are consequences of soil contamination, climate change, land overuse and soil-borne pathogens. This is proven in low agricultural production and food insecurity among the increasing human population globally.

There is a need for sufficient effort to be made to use and apply existing scientific knowledge for a better understanding of soil-plant-microbial associations and their modes of action in nutrient acquisition and plant growth promotion. With the recent increase in the awareness and demand to

use PGPR for a sustainable agroecological system, the need to substitute agrochemicals with the biological products made up of single or a consortium of elite PGPR are very crucial.

There are several benefits of microbial inoculant products with PGPR as active ingredients. These include increased mineral nutrients availability and biocontrol through antibiosis, ISR and competition for essential nutrients such as Fe through the chelation effect of siderophores (the competition must be carefully stated and understood by farmers for improved crop productivity). Genetic engineering of PGPR are an important constituent in modern food production that will mitigate soil contaminants, environmental alteration and destruction of soil flora and fauna when properly harnessed specifically in developing economies.

CHAPTER SIX

High-throughput sequencing survey of sunflower soils

Overview

For a very long period, the taxonomic description of microbial communities was an unsolvable issue with high material and labour expenses. There are restrictions on the number of recognized species and explored habitats due to the intricacy of the cloning process and additional nucleotide chain sequencing. The next-generation techniques used in molecular ecology, such as high throughput, made a significant advancement. The effectiveness of sequencing has increased from a few hundred (Sanger's method) to thousands of nucleotide sequences, enabling the most precise identification of intricate multicomponent systems like soil microbial communities. Characterization of the world's microbiome is currently the focus of various multinational efforts.

Due to the use of innovative sequencers, the Earth Microbiome Project (http://www.earthmicrobiome.org/) has already gathered information on the taxonomic organization of the microbiomes of various ecological niches. More than 9.000 samples, or over 800 million nucleotide sequences, have already been investigated within the framework of this project. However, for South Africa, this database only contains a few dozen soil samples. The work presented here is intended to fill a gap. This task is especially important in South Africa, whose territory contains almost every soil type and is the most promising experimental area of its kind on the planet.

In addition, studying soil microbial communities using modern sequencing methods is a practical step toward developing new optimized agricultural technologies. Such approaches can be developed using features of the soil microbiome as a universal and highly sensitive indicator of soil condition. A research study of soil microbiomes in various types of soils whose task was set in the investigation of this study is a starting point for the implementation of such projects. The following section (chapter 7) presents data on the bacterial communities structure linked with sunflower rhizospheric soils from Lichtenburg, North West, South Africa. The Illumina Miseq sequencing of the 16S rRNA gene and the distribution of taxonomic hits from USEARCH analysis revealed the diversity of bacterial communities at different levels of classification, also, the creation of sunflower rhizospheric soils metagenomic library to better understand the diversity and distribution.

Abstract

Plant rhizobiomes are responsible for major soil processes in the immediate plant environment, but our knowledge of the linkage between belowground microbiota diversity and plant health is limited. The bacterial and archaeal communities of sunflower rhizosphere organisms was studied by comparing the composition of these communities to bulk soils at three farms in the North West province of South Africa. A plethora of bacterial and archaeal taxa are evaluated and described in this chapter.

6.1. Introduction

South Africa, as one of the semiarid areas in the world, is a goldmine of important soil microorganisms with various functions that could be used for biofertilization. Sheila, Itsoseng and Kraaipan are towns located in the Ngaka Modiri Molema local district municipality in the North West Province of South Africa. North West Province is, after Free State, is the bedrock of sustainable sunflower production in the country, accounting for around 80% of sunflower plantations and contributing significantly to the economy (Nkuekam et al., 2018). The high productivity of the study areas can be attributed to the plant growth-promoting rhizobacteria present in the soil. This study was designed to determine the structure of sunflower rhizospheric soil bacterial and archaeal communities of the study areas.

6.2. Methodology

Soil samples were obtained from sunflower rhizosphere and bulk soils from three farms in North West Province. Farm soil samples came from Sheila (SH) (26° 2941.2020S, 25°57947.490E), Itsoseng (IT) (26°4923.0640S, 25°58937.1040E) and Kraaipan (KP) (26°17924.1860S, 25°13933.2580E). The rhizosphere soils were collected using a destructive sampling method in which sunflower plants were uprooted from an area of 2 by 4 m^2. Then, the soil that was attached to the plant roots after shaking the uprooted plant was collected and kept in a sterile plastic bag. The bulk soils were collected 10 m from each site where sunflower rhizosphere soils were collected and also kept in sterile plastic bags (Amoo and Babalola, 2019). The soil samples were immediately transported in an icepacked container to the laboratory and stored in a cold room at

4°C prior to analysis. A kit (Zymo, California, USA) was used to extract microbial DNA from 2 g of each soil sample, according to the manufacturer's instructions.

Sample sequencing was performed using an Illumina MiSeq instrument at Molecular Research LP (Shallowater, TX, USA). The purity and concentration of the DNA samples were analyzed using NanoDrop ND-2000 and Qubit DNA broad-range (BR) reagent assays. 16S rRNA libraries were obtained using the quality control (QC)-passed DNA samples with the PCR primers 515F (59-AATGATACGGCGACCACCACCGAGATCTACAC TATGGTAATTGTGTGCCAGCMGCCGCGGTAA-39) and 806R (59-CAAGCAGAAGACGGCAT ACGAGATTCCCTTGTCTCCAGTCAGTCAGCCGGACTACHVGGGTWTCTAAT-39) (Caporaso et al., 2010), with adapters and standard Illumina barcodes. The DNA samples were purified using AMPure XP beads. As a quality control measurement, the barcoded libraries were validated using the Agilent DNA 1000 bioanalyzer, and the concentration was quantified using a Qubit assay kit. The libraries were merged and sequenced on the Illumina MiSeq platform. The raw sequences were processed and analyzed on MGRAST server v4.0.3 (http://metagenomics.anl.gov/) (Meyer et al., 2008a). After demultiplexing of the paired-end reads, the raw data were uploaded as FASTQ files. Quality processing and deduplication by MG-RAST pipeline analysis generated reads which were subjected to taxonomic analysis (Meyer et al., 2008a). Then, the bacterial and archaeal abundances present in the Sheila, Itsoseng and Kraaipan soils were estimated (Meyer et al., 2008a). All bioinformatics tools were run with default parameters (Oberholster et al., 2018).

6.3. Results

At the domain level, the taxonomic descriptions of archaea and bacteria showed 7.0 to 13.4% and 86.59 to 99.30% mean read values, respectively. Bacterial phyla such as Firmicutes (17 to 51%), Proteobacteria (18 to 36%) and Actinobacteria (7 to 38%) were the most abundant. Archaeal reads were allotted to Thaumarchaeota (1 to 13%) and Crenarchaeota (1%) abundance. The numbers of raw sequence reads of each sample, which are higher than the corresponding numbers of reads that passed quality control (QC), are shown in Table 1. Quality control of the metagenomics data was done to remove low-sequencing reads and contaminating reads (e.g., reads of eukaryotic species). Since this study is based on the 16S amplicon, the number of reads that passed QC is lower than the number of raw reads, possibly because the samples contained more eukaryotic species, such as fungi, that were identified and removed during the quality control step.

Table 6.1: Sequence information from MG-RAST and NCBI databases for the analyzed soil samples.

Sample	Description	NCBI Bioproject no.	SRA accession no	No of raw sequence reads	No of reads that passed quality control	Mean sequence length (bp)
S1_A	SH rhizosphere soil replicate 1	PRJNA672856	SRR12960272	138,303	17,428	287 ± 17
S2_B	SH rhizosphere soil replicate 2	PRJNA672856	SRR12960271	112,226	11,684	288 ± 13
S3_C	SH bulk soil replicate 1	PRJNA672856	SRR12960268	116,529	12,217	290 ± 14
S4_D	SH bulk soil replicate 1	PRJNA672856	SRR12960267	57,330	8,511	268 ± 66
S5_M	IT rhizosphere soil replicate 1	PRJNA672856	SRR12960266	101,263	14,962	291 ± 10

S6_N	IT rhizosphere soil replicate 2	PRJNA672856	SRR12960265	82,616	14,313	291 ± 8
S7_O	IT bulk soil replicate 1	PRJNA672856	SRR12960264	81,580	14,921	291 ± 10
S8_P	IT bulk soil replicate 2	PRJNA672856	SRR12960263	95,189	16,961	291 ± 10
S9_Q	KP rhizosphere soil replicate 1	PRJNA672856	SRR12960262	123,996	17,947	287 ± 15
S10_R	KP rhizosphere soil replicate 2	PRJNA672856	SRR12960261	148,667	11,014	287 ± 19
S11_S	KP bulk soil replicate 1	PRJNA672856	SRR12960270	115,727	15,742	288 ± 14
S12_T	KP bulk soil replicate 2	PRJNA672856	SRR12960269	138,303	17,428	287 ± 17

6.4. Data availability

The raw sequence files (reads in FASTQ format) were deposited at the NCBI SRA database under the BioProject number PRJNA672856 for samples SH (samples S1_A, S2_B, S3_C and S4_D), IT (samples S5_M, S6_N, S7_O and S8_P) and KP (samples S9_Q, S10_R, S11_S and S12_T). The samples can be accessed under SRA accession numbers SRR12960272, SRR12960271, SRR12960268, SRR12960267, SRR12960266, SRR12960265, SRR12960264, SRR12960263, SRR12960262, SRR12960261, SRR12960270 and SRR12960269. The quality-filtered and annotated data for individual replicates have been released publicly in the MG-RAST database with the accession numbers mgs831279 (sample S1_A), mgs831282 (sample S2_B), mgs831285 (sample S3_C), mgs831300 (sample S4_D), mgs831303 (sample S5_M), mgs831306 (sample S6_N), mgs831309 (sample S7_O), mgs831312 (sample S8_P), mgs831270 (sample S9_Q), mgs831279 (sample S10_R), mgs831273 (sample S11_S) and mgs831276 (sample S12_T).

CHAPTER SEVEN

Amplicon sequencing data profiling of bacterial community connected with the rhizospheric soil from sunflower plants

Overview

Root-associated microbial communities play critical roles in plant health, growth and productivity. Rhizobacteria including nitrogen-fixing bacteria, for example, are liable for 40-50% of total nitrogen and up to 75% of total phosphorus acquired by plants each year. Thus, manipulating the rhizosphere microbial population has the possibility of boosting the yield of crops. Past studies have reported that soil type is the main factor of rhizospheric microbial communities, majority presumably due to the diverse microbial "inocula" available in each soil type. Other factors that influence microbial community composition include plant development stage, plant species, climatic conditions and the interactions between these factors.

Reports on the rhizosphere microbiota of some crops such as sunflower have revealed that plant species evaluated had very distinct microbiota from one another. The researchers have documented that while plants exerted strong selection on rhizospheric microbial populations, their structure and consistency were also affected by soil type. Overall, soil type and intensive agriculture that directly influences the soil is thought to be the primary drivers of rhizosphere microbial populations in agricultural settings, whereas plant species and environmental conditions are thought to be the most influential factors in natural ecosystems.

Plant roots are assumed to select for particular microbiota by producing a wide range of metabolites, such as secondary metabolites like antimicrobial substances and defense phytohormones that differ depending on plant species and age. Roots also alter the oxygen concentrations and pH in the surrounding soil the root, resulting in a distinct soil microhabitat. However, while the insight into rhizospheric microbial populations has recently increased, many factors of their assembly remain unknown, particularly for plants growing in the field. The majority of rhizosphere region studies are conducted on plants cultivated in controlled environments, with fewer studies performed in the field. Most indoor experiments use small containers, which have been shown to have a detrimental effect on root function, root microbial distribution and plant growth. The bacterial population composition and diversity in the rhizosphere of sunflower, grown in crop rotation under field conditions in South Africa, were investigated in this study and published as a dataset. The following section (chapter 8) highlighted the composition of rhizospheric microbial community from was subjected to next-generation sequencing using 16S amplicon sequencing technique and the community-level physiological profiling (CLPP) analysis was used to evaluate the functional of sunflower rhizosphere grown in the field in two different locations (Sheila and Itsoseng in North West province, South Africa).

Abstract

This chapter presents dataset on the bacterial communities structure associated with rhizospheric sunflower soils from Lichtenburg, South Africa. The Illumina Miseq sequencing of 16S rRNA gene amplicon unveiled the bacterial communities diversities as well as generated metagenomic

library from sunflower rhizospheric soils to understand the diversities and distribution better. The metagenome contained a total of 41,471 sequences with 45,975 operational taxonomic units (OTUs). Metagenome sequence information is obtainable at NCBI under the Bioproject assigned accession number PRJNA782103. Taxonomic hits distribution from USEARCH analysis at phylum level classification of BN_E discovered predominantly Actinobacteria (33.89%), followed by Proteobacteria (35.45%), Firmicutes (6.45%), Planctomycetes (4.12%), Chloroflexi (4.28%) and Gemmatimonadetes (2.40%). Also, USEARCH assisted analysis of BN_F sample also detected the prevalence of Actinobacteria (45.92%), Proteobacteria (23.23%), Firmicutes (3.84%), Planctomycetes (6.46%), Chloroflexi (4.94%) and Gemmatimonadetes (1.99%), which take part in vital ecological functions and biogeochemical activities needed for plant growth and health.

Table 7.1: Specifications table.

Subject	Microbiology
Specific subject area	Bacteriology, Next Generation Sequencing (NGS)
Type of data	Amplicon data (Text file and Figures)
How data was acquired	16S rRNA metagenome sequencing was performed on Illumina MiSeq platform (www.illumina.com) and OTU clustering analysis was conducted using USEARCH.
Data format	Raw data (FASTQ)
Parameters for data collection	Sunflower rhizospheric soils were collected, and bacterial diversity were analyzed from the extracted DNA using commercial DNA isolation kit (Zymo ®isolation kit, Zymo Research, USA).
Description of data collection	Sequence reads were processed through USEARCH (https://drive5.com/usearch) and taxonomy were assigned

	using Ribosomal Database Project's (http://rdp.cme.msu.edu/index.jsp) 16s database v16 database.
Data source location	Sunflower rhizospheric soils were collected from Lichtenburg (S 26°.4`31.398`` E25°.5`84.4286), North West Province, South Africa.
Data accessibility	The raw metagenomic DNA sequences has been deposited on NCBI repository with Bioproject ID: PRJNA782103. https://www.ncbi.nlm.nih.gov/bioproject/PRJNA782103. The Operational Taxonomic Units (OTUs) tables have been deposited into Mendeley Data repository "Operational Taxonomic Units of metagenomes from rhizospheric soils of sunflower plant" with DOI: 10.17632/nprdypz7c4.1. https://data.mendeley.com/datasets/nprdypz7c4/1.

7.1. Value of the data

The data presented in this study are important because exploiting sunflower-associated rhizosphere bacterial community structure with active novel genes and functions have promising potentials in promoting sustainable agriculture. The data information of culturable and unculturable bacterial population evaluated can be used by researchers and farmers for improved production of agricultural crops such as sunflower. The datasets can be employed to estimate the roles of various bacterial species in maintaining the rhizosphere which are important for plant growth and in solving the problem of food security.

7.2. Description of data

The dataset contains a raw metagenomic sequence obtained using amplicon sequencing of the sunflower rhizosphere bacterial community. The data files in FASTQ format can be found on National Center for Biotechnology Information (NCBI) portal with SRA accession number Bioproject ID: PRJNA782103.

A total of 26,854 (BN_E) and 20,617 (BN_F) reads were analyzed, and 25,842 (BN_E) and 20,133 (BN_F) operational taxonomic units (OTUs) were classified in order to reveal the diversity of bacterial community. All the resulting OTUs were then classified into phyla, classes, orders, families and genera. USEARCH analysis at phylum level classification discovered preponderantly Actinobacteria followed by Proteobacteria, Firmicutes, Planctomycetes, Chloroflexi and

Gemmatimonadetes. The data are obtainable in Figures 7.1 and 7.2, respectively. The categorized OTUs represent distinct classes dominated by Actinobacteria, Alphaproteobacteria, Gammaproteobacteria, Bacilli, Thermoleophilia Rubrobacteria Betaproteobacteria and Phycisphaerae. At orders level Actinomycetales, Pseudomonadales, Caulobacterales, Bacillales, Rhizobiales, Caulobacterales, Rhodospirillales and Rubrobacterales had higher OTUs. Families with the highest level of dominance were Caulobacteraceae Bacillaceae, Pseudomonadaceae, Rubrobacteraceae, Geodermatophilaceae, Micrococcaceae, Rubrobacteraceae, Geodermatophilaceae, Nocardioidaceae, Bradyrhizobiaceae and Acetobacteraceae. The analysis classified 10 most dominant genera, which were *Pseudoalteromonas*, *Arthrobacter*, *Rubrobacter*, *Balneimonas*, *Bacillus*, *Nocardioides*, *Acinetobacter*, *Rubrobacter*, *Pseudomonas*, *Brevundimonas* and *Modestobacter*. The 16S rRNA amplicon sequence information of the six most dominant OTUs in the bacterial species estimated in the rhizospheric soil of sun- flower are shown in Table 1.

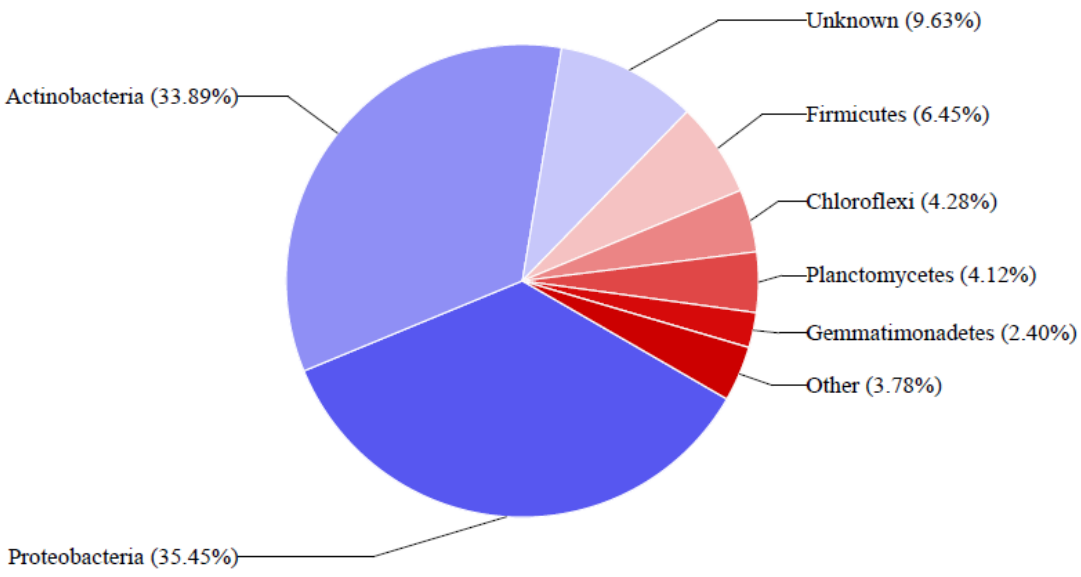

Figure 7.1: Percentage of key bacterial composition from sunflower rhizosphere soil samples (BN_E)

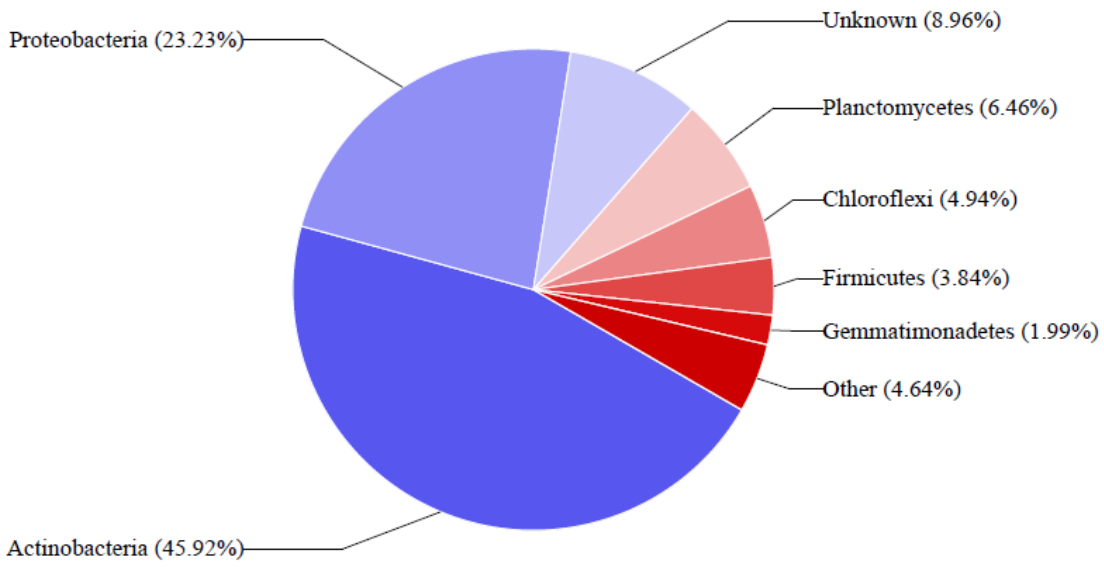

Figure 7.2: Percentage of key bacterial communities identified from sunflower rhizosphere soil samples (BN_F)

Table 7.2: Identities of the six most dominant OTUs in the bacterial communities from sunflower rhizosphere soil.

Relative abundance order	Taxa (Read count and % of abundance)	
	BN_E	BN_F
1	*Acinetobacter_johnsonii* (1482.0; 5.57%)	*Rubrobacter_* (1030.0; 5.06%)
2	*Rubrobacter_* (1071.0; 4.03%)	*Balneimonas_* (433.0; 2.13%)
3	*Acinetobacter_guillouiae* (720.0; 2.71%)	*Modestobacter_* (255.0; 1.25%)
4	*Brevundimonas_poindexterae* (639.0; 2.40%)	*Arthrobacter_pascens* (222.0; 1.09%)
5	*Bacillus_cereus* (575.0; 2.16%)	*Arthrobacter_* (158.0; 0.78%)
6	*Pseudomonas_fragi* (396.0; 1.49%)	*Solirubrobacter_* (150.0; 0.74%)

7.3. Experimental design, materials and methods

In this dataset, rhizosphere soil samples were collected from sunflower rhizosphere soils in Lichtenburg, South Africa (26.431398S: 25.5844286E) in March, 2020 at two different sites (BN_E and BN_F) and the metagenomic DNA extraction was performed using the Zymo® DNA isolation kit (Zymo Research, USA) following the manufacturer's instruction (Babalola et al., 2020). Metagenomic DNA purity and concentration were ascertained using a NanoDrop Lite Spectrophotometer (Thermo Fischer Scientific, CA, USA). Sequencing was performed using the Illumina Miseq platform at Inqaba Biotechnical Industries (Pty) Ltd, Pretoria, South Africa. The initial metagenomic DNA quality was evaluated using Qubit® dsDNA HS Assay Kit (Life Technologies).

The 16S rRNA library preparation was done using 16S rRNA gene universal primers (Forward Primer: ACTCCTACGGGAGGCAGCAG) and (Reverse Primer: GGACTACHVGGGTWTCTAAT) with standard Illumina adapters and barcodes (Babalola et al., 2021c). In contrast, the Ampure XP beads were used to further purify the Amplicons (Babalola et al., 2021a). The barcoded libraries were determined by Agilent DNA 1000 Bioanalyser and Qubit DNA BR reagent assay was used for quantification. The quantified libraries were pooled and sequenced using MiSeq (Edgar, 2010). Reads were processed through USEARCH (https://drive5.com/usearch) (Edgar, 2010) and taxonomic information was determined based on the Ribosomal Database Project's (http://rdp.cme.msu.edu/index.jsp) 16s database v16 database (Maidak et al., 1994). The seed sequence for each cluster was arranged by length and clustered

with a 3% divergence cut-off to create centroid clusters. Operational Taxonomic Units (OTUs) contributing less than 1% in size of the total data was excluded (Schmid et al., 2019).

CHAPTER EIGTH

Comparative study of microbial structure and functional profile of sunflower rhizosphere grown in two fields

Overview

Identifying the distribution of bacterial communities in rhizospheric soil has opened up new possibilities for utilizing their significance in agricultural systems. Different microbial communities colonize rhizospheric soil to maximize plant adaptive responses via various metabolic pathways. Although most microbial species have not been cultured and isolated, the characterization of rhizobacteria communities from numerous environmental samples is popular and commonly used in environmental researches. More importantly, advances in microbial biotechnology have revealed the benefit of amplicon metagenome sequencing in microbiological research. Besides, when compared to culture-dependent techniques, the use of 16S rRNA gene sequencing in assessing bacterial community structure in the sunflower rhizosphere region is progressing.

Bacteria species that colonize the rhizosphere are beneficial, but the majority have effect on plant fitness and development. However, little is known about the diversity and functionality of rhizobacteria communities in oil food crops like sunflower, necessitating this research. Rhizobacteria rapidly and extensively modify soil conditions within the rhizosphere. These rhizobacteria community activities have a significant impact on soil pH, soil aeration, soil texture,

soil nutrients, and thus influence decomposition rate, soil fertility and microbial diversity. Plant attributes to soil and microbial diversity in agrarian farmlands are required for soil to function properly. When plant contribution to soil is altered, changes in microbial ability to utilize carbon compounds are unavoidable.

Studies have shown that soil microbial populations can become familiar or altered to break down carbon substrates specific to their soil environment. Each microbial community has its own set of carbon sources that are essential to their survival. Carbon substrates vary from ecosystem to ecosystem and are hugely impacted by ecosystem disruption. As a result, soil microbes' ability to break down these substrates may suffer. Microbial societies are thought to be the primary indicators of several soil functions, including aggregate formation, nutrient mineralization, and plant health.

Evaluating soil functional diversity primarily entails evaluating microbial heterotrophic ability. This entails assessing soil respiration reactions in a short period of time to a variety of carbon substrates with varying structural complexity in order to generate community-level physiological profiling (CLPP). Because of the short duration of the assay methods and the processes (such as sieving) associated in soil preparation, CLPP assays have the potential to influence the quantification of microbial substrate reaction.

Under controlled laboratory conditions, the MicroRespTM method for CLPP provides an in-depth means of distinguishing sites or soil samples. The mechanism used by the MicroRespTM method is based on the estimation of microbial respiration obtained through a variety of carbon materials,

and the level to which microbial biomass used a particular carbon material indicates their richness. MicroRespTM offers an appropriate, quick, and effective technique for measuring whole soil CLPP, and this method is independent of microorganism cultivation and extraction. Its use in several research studies has affirmed its usefulness and advantages over other techniques. The diversity of the microbial community in sunflower rhizospheric soils from two different locations in South Africa (Sheila and Itsoseng) was assessed using 16S rRNA gene sequencing in this study.

The MicroRespTM technique was also used to estimate soil microbial functions. This will help to explain differences in the diversity of microorganisms at the structural and functional levels in soils from rhizospheric soil to their bulk soil samples. The next chapter (chapter 9) was designed to give insight into the impacts of soil physicochemical properties and carbon substrate utilization on the heterogeneity of bacterial community of sunflower rhizospheres grown in Ditsobottla and Kraaipan, North West Province, South Africa.

Abstract

Microbial communities inhabiting the rhizosphere play pivotal roles in determining plant health and yield. Manipulation of the rhizosphere microbial community is a promising means to enhance the productivity of economically viable and important agricultural crops such as sunflower (*Helianthus annuus*). This study was designed to gain insights into the taxonomic and functional structures of sunflower rhizosphere and bulk soil microbiome at two different locations (Sheila and Itsoseng) in South Africa. Microbial DNA extracted from the sunflower rhizosphere and bulk soils was subjected to next-generation sequencing using 16S amplicon sequencing technique.

Firmicutes, Actnobacteria and Proteobacteria predominated sunflower rhizosphere soils. Firmicutes, Cyanobacteria, Deinococcus-Thermus and Fibrobacteres were positively influenced by Na^+ and clay content, while Actinobacteria, Thaumarchaeota, Bacteroidetes, Planctomycetes, Aquificae and Chloroflexi were positively influenced by soil resistivity (Res) and Mg^{2+}. The community-level physiological profiling (CLPP) analysis showed that the microbial communities in SHR and ITR used the amino acids tryptophan and malic acid efficiently. The metabolisms of these carbon substrates may be due to the dominant nature of some of the organisms, such as Actinobacteria in the soils. The CLPP measurements of soil from sunflower rhizosphere were different from those of the bulk soil and the degree of the variations were based on the type of carbon substrates and the soil microbial composition. This study has shown the presence of certain taxa of rhizobacteria in sunflower rhizosphere which were positively influenced by Na^+ and Mg^{2+}, and taxa obtained from SHR and ITR were able to effectively utilized tryptophan and malic acid. Many unclassified microbial groups were also discovered and it is therefore recommended that efforts should further be made to isolate, characterize and identify these unclassified microbial species, as it might be plausible to discover new microbial candidates that can further be harnessed for biotechnological purpose.

8.1. Introduction

Microbial communities inhabiting the rhizosphere play pivotal roles in plant health and are responsible for sustaining soil health and functions (Philippot et al., 2013b, Oberholster et al., 2018). These microbes provide essential nutrients, such as N and P, required for plant use, and up

to 40-50% of N and 75% of P required by plants are supplied yearly (Udvardi and Poole, 2013). Hence, the manipulation of the rhizosphere microbial community is a promising means to improve the productivity of agricultural crops (Nwachukwu et al., 2021).

In previous studies, soil type and introduction of alien microbes have been shown to contribute to the distribution of rhizosphere microbial communities (Edwards et al., 2015, Igiehon et al., 2020). Other factors that are drivers of microbial community composition are plant species, plant developmental stages, climatic conditions and the interplay between these factors (Zarraonaindia et al., 2015, Enebe and Babalola, 2018). For example, studies on the rhizosphere of *Baphicacanthus cusia* (Zeng et al., 2018) and *Jacobaea vulgaris* (Yan et al., 2017b) showed that the rhizosphere zones are hotspots for microbial growth, abundance and diversity as they offer habitats with increased nutrient availability. The studies further showed that though plants have a strong selection impact on the rhizosphere microbiome, soil type has an effect on their stability and composition. Berlanas et al. (2019b) observed that the host genotype of grapevine rootstock genotypes did not predict any precise metrics of rhizosphere alpha and beta biodiversity in the young vineyard, whilst it was reported that various rhizosphere microbiome are associated with grapevine rootstock genotypes in the matured vineyard. Consequently, the latter could have been directly influenced by soil properties, age of the vineyard or agricultural management practices, which are key drivers of the rhizosphere microbiome in agricultural ecosystems.

Furthermore, plant roots select microbes inhabiting the rhizosphere region by secreting different metabolites, which vary with plant age and species, including secondary metabolites such as

phytohormones and antimicrobial compounds that induce defense against phytopathogens (Bais et al., 2006). To also select microbial species, plant roots create a discrete microenvironment by adjusting the oxygen and pH concentrations in the rhizosphere (Hacquard and Schadt, 2015). In recent times our knowledge of the rhizosphere microbiome has advanced. Many aspects of these rhizosphere microbial communities assemblages are less understood, especially in plants cultivated on the field (van der Heijden and Schlaeppi, 2015).

In addition, limited studies have been conducted on the rhizosphere of valuable agronomic crops such as sunflower plants (*Helianthus annuus*). Sunflower is essential for oil production and has many benefits to humans (Nwachukwu et al., 2021, Babalola et al., 2021b). It is, therefore, necessary to investigate the rhizosphere of sunflower plants in the field with the aim of determining microbial species with beneficial plant growth-promoting traits that can be used to improve the growth and health of sunflower plants. Thus, in this study, the functional and microbial diversity of sunflower rhizosphere grown in the field in two different locations (Sheila and Itsoseng in North West province, South Africa) are evaluated.

8.2. Materials and methods

8.2.1. Study sites and sample collection

The cultivar Pen 7011 Pannar was planted in the two sunflower fields. The study sites were located at Sheila (SH) (26°2′41.202″ S, 25°57′47.49″ E) and Itsoseng (IT) (26°4′23.064″ S, 25°58′37.104″ E) situated at Ngaka Modiri Molema district municipality, North West Province, South Africa

(Figure 8.1). North West Province has a mean annual rainfall of 300-700 mm annually. The sunflower farm at Sheila has been planted solely with maize for the past 5 years, a farming system called monoculture system. However, the Itsoseng farm has been subjected to crop rotation system for the past 5 years. In particular, maize-sunflower-pea-oat-sunflower have been rotationally planted on the Itsoseng farm. In addition, both sunflower farms have a history of maintaining conventional agronomic management practices, including pest control, weed control and NPK (15:8:4) fertilizer application.

The approval of the owners/farmers were obtained before the sunflower rhizosphere and bulk soil samples were collected from the farms. Sunflower rhizosphere soil samples were collected randomly from plants at different points on the farms by uprooting the sunflower plants. The roots were shaken to remove the loosely attached soil particles. The bulk soil samples, which serve as the control, were collected 10 m away at a depth of about 0-20 cm from the plant root using a soil auger. Two sites were selected for each of the two farms. At each of the two sites, three rhizosphere and three bulk soil samples were aseptically collected and pooled into labelled sterile plastic bags and were homogenized to obtain a single composite sample as described by Oberholster et al. (2018). Soil samples for the molecular analysis were stored -80 °C before processing.

8.2.2. Determination of soil physical and chemical properties

Rhizosphere and bulk soil samples analyses were performed at Agricultural Research Council-Soil, Climate and Water laboratory, Hatfield, Pretoria, South Africa, using standard procedures. Before soil analyses, soil samples were air-dried and sieved through a 2 mm mesh to remove roots

debris and large rocks and dried overnight at 50°C. The pH (1:3 soil/deionized water) of the soil samples were evaluated by a slurry technique using a Crison Bench pH meter (Crison Instruments, Barcelona, Spain) after allowing the soil to settle for 30 min. The soil particle size was classified through the hydrometer technique (Kettler et al., 2001). The total nitrogen and carbon were analyzed using the dry combustion method previously described by Santi et al. (2006).

Phosphorus was determined with a spectrophotometer (Zhou et al., 2020a). Organic matter (OM) in the soil was measured by Walkley Black method (Walkley and Black 1934). Soil nitrate was measured by KCI extraction method. Soil calcium, potassium, magnesium and sodium were evaluated using 1 M ammonium acetate at pH 7.0 (Deke et al., 2016).

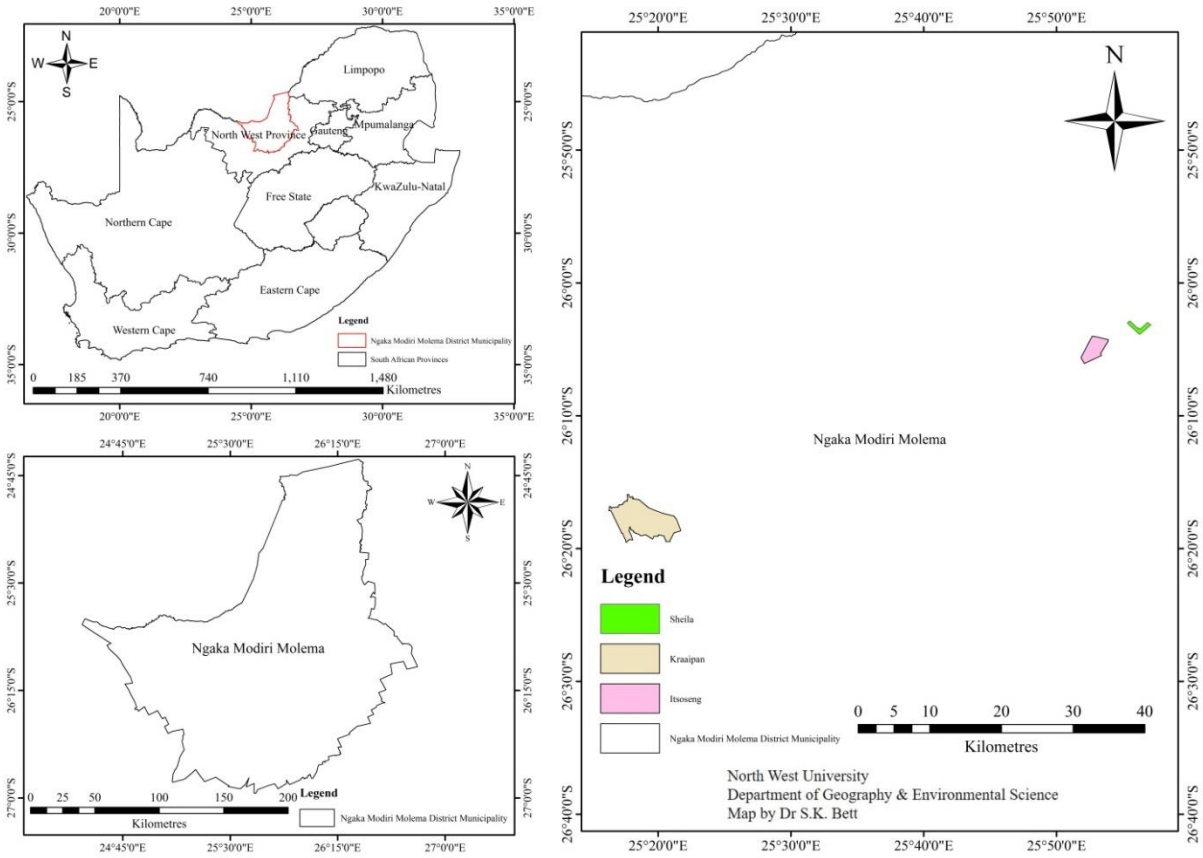

Figure 8.1: Map of the study area in South Africa showing Ngaka Modiri Molema district municipality (bottom left) where the two farms Sheila (green) and Itsoseng (pink) are located

Subsequently, magnesium, calcium and sodium in the extracts were determined using atomic absorption spectrophotometer, while a flame photometer was used to measure the potassium (Deke et al., 2016, Shi et al., 2011b).

8.2.3. DNA extraction and 16S rDNA amplicon sequencing

After removing the pebbles by sieving through 2 mm sieve, the microbial DNA was extracted from 0.5 g each of the soil samples collected from the sunflower rhizosphere and bulk soils using the Quick-DNA isolation kit (Zymo Research, Irvine, USA) following the manufacturer's instruction. DNA samples were sequenced at the Molecular Research Laboratory (Texas, USA).

Briefly, polymerase chain reactions (PCRs) were conducted in a single-step PCR using HotStarTaq Plus Master Mix Kit (Qiagen, USA) with primer pairs 515F (5´-AATGATACGGCGACCACCACCGAGATCTACAC TATGGTAATT GT GTGCCAGCMGCCGCGGTAA-3´) and 806R (5´-CAAGCAGAAGACGGCATACGAGAT TCCCTTGTCTCCAGTCAGTCAG CC GGACTACHVGGGTWTCTAAT-3´), which amplified both archaea and bacteria. The PCR products from all DNA samples were quantified using PicoGreen dsDNA assay. The samples were pooled together in equimolar concentration and then, purified using calibrated Ampure XP beads (Agencourt Bioscience Corporation, MA, USA). The pooled and then, purified PCR product was used to prepare an Illumina DNA library. Sequencing was performed on an Illumina MiSeq 2000 using a paired-end technique to obtain 312 bp paired-ends reads.

8.2.4. Functional measurements using community-level physiological profiling (CLPP) technique

MicroResp™, as described by (Campbell et al., 2008), was used to determine community respiration and substrate-induced respiration. Approximately 0.4 g of soil samples were placed in each well of 96-deep well plates, and incubated for two days at room temperature in the dark before the assay was performed. A total of 11 carbon substrates, including three amino acids (methionine, tyrosine and tryptophan), five carbohydrates (galactose, maltose, glucose, fructose and sucrose), and three carboxylic acids (D-pantothenic acid, citric acid and malic acid) were selected. The choice of substrates' was based on substrates complexity (i.e., carbon chain length) (Colombo et al., 2016, Klimek et al., 2016). All the carbon substrates used in this study were purchased from Sigma-Aldrich (Australia). Substrates were dissolved in deionized water, sterilized by filtering and introduced to four replicated wells. Preceding the addition of substrates, CO_2 detection microplates containing 12.5 μg mL^{-1} cresol red, 2.5mM sodium bicarbonate and 150 mM potassium chloride were pre-read at absorbance wavelength of 570 nm, then the deep-well plates were immediately placed on the airtight MicroResp™ seal and incubated at 25ºC for 6 h in the dark in a desiccator, as recommended by the manufacturer (Macaulay Scientific Consulting, UK). The differences in optical density after incubation was then estimated on a spectrophotometer microplate reader (EnSpire® 2300 Multilabel Reader, Perkin Elmer, USA) at a wavelength of 570 nm. The quantity of CO_2 respiration per gram of soil per well was calculated by adopting the formula in the MicroResp™ manual (James Hutton Ltd, UK), the rate of CO_2 respiration expressed per gram of soil was estimated.

8.2.5. Data processing and/or bioinformatics analysis

The raw sequenced data were processed using MR DNA analysis pipeline (MR DNA, Shallowater, TX, USA). In summary, paired ends of DNA sequences were combined, 'depleted of barcode' and sequences less than 150 base pairs and vague base call were jettisoned. DNA sequences were later denoised, operational taxonomic units (OTUs) were produced and chimeras were removed. OTUs were classified by grouping at 3% divergence (97% similarity) and defined into a taxonomy using 'BLASTn against curated database' obtained from RDPII and NCBI (http: //rdp.cme.msu. edu, https://www.ncbi.nlm.nih.gov).

Processed sequenced data were subsequently uploaded to MG-RAST for analyses (Caporaso et al., 2010). The analyzed data were downloaded from MG-RAST and pasted on a Microsoft Excel sheet. The species richness was estimated using rarefaction obtained from the MG-RAST pipeline. Alpha diversity was expressed using Shannon and evenness indices, while the Kruskal-Wallis test was used to describe the diversity indices across sites, and all the analyses were performed using PAST version 3.20 (Hammer et al., 2001). Taxonomic richness was represented as an OTU number. The heatmap expressing the relative abundance of microbial communities at the phylum level was plotted using Shinyheatmap (Khomtchouk et al., 2017). The principal coordinates analysis (PCoA) was used to analyze the abundance data of relative OTU, i.e. the microbial structure based on Bray-Curtis distances using CANOCO 5 (Microcomputer Power, Ithaca, NY, USA). The correlation between the measured physical/chemical properties and microbial communities was estimated using the canonical correspondence analysis (CCA) method on

CANOCO 5 software. The forward selection of environmental factors and the Monte Carlo permutation test were used to evaluate the environmental variables that best described the microbial composition. The 999 random permutations were employed for the significance test. The environmental variables itemized in Table 8.1 were used as explanatory variables in the CCA analysis.

All the sequences for this study were deposited in the Sequence Read Archive (SRA) of the National Center for Biotechnology Information (NCBI) under the bioproject number PRJNA672856.

8.2.6. Statistical analysis

The mean and standard errors of the physical and chemical properties and the functional measurement data were obtained in a Microsoft Excel sheet. Physical and chemical properties data were transferred to SPSS, where one-way ANOVA and Duncan Multiple tests were carried out. Graphs and one-way ANOVA for functional measurements were performed using GraphPad Prism 7, GraphPad Software, California. For the functional measurements, two composite samples were used per farm and each composite sample was replicated four times in the wells of MicroResp™ plates upon addition of the different carbon sources. The mean of the four replicates per composite sample was obtained; thereafter, the obtained mean values for the two composite samples per site were used for statistical analysis. Probability values less than or equal to 0.05 was considered to be statistically significant for all the data.

8.3. Results

8.3.1. Soil physicochemical properties of the sunflower soil samples

The soil's physical and chemical properties are presented in table 8.1. The amount of $N\text{-}NH_4$ in soil samples from SHR and SHB was not significantly different ($p>0.05$), but $N\text{-}NH_4$ content in soil from ITB was significantly different from soil from ITR ($p\leq0.05$). Similarly, rhizosphere soil did not influence soil pH in soil obtained from sunflower cultivated in Sheila since a significant difference was not observed in SHR and SHB. Also, ITR and ITB soil pH was not significantly different ($p>0.05$). In addition, Mg^{2+}, Na^{+} and silt (%) were not significantly different ($p>0.05$) in all the soil samples from the two sites, while the ITR rhizosphere soil significantly influenced percentage clay ($p\leq 0.05$) compared to ITR bulk soil.

8.3.2. Rarefaction analysis

The richness of the microbial diversity in the sunflower soil samples was measured by rarefaction analysis (Figure 8.2). From the results, the rarefaction curves for Sheila soils (SHR and SHB) were higher than the soils from Itsoseng (ITR and ITB). Most of the sample reads got to the saturation level, which indicates that the sampling methods were sufficiently covered (Figure 8.2). From the rarefaction curve, the highest read number, 120,000 (x-axis), aligned with SHB, while 1200-1400 (y-axis) were the highest species counts. It was possible to subsample 120,000 reads from the SHB sample when according to Table 8.2 only 10,364 sequences were obtained for SHB because Table 8.2 reports the means of two samples for SHB.

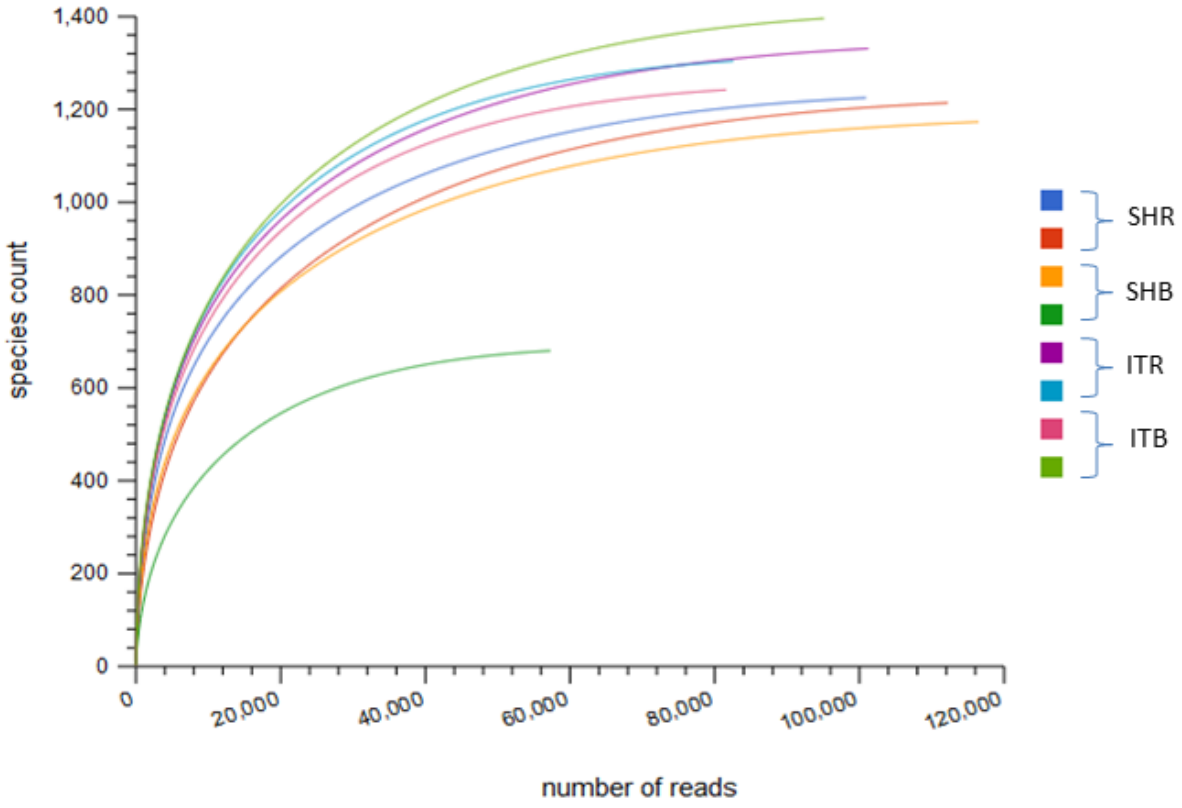

Figure 8.2: Rarefaction curves show the estimated richness in the rhizosphere soils and sampling effort. The total number of OTUs measured after sampling is represented on the y-axis, while the number of sequences is represented on the x-axis. **Legend:** SHR- Sheila rhizosphere soil, SHB- Sheila bulk soil, ITR- Itsoseng rhizosphere soil, ITB- Itsoseng bulk soil.

Table 8.1: Physical and chemical properties of the sunflower soils.

Sample	SHR	SHB	ITR	ITB
OM (%)	1.98 ± 0.0^{bc}	2.11 ± 0.1^{b}	1.78 ± 0.1^{c}	2.81 ± 0.0^{a}
$N\text{-}NO_3$ (mg/kg)	12.31 ± 2.8^{ab}	13.27 ± 0.2^{a}	5.54 ± 2.5^{bc}	4.30 ± 0.0^{c}
$N\text{-}NH_4$ (mg/kg)	4.93 ± 0.2^{c}	4.59 ± 0.1^{c}	6.67 ± 0.1^{b}	11.79 ± 0.1^{a}
pH	6.61 ± 0.1^{a}	6.69 ± 0.0^{a}	6.02 ± 0.1^{b}	6.05 ± 0.0^{b}
Res.(ohm)	1270 ± 3.0^{b}	1230 ± 30.0^{b}	2275 ± 155.0^{a}	2020 ± 20.0^{a}
P^{3-} (mg/kg)	68.50 ± 8.8^{a}	81.29 ± 2.6^{a}	14.09 ± 1.2^{b}	$79.84\pm3{\cdot}0^{a}$
Ca^{2+} (mg/kg)	560.50 ± 81.0^{ab}	659.50 ± 14.5^{a}	451.50 ± 3.5^{b}	651.00 ± 17.0^{a}
Mg^{2+} (mg/kg)	141.00 ± 3.0^{a}	138.50 ± 3.5^{a}	152.00 ± 8.0^{a}	135.50 ± 3.5^{a}
K^{+} (mg/kg)	161.50 ± 0.5^{a}	169.00 ± 3.0^{a}	119.00 ± 7.0^{b}	167.00 ± 1.0^{a}
Na^{+} (mg/kg)	78.70 ± 1.9^{a}	71.40 ± 3.4^{a}	71.95 ± 2.4^{a}	70.35 ± 3.4^{a}
C (%)	0.46 ± 0.0^{b}	0.56 ± 0.1^{b}	0.46 ± 0.0^{b}	1.06 ± 0.1^{a}
N^{3-} (%)	0.05 ± 0.0^{b}	0.05 ± 0.0^{b}	0.05 ± 0.0^{b}	0.09 ± 0.0^{a}
Sand (%)	78.00 ± 0.0^{b}	78.00 ± 0.0^{b}	79.00 ± 1.0^{b}	84.00 ± 0.0^{a}
Silt (%)	2.00 ± 0.0^{a}	3.00 ± 1.0^{a}	2.00 ± 0.0^{a}	3.00 ± 1.0^{a}
Clay (%)	20.00 ± 0.0^{a}	19.00 ± 1.0^{a}	19.00 ± 1.0^{a}	13.00 ± 1.0^{b}

Legend: SHR – Sheila rhizosphere soil; SHB- Sheila bulk soil; ITR- Itsoseng rhizosphere soil; ITB- Itsoseng bulk soil. Number of replicates (n) = 3. Data represent mean ± SE. Mean values having the same alphabets are considered not statistically significant ($P\geq0.05$) following Duncan's multiple range test.

8.3.3. Assessment of diversity indices

The RDP classifier was used to assign sequence reads of Sheila and Itsoseng soils into OTUs with 3% nucleotide cut-off values. The estimated Simpson, Shannon, evenness and Chao-1 indices are presented in table 8.2. The pre-processed analytical output unveiled different parameters like bp count, sequences count, mean sequence length (bp) and mean GC count (%). The post-process information similarly entails bp count, sequences count, mean sequence length (bp) and mean GC count (%), including predicted protein and rRNA features (Table 8.2).

Upon processing, a reduction in the bp count of the sequence reads was observed in all the soil samples with mean values corresponding to 4181225 (SHR), 2912336.5 (SHB), 4260016 (ITR) and 4645809 (ITB). A similar decrease was observed for the sequence count and mean sequence bp length in all the soil samples. However, 56±4.0, 54.5± 4.0, 56.5± 4.0 and 57± 4.0 were the % mean GC count of post-processed or quality control data for the SHR, SHB, ITR and ITB, respectively (Table 8.2). Out of the sequence reads that passed pre-processing test, 60, 60.5, 98.5 and 125.5 sequence reads corresponding to SHR, SHB, ITR and ITB have predicted protein features with known functions, while others have protein features with unknown functions. Also, 19312.5, 11693.5, 18265 and 20065 sequence reads from SHR, SHB, ITR and ITB were found to have predicted protein features that code for known rRNA (Table 8.2).

Table 8.2: Analysis of sequenced data of the amplicon metagenomes of the sunflower soil

Sampling site	SHR	SHB	ITR	ITB
	Uploading Information			
bp count	36938758	25321399	27104520	26066428
Sequences count	125264.5	86929.5	91939.5	88384.5
Mean sequence length (bp)	295± 5.0	289.5±29.0	295± 5.0	295± 5.0
Mean GC count (%)	55± 3.0	53.5± 3.0	56.5± 3.0	57± 3.0
	Post QC Information			
bp count	4181225	2912336.5	4260016	4645809
Sequences count	14556	10364	14637.5	15941
Mean sequence length (bp)	287.5± 24.0	279± 40.0	291± 9.0	291± 10.0
Mean GC count (%)	56±4.0	54.5± 4.0	56.5± 4.0	57± 4.0
	Processed Sequences			
Predicted proteins features	155.5	193.0	254.5	344.0
Predicted rRNA features	22730	13358.5	22051	25648
	Aligned Sequences			
Identified protein features	60	60.5	98.5	125.5
Identified rRNA features	19312.5	11693.5	18265	20065

Legend: SHR- Sheila rhizosphere soil, SHB- Sheila bulk soil, ITR- Itsoseng rhizosphere soil, ITB- Itsoseng bulk soil, GC- Guanine cytosine content, bp-base pair. Data represent the mean of the original samples

8.3.4. Alpha diversity assessment of the composition and abundance of taxonomic groups in sunflower rhizosphere and bulk soils

Statistical indicators such as Simpson, Shannon and evenness were used to describe alpha diversity (i.e., diversity within sunflower soil type) of the abundance of the microbial taxonomic groups in the different soil samples obtained from the different sunflower soil types. The Simpson, Shannon and evenness indicators reveal that the microbial taxonomic groups show no significant differences (Kruskal–Wallis, *p*-value = 0.77) as represented in Figure 8.3.

8.3.5. Beta diversity assessment of the composition and abundance of taxonomic groups in sunflower rhizosphere and bulk soils

On the other hand, a significant difference (ANOSIM, *p*-value = 0.01, R= 0.60) was observed in the beta diversity (i.e., diversity between the sunflower rhizosphere soil and bulk soil samples) of the soil microbial composition and abundance obtained from Sheila and Itsoseng as illustrated by Figure 8.4 using principal coordinates analysis (PCoA).

8.3.6. Microbial structures at the phylum and genus level observed in Sheila and Itsoseng rhizosphere and bulk soils

Sequence tags were assigned to Sheila and Itsoseng rhizosphere and bulk soil samples into different taxa via rapid metagenomic annotations using subsystems technology (MG-RAST) at The relative abundance of microbial phyla for each soil sample is presented in table 8.3.

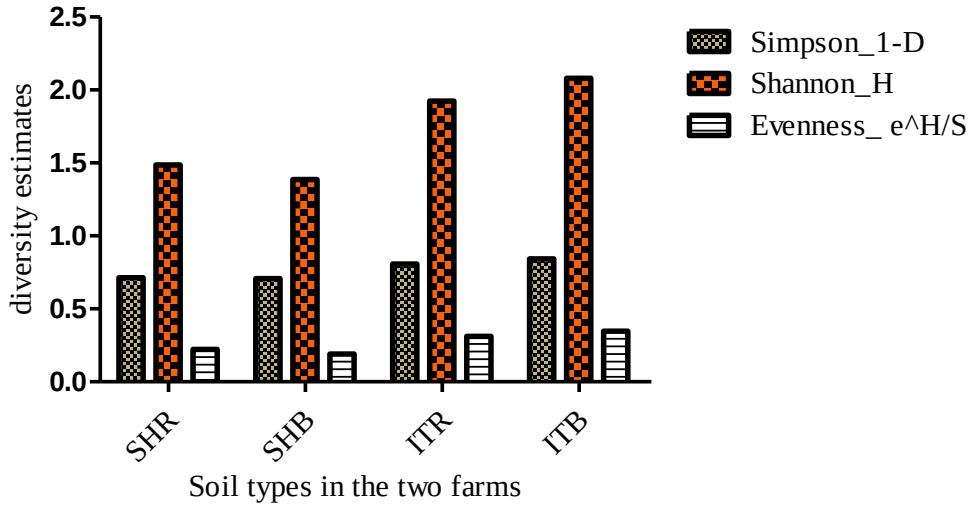

Figure 8.3: Diversity indicators of the abundance of the microbial taxonomic groups in sunflower rhizosphere and bulk soils. SHR- Sheila rhizosphere soil, SHB- Sheila bulk soil, ITR- Itsoseng rhizosphere soil, ITB- Itsoseng bulk soil.

Firmicutes and Proteobacteria were observed to be abundant in both SHR and SHB, whereas Actinobacteria and Proteobacteria dominated ITR, while Thaumarchaeota and Actinobacteria predominated in ITB. The mean relative abundant values of 39.74 and 31.82 were observed for Firmicutes in SHR and SHB, respectively, while 29.47 and 33.68 were recorded for Proteobacteria in SHR and SHB, respectively. Also, Actinobacteria had a mean relative abundant value of 26.41 for ITR, which was higher than that of ITB (19.50), while Thaumarchaeota in ITB had a mean relative abundant value of 19.40. However, the mean relative abundant values of the entire microbial community in table 8.3 were not significantly different ($p > 0.05$).

Furthermore, at the genus level, the microbes that were observed in the order of decreasing relative abundance include: *Bacillus*, *Pseudomonas*, *Staphylococcus*, *Vagococcus*, *Rubrobacter*, *Brevundimonas*, *Candidatus Nitrososphaera*, *Lysinibacillus*, *Terrimonas*, *Gemmatimonas*, *Paenibacillus*, *Mitsuaria*, *Clostridium*, *Streptomyces*, *Chroococcidiopsis*, *Sphingobacterium*, *Geodermatophilus*, *Alcaligenes*, *Oxalicibacterium*, *Candidatus Amoebophilus*, *Sphingomonas*, *Bradyrhizobium*, *Acinetobacter*, *Nocardioides*, *Cystobacter* and *Cellulomonas* (Table 8.3). A mean relative abundance of 34.65 for *Bacillus* was recorded in SHR as against 29.56 observed for *Pseudomonas* in SHB. Aligned to the microbial community structures at the phylum level, the mean relative abundant values of microbial species at the generic level did not show significance difference ($p > 0.05$).

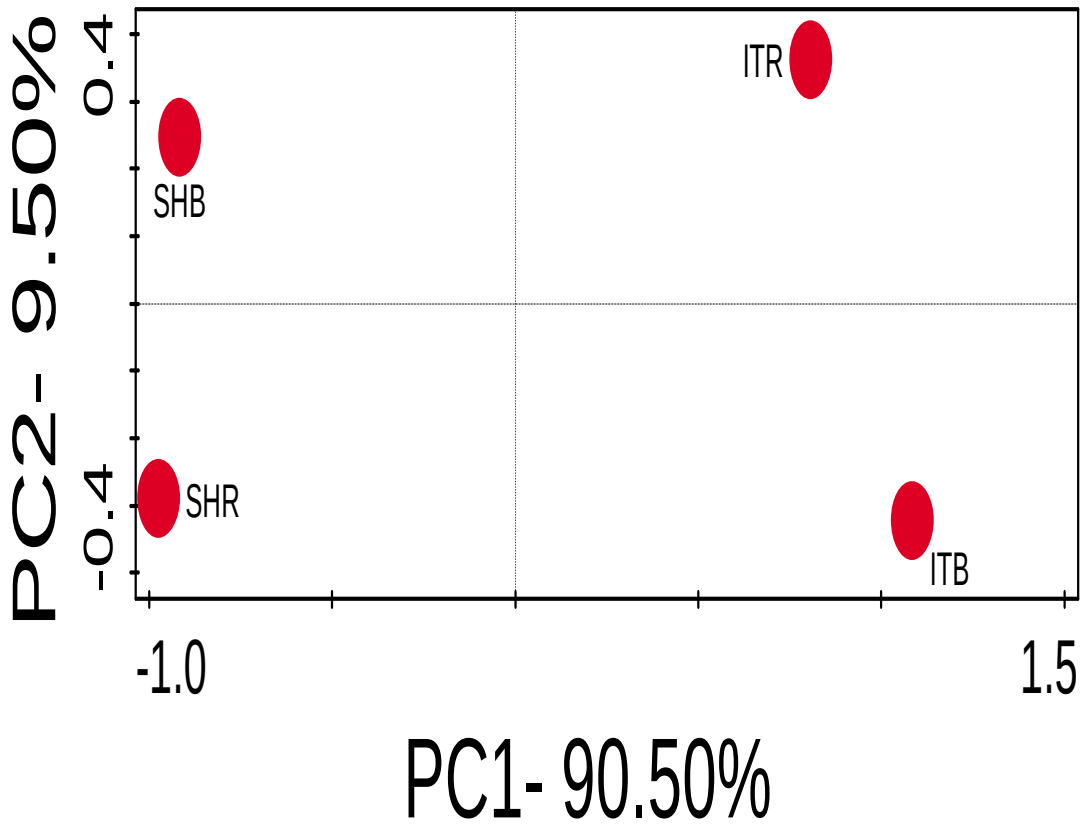

Figure 8.4: Principal coordinates analysis (PCoA) of the microbial (bacterial and archaeal) community composition and abundance at the operational taxonomic unit (OTU) level to illustrate beta diversity between sunflower rhizosphere soils and bulk soils obtained from Sheila and Itsoseng. SHR- Sheila rhizosphere soil, SHB- Sheila bulk soil, ITR- Itsoseng rhizosphere soil, ITB- Itsoseng bulk soil.

Table 8.3: The microbial taxonomic structure and mean relative abundance at phylum level from sunflower rhizosphere and bulk soils obtained from Sheila and Itsoseng farms.

Phylum	**Farm**			
	SHR	SHB	ITR	ITB
Firmicutes	39.74±6.54	31.82±9.93	12.11±8.56	9.54±0.08
Proteobacteria	29.47±0.55	33.68±18.81	12.67±8.96	11.49±1.19
unclassified (derived from Bacteria)	19.29±2.36	27.72±13.15	28.97±20.48	23.48±1.97
Actinobacteria	6.22±1.59	3.59±2.71	26.41±18.66	19.50±1.29
Bacteroidetes	1.58±0.91	0.83±0.29	3.14±2.22	3.75±0.29
Thaumarchaeota	0.92±0.08	0.49±0.44	8.25±5.83	19.40±2.82
Gemmatimonadetes	0.51±0.08	0.46±0.21	0.83±0.59	1.43±0.17
unclassified (derived from unclassified sequences)	0.36±0.24	0.23±0.17	1.35±0.95	1.16±0.06
Cyanobacteria	0.26±0.24	0.03±0.01	0.07±0.05	0.06±0.00
Acidobacteria	0.37±0.08	0.29±0.07	1.68±1.18	3.59±0.01
Verrucomicrobia	0.31±0.13	0.33±0.07	2.23±1.58	3.99±0.61
Planctomycetes	0.22±0.12	0.10±0.03	0.34±0.24	0.49±0.02
Chloroflexi	0.22±0.09	0.14±0.11	0.66±0.46	0.53±0.10
Nitrospirae	0.16±0.07	0.11±0.08	0.36±0.25	0.77±0.00

Crenarchaeota	0.23±0.03	0.07±0.06	0.66±0.47	0.11±0.05
Spirochaetes	0.06±0.00	0.04±0.03	0.20±0.14	0.53±0.12
Chlamydiae	0.02±0.02	0.01±0.00	0.01±0.01	0.05±0.01
Deinococcus-Thermus	0.03±0.01	0.03±0.02	0.02±0.01	0.01±0.00
Fibrobacteres	0.02±0.01	0.01±0.01	0.00±0.00	0.00±0.00
Tenericutes	0.01±0.00	0.01±0.01	0.01±0.01	0.01±0.00
Aquificae	0.00±0.00	0.00±0.00	0.01±0.01	0.01±0.00
Chlorobi	0.00±0.00	0.01±0.01	0.00±0.00	0.02±0.00
Dictyoglomi	0.00±0.00	0.00±0.00	0.01±0.01	0.07±0.01
Synergistetes	0.00±0.00	0.00±0.00	0.01±0.00	0.01±0.01

Legend: SHR- Sheila rhizosphere soil, SHB- Sheila bulk soil, ITR- Itsoseng rhizosphere soil, ITB- Itsoseng bulk soil, data represent mean ± standard error of the relative abundance of microbial phyla

8.3.7. Influence of environmental factors on microbial communities

Canonical correspondence analysis (CCA) was used to determine the influence of the environmental variables on the relative abundance of microbial species at the phylum level. *Tenericutes* and *Proteobacteria* correlated positively with Ca^{2+}, P^{3-}, K^{+}, pH, $N\text{-}NO_3$ but negatively correlated with silt, OM, C, N^{3-}, sand, $N\text{-}NH_4$, Res, Mg^{2+}, Na^{+} and clay. Dictyoglomi, Chlorobi, Spirochaetes, Chlamydiae, Nitrospirae, Actinobacteria, Gemmatimonadetes and Verrucomicrobia positively correlated with $N\text{-}NH_4$, sand, N^{3-}, C, OM and silt, and negatively correlated with Ca^{2+}, P^{3-}, K^{+}, pH, $N\text{-}NO_3$, clay, Na^{+}, Mg^{2+} and Res. Thaumarchaeota, Bacteroidetes, Planctomycetes, Actinobacteria, Aquificae and Chloroflexi positively correlated with Res and Mg, but negatively correlated with $N\text{-}NH_4$, sand, N^{3-}, C, OM, silt, Ca^{2+}, P^{3-}, K^{+}, pH, $N\text{-}NO_3$, clay and Na^{+}, while Firmicutes, Cyanobacteria, Deinococcus-Thermus and Fibrobacteria positively correlated with Na^{+} and clay, but negatively correlated with N-NO3, pH, K^{+}, P^{3-}, Ca^{2+}, silt, OM, C, N^{3-}, sand, $N\text{-}NH_4$, Res and Mg^{2+} (Figure 8.5).

From the above analysis it was also observed that Tenericutes and Proteobacteria were positively influenced by SHB, Dictyoglomi, Chlorobi, Spirochaetes, Chlamydiae, Nitrospirae, Actinobacteria, Gemmatimonadetes and Verrucomicrobia were positively influenced by ITB, Thaumarchaeota, Bacteroidetes, Planctomycetes, Actinobacteria, Aquificae and Chloroflexi were positively influenced by ITR, while Firmicutes, Cyanobacteria, Deinococcus-Thermus and Fibrobacteria were positively influenced by SHR.

This further shows that SHB correlated positively with Ca^{2+}, P^{3-}, K^{+}, pH, $N\text{-}NO_3$ but negatively correlated with silt, OM, C, N^{3-}, sand, $N\text{-}NH_4$, Res, Mg^{2+}, Na^{+} and clay. ITB positively correlated with $N\text{-}NH_4$, sand, N^{3-}, C, OM and silt and negatively correlated with Ca^{2+}, P^{3-}, K^{+}, pH, $N\text{-}NO_3$, clay, Na^{+}, Mg^{2+} and Res. ITR positively correlated with Res and Mg^{2+}, but negatively correlated with $N\text{-}NH_4$, sand, N^{3-}, C, OM, silt, Ca^{2+}, P^{3-}, K^{+}, pH, $N\text{-}NO_3$, clay and Na^{+}, while SHR positively correlated with Na^{+} and clay but negatively correlated with $N\text{-}NO_3$, pH, K^{+}, P^{3-}, Ca^{2+}, silt, OM, C, N^{3-}, sand, $N\text{-}NH_4$, Res and Mg^{2+}. The environmental variables that best described or explained the difference observed in the microbial community compositions observed in Fig. 8.5 were determined using the forward selection and the Monte Carlo permutation test with random permutations. It was observed that out of the 15 variables considered, $N\text{-}NO_3$ significantly ($p<0.1$) contributed 94.6% of the difference, while $N\text{-}NH_4$ significantly ($p<0.1$) contributed 72.9% of the difference to the microbial community composition and abundance of the different soil samples (Table 8.5). On the contrary, although the remaining 13 variables contributed to the difference in the microbial phyla, their contributions were not significant ($p\geq0.05$).

Table 8.4: The microbial taxonomic structure and relative abundance at genus and/or generic level from sunflower rhizosphere and bulk soils obtained from Sheila and Itsoseng farms.

Genus	**Farm**			
	SHR	SHB	ITR	ITB
Bacillus	34.65±9.95	29.50±10.39	9.91±2.69	6.95±0.68
unclassified (derived from Bacteria)	21.29±3.08	29.16±13.21	37.32±10.83	33.79±2.72
Pseudomonas	25.05±1.71	29.56±18.98	5.59±1.83	1.67±0.47
Staphylococcus	3.51±3.28	0.21±0.03	0.05±0.02	0.01±0.01
Vagococcus	2.02±0.14	1.39±0.28	0.53±0.33	0.09±0.01
Arthrobacter	1.56±0.33	1.19±1.03	8.30±3.00	1.64±0.03
Rubrobacter	1.07±0.36	0.77±0.59	10.74±8.13	7.46±1.74
Brevundimonas	1.21±0.01	1.47±0.39	0.50±0.34	0.08±0.02
Candidatus Nitrososphaera	1.02±0.11	0.54±0.48	10.48±0.94	27.16±2.22
unclassified (derived from *Betaproteobacteria*)	0.76±0.29	1.12±0.07	1.34±0.79	1.56±0.25
unclassified (derived from *Gammaproteobacteria*)	0.58±0.26	0.47±0.16	0.82±0.19	1.13±0.21
unclassified (derived from *Deltaproteobacteria*)	0.54±0.27	0.49±0.03	1.72±0.29	2.71±0.31

unclassified (derived from *Alphaproteobacteria*)	0.55±0.19	0.53±0.28	1.72±0.19	2.48±0.01
Lysinibacillus	0.48±0.22	0.47±0.04	0.18±0.09	0.37±0.08
Terrimonas	0.54±0.14	0.30±0.20	2.24±0.62	2.95±0.10
Gemmatimonas	0.57±0.11	0.49±0.24	1.06±0.16	2.01±0.21
unclassified (derived from unclassified sequences)	0.40±0.27	0.25±0.19	1.68±0.25	1.64±0.09
Paenibacillus	0.49±0.10	0.33±0.28	0.69±0.29	0.16±0.00
Mitsuaria	0.27±0.27	0.00±0.00	0.02±0.01	0.04±0.04
Clostridium	0.35±0.15	0.16±0.09	0.19±0.02	0.57±0.20
Streptomyces	0.32±0.12	0.19±0.16	1.12±0.35	2.79±0.47
Chroococcidiopsis	0.21±0.21	0.00±0.00	0.00±0.00	0.00±0.00
Sphingobacterium	0.22±0.19	0.00±0.00	0.02±0.02	0.08±0.07
Geodermatophilus	0.26±0.14	0.15±0.11	1.30±0.31	0.66±0.02
Alcaligenes	0.25±0.14	0.19±0.07	0.10±0.03	0.02±0.01
Oxalicibacterium	0.19±0.19	0.00±0.00	0.04±0.01	0.03±0.01
Candidatus Amoebophilus	0.19±0.18	0.02±0.01	0.05±0.03	0.25±0.04
Sphingomonas	0.18±0.15	0.01±0.00	0.15±0.07	0.11±0.03
Bradyrhizobium	0.20±0.12	0.09±0.06	0.62±0.47	0.84±0.43
Acinetobacter	0.19±0.12	0.22±0.05	0.10±0.05	0.01±0.00

unclassified (derived from *Nitrosomonadales*)	0.16±0.14	0.01±0.00	0.12±0.07	0.11±0.00
Nocardioides	0.23±0.07	0.18±0.13	0.91±0.11	0.20±0.04
unclassified (derived from *Enterobacteriaceae*)	0.16±0.14	0.19±0.07	0.16±0.13	0.20±0.02
Cystobacter	0.17±0.12	0.20±0.18	0.06±0.01	0.20±0.04
Cellulomonas	0.16±0.09	0.15±0.15	0.17±0.06	0.03±0.01

Legend: SHR- Sheila rhizosphere soil, SHB- Sheila bulk soil, ITR- Itsoseng rhizosphere soil, ITB- Itsoseng bulk soil, data represent mean ± standard error of the relative abundance of microbial genera

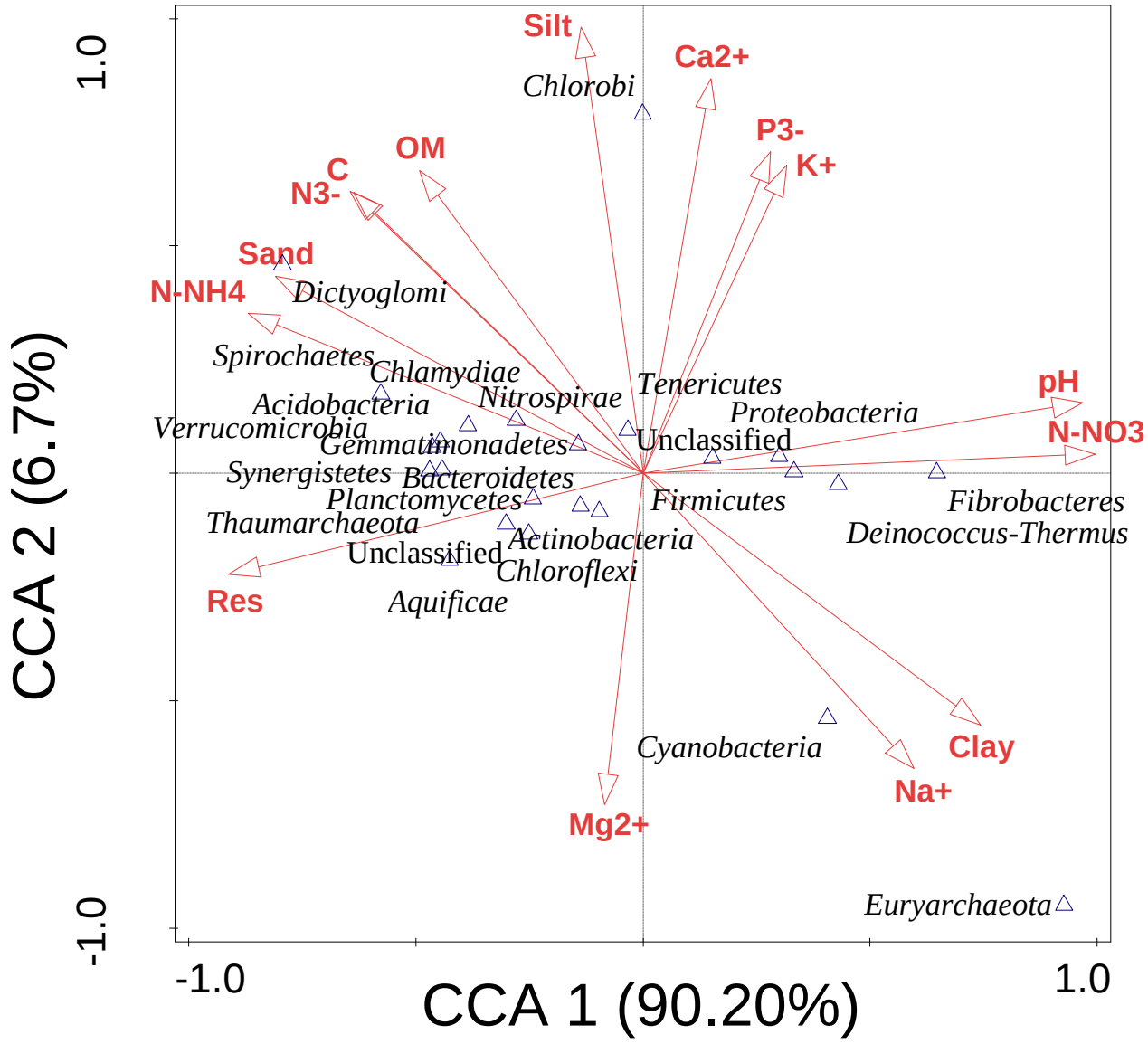

Figure 8.5: The influence of environmental variables on the microbial phyla from sunflower rhizosphere and bulk soils obtained from Sheila and Itsoseng using Canonical correspondence analysis.

8.3.8. Soil functional measurements

Individual substrate stimulated respiration, hence was determined by the respiration response of individual carbon compound which revealed the microbial communities present in the different soil types (SHR, SHB and ITR, ITB) showed different respiration rates (Figure 8.6, 8.7 and 8.8). However, the microbial respiration rates for all the substrates used in this study were not statistically significant (p >0.05). In particular, the respiration rate was higher under tryptophan amended soil for Sheila rhizosphere soil (2.33±0.51 0 µg g^{-1} h^{-1} CO_2-C) than Sheila bulk soil (1.42±0.06 µg g^{-1} h^{-1} CO_2-C), while Itsoseng rhizosphere soil (4.81±0.100 µg g^{-1} h^{-1} CO_2-C) was similarly higher than Itsoseng bulk soil (4.66±2.13 µg g^{-1} h^{-1} CO_2-C).

On the contrary, Sheila (3.97±0.44 µg g^{-1} h^{-1} CO_2-C) and Itsoseng bulk (4.81±0.10 µg g^{-1} h^{-1} CO_2-C) soils were both higher than their respective rhizosphere soils when tyrosine was used as a substrate (Fig. 8.6). The respiration rate of microbial communities in Itsoseng rhizosphere soil was higher than Itsoseng bulk soil, including Sheila rhizosphere and bulk soils under tyrosine amendment (Figure 8.6). When the amino acid, malic acid was applied as a substrate, the respiration rates of Sheila rhizosphere and bulk soils were similar since they both had related mean values of 4.38±1.25 µg g^{-1} h^{-1} CO_2-C and 4.35±1.33 µg g^{-1} h^{-1} CO_2-C respectively, while Itsoseng bulk soil with a mean value of 8.92±0.48 µg g^{-1} h^{-1} CO_2-C was higher than its rhizosphere soil with a mean value of 5.53±0.84 µg g^{-1} h^{-1} CO_2-C (Figure 8.7).

Table 8.5: Forward selection of environmental variables that best described difference in microbial species between soil samples.

Environmental variable	Explains %	Contribution %	Pseudo-F	*p* value
N-NO_3 (mg/kg)	89.7	94.6	17.4	0.052
pH	85.0	90.8	11.3	0.18
Res (ohm)	75.8	82.9	6.3	0.276
N-NH_4 (mg/kg)	69.3	72.9	4.5	0.048
Sand (%)	60.8	63.5	3.1	0.078
Clay (%)	52.1	53.3	2.2	0.18
Total N (%)	40.4	39.8	1.4	0.32
Total C (%)	39.7	39.0	1.3	0.33
Na^+ (mg/kg)	35.3	29.9	1.1	0.454
OM (%)	25.7	23.1	0.7	0.646
K^+ (mg/kg)	13.5	18.4	0.3	0.96
P^{3-} (mg/kg)	11.7	15.9	0.3	0.964
Ca^{2+} (mg/kg)	7.1	8.5	0.2	0.886
Silt (%)	7.1	3.4	0.2	0.668
Mg^{2+} (mg/kg)	5.5	6.0	0.1	0.966

A similar result pattern was observed for both rhizosphere and bulk soils in Sheila and Itsoseng when citric acid was used as a carbon substrate. Contrarily, the resulting pattern observed in D-pantothenic acid and sucrose amended soil was almost similar, except that D-pantothenic acid amended bulk soil of Itsoseng was relatively lower than the other soil types (Figure 8.7). When carbon substrates maltose, fructose, glucose and galactose were used, a different set of results was obtained (Figure 8.8). In that it was only in the galactose amended rhizosphere soil (0.43±0.25 µg g^{-1} h^{-1} CO_2-C) of Sheila, that had a higher microbial respiration rate than its bulk soil (0.24± 0.03 µg g^{-1} h^{-1} CO_2-C) (Figure 8.8). CCA was used to determine the influence of the carbon substrates on the relative abundance of microbial species at the phylum level (Figure 8.9).

Spirochaetes, Chlamydiae, Nitrospirae, Gemmatimonadetes, Acidobacteria, Thaumarchaeota, Dictyoglomi and Viromicrobia positively correlated with tyrosine, glucose, maltose, fructose, malic acid, citric acid, galactose and sucrose, but negatively correlated with tryptophan, methionine, D-pantothenic acid and distilled water. Synergistetes, Actinobacteria, Chloroflexi, Aquificae and Bacteriodetes positively correlated with tryptophan, methionine and D-pantothenic acid, but negatively correlated with tyrosine, glucose, maltose, fructose, malic acid, citric acid, galactose, sucrose and distilled water, while Euryarchaeota, Cyanobacteria and Deinococcus-Thermus positively correlated with distilled water, but negatively with tyrosine, glucose, maltose, fructose, malic acid, citric acid, galactose, sucrose, tryptophan, methionine and D-pantothenic acid. However, none of the carbon substrates influenced the abundance of Fibrobacteres, Firmicutes, Tenericutes and Proteobacteria (Figure 8.9).

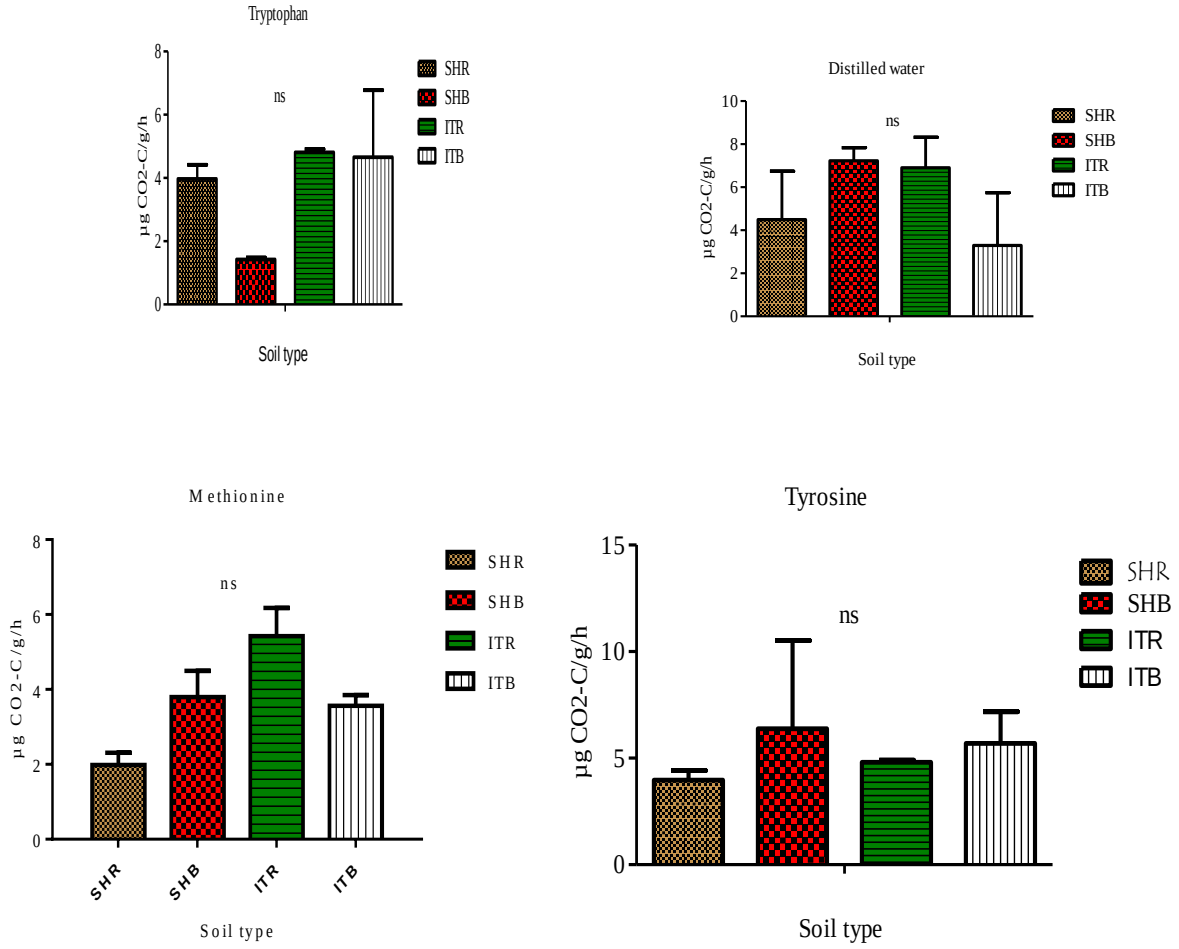

Figure 8.6: Effects of soil type on soil functional properties as measured for distilled water i.e., control and tryptophan, tyrosine and methionine using MicroResp assay. Number of replicates (n) = 2. Data represent mean ± SE, ns = not statistically significant following Duncan's multiple range test. SHR= Sheila rhizosphere soil, SHB=Sheila bulk soil, ITR= Itsoseng rhizosphere soil, ITB=Itsoseng bulk soil.

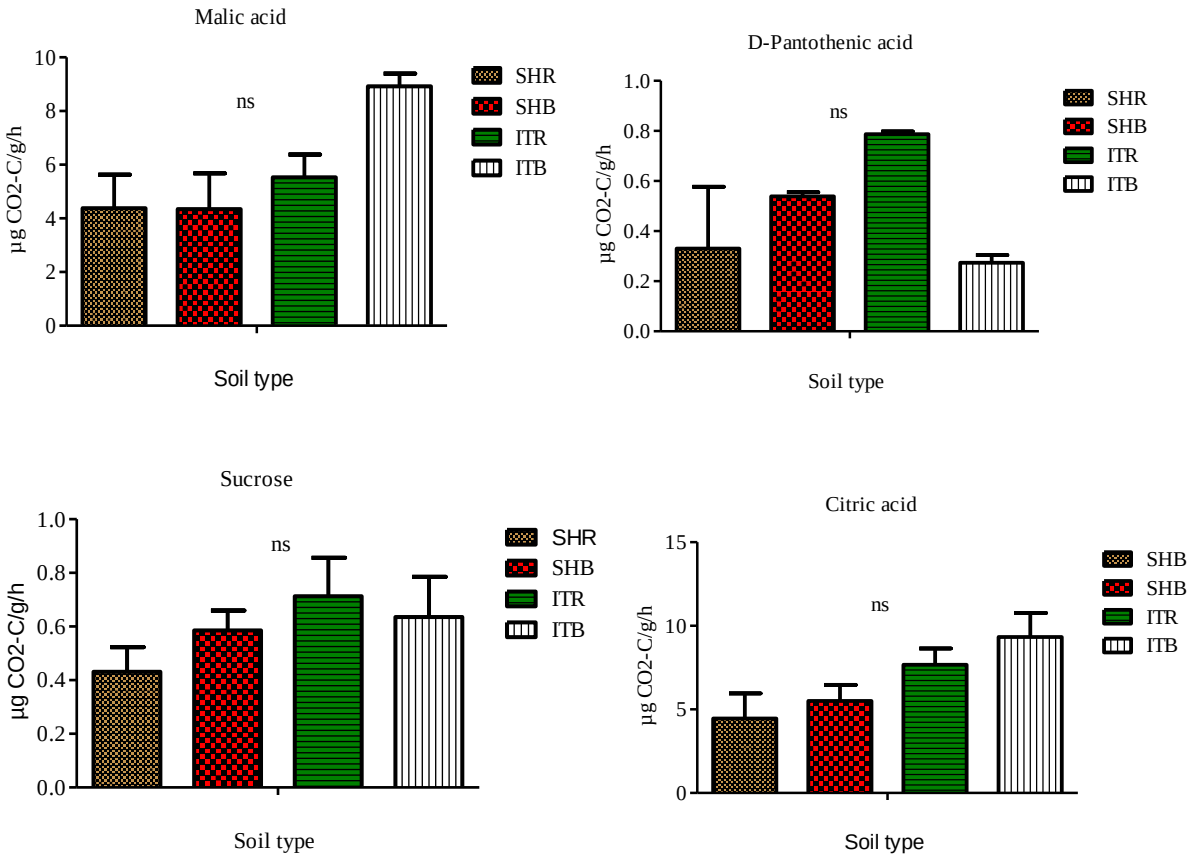

Figure 8.7: Effects of soil type on soil functional properties as measured for malic acid, D - pantothenic acid, citric and sucrose using MicroResp assay. Number of replicates (n) = 2. Data represent mean ± SE, ns = not statistically significant following Duncan's multiple range test. SHR= Sheila rhizosphere soil, SHB=Sheila bulk soil, ITR= Itsoseng rhizosphere soil, ITB=Itsoseng bulk soil.

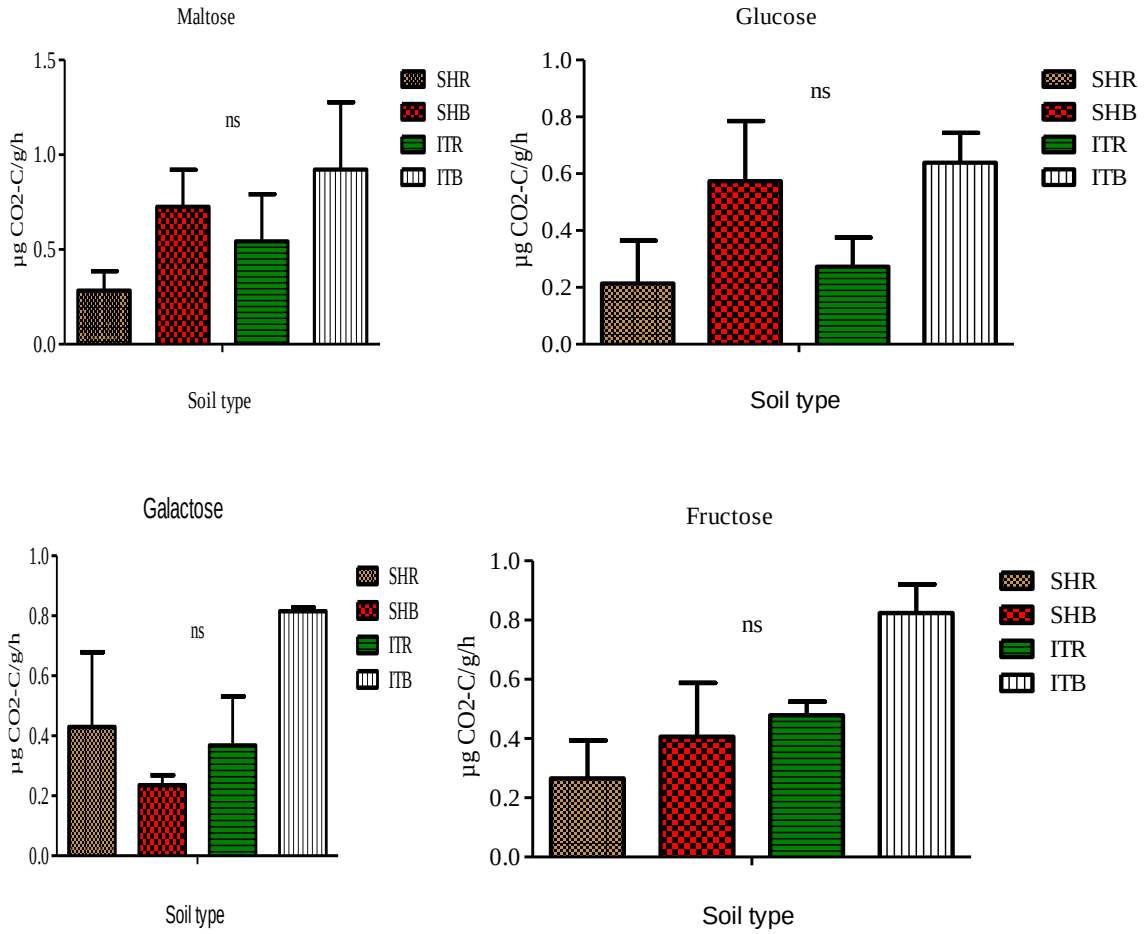

Figure 8.8: Effects of soil type on soil functional properties as measured for maltose, fructose, glucose and galactose using MicroResp assay. Number of replicates (n) = 2. Data represent mean ± SE, ns = not statistically significant following Duncan's multiple range test. SHR= Sheila rhizosphere soil, SHB=Sheila bulk soil, ITR= Itsoseng rhizosphere soil, ITB=Itsoseng bulk soil.

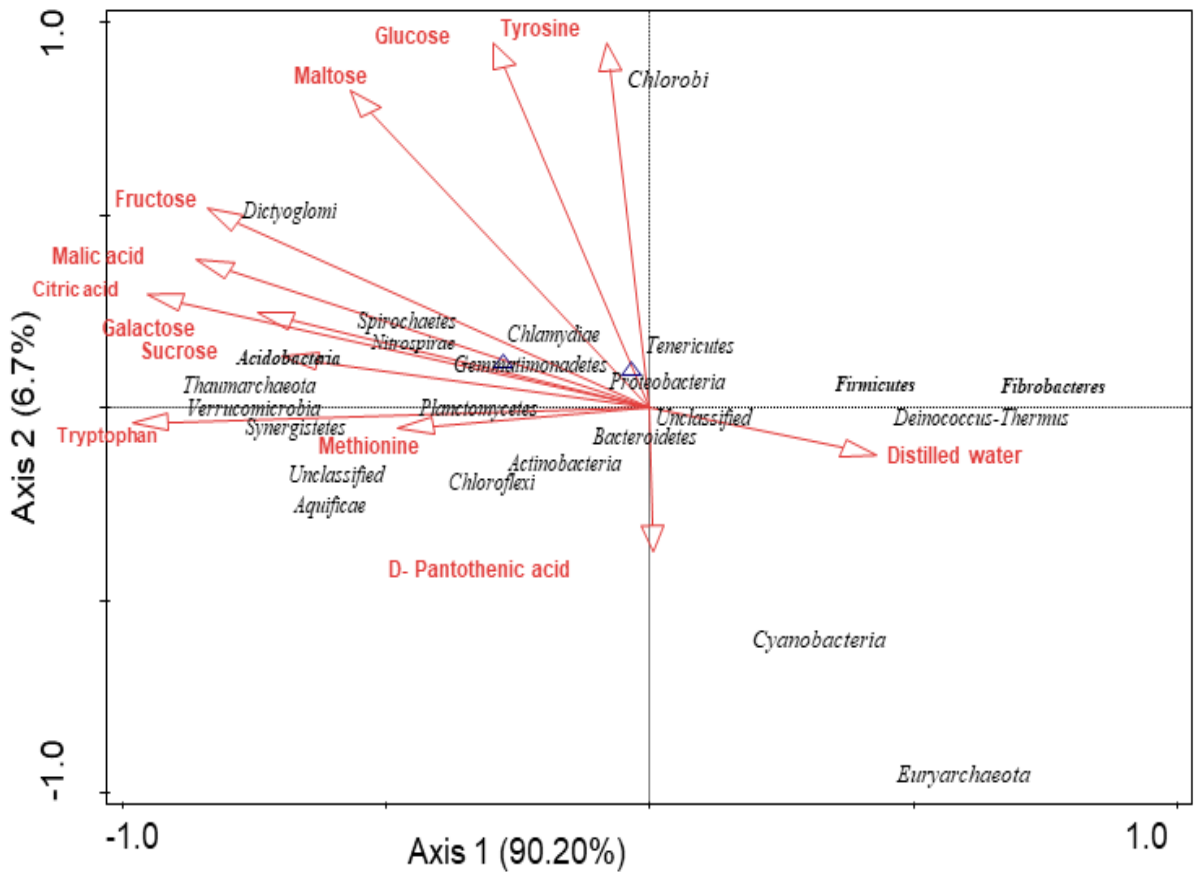

Figure 8.9: The influence of carbon substrates on the microbial phyla from sunflower rhizosphere and bulk soils obtained from Sheila and Itsoseng using Canonical correspondence analysis (CCA)

8.4. Discussion

In this present study, the differences between two soil types from Sheila and Itsoseng were shown by profiling the microbial diversity associated with sunflower rhizosphere soils and bulk soils using 16S rDNA amplicon sequencing technique. Differences in functional measurements between the two soil types from the different locations were determined using CLPP approach. Community respiration and substrate-induced respiration have been constantly used to assess ecosystem function because it is broadly distributed among diverse groups of microbes and are considered as a suitable alternative for total biological activity (Creamer et al., 2016, Ruiz-Dueñas and Martínez, 2009).

Considering the microbial diversity in this study, rarefaction curves were plotted to depict the microbial richness of each sample. The two soil samples with the highest species counts were obtained from ITB (light green curve) and ITR (purple curve), even though soil sample from SHB (orange curve) had the highest number of reads (120,000). In agreement with Enagbonma et al. (2020), the analysis from the rarefaction curves obtained in this study had shown that the majority of the read numbers from all the soil samples reached saturation, indicating that the sampling method sufficiently captured a high proportion or percentage of the microbial species from the different sampling sites. In addition, reductions in bp count of the sequence read in all the soil samples were observed (Table 8.2). This reduction in bp count is as a result of the removal of low quality and contaminating sequences, which could affect downstream analysis (Zhou et al., 2014).

It was further observed that the sampling sites vary in physical and chemical properties and alpha diversity. Variations in the microbial community composition among soil types using PCoA were also observed. This indicates that the microbial community structures are perhaps unique to the soil type. This was further buttressed statistically by the analysis of similarity (ANOSIM), which shows that the separation was significant (p-value = 0.01, R = 0.60). This significant variation can be attributed to the significant differences ($p \leq 0.05$) recorded for most of the physical and chemical properties of the soil types, similar to the result reported by Oberholster et al. (2018). The uniqueness of microbial species in the different soil types could be due to restricted dispersal mechanisms between the two sunflower farms as well as local assembly mechanisms that may remove or introduce certain microbial species within a particular soil type (Hanson et al., 2012). The alpha diversity of microbial taxonomic groups (Simpson, Shannon and evenness indicators) of the two sunflower farms soil types were not significantly different, and this is similar to the report of Enagbonma et al. (2020).

Previous studies on microbes associated with plants have demonstrated that soil type plays a major role in shaping microbial diversity structure (Bulgarelli et al., 2012, Edwards et al., 2015, Lundberg et al., 2012, Peiffer et al., 2013). Findings from this study however differ from the findings of Makonde et al. (2015), who highlighted no difference or separation in beta diversity of soil types or samples when they considered the conserved regions of the microbial species through next generation sequencing approach.

At the phylum level, it was observed that Firmicutes predominated in the soil type SHR with a mean relative abundant value of 39.74. Also, abundant at the phylum level included Actinobacteria with a mean relative abundant value of 26.41 in the soil type ITR (Table 3). The high abundance of Firmicutes and Actinobacteria can partly be explained by the influence of the sunflower root exudates within the sunflower rhizosphere region (Philippot et al., 2013b). These two phyla were among the core microbiome reported by Igiehon et al. (2020b) in soybean plants' rhizosphere inoculated with *Rhizobium* species and mycorrhizal fungal consortium. Most of the bacteria in these two groups possess the particular traits of promoting plant growth because they are endowed with plant growth-promoting traits (Igiehon et al., 2020, Nwachukwu et al., 2021).

These plant growth-promoting traits may include, but not limited to, siderophores and exopolysaccharide production, phosphate solubilization, hydrogen cyanide (HCN) production and indole-acetic-acid (IAA) production (Nwachukwu et al., 2021, Igiehon and Babalola, 2018b, Igiehon et al., 2019). In a study conducted by Offre et al. (2013) using Illumina sequencing approach, Actinobacteria, Acidobacteria, Verrucomicrobia, Planctomycetes, Proteobacteria and Bacteroidetes were present in both rhizosphere and bulk soils of sunflower plants. This is, however, in contrast with the work of Oberholster et al. (2018), who reported Proteobacteria as the dominant keystone microbe in the rhizosphere of sunflower plants.

Microbes with plant growth-promoting functions were also revealed at the genus level. In particular, SHR and ITR predominantly harbored more *Bacillus* and *Arthrobacter*, respectively, than their corresponding SHB and ITB (Table 8.4). It was, however, observed that the genus

Candidatus Nitrososphaera was low in SHR and SHB compared to ITR and ITB (Table 8.4). This result is partly similar to the work of Oberholster et al. (2018) who reported a significant reduction of *Nitrososphaera* in the rhizosphere of sunflower compared to the bulk soil. *Nitrososphaera* is known to be involved in nitrification process (Alawiye and Babalola, 2021). Nitrification may either be induced or inhibited in the rhizosphere (Philippot et al., 2013a), which can eventually affect nitrate and ammonium availability in the rhizospheres. Also, the genera *Aspergillus, Alternaria, Penicillium* and *Fusarium* were the dominant fungi found in the rhizosphere of sunflower plants amended with organic manure (Parfeniuk et al., 2021).

Interestingly, in this study, a relatively high proportion of unclassified microbes were discussed at the phylum and genus levels, even after using other bioinformatics and clustering tools besides MG-RAST such as Quantitative Insights into Microbial Ecology (QIIME) and mothur. It is possible that these unclassified microbes would contain useful plant-microbial functions. Thus, isolating and cataloging these microbes may lead to the discovery of new species with novel plant growth functions. This will help to bridge the existing lacuna in food production, as such new species could be used to complement and/or replace conventional chemical fertilizer application for sustainable agriculture.

The CCA results showed that the phylum Firmicutes, as well as other phyla like Cyanobacteria, Deinococcus-Thermus and Fibrobacteres, positively correlated with Na^{+} and clay but negatively correlated with $N\text{-}NO_3$, pH, K^{+}, P^{3-}, Ca^{2+}, silt, OM, C, N^{3-}, sand, $N\text{-}NH_4$, Res and Mg^{2+}. This means that an increase in the amount of Na^{+} and clay in the soil would directly translate to an increase in

the abundance of these microbes, while an increase in the amount of $N\text{-}NO_3$, pH, K^+, P^{3-}, Ca^{2+}, silt, OM, C, N^{3-}, sand, $N\text{-}NH_4$, Res and Mg^{2+} would lead to a decrease in the abundance of the microbes. Therefore, the relatively high amounts of Na^+ (78.70%) and clay (20%) that were present in SHR (Table 8.1) could have contributed to the dominance of Firmicutes in SHR. It may appear that these two environmental variables (Na^+ and clay) did not have an influence on Cyanobacteria, Deinococcus-Thermus and Fibrobacteres because their mean relative abundance values were relatively low in this study.

The effects of Na^+ and clay on the latter microbes may not be obvious due to other external factors that might have played crucial roles in determining their occurrence and/or abundance, as was previously reported by Schreiter et al. (2014). Disentangling the impact of soil type on microbial composition is still a challenge under field conditions. The soil's physical and chemical properties affect the soil microbiota as well as agricultural management practices, weather conditions (including relative humidity, temperature and amount of rainfall) (Igiehon et al., 2020), and cropping history.

This study also showed that the occurrence and/or abundance of Actinobacteria, Thaumarchaeota, Bacteroidetes, Planctomycetes, Aquificae and Chloroflexi were positively influenced by Res and Mg^{2+}, and negatively affected by $N\text{-}NH_4$, sand, N^{3-}, C, OM, silt, Ca^{2+}, P^{3-}, K^+, pH, $N\text{-}NO_3$, clay and Na^+. A similar result was observed in another study where Mg^{2+} influenced phylogenetic diversity of sunflower rhizosphere, but not in sorghum rhizosphere soils (Oberholster et al., 2018). However, this study is different from the study of Oberholster et al. (2018) given that, in this

present study, Ca^{2+}, P^{3-} and K^{+} negatively impacted the microbial community composition of the sunflower rhizosphere, whereas Ca^{2+}, P^{3-} and K^{+} positively contributed to the sunflower rhizosphere microbial community reported by Oberholster et al. (2018).

However, from the CCA results, it was found that N-NO_3, pH, Res, sand, clay, total N^{3-}, total C, Na^{+}, OM, K^{+}, P^{3-}, Ca^{2+}, silt and Mg^{2+} did not significantly (p>0.05) enhance soil microbial composition, while N-NO_3 and N-NH_4 were the only environmental variables that significantly improved microbial community structure and composition since they respectively showed 94.6% and 72.9% significant contribution (p<0.1) to microbial species (Table 8.5), particularly in ITB. These findings contradict the findings of Enagbonma et al. (2020), who reported significant contributions of 46% (from pH) and 11.50% (from sand) to soil bacterial communities. Nevertheless, all the soil physical and chemical properties considered played some roles in shaping the microbial communities.

Considering the functional diversity of the microbial communities in sunflower rhizosphere and bulk soils, the soil microbial communities utilized the various carbon substrates at different rates. The consumption rate of these substrates is used for characterizing microbial species (Lladó and Baldrian, 2017b). Using the relative abundance values at the phyla level and the measured respiration rate or functional property values, the influence of carbon source on the community composition was done using the statistical software canonical correspondence analysis. From the analysis, the data show the ability of some microbes to metabolize the incorporated carbon substrates. The analysis unveiled that the microbial communities in SHR and ITR used the amino

acid tryptophan more efficiently, but malic acid was more effectively utilized by microbial species in SHR. Similarly, carbohydrate substrates were observed to be proficiently used by the microbial communities, particularly those of SHR, which metabolized galactose more effectively. The CCA analysis further revealed that the effectiveness in the metabolism of tryptophan by the microbial communities could have alluded to the relatively high abundance of Actinobacteria that was present in ITR.

Besides Actinobacteria, other phyla that positively contributed to the metabolism of tryptophan included Synergistetes, Chloroflexi, Aquificae and Bacteriodetes. The CCA analysis however showed that these microbial phyla did not positively contribute to the metabolism of tyrosine, glucose, maltose, fructose, malic acid, citric acid, galactose and sucrose. The analysis also showed that Spirochaetes, Chlamydiae, Nitrospirae, Gemmatimonadetes, Acidobacteria, Thaumarchaeota, Dictyoglomi and Viromicrobia positively correlated with tyrosine, glucose, maltose, fructose, malic acid, citric acid, galactose and sucrose, but negatively correlated with tryptophan, methionine, D-pantothenic acid and distilled water.

These positive correlations may partly be due to the usage of the carbon substrates as an energy source by soil microbes (Gunina and Kuzyakov, 2015). Some of these carbon substrates, such as carbohydrates, are the abundant organic compounds in the soil. It has been postulated that the most critical roles of sugars in the soil are the preservation and stimulation of microbial activities for priming results (Gunina and Kuzyakov, 2015, Struecker and Joergensen, 2015). Amino acid, which is majorly used for building up microbial biomass, was the least respired among some of

the substrates used. The low amounts of nitrogen in the soils give an explanation for this. Consequently, it can be considered that a major percentage of the amino acids available in the soils were assimilated for building up microbial biomass, and only a small quantity was used for respiration since there was a low level of N content available in the soils (Struecker and Joergensen, 2015, Klimek et al., 2016).

8.5. Conclusion

Using 16S rDNA amplicon sequencing approach, this study has shown that Firmicutes, *Actinobacteria* and *Proteobacteria* predominated sunflower rhizosphere soils. Firmicutes, as well as other phyla like Cyanobacteria, Deinococcus-Thermus and Fibrobacteres were positively influenced by Na^{2+} and clay, while Actinobacteria, Thaumarchaeota, Bacteroidetes, Planctomycetes, Aquificae and Chloroflexi were positively influenced by Res and Mg^{2+}. It was reported that $N\text{-}NO_3$ and $N\text{-}NH_4$ significantly contributed to the microbial communities resulting in the differences observed in the sunflower rhizosphere and bulk soils.

The CLPP analysis further demonstrated that microbial communities in SHR and ITR used the amino acids tryptophan and malic acid more efficiently and that the relatively high metabolisms of these carbon substrates may be due to the dominant nature of some of the microbes (e.g., Actinobacteria) in the soils. It was also observed that the number of identified rRNA features was larger than the number of sequences. This could be linked to the high proportion of unclassified bacteria observed in this study since it has been reported that application of rRNA genetic information helps to identify novel sequences, predict the nutritional composition for bacteria that

are un-culturable and improve media formulation or development (Boomer et al., 2002, Hugenholtz et al., 1998). With the high proportion of unclassified microbes observed in the studied soils, it was therefore recommended that efforts should further be made to isolate, characterize and identify these unclassified microbial species as it might be plausible to discover new microbial candidates that can further be harnessed for biotechnological purpose.

CHAPTER NINE

Effects of soil properties and carbon substrates on bacterial diversity of two sunflower farms

Overview

Soil and plant resources are essential for human development. Farmland expansion has grown globally over the past centennial to feed a constantly growing human population. As the world's population continues to grow, so does the demand for land. Understanding how farmland activities manipulate multiple soil ecosystem functions, as well as the structure and function of soil microorganisms, is critical for assessing the potential influences of human growth on ecological systems. The group of ecosystem functions linked to carbon cycling is one of the most important. The majority of carbon in the terrestrial ecosystem is organic carbon.

Soil organic matter (SOM) plays an important role in ecosystem functioning, nutrient retention, water retention and soil structure stabilization. Soils are important terrestrial biomes because they act as carbon sinks. The breakdown of SOM results in the release soil mineral nutrients that are then available for plant growth. Consequently, the potential of soil bacterial communities to degrade a wide range of organic compounds aids in controlling the rate of transformation of these compounds. As a result, the bacterial community’s structural and functional diversity is extremely important for the proper functioning of soils. Understanding how these ecosystems work requires insight into the utilization of carbon substrate by the bacterial

communities inhabiting the rhizosphere and bulk soils of sunflower. Hence, to unveil the importance of the role by diverse soil microorganisms and their functions, the respiratory response of soil microbiota to important environmental substrates, particularly those linked to plant-bacterial interactions, must be understood. Using amplicon sequencing and community level physiological profiles (CLPP), this study is aimed to reveal the impact of carbon respiration on the structural and functional qualities used by the bacterial community associated with sunflower plants rhizosphere.

The succeeding chapter (chapter 10) provides evidence that beneficial rhizosphere-microorganisms connections and crop rotation can help to maintain a stable ecosystem. When compared to nearby bulk soils, the use of a next-generation sequencing technique will aid in characterizing the entire bacterial species in sunflower rhizosphere soils. The possibility for predictive functional features of the bacterial community structure of sunflower rhizosphere and bulk soils grown under different agricultural practices was investigated at Lichtenburg and Krayburg, both in North West Province, South Africa.

Abstract

The sustainable production of sunflower (*Helianthus annuus*) is crucial and one way to accomplish this feat is to have an understanding of the beneficial bacteria of sunflower rhizosphere. Similarly, the respiratory response of these bacteria needs to be studied to understand their roles in the ecosystem. This study was therefore conceptualized to gain insights into the effects of soil properties and carbon substrate utilization on bacterial community diversity of sunflower

rhizosphere grown in Ditsobottla and Kraaipan, North West Province, South Africa. Extracted DNA from sunflower rhizosphere and bulk soils was subjected to 16S amplicon sequencing. Significant differences were observed for the alpha and beta diversity of the soil bacterial communities ($P < 0.05$). At the order level, among all the bacterial taxa captured in the farms, Bacillales were the most dominant. The abundance of Lactobacillales, Bacillales, Rhizobiales, Enterobacteriales, Burkholderiales, Flavobacteriales, Sphingomonadales, Myxococcales and Nitrosomonadales obtained from Ditsobottla rhizosphere soil (R1) was positively influenced by organic matter (OM), while the abundance of Planctomycetales, Cytophagales, Gemmatimonadales, Nitrospirales and Caulobacteriales from Kraaipan rhizosphere soil (R2) was positively influenced by total N and pH. Bacterial communities of all the soil samples utilized the different carbon substrates (three amino acids, six carbohydrates and three carboxylic acids) as an energy source. Significant differences ($P < 0.05$) were only observed in tryptophan and methionine amended soils. Unclassified bacteria were also captured in this study. Such bacteria can further be harnessed for sustainable production of sunflower and other agricultural crops.

Keywords: Bacterial community, Functional profile, Sustainable agriculture, 16S amplicon sequencing

9.1. Introduction

Over a decade, studies have revealed that plant-microbial interactions are crucial for plant growth and health, and these interactions are of immense importance in comprehending nature conservation and agricultural sustainability (Nwachukwu and Babalola, 2021). Similarly, recent

attempts to understand the belowground activities have shown that the rhizosphere bacterial communities play a key role in improving ecosystem functions and plant yield (Agomoh et al., 2020, Igiehon and Babalola, 2018a). The richness and diversity of rhizosphere bacterial communities significantly contribute to the resilience and resistance of the ecosystem to biotic and abiotic stresses (Shrestha et al., 2019). Interestingly, awareness into plant–soil interactions and processes are complex and still require further studies (Yee et al., 2021).

Soil microorganisms play important roles in many soil processes, including nutrient acquisition, nitrogen and carbon acquisition, and transformation of plant deposits to soil organic matter (Jiang et al., 2021, Enebe and Babalola, 2021). In plants, nutrient distributions and accessibility differ significantly depending on the soil type, plant species and plant age (Nwachukwu and Babalola, 2021, Lambers et al., 2008). Generally, plants influence the rhizosphere bacterial communities through the release of metabolites that stimulate or reduce the proliferation of a particular species of the microbiota, thus creating an environment favorable for the plant species (Igiehon et al., 2019). Other plant species harbors species-specific microorganisms that are strictly linked with them in the rhizosphere (Yee et al., 2021). Rhizosphere bacterial communities likewise show an advanced level of host interaction and specificity with plant species (Schlatter et al., 2019).

On the contrary, cropping system and agricultural practices have a strong influence on soil microbiome diversity, crop benefits and soil properties (Zhang et al., 2019a). Various studies have assessed soil bacterial diversity. Limited studies have, however, investigated the impact of carbon substrate utilization on their functions. Nevertheless, considering the multifaceted dynamics that

regulate rhizosphere bacterial communities, the fundamental significance and functions of rhizosphere microbiome are still not fully understood (Shrestha et al., 2019).

Furthermore, physicochemical properties of soils and other abiotic components can regulate the interactions between soil bacterial communities and plants (Igiehon et al., 2019). The ability of soil bacterial communities to breakdown numerous organic compounds helps to control the transformation rate of these compounds. Therefore, for proper functioning of soils, the structural and functional diversity of bacterial communities are of enormous significance (Chukwuneme et al., 2021, Babalola et al., 2021c). Insight into carbon substrate utilization of the bacterial communities inhabiting sunflower rhizosphere and bulk soils is also important to comprehend how these ecosystems function. To the best of my knowledge, limited studies exist on the processes and effects of different carbon respiration on the diversity, structural and functional characteristics of the rhizosphere microbiome associated with sunflower plants.

The respiratory response of soil microorganisms to environmentally relevant substrates, especially those involved in plant-bacterial activities, needs to be understood to reveal the role played by diverse soil microorganisms and their functional diversities. This study, therefore, aimed to unveil the influence of rhizosphere carbon respiration on the structural and functional attributes used by the rhizosphere bacterial community associated with sunflower plants using amplicon sequencing and community level physiological profile (CLPP).

The hypothesis is that the bacterial community structure and CLPP of sunflower rhizosphere soils would be different from bacterial community structure and CLPP of their corresponding bulk soils.

These projected variations in bacterial community structure and carbon utilization between the soil habitats may be alluded to a significant increase in the amount of root exudates, plant-bacterial interactions and greater utilization of highly structurally complex carbon substrates in the rhizosphere soil compared to their corresponding bulk soils.

9.2. Materials and methods

9.2.1. Farm history and sample collection

This study was performed in North West Province of South Africa. The province has a summer period between December and February characterized by short thundershowers in the afternoons. The mean rainfall is between 300-700 mm per annum, while average maximum temperatures during summer and winter are 34 and 22 ^{o}C respectively. The annual mean temperature during winter (June-August) is 2-20 ^{o}C, while autumn (March-May) and spring (September-November) annual temperatures are mainly between 13 and 25 ^{o}C and 19 and 30 ^{o}C respectively.

The two major sunflower plant farms in Ditsobottla also called Sheila (26^{o} 2′41.202″ S 25^{o} 57′ 47. 49″ E) and Kraaipan (26^{o} 17′24.186″ S 25^{o} 13′33.258″ E) were cautiously selected based on the agricultural practices of the farms (Table S9.1). Approval was obtained from the farm owners. Sunflower seed (Pen 7011 Pannar) was the cultivar planted on both farms. Over the years, both farms have history of fertilizer (NPK 15:8:4) application.

After precisely 8 weeks of cultivation, rhizosphere and bulk (control) soils were collected from both farms. Three rhizosphere and three bulk soil samples each were obtained for Ditsobottla and

Kraaipan rhizosphere soils, and each replicate was composed of soils collected from 20 plants after germination at a depth of 0–15 cm on different plots having radius of 3 cm away from the plants and afterward pooled together to make a composite sample as described by Oberholster et al. (2018). Similarly, Ditsobottla and Kraaipan bulk soils were collected from the surrounding soil at a distance of 10 m away from the sunflower rhizospheres.

The soil samples were collected into sterile plastic bags, then transferred into an ice packed container and conveyed to the laboratory immediately. A mesh sieve (2-mm pore size) was used to sieve the soil samples, while the sieved soil samples were divided into three portions: one portion was used for 16S amplicon sequencing, which was stored at –20 ^{o}C, the second portion was stored at 4 ^{o}C for physiochemical analyses, while the third portion was used for CLPP.

9.2.2. Soil physiochemical analyses

A Jenway 3520 pH-meter (Cole-Parmer Instruments, Staffordshire, UK) was used to determine soil pH after mixing 2 g of the soil in 10 ml deionized water. Soil moisture content was quantified by oven-drying the soil samples at 105 °C for 24 hours (Colombo et al., 2016). The total nitrogen was determined using dry combustion method, while Walkley Black method was used to ascertain the organic carbon (Walkley and Black, 1934, Jacoby et al., 2017).

9.2.3. Metagenome DNA extraction and amplicon sequencing

Zymo DNA isolation kit (Zymo Research, Irvine, USA) was used for the extraction of the microbiome DNA, following manufacturer's instruction. The extracted DNA samples were sent

to MR DNA Laboratory (Texas, USA) for 16S amplicon sequencing. The 16S rRNA gene variable region V4 for bacterial community was sequenced using an Illumina MiSeq sequencer (Illumina NovaSeq 6000 system). The PCR primers 515F and 806R were used, and then paired-ends reads of 312 bp were obtained.

9.2.4. Annotation, data and statistical analyses

The forward and reverse sequences were obtained after sequencing was performed on the Illumina MiSeq sequencer using a paired-end technique. The raw sequence reads were uploaded into MG-RAST, an online server (Meyer et al., 2008b). After joining the paired ends on the MG-RAST to generate consensus sequences, quality control (QC) processes were carried out. The sequence reads were then pre-processed (to remove the artificial sequences, ambiguous base pairs and host specific-species sequences), followed by filtering of the read length. Then, the sequences were denoised and screened for the presence of chimeras. The processed sequences were annotated using BLASTn (Mohammed et al., 2020) against RDPII and NCBI databases (Garcia-Mazcorro et al., 2019), permitting non-redundant integration of numerous databases. The RDP was used to classify the bacteria.

Further analyses were not conducted on sequences that failed annotation. Then focus was on bacteria, hence, sequences obtained from eukaryotes were discarded. The normalized data option of MG-RAST was used to reduce the effect of experimental error/ noise. The bacterial abundance values, which were assembled according to the taxa, and unclassified bacteria were reserved for statistical purposes. Afterward, the taxa abundance values were transformed into percentages.

Binning was carried out on the analogous sequences into operational taxonomic units (OTUs). OTUs were clustered at 3% divergence (97% similarity). Equitability_J, Fisher_alpha, Berger-Parker and Chao-1 were used to estimate species richness. Taxonomic richness was expressed as OTU number. Furthermore, statistical analysis was carried out using the mean of the relative abundance for all the replicates for each sampling site. The sequences have been deposited on the National Center for Biotechnology Information SRA dataset under the accession number PRJNA672856.

9.2.5. Determination of carbon substrate utilization by soil bacterial components using CLPP technique —MicroPlate™

The bacterial community level physiological profiles of the soil samples were determined using the MicroResp™ method (Moscatelli et al., 2018). Soil samples were adjusted to 40% of their maximum water holding capacity and loaded into 96-well of 1.2 ml deep-well microplate. Prior to carrying out MicroResp™ method, 0.4 g of soil was distributed to each well and incubated in the dark at 25 °C for 5 days.

Eleven (11) different carbon substrates, consisting of 3 amino acids (L-methionine, L-tyrosine, S-tryptophan), 6 carbohydrates (D-galactose, D-glucose, D-fructose, D-maltose, Sucrose) and 3 carboxylic acids (D-pantothenic, citric acid, malic acid) were used to determine the physiological profiles, while distilled water served as the control. Sterile deionized water was used to measure individual basal respiration. Each substrate dissolved in sterile deionized water was added to 4 replicated wells.

Detection plates containing cresol red (12.5 μg/ml), potassium chloride (150 mM) and sodium bicarbonate (2.5 mM) were read at absorbance wavelength 570 nm, and immediately placed on the MicroResp™ seal, and then incubated at 25 °C in the dark for 6 hours, as recommended in the instruction manual. After incubation, the modification in optical density (OD) was determined on a spectrophotometer microplate reader (AccuReader M965+, Taipei, Taiwan) at a wavelength of 570 nm. The rate of individual CO_2 respiration per gram of soil was calculated using the formula provided in the MicroResp™ manual (James Hutton Ltd, UK). Total respiration rate was obtained by following the method of Colombo et al. (2016) with little modification.

9.2.6. Statistical analyses

Microsoft Excel Software was used to analyze the abundance and distribution of the key rhizosphere bacterial communities at the class and order level. Also, Shinyheatmap online tool (www1.heatmapper.ca/expression/) was used for the plotting of heatmaps using the relative abundance values. Equitability_J, Fisher_alpha, Berger-Parker and Chao-1 indices for diversity assessment were used for samples across rhizosphere and bulk soil samples, and the comparison of these indices was performed using Kruskal–Wallis test**.** The analyses were carried out using PAST version 3.20 (Hammer et al., 2001). Beta (β) diversity of the bacterial communities of the samples was determined using the Euclidean-based principal coordinate analysis (PCoA) and ANOSIM through 999 permutations. $P < 0.05$ was considered statistically significant for all datasets. Both PCoA and Principal Component Analysis (PCA) graphs were used to evaluate the relationship between bacterial communities and the measured physiochemical parameters which

were plotted using CANOCO version 5.0 (Microcomputer Power, Ithaca, NY). The effects of pH, total N (%) and OM (%) on the bacterial community distribution were determined using Canonical Correspondence analysis (CCA). Graphs and one-way ANOVA for functional measurements were achieved using GraphPad Prism 7 (GraphPad Software, California).

9.3. Results

9.3.1. Amplicon metagenome sequencing, quality control and protein annotation

An estimated mean number of sequences uploaded were 73,877,515 (R1) and 80,404,565 (R2) sequence reads for sunflower rhizosphere soils and 50,642,798 (B1) and 74,956,208 (B2) sequence reads for bulk soil samples. After performing quality control (QC) using MG-RAST software, the estimated sequence was 8,362,450 (R1) with a mean G + C content of 56%, and R2 had a mean sequence of 8,310,524 with a mean G + C content of 55.5%, while the mean sequence of B1 sample was 5,824,673 with a mean G + C content of 54.5%. Whereas, B2 had a mean sequence of 9,525,300 with a mean G + C content of 57%.

Additionally, 29,112 (R1) and 272,663 (R2) sequence reads contained predicted proteins with unknown functions from the sunflower rhizosphere soils, while 173859 (B1) and 254,030 (B2) sequence reads contained predicted proteins with unidentified functional categories from sunflower bulk soil samples. OTU sequences were clustered at a similarity of 97% and the abundant values for R1, R2, B1 and B2 were obtained. A Venn diagram http://bioinformatics.psb.ugent.be/cgi-bin/liste/Venn/calculate_venn.htpl (Oliveros, 2017)

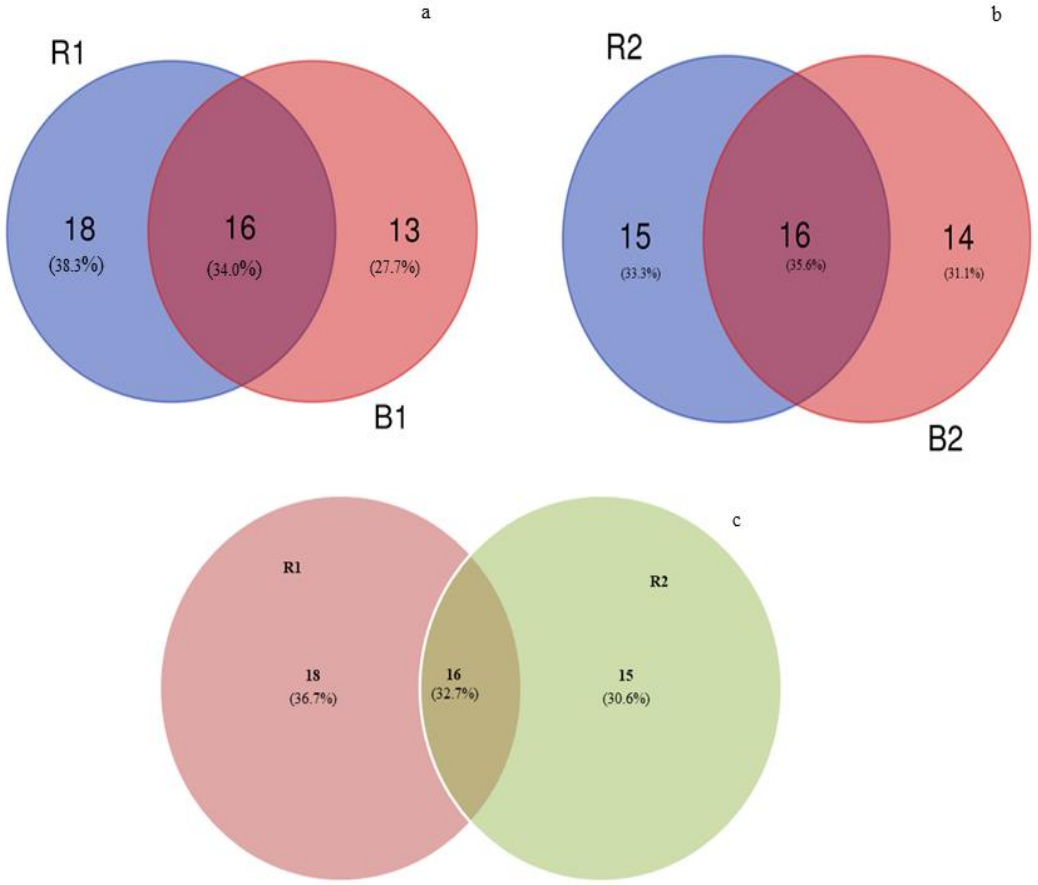

Figure 9.1: Venn diagram of shared operation taxonomic units between the bacterial components (at the order level) of sunflower rhizosphere and bulk soils obtained from Ditsobottla and Kraaipan farms. R1- Ditsobottla rhizosphere soil, B1- Ditsobottla bulk soil, R2- Kraaipan rhizosphere soil, B2- Kraaipan bulk soil.

(Figure 9.1) revealed that R1 and B1 shared 34.0% of OTUs, while R2 and B2 shared 35.6% of OTUs. However, 32.7% of OTUs was shared between R1 and R2.

9.3.2. Alpha and beta diversity assessment of the bacterial communities across the sampling sites

The diversity of the structural categories was examined using the equitability_J, Fisher_alpha, Berger-Parker and Chao-1, and the results revealed that they differ significantly ($P < 0.05$) (Table 9.1). Through Kruskal-Wallis evaluation, the level of the differences in structural diversity across the sites was estimated and was found to be significant ($p = 0.01$). The PCoA graph revealed a clear difference in the relative abundance of bacterial groups between R1 and B1 sites as compared to R2 and B2 sites (Figure 9.2). One-way ANOSIM was used to test for similarity across the sites. The results showed a significant difference in the identified bacterial communities from all samples across the cropping sites (R = 1.167 and $p = 0.01$).

Table 9.1: Alpha diversity indices of the bacterial components of sunflower rhizosphere and bulk soils.

Alpha diversity indices	**Soil type**				***P* value**
	R1	B1	R2	B2	
Equitability_J	0.4894	0.4366	0.5861	0.5579	0.01
Fisher_alpha	11.9	11.51	10.67	9.571	
Berger-Parker	0.512	0.4373	0.3552	0.4314	
Chao-1	23	22	22	22	

Legend: R1- Ditsobottla rhizosphere soil, B1- Ditsobottla bulk soil, R2- Kraaipan rhizosphere soil, B2- Kraaipan bulk soil, p – probability value

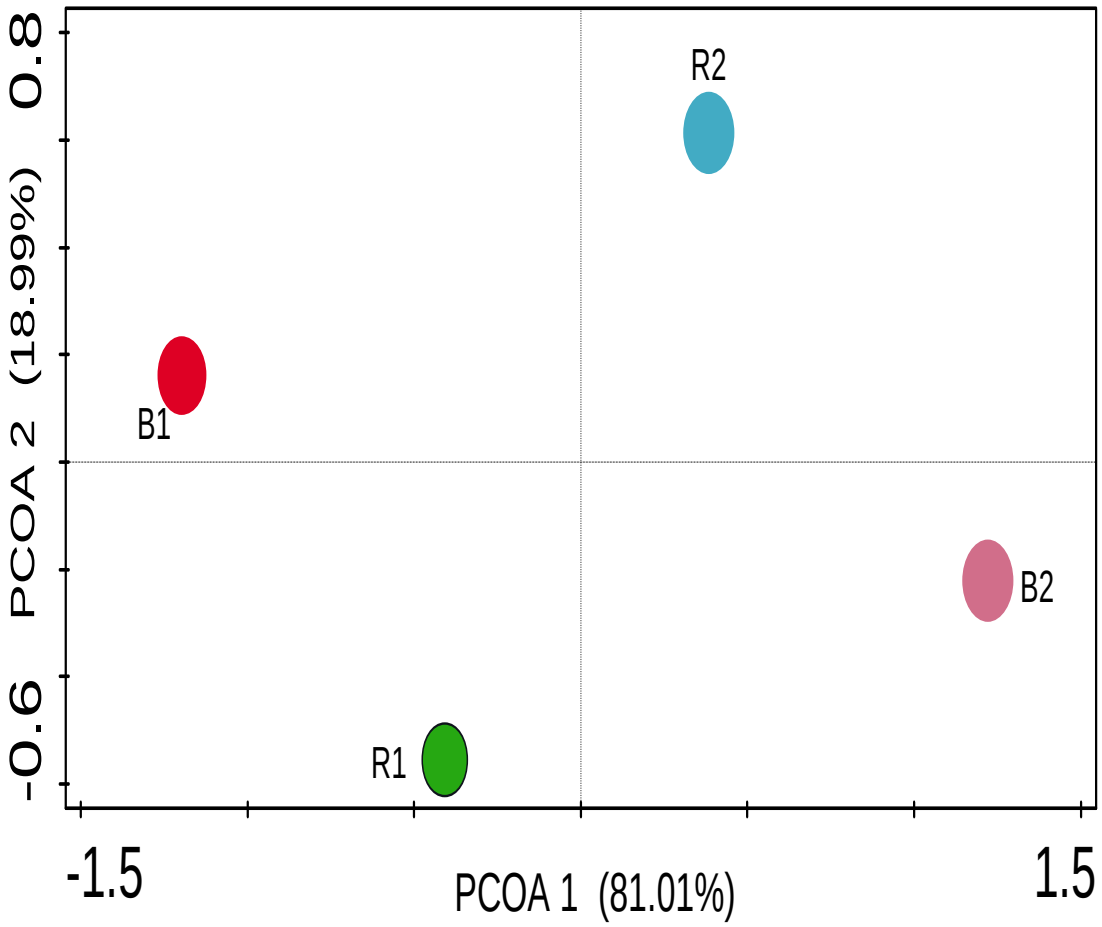

Figure 9.2: PCoA of the bacterial components (at the order level) of sunflower rhizosphere and bulk soils obtained from Ditsobottla and Kraaipan farms using Canonical correspondence analysis. PCoA – principal coordinates analysis, R1- Ditsobottla rhizosphere soil, B1- Ditsobottla bulk soil, R2- Kraaipan rhizosphere soil, B2- Kraaipan bulk soil

9.3.3. Bacterial structural composition at the class and order level across the rhizosphere and bulk soil samples

Sequence tags were assigned to rhizosphere and bulk soil samples into diverse taxa via rapid metagenomic annotations using MG-RAST software. At the class level, the relative abundance of bacterial class for each soil sample is presented in Figure 9.3. Relative abundance of Bacilli, Bacteroidia, Acidobacteria, Planctomycetacia, Flavobacteria and Betaproteobacteria was higher in R1. Gammaproteobacteria and Opitutae were observed to be more abundant in the corresponding bulk soil (B1). Bacilli were higher in all soil samples (R1, B1, R2 and B2) with relative abundance values of 38.18, 30.66, 28.85 and 37.43 respectively.

Gammaproteobacteria were dominant in R1, B1 and R2 soil samples with relative abundance values of 23.74, 28.36 and 21.96 respectively. While Actinobacteria (18.05) were recorded to be more abundant in bulk soil sample (B2). Cytophagia was exclusively detected in the rhizosphere soil (R2) but, Actinobacteria, Clostridia, Spingobacteria, Gemmatimonadetes, Deltaproteobacteria, Negativicutes, Thermamicrobia, Solibacteres, Nitrospira and Spartobacteria were abundant in the bulk soil sample (B2).

At the order level, the relative abundance of Bacillales was higher in R1 (36.24) than the bulk soil (B1 = 29.28). On the other hand, B2 (37.00) had higher mean relative abundance than the rhizosphere soil (R2 = 26.99). Pseudomonadales were higher in bulk soil (B1= 26.94) than in the R1 (22.64). Also, Pseudomonadales in R2 (21.10) were more than those of B2 (7.35). Actinomycetales were higher in B2 (7.41) than R1, B1 and R2. From the heatmap

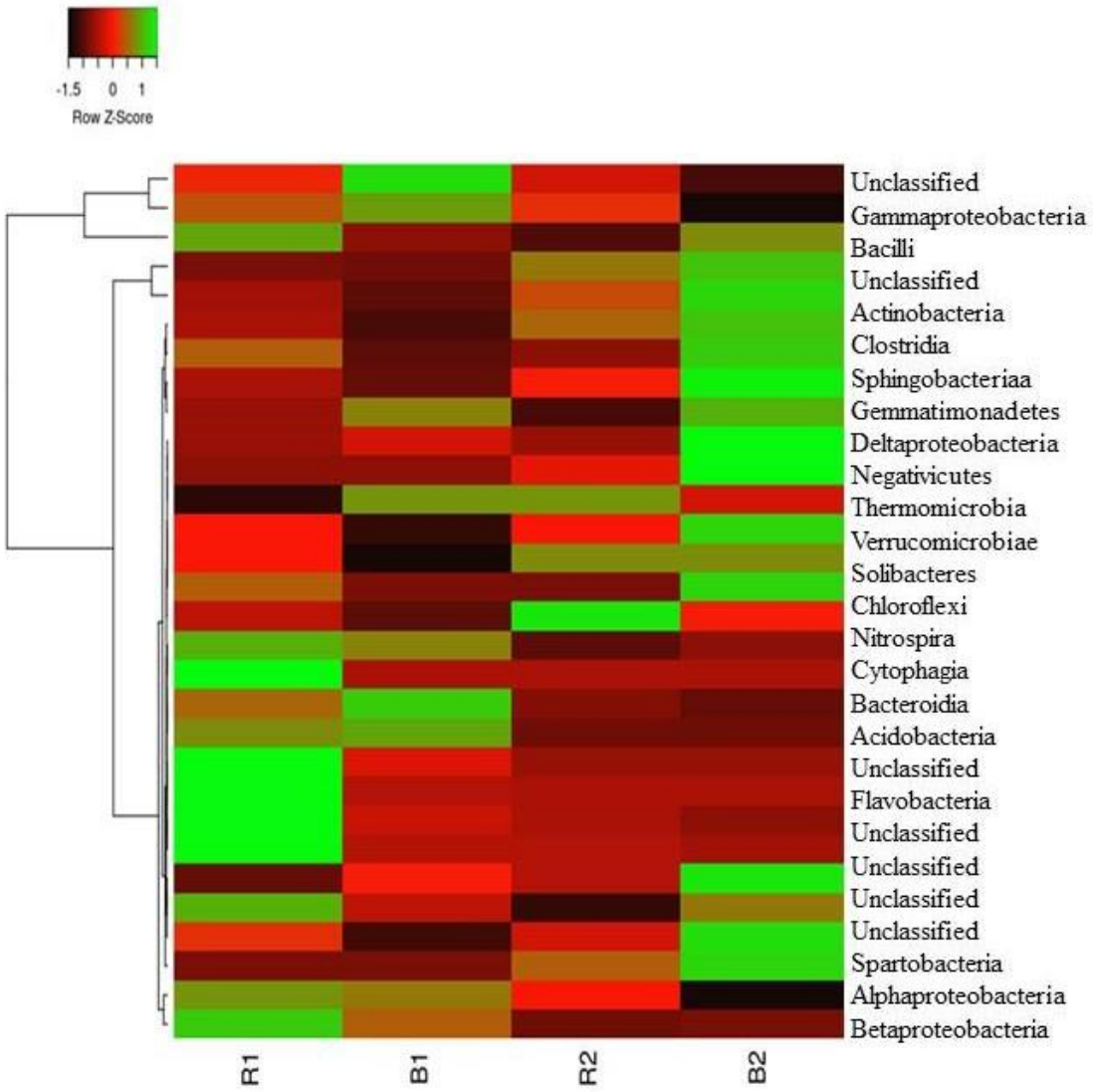

Figure 9.3: A heat-map of the bacterial taxonomic structure and relative abundance at class level from sunflower rhizosphere and bulk soils. The scale bar signifies colour saturation gradient established by the relative abundances with z-score. Bacterial groups with more intense color have higher relative abundant values. R1- Ditsobottla rhizosphere soil, B1- Ditsobottla bulk soil, R2- Kraaipan rhizosphere soil and B2- Kraaipan bulk soil.

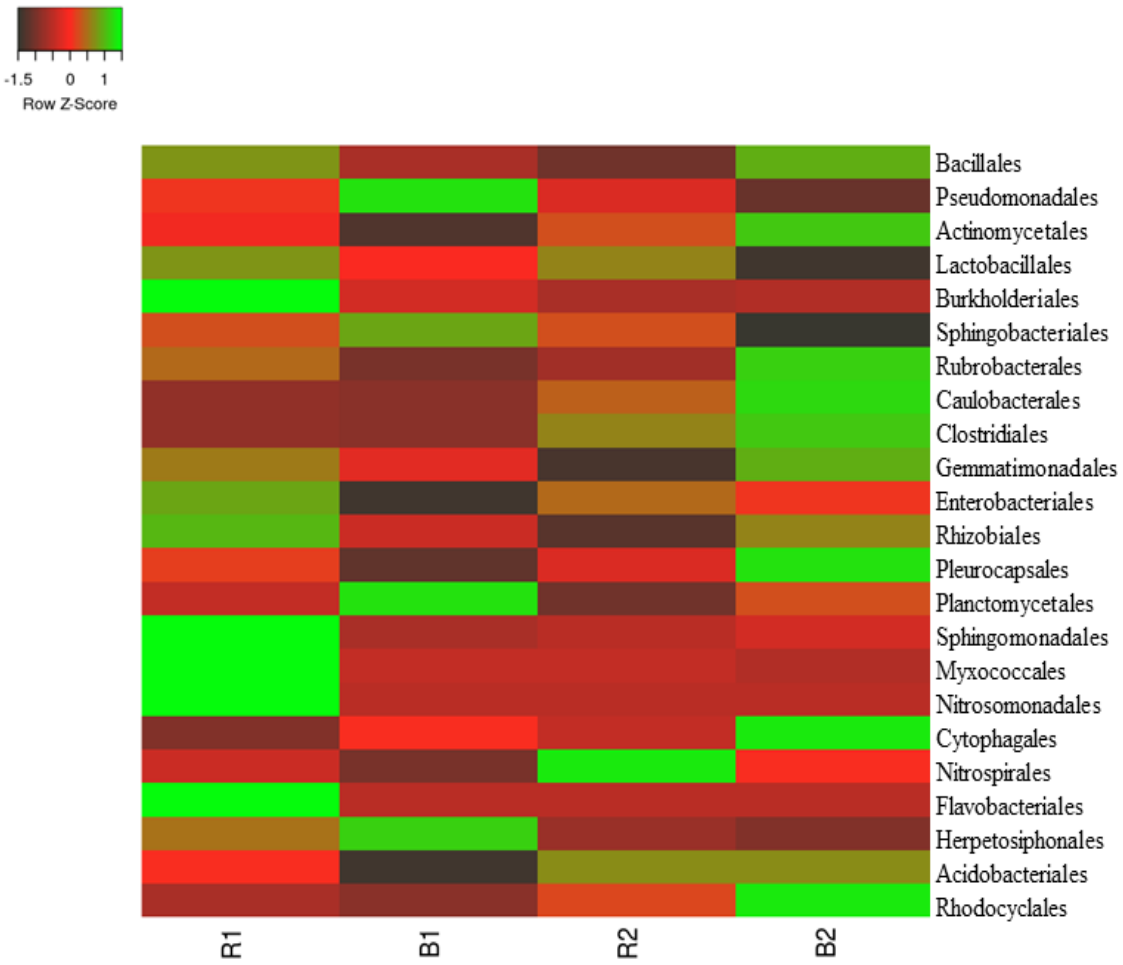

Figure 9.4: Relative abundance of the bacterial taxonomic structure at order level from sunflower rhizosphere and bulk soils. Bacterial groups with high colour intensity have higher relative abundant values. R1- Ditsobottla rhizosphere soil, B1- Ditsobottla bulk soil, R2- Kraaipan rhizosphere soil, B2- Kraaipan bulk soil

Z score color intensity. Lactobacillales, Burkholdeiales, Enterobacteriales, Rhizobiales, Sphingomonadales, Myxococcales, Nitrosomonadales and Flavobacteriales were higher in the R1 than in other soil samples. Spingobacteriale and Herpetosiphonales were relatively more abundant in B2. Lactobacillales and Nitrospirales were more dominant in R2. Rubrobacterales, Caulobacterales, Gemmatimononadales, Pleurocapsales, Cytophagales and Rhodocyclales were highly abundant in the corresponding bulk soil (B2) (Figure 9.4). There were significant differences ($P < 0.05$) between the mean relative abundance of the bacterial communities of the two farms at the class and order level.

9.3.4. Effect of soil properties on bacterial communities

The CCA plot showed that the composition of bacterial communities was influenced by the soil properties. The vector length of pH and total N (%) were (on axis 1) positively correlated with Planctomycetales, Cytophagales, Gemmatimonadales, Nitrospirales and Caulobacterales. On axis 2, the vector length of organic matter (%) positively correlated with Lactobacillales, Bacillales, Rhizobiales, Enterobacteriales, Burkholderiales, Flavobacteriales, Sphingomonadales, Myxococcales and Nitrosomonadales, but negatively correlated with Actinomycetales, Clostridiales, Acidobacteriales, Rubrobacterales, Pleurocapsales and Rhodocyclales (Figure 9.5).

The soil properties that best explained the variance detected in the bacterial community structures observed in Figure 9.5 and 9.6 were ascertained using the forward selection and the Monte Carlo permutation test with random permutations. It was noted that all the soil variables contributed to the difference in the bacterial composition and abundance of the different soil samples. Their

contributions were, however, not significant (P ≥0.05) (Table S9.2). pH had the highest contribution of 71.4%, followed by total N (%) with 20.0% contribution and 8.6% contribution from organic matter (%).

9.3.5. Carbon substrate utilization by the sunflower rhizosphere soil isolates

Different soil types (R1, B1, R2 and B2) showed different carbon substrate utilization and respiration rates as depicted in Figure 9.6, 9.7 and 9.8. In tryptophan amended soils, the bacterial community of B2 had the highest respiration rate, which was significantly different ($P < 0.05$) from those of R1 and B1 (Figure 9.6).

Similarly, the bacterial community of B2 significantly ($P < 0.05$) utilize methionine more than those of R1, B1 and R2 with a concomitant higher respiration rate as depicted by the amount of CO_2 produced (Figure 9.6). However, the bacterial respiration rates in the soils (that is, R1, B1, R2 and B2) amended with distilled water (control), tyrosine, malic acid, citric acid, D-pantothenic acid, sucrose, maltose, fructose, glucose and galactose showed no significant difference ($p > 0.05$) (Figure 9.6, 9.7 and 9.8).

For the two rhizosphere soils, R2 showed a higher total respiration rate (16.83 µg g^{-1} h^{-1} CO_2-C), while for the two bulk soils, B2 showed a higher total respiration rate (23.80 µg g^{-1} h^{-1} CO_2-C). The respiration responses from distilled water utilization (basal respiration) were 4.50, 7.23, 6.38 and 8.19 µg g^{-1} h^{-1} CO_2-C for R1, B1, R2 and B2 respectively. The influence of the carbon substrate

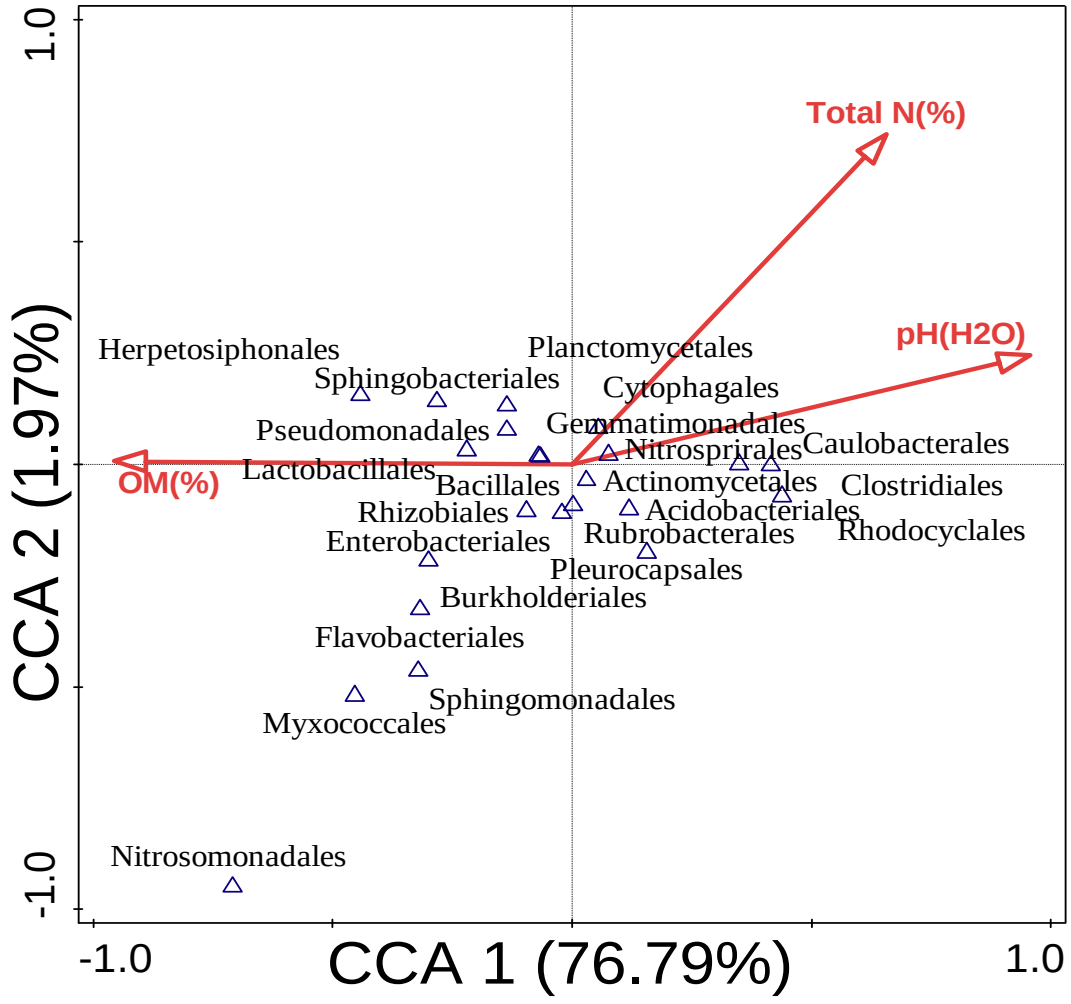

Figure 9.5: The influence of pH, total N (%) and OM (%) on the bacterial components (at the order level) of sunflower rhizosphere and bulk soils obtained from Ditsobottla and Kraaipan farms using Canonical correspondence analysis. N – nitrate, % - percentage, OM – organic matter, R1- Ditsobottla rhizosphere soil, B1- Ditsobottla bulk soil, R2- Kraaipan rhizosphere soil, B2- Kraaipan bulk soil.M

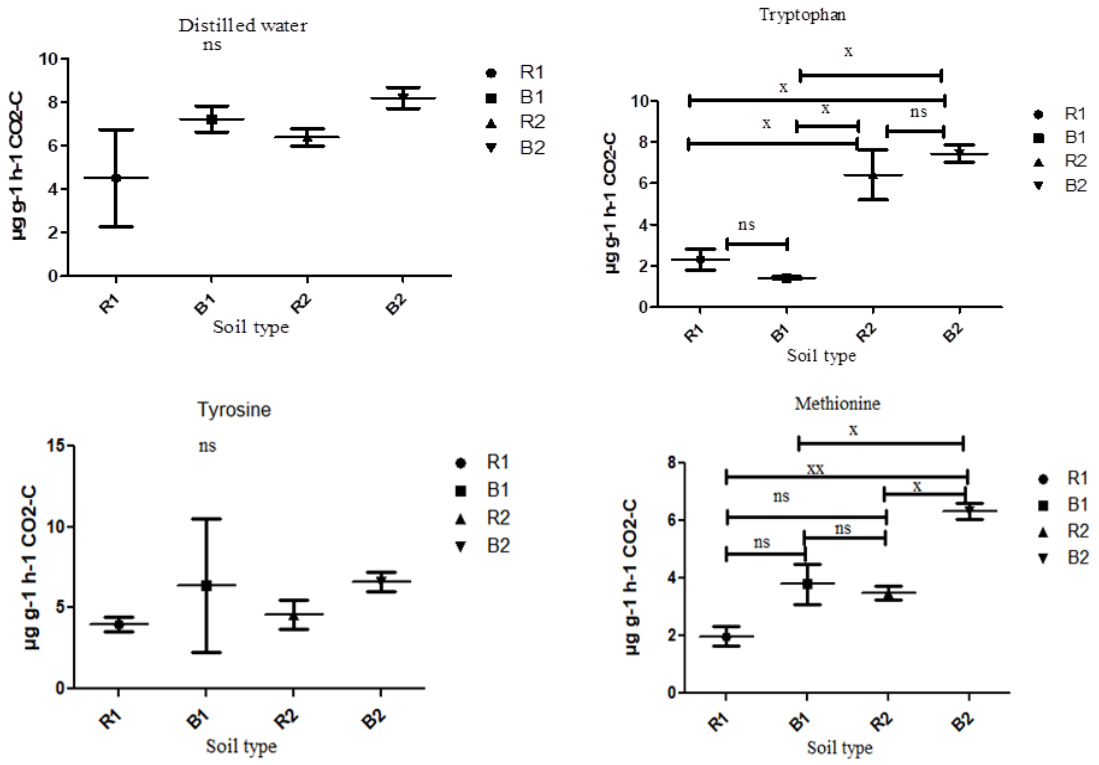

Figure 9.6: Effects of soil type on soil functional properties as measured for distilled water i.e. control, tryptophan, tyrosine and methionine using MicroResp assay. Number of replicates (n) = 2. Data represent mean ± SE, ns = not statistically significant, x = statistically significant, xx = more statistically significant. R1= Ditsobottla rhizosphere soil, B1= Ditsobottla bulk soil, R2= Kraaipan rhizosphere soil, B2 = Kraaipan bulk soil.

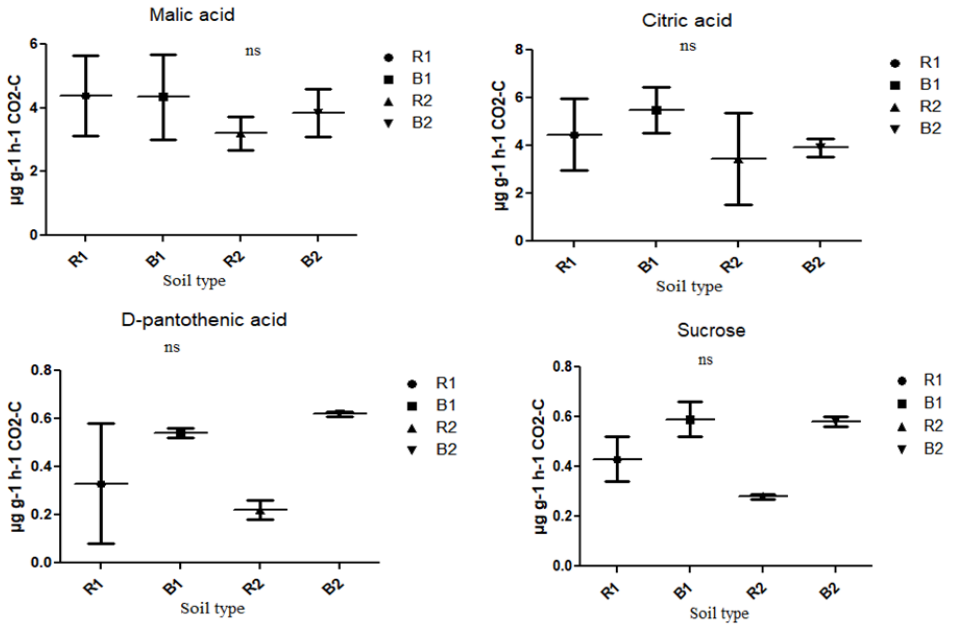

Figure 9.7: Effects of soil type on soil functional properties as measured for malic acid, citric acid, D- pantothenic acid and sucrose using MicroResp assay. Number of replicates (n)= 2. Number of replicates (n)= 2. Data represent mean ± SE, ns= not statistically significant. R1= Ditsobottla rhizosphere soil, B1= Ditsobottla bulk soil, R2= Kraaipan rhizosphere soil, B2 = Kraaipan bulk soil.

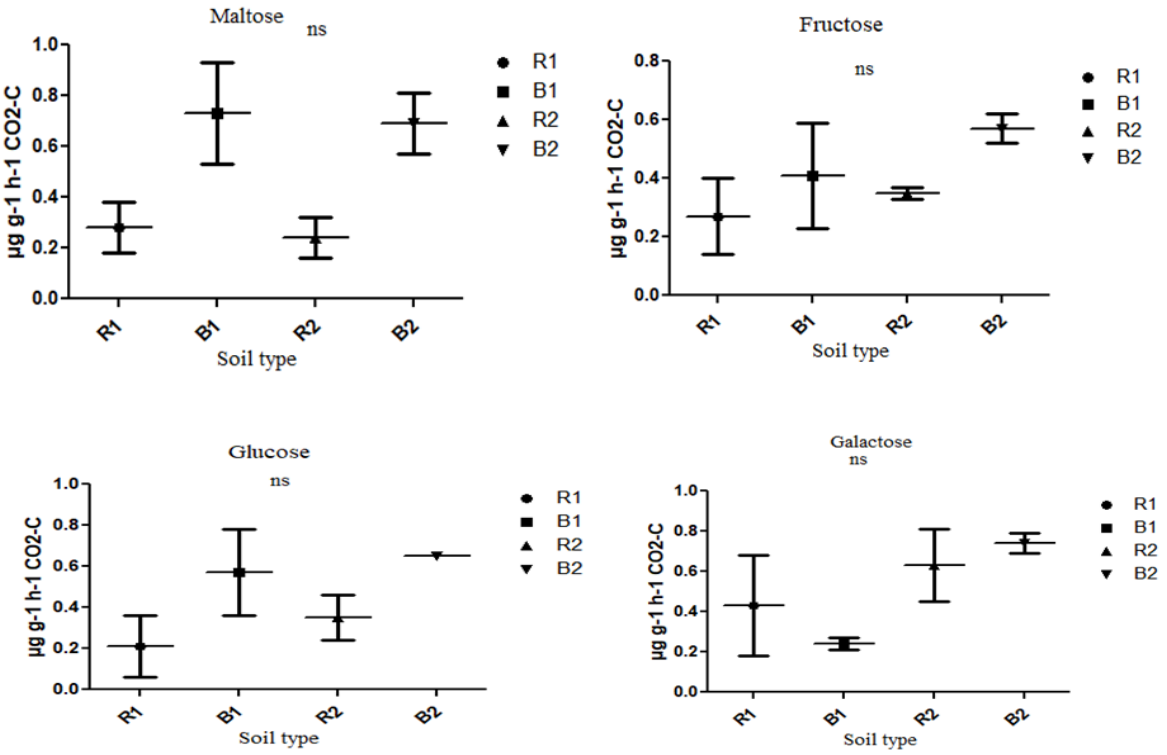

Figure 9.8: Effects of soil type on soil functional properties as measured for maltose, fructose, glucose and galactose using MicroResp assay. Number of replicates (n) = 2. Data represent mean ± SE, ns = not statistically significant. R1= Ditsobottla rhizosphere soil, B1= Ditsobottla bulk soil, R2= Kraaipan rhizosphere soil, B2 = Kraaipan bulk soil.

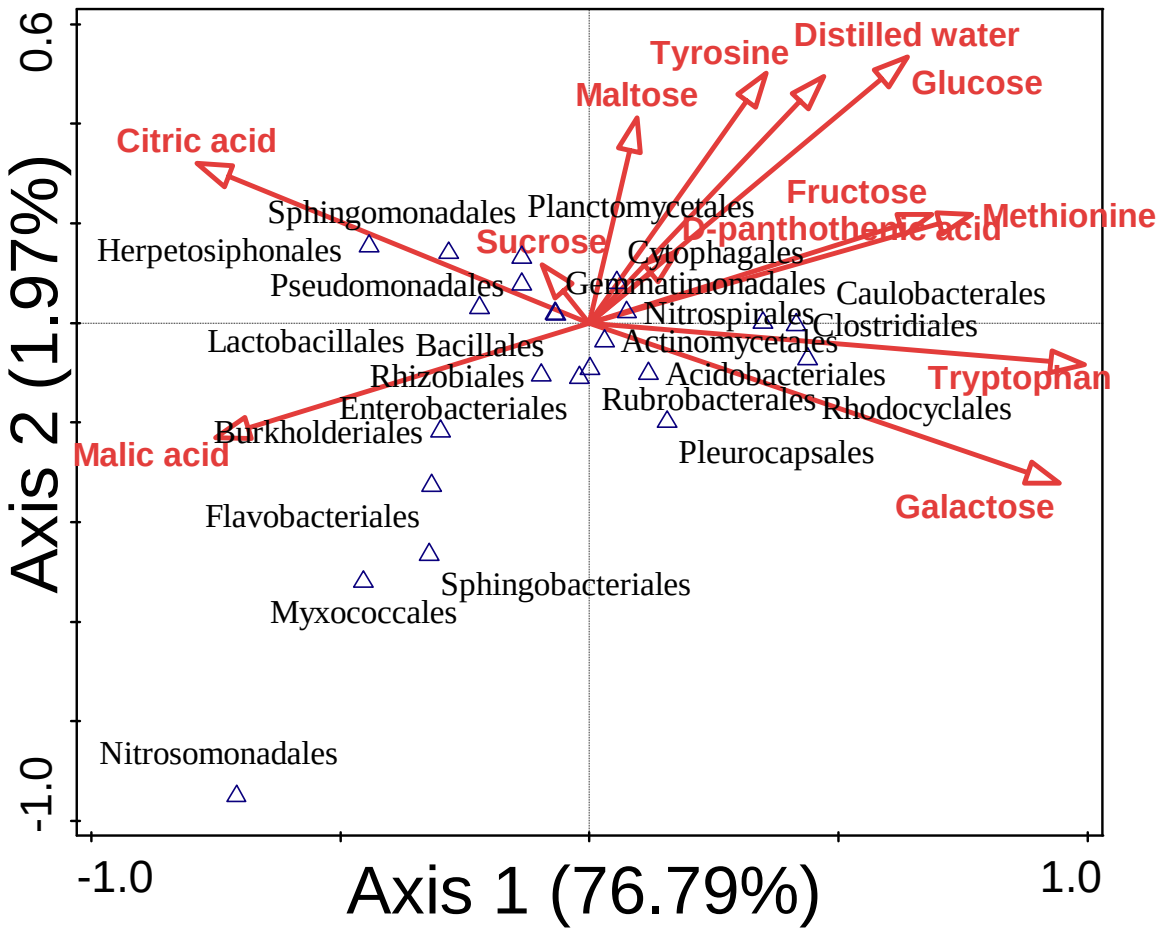

Figure 9.9: The influence of carbon substrate utilization on the bacterial components (at the order level) of sunflower rhizosphere and bulk soils obtained from Ditsobottla and Kraaipan farms using canonical correspondence analysis.

utilization on the relative abundance of bacterial species at the order level was determined using CCA (Figure 9.9). Herpetosiphonales, Sphingobacteriales, Pseudomonadales and Planctomycetales were positively correlated with citric acid and sucrose, but negatively correlated with tyrosine, glucose, maltose, fructose, galactose tryptophan, methionine, D-pantothenic acid and distilled water. Cytophagales, Gemmatimonadales, Nitrospirales and Canulobacterales were positively correlated with maltose, tyrosine, D-pantothenic acid, methionine, glucose, fructose and distilled water, but negatively correlated with tryptophan, galactose, malic acid, sucrose and citric acid, while Actinomycelales, Acidobacteriales, Rhodocyclales, Rubrobacteralesand Pleurocapsales were positively correlated with tryptophan and galactose, but negatively correlated with tyrosine, distilled water, glucose, maltose, fructose, malic acid, citric acid, sucrose, methionine and D-pantothenic acid.

However, only malic acid influenced the abundance of Lactobacillales, Bacillales, Rhizobales, Enterobacteriales, Burkhoderiales, Flavobacteriales, Myococcales, Nitrosomonadales and Sphingomonadales (Figure 9.9). Furthermore, the effect of sucrose, maltose and D-pantothenic acid on the bacterial community were lower than those of glucose, fructose, malic acid, citric acid, tryptophan, tyrosine, methionine, galactose and distilled water as shown by the lengths of the vectors. The total variation is 0.12110 and permutation test results on all axes presented as pseudo-$F<0.1$ and $P=1$.

Out of all the carbon substrates used for this study (Figure 9.9), tryptophan had the highest contribution of 75.9% ($p = 0.102$), followed by galactose (16.7%). Thus, from Table S9.3,

tryptophan best explained (75.9%) the variation that occurred in the soil bacterial (specifically Actinomycetales, Acidobacteriales, Rubrobacteriales, Rhodocyclales and Pleurocapsules) composition and abundance (Figure 9.9). Citric acid, however, had 7.5% effect ($p = 1.000$) on the soil bacterial components.

From figure S9.3, the OM positively correlated with malic acid, citric acid, tyrosine and sucrose, but negatively correlated with other carbon substrates. Total N and pH positively correlated with galactose and tryptophan, but negatively correlated with other carbon substrates.

It was noted that all the soil variables contributed to the difference in carbon substrate utilization by the bacterial components of the soils (Table S9.4). Organic matter showed 51.4% contribution ($p = 0.052$) to malic acid, citric acid, tyrosine and sucrose utilization by the bacteria. pH and total N on the other hand, respectively showed 32.5% ($p = 0.212$) and 16.2% ($p = 1.000$) contribution (Table S9.4) to both galactose and tryptophan utilization by the bacterial components of the soil (Figure S9.3).

9.4. Discussion

In this study, the influence of soil properties and carbon substrate utilization on the bacterial components and diversity associated with sunflower rhizosphere and bulk soils obtained from two farms with different history of agricultural practices in North West Province of South Africa were investigated. MG-RAST analyses of 16S rRNA amplicon sequenced data was done prior to determination of mean relative abundance, alpha/beta diversity. Soil properties were determined

as previously described, while the ability of the bacterial communities of the sampled soils to utilize carbon substrates was ascertained by using CLPP.

With respect to the structural bacterial diversity, the sharp reduction in mean sequence reads of R1, R2, B1 and B2 after QC is due to the exclusion of low quality and contaminated sequences that could have affected downstream analyses (Zhou et al., 2014). Furthermore, analysis using a Venn diagram showed that at the OTU level, the read sequences of R1 and B1 had 34.0% similarity, R2 and B2 had 35.6% similarity, and R1 and R2 had 32.7% similarity (Figure 9.1). This result contradicts the result of Molefe et al. (2021), who reported 10.9% bacterial similarity between maize rhizosphere and bulk soils obtained from Ventersdorp, and 17.2% bacterial similarity between maize rhizosphere and bulk soils obtained from Mafikeng in South Africa. The contradiction between the results of the two studies could be due to the different sampling locations and plant type.

The bacterial diversity within each sample, that is, R1, R2, B1 or B2 was determined using different diversity indices such as Equitability_J, Fisher_alpha, Berger-Parker and Chao-1 (Table 9.1). At a probability level of 0.05, it was observed that the bacterial diversity 'within' each sampling site showed significant difference ($p = 0.01$, $R = 0.06$). The uniqueness 'between' samples (beta diversity) was further determined in this study by PCoA analysis (Figure 29.). At a probability level of 0.05, the analysis revealed significant differences ($p = 0.01$, $R = 1.167$) in the structural diversity 'between' samples. The significant variation in the bacterial diversity observed between samples in this study can partly be attributed to the differences in the agricultural practices

(Table S9.1) of the two farms. For instance, herbicides, pesticides and bio-fertilizer were applied on Ditsobottla farm, but not on Kraaipan farm. The effects of these components on the bacterial communities of sunflower and other plants rhizospheres have been reported previously (Kostyuchenko et al., 2021, Thokchom et al., 2017, Igiehon et al., 2020, Li et al., 2022).

However, chemical fertilizer (NPK 15:8:4), which was applied on both farms, has also been reported to influence changes on the relative abundances of bacterial components of agricultural crop rhizospheres (Li et al., 2020, Ai et al., 2012, Sun et al., 2018). Thus, the cumulative effects resulting from herbicide, pesticide, bio-fertilizer and chemical fertilizer applications on Ditsobottla farm may have triggered the significant bacterial composition variations observed between Ditsobottla farm and Kraaipan farm (which was only applied with chemical fertilizer).The results of the bacterial structural diversity of sunflower rhizosphere and bulk soils in the two farms revealed different bacterial taxa both at the class and order level.

The relative high abundance of the bacteria in R1 may be linked to the exudates from the sunflower roots. Root exudates have been reported to attract bacteria and other biotic components to the rhizosphere, rhizoplane and endosphere of plant roots (Philippot et al., 2013a, Gkarmiri et al., 2017a, Jacoby et al., 2017). Similarly, the abundance of the bacteria might be traceable to the bio-fertilizer that was previously applied to the soil (Table S9.1), since bio-inoculant applications can influence the bacterial diversity of plant rhizosphere (Igiehon et al., 2020, Mayer et al., 2021, Mohammed et al., 2020). Considering the unclassified bacteria, one possibility, is that, the unclassified bacterial group might contain novel bacterial species that have not been previously

identified, probably because the nutritional compositions for the cultivation of such bacteria are unknown (Enagbonma et al., 2020). The unclassified bacterial group may harbor species with novel plant growth promoting traits that may be useful to improve the growth of sunflower crops and, perhaps, other agricultural crops.

At the order level, among all the bacterial taxa captured in the two farms, Bacillales were the most dominant. Bacillales was more dominant in R1 and B2 with mean relative abundance values of 36.24 and 37.00 respectively (Fig. 4). Bacillales dominance in these soils may be attributed to the fact that members of this group possess heat-resistant components (that is, endospores) in their cellular structures (Filippidou et al., 2016). Possession of endospores is a survival strategy for bacteria to strive in environments with high temperatures (Filippidou et al., 2016, Gray et al., 2019). The two sunflower farms used in this study were located in semi-arid region of South Africa, which is characterized by hot weather conditions because of its relatively low amount of rainfall. Low detection of endospore forming bacteria was, however, recorded in metagenomics datasets using *spo0A* and *gpr* molecular markers because these markers were absent in common environments (such as soil) of these endospore-forming bacteria, with *spo0A* present in the microbiome of mammals (Filippidou et al., 2015).

Furthermore, the positive influence of total N (%) and pH on five bacteria groups at the order level indicates that an increase in the soil total N and pH would lead to a direct increase in the bacterial species abundance of the five taxa. These bacteria were captured from R2 (Figure 9.6). From Figure 9.5, the abundance of Lactobacillales, Bacillales, Rhizobiales, Enterobacteriales,

Burkholderiales, Flavobacteriales, Sphingomonadales, Myxococcales and Nitrosomonadales obtained from R1 (Figure S9.1) was positively influenced by OM. Organic matter was positively related with these bacterial orders and not others since the components of the organic matter supported the growth and activities of the bacterial orders (Esmaeilzadeh and Ahangar, 2014, Mohammadi et al., 2011). In addition, figure S9.1 revealed that the bacterial species from B1 and B2 were slightly influenced by soil total N, pH and OM when compared to R1 and R2. Therefore, the greater positive influence of soil N, pH and OM on certain bacterial communities of R1 and R2 (rhizosphere soils of Ditsobottla and Kraaipan farms respectively) may be connected to the high biological activities that might have occurred in the root regions of the sunflower plants (Philippot et al., 2013a).

For instance, increase in OM resulting from the decomposition of debris (Kotroczó et al., 2014, Shahbaz et al., 2017) might have led to the proliferation of Planctomycetales, Cytophagales, Gemmatimonadales, Nitrospirales and Caulobacteriales observed in R1. Although, the physiochemical parameter that mostly contributed and best explained the differences in bacterial communities was soil pH (Table S9.2). The pH showed 71.4% ($p = 0.184$) contribution to the bacterial community profile of Planctomycetales, Cytophagales, Gemmatimonadales, Nitrospirales and Caulobacteriales.

In addition, the community respiration among various groups of microorganisms has made it possible to measure their functions in the ecosystem (Di Salvo et al., 2018). The functionality of bacterial community of soil samples from the two farms was further ascertained by determining

their ability to utilize different carbon substrates using CLPP technique (Figure 9.6, 9.7 & 9.8). Increase in carbon substrate utilization is directly proportional to increase in CO_2 released or produced (respiration rate) (Creamer et al., 2016). Carbon substrates have huge ability to distinguish diverse soil bacterial communities through carbon mineralization capacity (Amoo et al., 2021). Bacterial communities of R1, B1, R2 and B2 soils were found to use the different carbon substrates as an energy source, but significant differences were only observed in tryptophan and methionine amended soils. In particular, in tryptophan amended soils, the bacterial components of R2 and B2 showed significant ($P < 0.05$) higher carbon substrate utilization and respiration rates than those of R1 and B1 (Figure 9.6). Also, in soils amended with methionine, bacterial components of B2 significantly ($P < 0.05$) utilized the carbon substrate and produced more CO_2 than those of R1, B1 and R2 (Figure 9.6).

The total respiration rate resulting from the summation of the 11 carbon substrates without basal respiration (Figure S9.2) was further extrapolated. The quantity of carbon substrate utilized shows the abundance of the microbial communities that are able to utilize a particular carbon source (Creamer et al., 2016). The higher the total quantity of carbon source utilized, the greater the metabolic properties and functional diversity of the bacterial communities (Jones et al., 2018, Deng et al., 2011). From the extrapolation, the results showed that rhizosphere soils had higher respiration rate compared with the basal respiration. However, the higher microbial activity (respiration function) observed in the bulk soil (B2) may have been caused by the contributions of extraneous abiotic factors in B2, since it was reported by (Leckie et al. (2004), Hannam et al.

(2006), He et al. (2006)) that the activities (e.g. respiration) of soil microbial communities are not only affected by biotic factors but also by the abiotic components of the soil.

In addition, it was observed that utilization of most of the carbon substrates positively influenced bacterial abundance and composition. In particular, the organic acid (citric acid) and a disaccharide (sucrose) had a positive influence on Herpetosiphonales, Sphingobacteriales, Pseudomonadales and Planctomycetales (Fig. 9.9). Citric acid is an intermediate of the Krebs cycle, which is a chain of biochemical reactions utilized by aerobic organisms to produce energy in the form of ATP via the oxidation of acetate (obtained from carbohydrate, fatty acid and protein oxidation) (Gasmi et al., 2021, Kumar and Dubey, 2019).

The carbon substrate that best explained these differences in bacterial components was an essential amino acid (tryptophan) (Table S9.3). This amino acid showed 75.9% ($p = 0.102$) contribution to the bacterial profile of Actinomycelales, Acidobacteriales, Rhodocyclales, Rubrobacterales and Pleurocapsales (Figure 9.11).

Also, the influence of OM, total N and pH on carbon substrate utilization was investigated. It was observed that OM of the sunflower soils positively had influence on the utilization of malic acid, citric acid, tyrosine and sucrose utilization by the soil bacterial species, while the utilization of disaccharide (galactose) and essential amino acid (tryptophan) by the soil bacterial species was positively influenced by both the soil total N and pH (Figure S9.3). The soil parameter that best explained the differences in soil bacterial utilization of carbon substrates was OM. Specifically, soil OM showed 51.4% contribution ($p = 0.052$) to the carbon substrate utilization (Table S9.4).

However, soil OM, total N and pH did not positively influence bacterial utilization of other carbon substrates such as methionine, fructose, glucose, maltose, D-pantothenic acid and distilled water (Figure S9.3).

9.5. Conclusion

At a probability level of 0.05, significant differences were observed for the alpha and beta diversity of the soil bacterial communities. At the class level, Bacilli, Bacteroidia, Acidobacteria, Planctomycetacia, Flavobacteria and Betaproteobacteria were relatively higher in R1 than the corresponding bulk soil samples (B1), while at the order level, among all the bacterial taxa captured in the two farms, Bacillales were the most dominant. Bacterial communities of R1, B1, R2 and B2 soils were found to use the different carbon substrates as an energy source, but significant differences were only observed in tryptophan and methionine amended soils. The utilization of most of the carbon substrates positively influenced the soil bacterial communities. The presence of unclassified bacteria in this study, calls for a further effort to capture and identify these unknown bacteria, since it may be possible to discover novel bacterial species with plant growth promoting functionality.

CHAPTER TEN

Structural diversity of bacterial communities in two divergent sunflower rhizosphere soils

Overview

Root exudates have an impact on the rhizosphere, which is the zone of soil that surrounds plant roots. Because of the availability of malleable carbon and other nutrients in this region, activities such as nutrient and moisture utilization, respiration, gas exchange, as well as other bioprocesses are more intense in the root region. Rhizodeposition is a major event that take place in the rhizosphere. This activity involves the release of many low- and high-molecular-weight compounds into the surrounding soil, including organic acids, secondary metabolites, sugars, polysaccharides, amino acids and vitamins. Plant roots release organic substances in form of exudates which increase microbial and functional diversity, which leads to the formation of more effective and unique microbial communities linked to roots than in bulk soils. In addition, plant microbiota, and microbial community in the rhizosphere contribute significantly to crucial plant beneficial functions such as plant metabolism and nutrient uptake, as well as tolerance to abiotic and biotic stress.

Microbiota interaction in the root region may involve associations with those microorganisms that are heavily influenced by exudates and are located near the surrounding soil. As most nutrients in the soil are inaccessible to plants, the plants rely on the microbial communities for nutrients. The

rhizosphere microbiome as well as promotes plant developement through the production of phytohormones, which protect plants from abiotic stresses like salt, heat, drought, and flood, as well as pathogen invasion. Plants nurture their microbial community by supplying energy sources in the form of carbon-rich rhizodeposits, reducing competition and modifying soil pH for beneficial microbes by choosing microorganisms that aid in their growth and survival, particularly in poor environmental conditions. To address the numerous challenges that crop production faces, a better knowledge of the functional traits of microbiota that inhabit the plant root zone is required. Understanding the functional distinctiveness of the microbial community in the region surrounding the root will aid in the discovery of appropriate and better approaches to increasing crop production, particularly in nutrient-scarce and semi-arid regions around the world where inorganic fertilizers are used to augment farming.

Sunflower is a valuable oil-seed crop with distinct phenotypic and molecular variance. The crop is heavily manipulated by environmental distinctions in genetic heterogeneity. Given that sunflower commonly cultivated as a monoculture, the crop can be viewed as an ecosystem engineer with the optimal capacity to for its plant-root region for cohabiting microorganisms. Plant roots release exudates, including aromatic acids, enzymes, organic acids and sugars, and interact with resident microbial communities and soil properties, including soil texture, moisture, organic matter, pH and available nutrients to promote sunflower plant growth and health. Categorizing the predictive functional traits and diversity of microbiome in the sunflower root is therefore critical for connecting microbial communities with various functional abilities that enhance soil and plant development and health in semi-arid regions. As a result, it is expected that the microbial

functional traits in the soil samples to differ as result of variations in the physical and chemical properties of the soils observed at the study areas.

Agricultural practices such as soil tillage, land use, organic land management practices and fertilizer application change the soil microbiome composition. Long-term farming practices, such as alter soil properties, like organic matter content and aeration as well as soil tillage, the soil microbial community composition and structure. Most lands, such as forest lands and grasslands, have been transformed for agricultural use because of the increasing demand for food production for the world's rapidly growing population. There have been reports on the effects of land-use change on the composition and the structural diversity of the rhizosphere microbial community. However, modification in the metabolic and functional potential of rhizosphere microbiota following transformation from grasslands to agricultural lands is under reported. It is not certain if microbial communities preserve their function traits in the rhizosphere after land use change. As a result, sustainable sunflower production necessitates the understanding of the metabolic and functional prospects of microorganisms in the environment, notably those associated directly and/or indirectly in plant growth enhancement. The operation of the ecosystem. The operation of the ecosystem. Secondly, as a result, it is assumed that the functional variation and metabolic profiles of the study areas will be distinct due to the farming history.

To put the presumptions to the test, the amplicon sequencing method was used, which involved the direct extraction of entire metagenomes from the soil samples. Next-generation sequencing approaches have simplified the process of gathering information on the genetic composition of

many microbes and increased the understanding of microbial processes and activities in their respective environments. As a result, the goal of this study was to compare the predictive functional traits and metabolic pathways of the microbial community in a sunflower rhizosphere using the amplicon sequencing technique on the Illumina platform. The predictive functions and metabolic processes of rhizosphere microbial population to those of their bulk soils.

Abstract

Farming practices on farmlands aim to improve nutrients in the fields or crops, soil quality and functions, and boost and sustain crop yield; however, the effects of loss of ecological diversity and degradation have impacted ecosystem functions. The beneficial rhizosphere-microorganism networks and crop rotation may enhance a stable ecosystem. The use of next-generation sequencing technique will help characterize the entire bacterial species in the sunflower rhizosphere compared with the nearby bulk soils. The potential for predictive functional attributes of the bacterial community structure of sunflower rhizosphere and bulk soils cultivated under different agricultural practices at two geographical locations in the North West Province of South Africa was investigated. DNA was extracted from rhizosphere and bulk soils associated with sunflower plants from the crop rotation (rhizosphere soils from Lichtenburg [LTR]) and bulk soils from Lichtenburg (LTB) and mono-cropping (rhizosphere soils from Krayburg [KRPR] and bulk soils from Krayburg [KRPB] sites), and sequenced employing 16S amplicon sequencing. Bioinformatics tools were used to analyse the sequenced dataset. Proteobacteria and Planctomycetes dominated the rhizospheric soil, while Firmicutes and Actinobacteria were

predominant in bulk soils. Significant differences in bacterial structure at phyla and family levels and predicted functional groups between soils ($P< 0.05$) across the sites were discovered. The effect of physicochemical parameters was observed to influence bacterial dispersal across the studysites. This research study provides evidence on the predominant bacterial community structure in sunflower soils and their predictive functional attributes at the growing stage, which suggest their future study for imminent crop production and management for enhanced agricultural yields.

Keywords: Bacterial diversity, *Helianthus annuus*, Soil metagenomics, Sustainable agriculture, 16S rRNA gene sequencing.

10.1. Introduction

Comprehending the rhizosphere's geographical distribution of microbial communities has opened up several possibilities for exploiting their agricultural potential. Various microbial communities inhabit the rhizosphere, each with the ability to induce maximal adaptive responses in the plant via specific metabolic pathways. The rhizosphere is the area near the plant's roots where exudates containing various metabolites are discharged, as well as a variety of microorganisms (Agomoh et al., 2020, Ai et al., 2012). Roots are engaged in the release of exudates of various chemical components into the rhizosphere, in addition to providing nutrients and anchoring the entire plant. Through the secreted root exudates, the rhizosphere plays an important role in the modification of its microbiome component.

Plant-microbial interactions are complicated and can enhance plant growth and development (Igiehon and Babalola, 2018a, Berlanas et al., 2019a). Bacteria are the predominant microorganisms in the rhizosphere and are pointers of good soil quality, health and fertility due to their responses to biotic and abiotic pressures (Igiehon et al., 2019). The activities of bacteria are dynamic because they accelerate most biogeochemical processes, thus inducing mineral nutrient availability in soil (Nwachukwu and Babalola, 2021). Bacterial communities in the rhizosphere can resist pathogens and stimulate tolerance to abiotic stressors, hence enhancing plant growth, health and yield (Li et al., 2020, Meena et al., 2014). Bacterial communities that colonize the rhizosphere could be valuable, however most do not affect plant health (Nwachukwu et al., 2021, Maquia et al., 2020).

Although, various researchers have explored the microbiome of oil food crops such as sunflower root microbiome, studies on the impact of plants on microorganisms are still ongoing; thus, necessitating this study. The sunflower (*Helianthus annuus*), a major oilseed crop in modern agriculture, is used for various food and industrial purposes (Majeed et al., 2018a). Owing to its growing agricultural importance, some continents, such as Europe, South America and Africa, especially South Africa, have exploited the potential for its usage (Pandey et al., 2013, Majeed et al., 2018a).

Reports on the plant growth-promoting bacteria linked with sunflower plants for improved productivity in South Africa are limited, perhaps due to the inadequate studies on sunflower plants using the next-generation sequencing techniques. Hence, it is imperative to determine bacterial

community structures that are resident in the sunflower rhizosphere soils using the 16S rRNA gene and their associated predictive functions (Yadav et al., 2017, Lu et al., 2020). Given this, to distinguish the effects of plants, the bacterial communities in the rhizopheric soil of sunflower and the corresponding bulk soils were evaluated. Furthermore, the dissimilarities in the associated predicted functional compositions of the soils were explored.

It was postulated that the soil properties and agricultural practices, such as the use of chemical fertilizer, cropping type (mono-cropping and mixed cropping) and organic manure would influence the structure and metabolic potential of sunflower rhizosphere bacterial communities compared to the bulk soil. A good knowledge of the predicted metabolic pathways of bacterial communities in the rhizosphere region is essential since functional heterogeneity is a delicate signal of the quality elements of the soil management. It also speeds up the amplification of bacterial community functions as a comprehension of biochemical and molecular components in the rhizosphere zone and controls particular bacterial enhancement.

10.2. Materials and methods

10.2.1. Site location, sampling and climatic conditions

In March 2020, the rhizosphere and bulk replicate soil samples from the two commercial sunflower fields (at the growing stage) of different cultivars, PAN 7160 CLP and PAN 7011 Pannar, were collected from Lichtenburg (LT) (S26°4′31.266″ E25°58′44.442) and Krayburg/Kraaipan (KRP) (S26°17′24.186″ E25°13′33.258), North West Province, South Africa. A total of 12 samples each

for the rhizosphere and bulk soil were collected from 4 points of sunflower plant and 15–20 cm depth from the two farms and pooled into labelled zip lock bags and were homogenized to get a composite sample as described by Oberholster et al. (2018). The soils were immediately transported to the Microbial Biotechnology Research Laboratory, North-West University, South Africa. The soils were placed separately, sieved and stored in zip lock bags in the dark at -80°C for DNA extraction and high throughput sequencing.

Usually, North West Province has a summer temperature ranging between 17°C to 31°C and a winter temperature ranging from 3°C to 21°C. The annual rainfall ranges between 300 and 600 mm. According to the farm owner, the farmland in Lichtenburg has been cultivated for over 40 years. Sunflower has been rotationally cultivated with other agricultural crops, such as soybean and maize. Water supply in the farms is mainly by rainfall during the summer and irrigation during winter. The main farm activities are clearing, tilling, plowing and ridging.

Also, the application of chemical fertilizers (NPK 15:8:4), pre-emergence and post emergence herbicides (Metagon Gold and Judo 50EC) were conducted to the farm soil before and after planting. Foliar insecticide spray (Max-Foliar) was applied to the leaves after the plants germinated. In Krayburg, maize was the only crop previously cultivated on the farmland. Soil amendments include the application of urea and organic manure.

10.2.2. Soil physicochemical parameters analysis

The physicochemical parameters analyses of the rhizosphere and bulk soil samples were performed using standard procedures, and 30 g of pulverized and sieved soil was taken from each sample. The soil pH in distilled water was measured using a pH meter (ratio 1:2.5, soil to water), the organic matter (OM) was determined using the Walkley-Black method (Walakley and Black, 1934), while phosphorus (P) was extracted from the samples following the method of Bray and Kurtz (1945). Potassium (K) was assessed using 1 M acetate at pH 7.0 (Gutierrez Boem et al., 2011). The soil total carbon (C) and total nitrogen (N) were determined using the dry combusting technique as described by (Craft et al., 1991). The nitrate ($N–NO_3$) and ammonium-N ($N–NH_4$) were calculated using the KCl extraction method by Nelson et al. (1996).

10.2.3. Metagenomic DNA extraction and 16S rRNA amplicon sequencing

The DNA was extracted from 5 g of each sieved rhizosphere and bulk soil samples using a Zymo DNA isolation kit (Zymo Research, Irvine, USA) following the manufacturer's instructions. All the data are products of 16S amplicon sequencing at the Molecular Research Laboratory (MR DNA, Shallowater, TX. USA). The polymerase chain reactions (PCRs) were performed in a single-step PCR using the HotStarTaq Plus Master Mix Kit (Qiagen, USA) primer pairs 515F (5′-AATGATACGGCGACCACCACCGAGATCTAC AC TATGGTAATT GT GTGCCAGCMGCCGCG GTAA-3′) and 806R (5′-CAAGCAGAAGACGGCATAC GAGAT TCCCTTGTCTCCAGTCAGTCAG CC GGA CTACHVGGGTWTCTAAT-3′).

The PicoGreen dsDNA assay was used to quantify the PCR products from the DNA samples. The samples were combined in an equimolar concentration. Then, for purification, calibrated Ampure XP beads (Agencourt Bioscience Corporation, MA, USA) were used. The Illumina DNA library was prepared from the pooled and purified PCR products. Sequencing was performed on an Illumina MiSeq 2000 using a paired-end approach to obtain 312 bp paired-ends reads.

The Quantitative Insights Into Microbial Ecology (QIIME 2) 16S pipeline (version 2020.11) was used to process the sequence reads (Caporaso et al., 2010) performed on Nephele microbial bioinformatics platform (version 1.8) (https://nephele.niaid.nih. gov/) (Weber et al., 2018). Preprocessing steps involved read pair joining using default parameters (a minimum overlap of 10 and percentage maximum difference of 25), an average Phred score of ≤ 20 was removed, while chimeras were removed using VSEARCH (Edgar et al., 2011), while clustering was done using Open Reference Method and SILVA 99 version 132 (Wang et al., 2007). SILVA version 132 was used to assign taxa, with a sequence similarity of 0.99, and then chimeric sequences, including mitochondria, singleton and chloroplast reads were eliminated.

10.2.4. Statistical analysis

Microsoft excel sheet was used to derive the mean and standard errors of the soil physicochemical properties. Soil physicochemical data were transferred to the Statistical Package for the Social Sciences (SPSS), where one-way analysis of variance (ANOVA) and Duncan multiple tests were performed. The relative abundance graph of the bacterial community between the sunflower rhizosphere and bulk soil was plotted using the Shinyheatmap (version 0.12.2) online tool

(www1.heatmapper.ca/expression/) (Khomtchouk et al., 2017). The alpha diversity (diversity within the samples) of the bacterial community structure across each sampling sites, diversity indices (Simpson, Evenness and Shannon_H) and Kruskal-Wallis test in the paleontological statistics software package (PAST version 4.0) was used to assess bacterial richness (Hammer et al., 2001). The rhizosphere and bulk soils were also compared using these indices.

The beta diversity was calculated using the principal coordinate analysis (PCoA) on a Bray-Curtis dissimilarity matrix, and the variances in community structure and composition were calculated using the one-way analysis of similarities (ANOSIM) (Clarke and Green, 1988). Principal component analysis (PCA) using Euclidean matrix was employed to identify the distribution of bacteria across the sunflower sites. Also, PCA using Euclidean matrix was used to assess the environmental variables that best described the composition of the obtained bacteria, as well as the potential correlations between bacterial communities and the measured environmental variables.

A forward selection of environmental variables was employed to conduct a significance test. The PCoA and PCA plots were designed using CANOCO version 5 (Microcomputer Power, Ithaca, NY, USA) software. The predictive functional annotation of the bacterial categories in the sampling farm site was assessed on Phylogenetic Investigation of Communities by Reconstruction of Unobserved States (PICRUSt); the predicted functional classifications at the different levels (i.e. first, second and third) were obtained.

10.3. Results

10.3.1. Physical and chemical analysis of sunflower rhizosphere and bulk soils

Soil analysis showed that OM, N-NH_4 and total N were higher in rhizosphere soils from Lichtenburg (LTR) than in rhizosphere soils from Krayburg (KRPR) as shown in Table 10.1. The pH values of the soil samples from the LT site had low pH values (acidic) compared to the pH values of the soils from the KRP site.

10.3.2. Sequence data and beta analyses of the rhizosphere and bulk soil samples

The taxonomic groups were assigned using the SILVA reference database. The total number of uploaded sequences varied between samples and across the sites. Sequence base pair count of 87,446 (LTR), 80,404 (KRPR), 100,988 (bulk soils from Lichtenburg- LTB) and 74,956 (bulk soils from Krayburg- KRPB) sequence reads for the soil samples. Consequently, quality control (QC) check revealed the sequence read count for LTR- 47,471, LTB−9,628, KRPR−16,621 and KRPB – 19,050 between the samples and across the sites. Sequences were clustered at 97% similarity according to their connection to one another by Operational Taxonomic Units (OTUs) and the different OTU abundances in all samples were obtained based on the similarity threshold.

The PCoA graph of the bacterial diversity at the phyla level in the soil samples across the sites is presented in figure 10.1, which indicated that samples from LTB differ significantly from LTR, KRPR and KRPB soil samples. The vector length of the PCA graph revealed the most dominant bacterial phyla in the individual soil niche. Specifically, this is the bacterial phyla having the

longest vector length of PCA. The vector length was used as an indicator, notably in LTR Acidobacteria, Planctomycetes, Chloroflexi, Armatimonadetes, Gemmatimonadetes and Cyanobacteria dominated, whereas Actinobacteria, Elusimicrobia and Nitrospirae were predominant in LTB, whereas Proteobacteria, Verrucomicrobia, Bacteroidetes and unclassified sequences were prevalent in KRP, and the main bacterial phyla in KRPB were Spirochaetes and unclassified bacteria (Figure 10.2).

The bacterial phyla selected for PCoA and PCA plots were established on the level of significance. Analysis of similarities (ANOSIM) revealed that the differences in the beta diversity of the bacterial communities across the sites differed significantly ($P = 0.01$ and $R = 0.58$).

10.3.3. Structural composition of the bacterial community

At the phylum level, the dominant rhizospheric bacteria in LTR were Proteobacteria, Choloflexi Planctomycetes, Gemmatimonadetes, Acidobacteria, Armatimonadetes and Cyanobacteria, while Actinobacteria, Nitrospirae and Elusimicrobia predominated LTB. Interestingly, unclassified bacteria dominated KRPR, while Firmicutes, Bacteroidetes, Verrucomicrobia and unclassified sequences, and Spirochaetes were abundant in KRPB (Figure 10.3).

Table 10.1: Mean _ standard error values of the physical and chemical properties of the sunflower rhizosphere soils.

Site	LTR	LTB	KRPR	KRPB
Organic matter (OM) (%)	1.85±0.1[a]	1.81±0.0[a b]	1.19±0.0[c]	1.27±0.0[c]
Nitrate (N-NO3) (mg/kg)	11.54±2.5[ab]	9.3±0.0[bc]	9.695±0.3[bc]	13.14±0.0[a]
Ammonium-N (NH4-N) (mg/kg)	9.875±0.1[a]	8.723±0.1[b]	6.255±1.1[bc]	5.01±0.0[c]
pH (N/A)	6.92±0.1[b]	6.91±0.0[b]	6.94±0.2[a]	6.93±0.0[a]
Resistivity conductivity (ohm)	2365±135.0[a]	2120±21.0[a]	855±85.0[b]	890±80.0[b]
Phosphorus (P) (mg/kg)	23.095±1.2[b]	7.84±3.1[c]	6.315±0.9[c]	72.88±2.3[a]
Calcium (Ca) (mg/kg)	1752.5±3.5[b]	781±1.5[b]	1680±250.0[a]	659.5±17.5[b]
Magnesium (Mg) (mg/kg)	350±7.0[a]	145.5±3.5[c]	311±12.0[b]	148.5±3.5[c]
Potassium (K) (mg/kg)	230±7.0[a]	183±1.0bc	228±18.0 [ab]	179.5±2.5[c]
Sodium (Na) (mg/kg)	72.85±2.4[a]	69.25±3.3[ab]	7.52±0.18 [b]	73.85±0.9[a]
Total carbon (%)	0.685±0.0 [a]	0.6±0.0 [ab]	0.5975±0.0[b]	0.589±0.1[b]
Total nitrogen (%)	0.057±0.0 [a]	0.055±0.0 [b]	0.056±0.0[b]	0.0535±0.0[b]
Sand (%)	85±1.0 [a]	87±0.0 [b]	76±2.0[b]	77±1.0[b]
Silt (%)	5±0.0 [a]	4±1.0 [a]	3±1.0[a]	2±0.0[a]
Clay (%)	20±1.0 [a]	19±1.0 [a]	21±1.0[a]	21±1.0[a]

Legend: % - percentage, LTR- Rhizosphere soil from Lichtenburg, LTB- Bulk soil from Lichtenburg, KRPR- Rhizosphere soil from Krayburg, KRPB- Bulk soil from Krayburg.

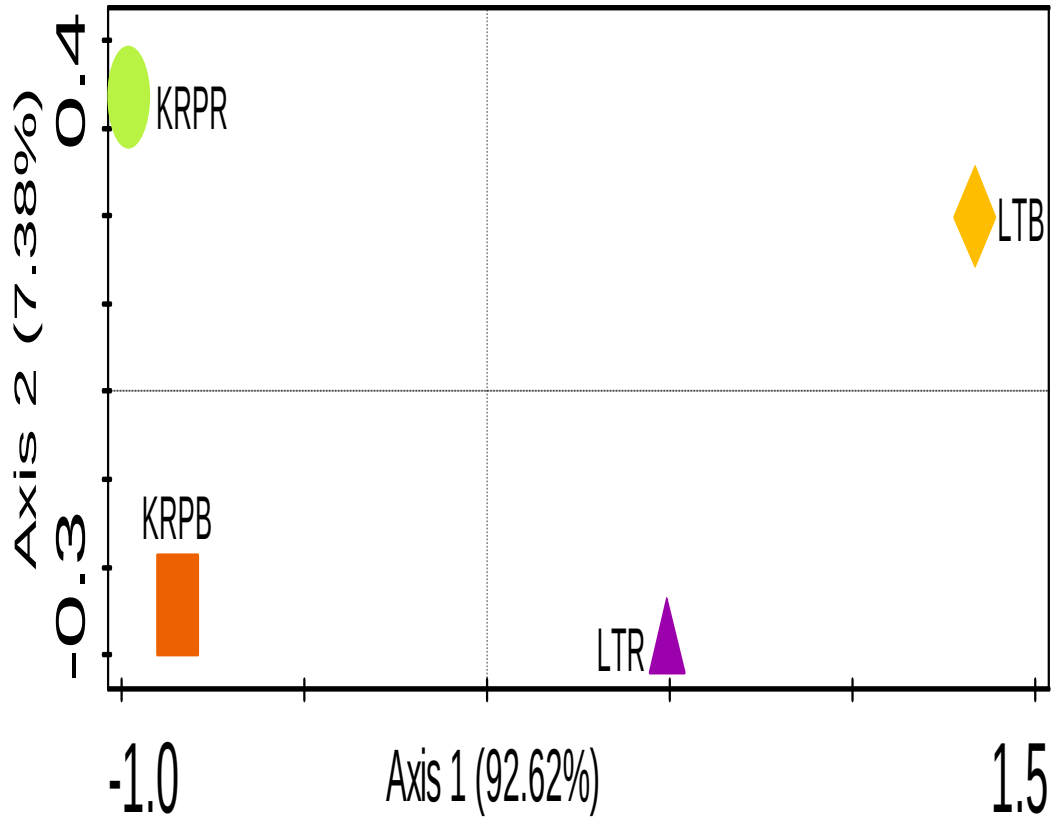

Figure 10.1: Principal coordinate analysis (PCoA) of shared OTUs between the rhizosphere and bulk soils from Lichtenburg and Krayburg at phylum level. (LTR= Rhizosphere soils from Lichtenburg, LTB= Bulk soils from Lichtenburg, KRPR= Rhizosphere soils from Krayburg, KRPB= Bulk soils from Krayburg).

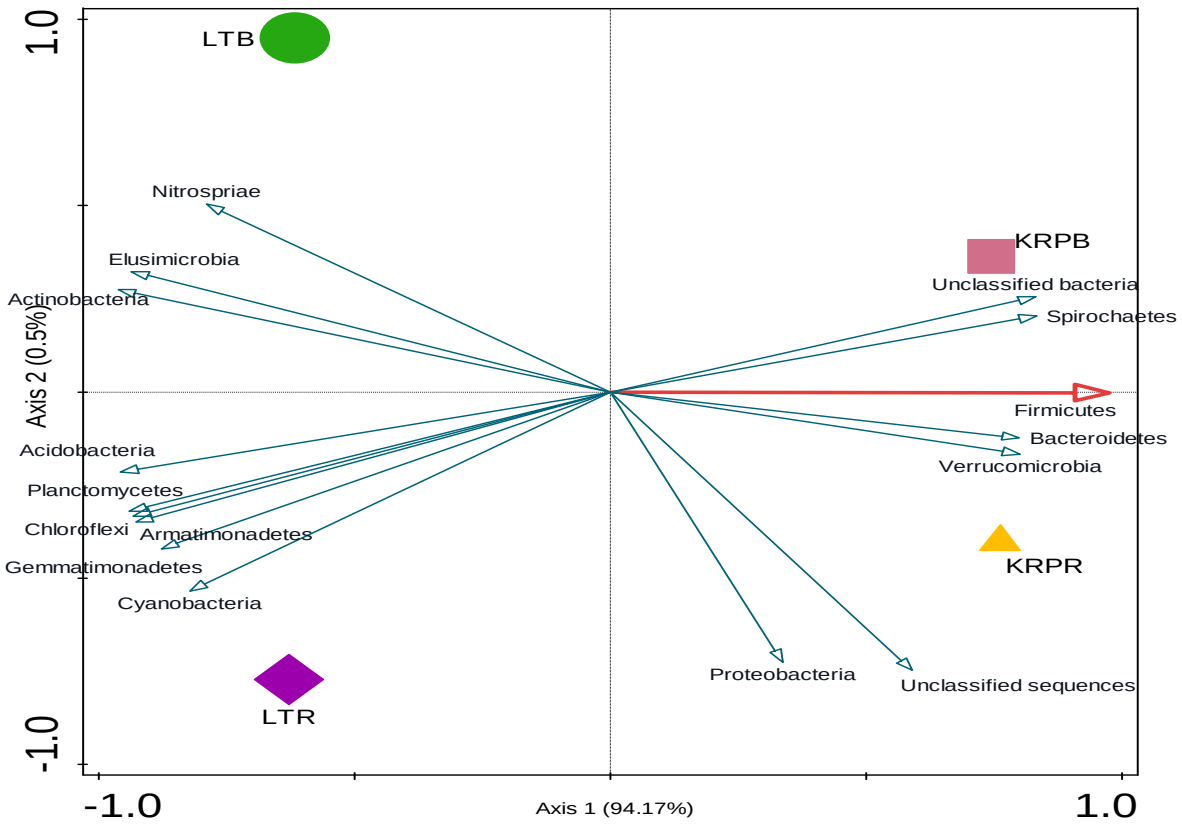

Figure 10.2: Principal component analysis (PCA) of common OTUs between the rhizosphere and bulk soils from Lichtenburg and Krayburg at phylum level. (LTR= Rhizosphere soils from Lichtenburg, LTB= Bulk soils from Lichtenburg, KRPR= Rhizosphere soils from Krayburg, KRPB= Bulk soils from Krayburg).

At the family level (Figure S10.1), Moraxellaceae, Caulobacteraceae, Geodermatophilaceae, Solirubrobacteraceae, Streptomycetaceae, Acetabacteraceae, Bradrhizobiaceae, Comamonadaceae, Micromonosporaceae and Chitinophagaceae were predominant in LTR. Unknown bacteria, Micrococcaceae, Nocardioidaceae, Rhodospirillaceae, Pseudonocardiaceae, Sphingomonadaceae, Thermomonosporaceae and Microbacteriaceae were dominant in LTB. Pseudomonadaceae, Streptomycetaceae, Paenibacillaceae and Planococcaceae influenced KRPR, while Baccilaceae, Rubrobacteraceae, Oxalobacteraceae and Clostridiaceae dominated KRPB.

10.3.4. Effect of environmental factors on the bacterial community structure

The PCA (Figure 10.4) was used to determine the correlation between the soil physical and chemical properties (Table 10.1) on the bacterial community distribution at the phylum level. The six soil physical and chemical parameters that best explained (Table 10.1) were considered for the PCA plot (Figure 10.4). The PCA plot indicated that the bacterial community structure was influenced by the soil physicochemical properties. The total variation was 0.14385 and explanatory variable accounted for 100%. Using the vector length as an indicator, it is obvious that OM was at the mid-

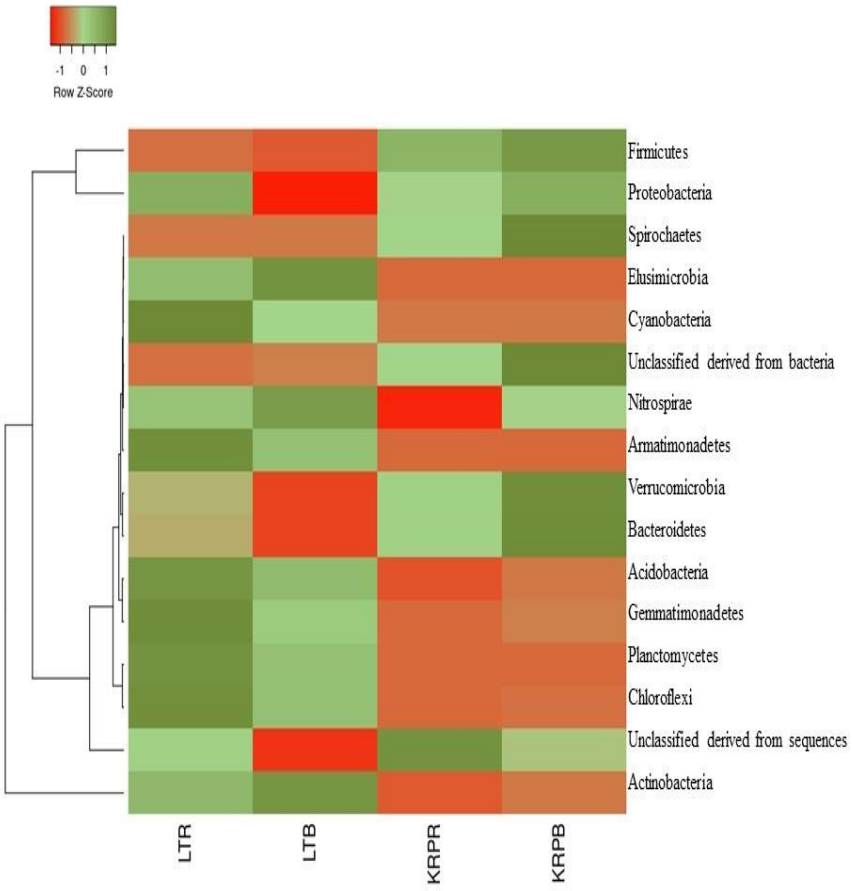

Figure 10.3: Taxonomic classification of the relative abundance of bacterial phylum from rhizosphere and bulk soils from Lichtenburg and Krayburg. (LTR= Rhizosphere soils from Lichtenburg, LTB= Bulk soils from Lichtenburg, KRPR= Rhizosphere soils from Krayburg, KRPB= Bulk soils from Krayburg). The colour permeation gradient is designated as the scale bar based on the relative abundances and a row z-score of the bacterial communities' relative abundance.

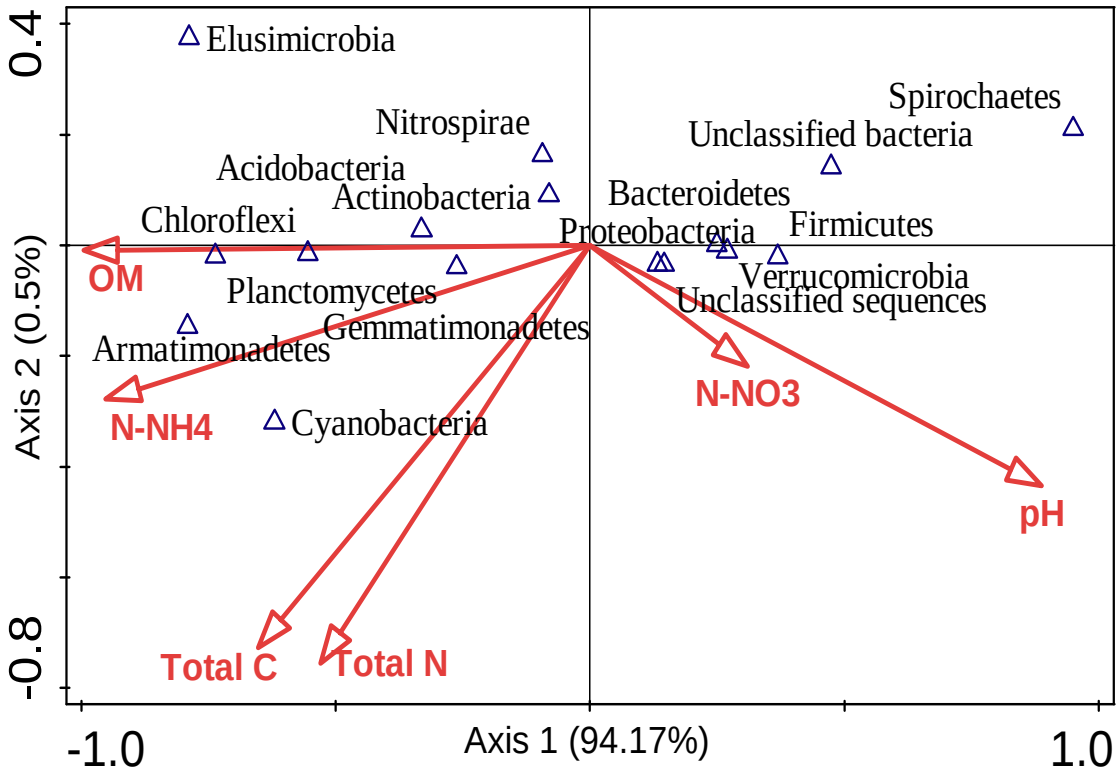

Figure 10.4: Principal component analysis (PCA) plot of the bacterial phyla distribution and soil environmental variables of both rhizosphere and bulk soils from Lichtenburg and Krayburg. (OM= Organic matter, N-NH4= Ammonium-N, N-NO3= Nitrate, Total N= Total nitrogen, Total C= Total carbon).

point. The vector lengths of total C, total N and N-NH_4 positively correlated with Planctomycetes, Armatimonadetes, Cyanobacteria and Gemmatimonadetes from LTR. The vector length of N-NO_3 and pH was positively correlated with Verrucomicrobia and unclassified sequences from KRPR.

10.3.5. Predictive functional information analysis of bacterial community in the rhizosphere and bulk soils

The predictive functional categories of bacterial community composition with differences in their relative abundances across the sunflower farms at three different levels were analyzed employing PICRUSt. At level 1 functional classification, the bacterial predictive functions were categorized into 6 major predicted functions in both rhizosphere and bulk soils of the farms, including cellular processes, genetic information processing, human diseases, environmental information processing, metabolism and organismal systems (Figures 10.5a and 10.5b). Also, unclassified predicted functions were categorized (Figure 10.5b).

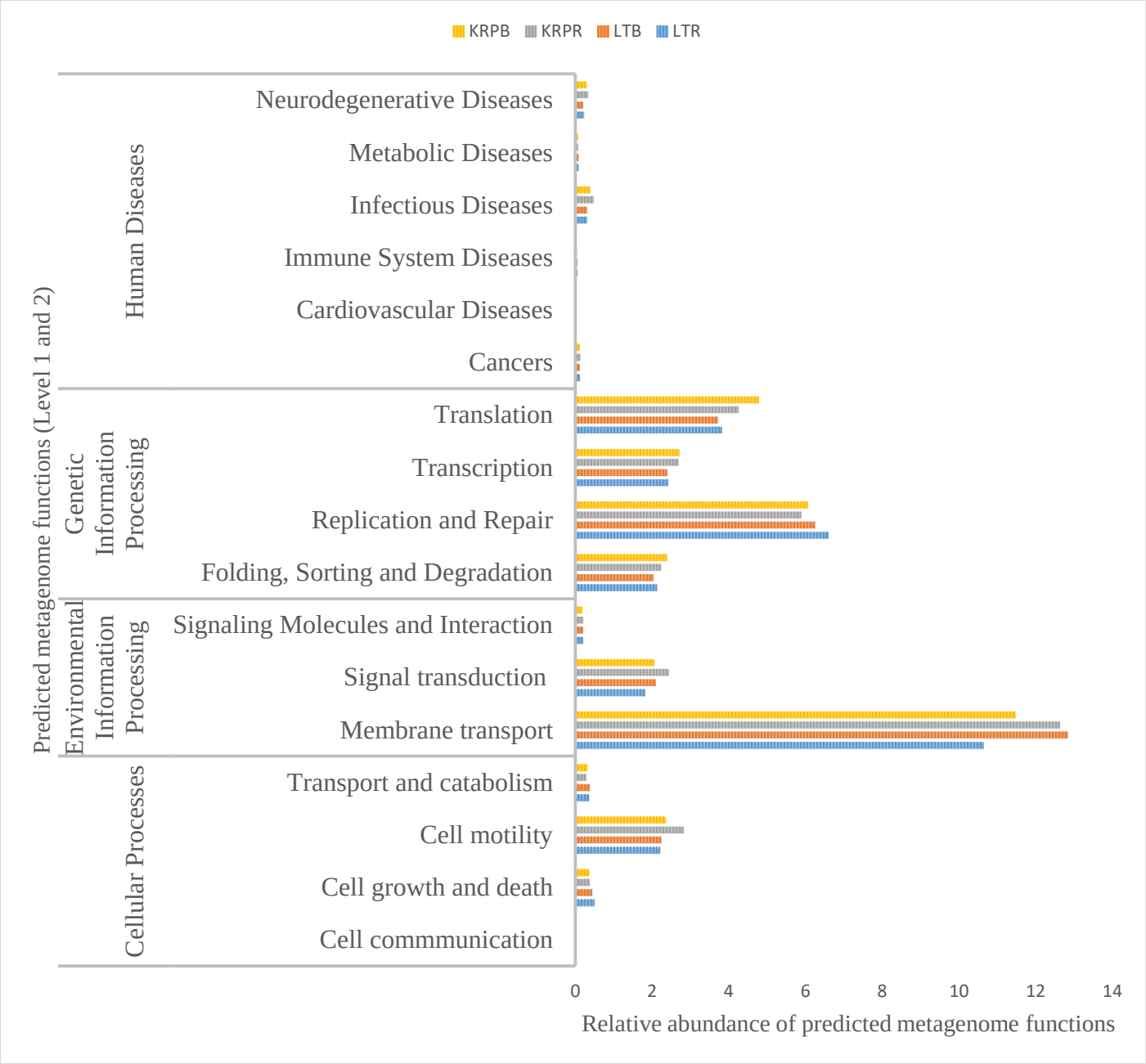

Figure 10.5a: Major bacterial community metabolisms in the sunflower rhizosphere and bulk soil samples from Lichtenburg and Krayburg at level 1 and level 2.

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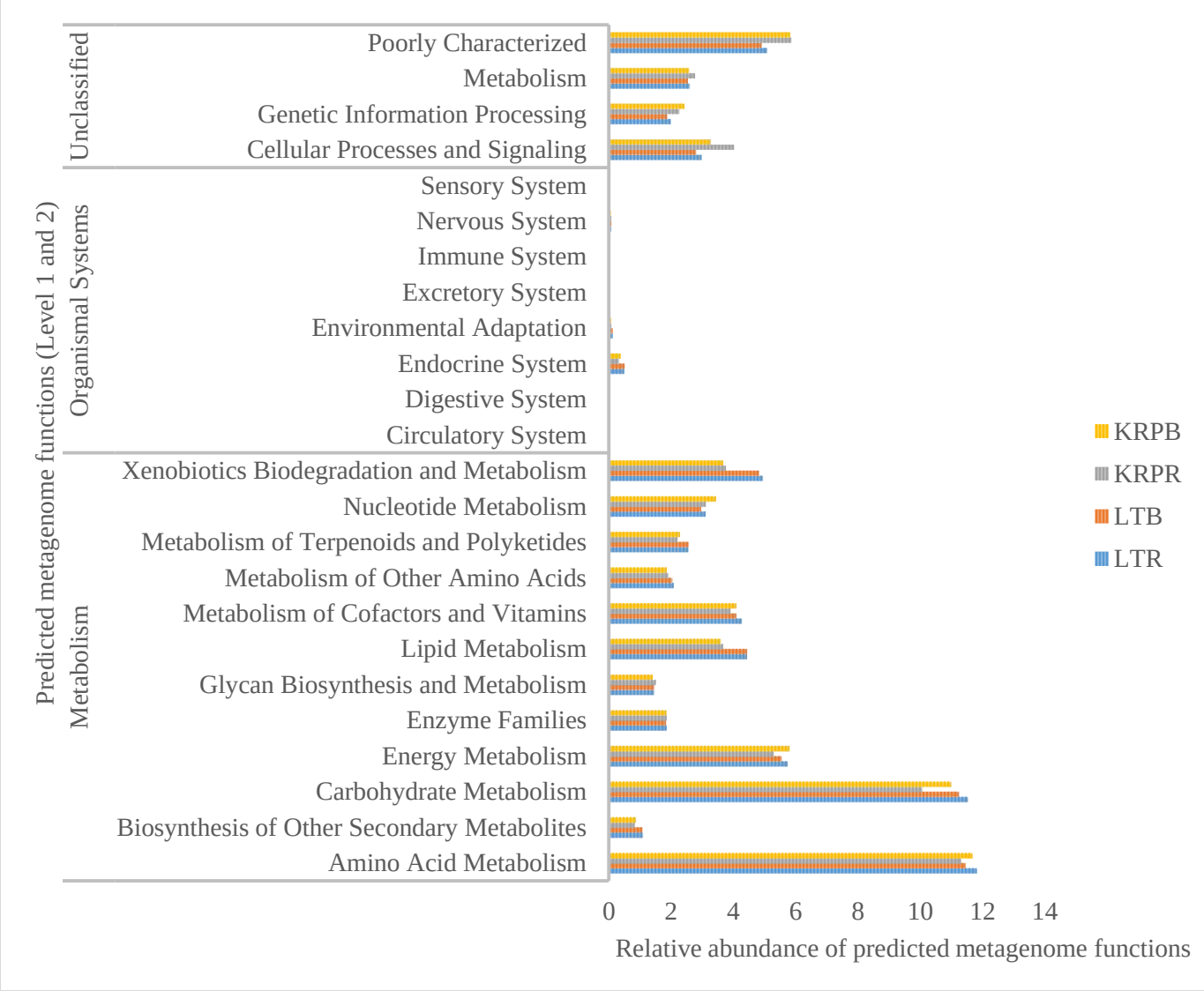

Figure 10.5b: Major bacterial community metabolisms in the sunflower rhizosphere and bulk soil samples from Lichtenburg and Krayburg at level 1 and level 2.

Furthermore, the predicted functions revealed at second-level classification (Figures 10.5a and 10.5b), 16 predicted functions, including cell communication, cell growth and death, replication and repair, immune system diseases, metabolic diseases, amino acid metabolism, biosynthesis of other secondary metabolites, carbohydrate metabolism, lipid metabolism, metabolism of cofactors and vitamins, metabolism of amino acids, xenobiotic biodegradation and metabolism, environmental adaptation and immune system were more predominant in LTR, whereas the predicted functions, including cell motility, signal transduction, signal molecules and interaction, cancers, infectious diseases, neurodegenerative disease, N-Glycan biosynthesis and metabolism, circulatory system and nervous systems predominated the KRPR. The abundance of enzyme families in the soils across the sites were the same (1.86), except for LTB whose enzyme family's relative abundance was 1.85. N-Glycan biosynthesis and metabolism relative abundance (1.47) were the same in LTR and LTB whereas KRPR and KRPB were 1.52 and 1.41 respectively.

The predicted functions revealed that the highest predicted functional profiling of bacteria was in KRPR at third-level selection (Figure 10.6). The abundance of bacterial motility proteins was predominant followed by ABC transporters (KRPR), whereas the least predicted function was the biosynthesis of steroid hormone (0.04) recorded in both the rhizosphere; bulk soils from Krayburg. Nitrogen (N) and sulfur (S) metabolism were higher in KRPR. Beta-Lactam resistance was the same (0.07) across all sites and samples.

Amino acids and derivatives pathways, including alanine, aspartate and glutamate metabolism, phenylalanine metabolism, tryptophan, cyanoamino acid metabolism and taurine and hypotaurine

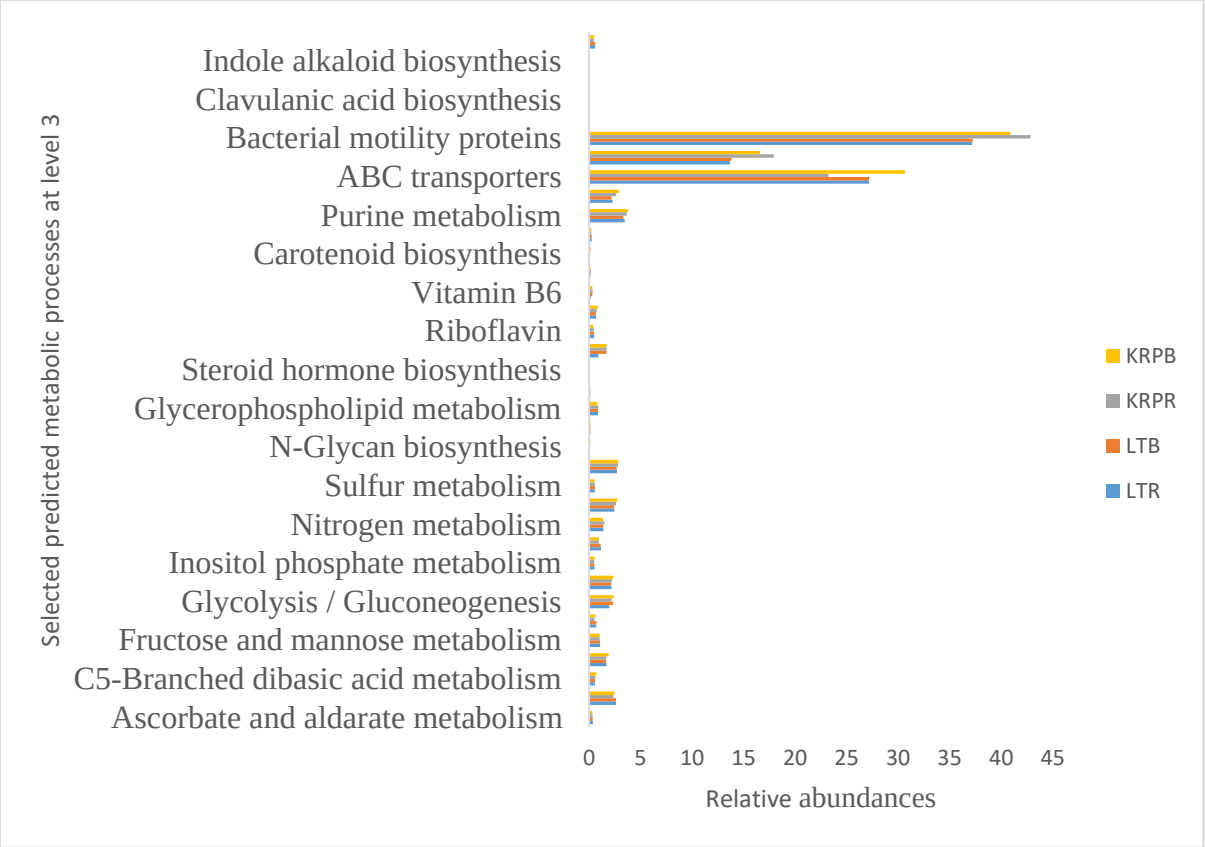

Figure 10.6: Selected predictive metabolic pathways of bacterial communities in the rhizosphere and bulk soils of sunflower from Lichtenburg and Krayburg at level 3. (LTR= Rhizosphere soils from Lichtenburg, LTB= Bulk soils from Lichtenburg, KRPR= Rhizosphere soils from Krayburg, KRPB= Bulk soils from Krayburg).

metabolism, were more abundant in LTR than in other samples (Figure S10.2). Alternatively, the relative abundances of amino acid related enzymes, cysteine and methionine metabolism, arginine and proline metabolism, glycine, serine and threonine metabolism, tyrosine metabolism, lysine degradation, D-alanine metabolism, D-arginine and D-ornithine metabolism, histidine metabolism, D-glutamine and D-glutamate metabolism, glutathione metabolism and phosphonate and phosphinate metabolism were more in KRPR than in other samples (Figure S10.2).

10.3.6. The predictive functions and bacterial community distribution in the rhizosphere and bulk samples

The PCA (Figure 10.7) was used to illustrate the correlation between the predictive functional categories (Level 1) on the bacterial community distribution at the phylum level. The PCA plot indicated that axis 1 had 94.17% and axis 20.5%. The vector length of environmental information processing positively correlated with Spirochaetes, Verrucomicrobia, Firmucutes, unclassified sequences and unclassified bacterial community structure.

The vector length of organismal systems positively correlated with Elusimicrobia, Nitrospirae, Acidobacteria and Actinobacteria.

10.3.7. The impact of soil physical and chemical properties on bacterial predictive functions

To investigate the link between the predictive functional categories of bacterial communities in LT and KRP samples and soil physical and chemical properties, PCA was used (Figure S10.3). Only the p-value of N-NH4 at the structural categories was statistically significant in the forward

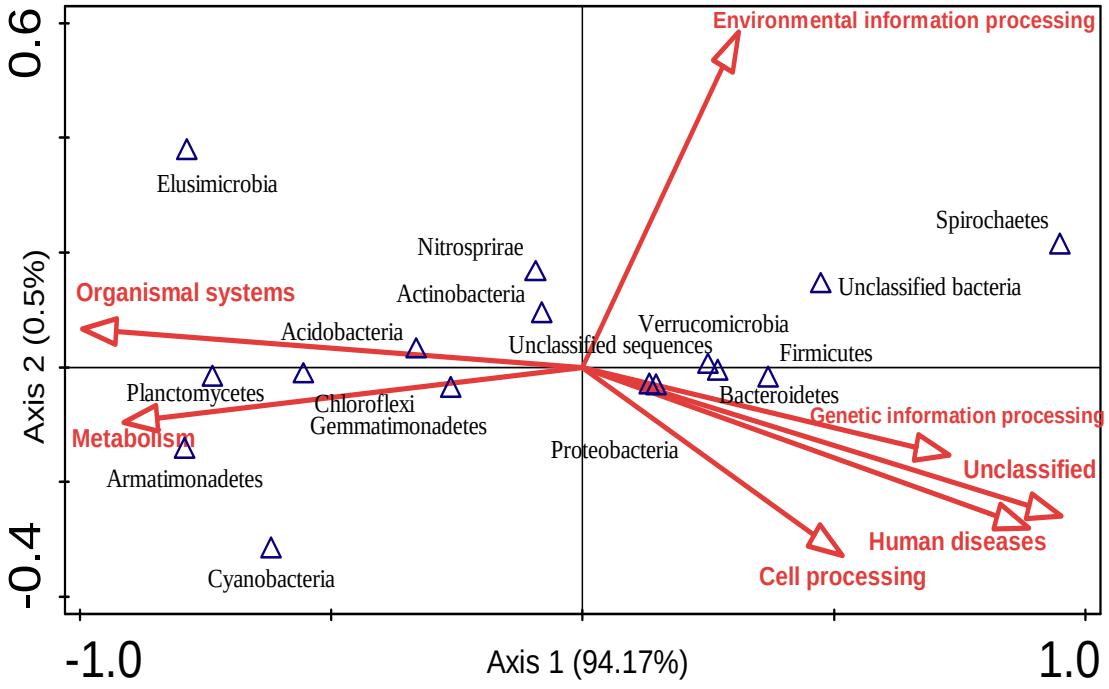

Figure 10.7: Principal Component Analysis (PCA) of major predictive functional information (Level 1) of bacterial communities in the soil samples from Lichtenburg and Krayburg. The vector lengths depict the strength of the bacterial metagenomes dominance.

Table 10:2: The forward selection results of environmental variables that best explains the variations in bacterial structure and predictive functions from the soil samples using the canonical correspondence analysis.

	Soil property	Explains %	Contribution %	F	P
Bacterial structure	OM (%)	93.4	93.4	28.3	0.102
	N-NH4 (mg/kg)	85.7	85.7	12.0	0.048
	pH	75.0	75.0	6.0	0.292
	Total N (%)	28.7	28.7	0.8	0.354
	Total C (%)	42.0	42.0	1.4	0.054
	N-NO3	11.2	11.2	0.3	0.522
Predictive functional category	OM (%)	74.9	74.9	6.0	0.31
	N-NH4 (mg/kg)	53.8	53.8	2.3	0.35
	pH	75.7	75.7	6.2	0.338
	Total N (%)	7.7	7.7	0.2	1
	Total C (%)	27.3	27.3	0.8	0.696
	N-NO3	14.0	14.0	0.3	0.842

Legend: Organic matter, % - percentage, p – probability value

selection of environmental factors that best explain variations' in bacterial structural composition and predictive functional categories (Table 10.2). The total variation was 0.00093 and explanatory variable account for 100%. The results revealed that OM had the most explained variable and contribution of 93.4% at the structural classification, whereas pH had the most explained variable and contribution of 75.7% at the predictive functional categories, which is depicted by the length of the vector arrows, as shown in Figure S10.3 and Table 10.2.

10.3.8. Alpha diversity assessments of bacterial communities and predictive functions in rhizosphere and bulk soils

The Simpson, Shannon_H and Evenness diversity index values within the samples were used to describe the alpha diversity of the bacterial communities at the taxonomic level presented in Table 10.3 across the sites. High Shannon H diversity index values were obtained between samples at the phylum and family levels when compared to other diversity indices measured across farm (Table 10.3). These diversity indices at the phylum and family levels revealed no significant differences ($p > 0.05$) in the bacterial composition's alpha diversity. Based on Shannon_H diversity indices, LTR had the highest alpha diversity index observed at the family level, and the least Shannon_H diversity index values were observed at the phylum level in LTB (Table 10.3).

Also, the result from the predictive functional categories analysis (Kruskal–Wallis, *p*-value = 0.51) (Table 10.3) showed that Shannon_H in LTR had a higher alpha diversity index compared to other samples. The alpha diversity indicated that bacterial diversity and predictive functions did not differ significantly (p-value > 0.05) between the LTR, LTB, KRPR and KRPB (Table 10.3).

Table 10.3: Alpha diversity indices of bacterial community and predictive functions from sunflower rhizosphere and bulk soils.

	Diversity indices	LTR	LTB	KRPR	KRPB	*p*-value
Bacterial taxonomic level	Simpson_1-D	0.7376	0.5639	0.7364	0.7201	0.50
	Shannon_H	1.67	1.333	1.488	1.471	
	Evenness_e^H/S	0.3542	0.2529	0.3163	0.3109	
Family	Simpson_1-D	0.7798	0.736	0.7794	0.7576	0.51
	Shannon_H	2.286	2.105	1.887	1.954	
	Evenness_e^H/S	0.3172	0.2736	0.2276	0.2433	
Predictive functional categories	Simpson_1-D	0.9368	0.9346	0.9373	0.9368	0.50
	Shannon_H	3.028	3.003	3.026	3.015	
	Evenness_e^H/S	0.4998	0.4916	0.5027	0.4975	

Legend: p– probability value, LTR- Rhizosphere soil from Lichtenburg, LTB- Bulk soil from Lichtenburg, KRPR- Rhizosphere soil from Krayburg, KRPB- Bulk soil from Krayburg.

10.4. Discussion

Sunflower is a significant oil-seed plant, hence, expanding its production is a major step toward achieving food availability and sustainable agriculture. Improving sunflower yield necessitates a better understanding of the functional, structural and metabolic potentials of the diverse bacterial communities abundant in their rhizosphere, especially those involved in conservation of ecosystem function, plant-growth promotion, biogeochemical cycles and sustainable agriculture (Li et al., 2022). In a bid to comprehend the activities in the plant rhizosphere, the bacterial community structure in the rhizosphere and bulk soil of sunflower was evaluated using a next-generation sequencing technique during the growing stage.

According to the information received on the farm history, it was postulated that soil physicochemical properties and agricultural practices, including the use of organic manure, chemical fertilizer and cropping type (mixed cropping and mono-cropping) may influence the bacterial diversity and their functions in the sunflower rhizosphere, which compelled the choice of the sampling sites. The application of organic manure and chemical fertilizer to enhance soil nutrients and plant growth may, in reverse, may incite a shift in the bacterial community structure and soil properties (Li et al., 2017a). The next-generation sequencing technique (amplicon-based approach) has been used successfully in studies to evaluate the diversity of bacterial communities in the rhizosphere soil of maize, soybean and sunflower (Kielak et al. 2016; Naumoff and Dedysh 2012).

In the present study, predominant bacterial phyla were identified in the rhizosphere and bulk soil of sunflower at the growing stage. The existence of these bacterial phyla might be attributed to their attraction to form a community in the rhizosphere. Most of the identified rhizosphere bacterial phyla have been previously reported in the rhizosphere of sunflower, soybean, wheat and maize (Alawiye and Babalola, 2021, Igiehon et al., 2020, WEN et al., 2016). Firmicutes contributes a significant quantity of nitrogen to plant nutrition resulting to increase in agricultural crop yield (Ichihashi et al., 2020). Acidobacteria have previously reported to play a significant role in carbon cycling because of their potential to degrade complex plant tissues, as well as lignin and cellulose, though their role in the rhizosphere is not well recorded (Ward et al., 2009), whereas some Bacteroidetes species participate in nitrogen cycling via denitrification (Chaparro et al., 2014).

The abundance of the identified bacterial phyla in sunflower soils from Lichtenburg has been reported to be significant in improving plant growth, disease suppression and soil health (Kielak et al., 2016, Naumoff and Dedysh, 2012, Li et al., 2017b). The phylum Armatimonadetes is relatively novel and was formerly recognized as a member phylum OP10 (Hu et al., 2014, Jiménez et al., 2020). There is limited information on its role in the rhizosphere or of the phylum Planctomycetes (Jiménez et al., 2020). The unclassified bacterial phyla and identified unclassified sequences may create insights for further research into their novel distinctiveness.

At the family level, the dominance of the bacterial community in LTR compared its corresponding samples may indicate the bacterial family's agricultural relevance, whereas the bacterial community dominant in the KRPR site has been reported as important plant growth-promoting

bacteria. Similarly, most of these families have previously been reported to positively influence the growth of sunflowers (Tseng et al., 2021, WEN et al., 2016, Majeed et al., 2018a).

Furthermore, the differences in the relative abundance of most bacterial community compositions in the rhizosphere soil of sorghum, maize, mustard and cucumber plants have been reported to positively improve agrobiodiversity (Agomoh et al., 2020, Ali et al., 2019, Wang et al., 2012). Moreover, the effect of climatic conditions and soil management practices impact the distribution of bacterial communities in the rhizosphere (Igiehon and Babalola, 2018a). Intriguingly, the variation in the bacterial community observed in the rhizosphere soil from Lichtenburg compared to the rhizosphere soil from Krayburg supports the study's hypothesis on the influence of mixed and crop rotational farming systems on the variety of bacterial communities in varied agricultural practices.

In the current study, the bacterial diversity indices at the phylum and family level revealed a significant variance in bacterial distribution across sites, which explained why mixed cropping and crop rotational practice produced more bacterial diversity in Lichtenburg than the mono-cropping system in Krayburg. Gentsch et al. (2020) documented the use of crop rotation systems in sustaining stable biodiversity and bacterial activities. The series of mixed cropping systems can increase nutrient acquisition and nutrient bioavailability, which directly increases rhizospheric bacteria and selectively attracts diverse plant growth-promoting bacteria into the region (Tyler, 2021, Couëdel et al., 2018).

The bacterial phylum identified in this research, such as Elusimicrobia predominantly in soils from Lichtenburg, has not been reported in the soil of any oil-seed crops, indicating its bioprospecting potential in agriculture. However, a study by Gkarmiri et al. (2017a) revealed an abundance of Verrucomicrobia, Gemmatimonadetes, Planctomycetes, Proteobacteria, Acidobacteria, Actinobacteria and Chloroflexi in the rhizosphere soil of oilseed rape . The most active phylum Proteobacteria from the LTR corroborates with that of Saleem et al. (2016), who discovered a related bacterial phylum in the burley tobacco plant's rhizosphere and roots.

Fascinatingly, the different families of rhizospheric bacteria in the LTR and KRPR can highlight their relevance in agriculture in improving plant growth and health. Because of high number of unclassified bacteria phyla from sunflower using amplicon sequencing, the findings from this study can serve as a model in future research of plant growth-promoting rhizospheric bacteria associated with oilseed crops, including sunflower. Exposing farming soils to continuing fertilization may change the bacterial diversity and nutritional profile (Zhang et al., 2020, Xiong et al., 2021).

Correspondingly, researchers have documented that OM is an important factor determining the diversity of bacterial in different soils, including sunflower and other agricultural soils (Cordero et al., 2020, WEN et al., 2016). The diversity, abundance and richness of bacterial communities are largely dependent on the soil OM content. This study revealed that the soil OM influenced the relative abundances of the major phyla differently across the sites.

In view of the vector lengths of the environmental variables in the PCA plot, it showed that not only OM determines the modelling of the bacterial communities and their functional diversity. The pH is a major determinant factors manipulating soil bacterial community structure (Qu et al., 2020). The pH values of sunflower rhizosphere and bulk soils ranged from 6.91 to 6.94 and this validates the findings of Alawiye and Babalola (2021), who documented pH values, range from 5.8 to 6.6 on rhizosphere soils from four sunflower farms in South Africa. The effect of these factors on rhizospheric bacterial structure diversity and their functional potentials has been reported (Chen et al., 2021), though, may form the bacterial community structure and selection of soil for agricultural purposes.

In accordance with the report of Jacoby et al. (2017), potassium, phosphorus and sodium available in the rhizosphere also contribute to the microbial community structure and function in mineralization processes vital for plant nutrition in natural ecosystems. The secretion of root exudates released by plants is linked to the modulation of microbial communities and their functions in the rhizosphere (Bargaz et al., 2018). Also, root exudates initiate's connections between the plant roots and soil microbes. The alpha diversity revealed no significant difference (p-value > 0.05) between bacterial diversity and predictive functions of the soils from LTR, LTB, KRPR and KRPB. The sunflower rhizosphere effect is the primary driver of alpha diversity in this study.

After secreting different profiles of bioactive compounds and nutrients into the rhizosphere, sunflower root exudates have the potential to influence bacterial diversity and processes in the

rhizosphere and bulk soils (Reavy et al., 2015, Wei et al., 2019). Only the predictive functional diversity represented by the bacterial metagenomes of the rhizosphere and bulk soils exceeded the theoretical limit of 2.81, according to the alpha diversity indices (Shannon_H) (Dinsdale et al., 2008, Rygaard et al., 2017), suggesting that bacterial metagenomes were most characterized in both soils from LTR, LTB, KRPR and KRPB. The Simpson and Evenness diversity indices for the bacterial metagenomes across all soil samples were < 1, indicating that there are a few predominant bacterial taxa, (e.g. Moraxellaceae, Solirubrobacteraceae, Chitinophagaceae and Streptomycetaceae) and the predictive functional categories (At level 1), cell processing, environmental information processing, genetic information processing, organismal systems, metabolisms and human diseases) in each soil samples.

The relative abundance of bacterial predictive functional categories at the second-level was used to distinguish the particular predictive functions that are of greater benefit to the bacteria present in a given habitat. Bacteria from soil use carbons as a source of energy for metabolism and growth, so they rely on diverse carbon sources such as maltose, inositol, glucose and mannose for their growth and survival. Evidently, this is distinguished in the abundance of predictive functional groups involved in carbon dioxide, di- and oligosaccharides fixation and carbohydrate metabolism at level 2, as well as the sufficient amount of metabolic pathways related to sugar usage, galactose metabolism, fructose and mannose metabolism, starch and sucrose metabolism, pentose phosphate pathway and TCA cycle in the samples.

Also, bacteria use amino acids as an energy source for persistence in environments with poor nutrient and in environments with little OM content (Gianoulis et al., 2009). This is in agreement with the results for the soil physicochemical properties, higher amounts of OM, P, N-NO_3 and Na were found in the LTR samples than in the samples from the Krayburg site. The decrease in soil nutrients in KRPR soil samples could be attributed to cropping systems and land-use practices, this substantiate the report from previous studies that continuous cropping of a particular crop depletes soil nutrients (Kumar Behera et al., 2009, Dhaliwal et al., 2019, Foley et al., 2005, El-Fouly et al., 2015, Chen et al., 2018). Therefore, as a response mechanism for bacterial survival in nutrient-poor soils, the richness of genes linked with cell motility (bacterial mobility proteins and bacterial chemotaxis) is necessary.

The findings demonstrated that the bacterial communities found in soil samples support plants in acquiring carbon through various metabolic pathways (Wang et al., 2019b, Xu et al., 2020b, Prabha et al., 2019). In addition, sequences linked to the metabolism of important mineral nutrients like S and N were discovered in soils. LTR and KRPR had larger relative abundances of these predictive functions than LTB and KRPB. Mineral nutrients are required for plant growth and health. As a result, the findings suggest that the bacterial communities found in the samples support sunflower plants in obtaining essential nutrients for growth and development.

Similarly, at level 3, the presence of selected predicted metabolic functions such as ABC transporters, oxidative phosphorylation, glycerophospholipid and nitrogen and sulfur metabolism in the samples indicate that bacterial communities inhabiting these soils play a key role in

promoting nutrient cycling, plant health and growth (Tang et al., 2016, Reed et al., 2011, Lindsay et al., 2010). The metabolism of sulfur in level 3 disclosed that the samples were also controlled by S. Many studies have shown that bacteria found in various environments, including sunflower soil, play critical roles in enzymatic sulfur metabolism (Wrighton et al., 2012, Yin et al., 2014, Zhang et al., 2016). Hence, the presence of sulfur metabolism genes in the samples proposes that the bacteria present in the soil samples can help maintain a balanced sulfur metabolism in their environments.

Moreover, the presence of metagenome sequences involved in the biosynthesis of chelating and iron compounds, for instance siderophore in the results was observed. Iron is a micronutrient that is important in the initiation of metabolic pathways and a constituent of the prosthetic group in living organisms (Dimkpa et al., 2009a). Abiotic stresses in plants caused by iron can be eased by bacteria through high-affinity transport systems linking the biosynthesis of siderophores. The transport systems play critical roles in many soil environments, including aiding the competitive discovery of iron for plant usage (Prabha et al., 2019, Mohapatra et al., 2021).

The sequences associated with metabolic activity, ABC transporters, were discovered at level 3. Most of these metabolic genes were found to be abundant in the Kraybrug samples. As a result, it was predicted that the samples will be dominated by bacteria that aid in the acquisition of minute iron, hence increasing iron bioavailability in the soil samples. At level 3, the predicted metabolic processes, such as those involved in virulence, disease and defense, stress response, secondary metabolism and aromatic chemical metabolism were observed. The abundance of sequences

relevant to antibiotic and hazardous chemical resistance, plant hormones, siderophores, oxidative stress and virulence regulation at level 3 support these findings.

The metabolic pathways involved in indole alkaloid biosynthesis, flavonoid biosynthesis, clavulanic acid biosynthesis, steroid hormone biosynthesis, inositol phosphate metabolism, linoleic acid metabolism and N-Glycan biosynthesis were all shown to be abundant at level 3. Sequences associated to streptomycin biosynthesis and antibiosis resistance, particularly beta-lactam resistance, were discovered once more. Secondary metabolism, which includes the bacterial community's biosynthesis of numerous metabolites (low molecular-weight compounds) as a sign of metabolic complexity, is an important feature of bacteria from soil (Berdy, 2005, Barka et al., 2016). This defense mechanism is used by bacteria to defend themselves against pathogens. As a result, they are important in medical practice, serving as antimicrobials and antibiotics (Newman and Cragg, 2016).

Many of the bacterial phyla identified, such as Proteobacteria, Actinobacteria, Firmicutes and Bacteroidetes, produce a variety of bioactive compounds, like siderophores, that also act as antifungal, antibacterial and biosurfactant (Hwang et al., 2014, O'Connor, 2015, Ludwig-Müller, 2015, Gómez Expósito etfc x al., 2017a). Because secondary metabolism is so important to plant growth, competent culturing methods must isolate and identify bacterial strains from the soil bacterial community that can perform secondary metabolic functions efficiently for increased crop yield. As a result, examining the soil bacterial community for bacteria that have these different inheritable factor can lead to the classification of unique secondary metabolic traits that could be

used as biofertilizers in soils and plants to enhance resistance against pathogenic attacks. The findings are in accordance with previous studies that show the diversity and abundance of genes linked to antibiotic resistance (Wang et al., 2013, Enagbonma and Babalola, 2020).

The metabolic pathways of amino acids at level 3 revealed the samples were also dominated by amino acids and derivatives. The bacterial communities inhabiting the fields can produce amino acids such as glutathione and Lysine involved in the protection against oxidative stress in the crops such as sunflower plants as indicated in the results (Takagi and Ohtsu, 2016). The high richness of the unclassified predicted functions and poorly characterized predicted functions at level 1 and level 2 respectively, indicates many bacterial genes predicted functions in soils are still unknown. Though, their presence indicates that they play a part to important functions in soils that can be useful to plant growth and survival.

Another hypothesis of this study was that the physicochemical parameters would influence the predictive functional attributes of the bacterial communities. The pressure of selection on sunflower roots, which constantly emit exudates containing amino acids and carbohydrates into the rhizosphere, causes the aggregation of various bacterial communities. In various agricultural plants, namely maize, wheat and peas, the effect of host plants on bacterial diversity in the rhizosphere has been observed (Mohammadi et al., 2011, Gentsch et al., 2020). According to Hamel et al. (2006), in a study of high-frequency pea production, there was an increase in bacterial diversity in pea rhizosphere soil with mineral nitrogen levels compared to the bulk soil.

Furthermore, metagenomic analysis of wheat rhizosphere and bulk soil found that the rhizosphere soil has higher bacterial diversity than the bulk soil (Priya et al., 2018, Velázquez-Sepúlveda et al., 2012). Moreover, several research focused mostly on selected rhizospheric isolates have found that the rhizosphere region of young plants is a more random environment than the rhizosphere region of mature plants during the developing phases of maize (Tiemann et al., 2015). This is consistent with the observations of García-Salamanca et al. (2013), who found that the rhizosphere is a more nutrient-dense ecosystem than bulk soil, and that the activity levels of some enzymes from bacterial cells in the maize rhizosphere, such as dehydrogenase, β-glucosidase and alkaline phosphatase, were higher in the rhizosphere than in bulk soil.

Furthermore, it was discovered that physicochemical parameters influenced the predicted functionalities. The primary factors to the distinctiveness exhibited in soil bacterial structural diversity have been identified as soil physicochemical characteristics (Shi et al., 2011a, Hanson et al., 2012). The functional diversity of bacterial communities is driven by soil characteristics, according to previous studies (Shi et al., 2011a, Hanson et al., 2012). The present study's findings show that the physical and chemical properties of the soil impacted the relative abundance of bacterial predicted functions in the study sites.

10.5. Conclusion

Understanding the roles of various rhizospheric bacterial communities in plant growth and health promotion using 16S rRNA gene sequencing creates novel prospects for enhancing effective and eco-friendly methods for improving agricultural yield through the manipulation of

microorganisms. Dissimilarities in predominant bacterial communities were documented between the soil samples across the sites. The dominance of unclassified bacteria and sequences in the samples proposes further research into developing culturable approaches for their classification and discovery of new genes that can be exploited as bioinoculants in the development environmentally friendly agriculture.

Across the sites bacterial diversity was positively and negatively influenced by environmental variables. The predicted functional attributes of these bacteria propose their agricultural significance, which can be discovered in emerging biofertilizers as a substitute to chemical fertilizer. Because of the economic importance of sunflower, it is recommended to employ culture-dependent methods, invitro inoculation of seeds and fields and greenhouse planting to study the potential of rhizospheric bacteria on sunflower crops. Also, mining the metagenomes using more advanced techniques is important to identify novel genes that encode valuable metabolic pathways with numerous essential functions crucial for plant development and enhancement of sustainable agriculture.

Likewise, understanding plant-associated microorganisms in various cropping systems will aid in determining their functional roles in nutrient cycling, plant nutrition, development and survival. Interestingly, this research offers proof of the effect of agricultural practices, crop rotation and physicochemical properties on the bacterial diversity in sunflower soils from the two sites (Lichtenburg- LT and Krayburg- KRP). Conclusively, this study will assist the agricultural sector

to enhance economic, agricultural and environmental sustainability by taking strategic soil management decisions.

CHAPTER ELEVEN

Conclusion

11.1. Concluding remarks

In this study, soil physical and chemical properties in the rhizosphere and bulk soils of different sunflower farms (Sheila/ Ditsobottla, Itsoseng, Lichtenburg and Kraaipan/Krayburg) were successfully investigated, also the impacts of these soil parameters on bacterial diversity and functioning of the soil environment were determined. The distribution and variations of rhizospheric soil bacterial community structure and their function was also evaluated. The soil physical and chemical properties were observed to be key drivers of bacterial diversity and their functioning.

Describing the soil abiotic factors provide understandings on the effect of these factors on the composition of bacterial community and the level of modifications these bacterial communities undergo. From the results, soil variables directly influenced soil functioning. The confirmation that microorganisms are critical drivers of the functioning of soil ecosystems was established. The rhizospheric soil bacterial community structure and distribution undergo spatial distribution because of these environmental variables. The disparity in rhizospheric soil bacterial diversity, community structure and functioning were observed across the farm locations. Furthermore, there were clear distinctions between the microbial communities identified in the rhizospheric soils and their bulk soils across the study sites. Bacterial phyla such as Firmicutes, Proteobacteria and

Actinobacteria were the most abundant in the rhizospheric soils from Sheila, Itsoseng and Kraaipan, while Thaumarchaeota and Crenarchaeota were the predominant archaeal species identified. Lichtenburg taxonomic hits distribution at phylum level classification of rhizospheric soils discovered predominantly Actinobacteria, followed by Proteobacteria, Firmicutes, Planctomycetes, Chloroflexi and Gemmatimonadetes. Moreover, Firmicutes, Actinobacteria and Proteobacteria predominated sunflower rhizosphere soils from Sheila and Itsoseng farms than the bulk soils from the farm sites. At the order level, the bacterial communities from the rhizospheric soils in Ditsobottla and Kraaipan, it was Bacillales were the most abundant. Lactobacillales, Bacillales, Rhizobiales, Enterobacteriales, Burkholderiales, Flavobacteriales, Sphingomonadales, Myxococcales and Nitrosomonadales were more predominant from Ditsobottla rhizosphere soils than the bulk soils, while the abundance of Planctomycetales, Cytophagales, Gemmatimonadales, Nitrospirales and Caulobacteriales were from Kraaipan rhizosphere soil. In soils from Lichtenburg and Krayburg, Proteobacteria and Planctomycetes dominated the rhizospheric soil, while Firmicutes and Actinobacteria were predominant in bulk soils. Also, agricultural practices, including cropping type (mono-cropping and mixed cropping), chemical fertilizer use and organic manure influenced the bacterial composition, diversity, interactions, activities and their functions.

Bacterial communities exhibited structural pattern of distributions at the different local scales. Bacterial species in the soil are stimulated by root exudates through chemical pointers. This study revealed that soil bacterial community structure and functional diversity are related with variations associated to predictable periodical changes in nutrients and resource patterns. The differences in the bacterial community composition therefore impacts soil ecosystem functions as observed in

community level respiration and predictive functional profile. Also, there were notable differences in the functionality of the bacterial community in the rhizospheric and bulk soils in the study locations. Also, there were notable differences in the functionality of the bacterial community in the rhizospheric and bulk soils in the study locations. The community-level physiological profiling (CLPP) analysis showed that the microbial communities in Sheila (SHR) and Itsoseng (ITR) used the amino acids (tryptophan and malic acid) effectively. The metabolisms of these carbon substrates may be due to the dominant nature of some of the organisms, such as Actinobacteria in the soils. The measurement of CLPP from the sunflower rhizospheric soils were different from those of the bulk soil and the level of the differences were based on the soil microbial composition and the type of carbon substrates. The presence of certain taxa of rhizobacteria such as Bacillales in the sunflower rhizosphere were positively influenced by Na^{+} and Mg^{2+}, and taxa obtained from SHR and ITR were able to efficiently utilized tryptophan and malic acid. Further, the bacterial community from Ditsobottla rhizosphere soil (R1) was positively influenced by organic matter (OM), while Kraaipan rhizosphere soil (R2) was positively influenced by total N and pH. Bacterial communities of the rhizospheric soil samples utilized more of the different carbon substrates (six carbohydrates, three amino acids and three carboxylic acids) as an energy source than their corresponding bulk soil samples. Significant differences ($P< 0.05$) in bacterial predicted functional groups at phyla and family levels between soils from Lichtenburg rhizosphere (LTR) and bulk soils (LTB), and rhizosphere soils from Krayburg [KRPR] and bulk soils from Krayburg [KRPB] sites, were observed.

Assessment of the differences in the rhizospheric soil provides an understanding of the factors controlling the dispersal of microorganisms and structural variations. The diverse microbial community identified from the sunflower soils were similar to the microbial community reported from the soils of other plants, including maize, sorghum and soy bean. This may be due to the influence of factors such as cropping system, soil properties and chemical compositions. The patterns of microbial composition, diversity and/or abundance can be related to heterogeneity in biogeochemical processes. The differences of soil microbial diversity composition and variability have clear significances for many ecosystem processes, soil fertility and plant nutrition. More studies on the influence of agricultural practices on soil bacterial communities are essential as soil biodiversity regulate the main biogeochemical pathways and not paying attention to them could result in food insecurity, hunger, malnutrition and global warming.

REFERENCES

ABRAM, F. 2015. Systems-based approaches to unravel multi-species microbial community functioning. *Computational and Structural Biotechnology Journal,* 13**,** 24-32.

ACKERMAN, K., CONARD, M., CULLIGAN, P., PLUNZ, R., SUTTO, M.P. & WHITTINGHILL, L. 2014. Sustainable food systems for future cities: The potential of urban agriculture. *The Economic and Social Review,* 45**,** 189–206-189–206.

ADEDEJI, A. A., HÄGGBLOM, M. M. & BABALOLA, O. O. 2020. Sustainable agriculture in Africa: plant growth-promoting rhizobacteria (PGPR) to the rescue. *Scientific African,* 9**,** 1-14.

ADEGBOYE, M. & BABALOLA, O. 2013. Phylogenetic characterization of culturable antibiotic producing *Streptomyces* from rhizospheric soils. *Molecular Biology,* 1**,** 1-7.

ADEGBOYE, M. F., LOBB, B., BABALOLA, O. O., DOXEY, A. C. & MA, K. 2018. Draft genome sequences of two novel cellulolytic *Streptomyces* strains isolated from South African rhizosphere soil. *Genome Announcements,* 6**,** e00632-18.

ADELEKE, B. S. & BABALOLA, O. O. 2020a. The endosphere microbial communities, a great promise in agriculture. *International Microbiology***,** 1-17.

ADELEKE, B. S. & BABALOLA, O. O. 2020b. Oilseed crop sunflower (*Helianthus annuus*) as a source of food: Nutritional and health benefits. *Food Science and Nutrition,* 8**,** 1-19.

ADESEMOYE, A. O. & EGAMBERDIEVA, D. 2013. Beneficial effects of plant growth-promoting rhizobacteria on improved crop production: prospects for developing economies. In *Bacteria in agrobiology: Crop productivity* (pp. 45-63). Springer, Berlin, Heidelberg.

ADESEMOYE, A. O., YUEN, G. & WATTS, D. B. 2017. Microbial inoculants for optimized plant nutrient use in integrated pest and input management systems. In *Probiotics and plant health* (pp. 21-40). Springer, Singapore.

AGLER, M. T., RUHE, J., KROLL, S., MORHENN, C., KIM, S.T., WEIGEL, D. & KEMEN, E. M. 2016. Microbial hub taxa link host and abiotic factors to plant microbiome variation. *Public Library of Science Biology,* 14**,** 1-20.

AGOMOH, I. V., DRURY, C. F., PHILLIPS, L. A., REYNOLDS, W. D. & YANG, X. 2020. Increasing crop diversity in wheat rotations increases yields but decreases soil health. *Soil Science Society of America Journal,* 84**,** 170-181.

AHMAD, I., AKHTAR, M. J., MEHMOOD, S., AKHTER, K., TAHIR, M., SAEED, M. F., HUSSAIN, M. B. & HUSSAIN, S. 2018. Combined application of compost and *Bacillus* sp. CIK-512 ameliorated the lead toxicity in radish by regulating the homeostasis of antioxidants and lead. *Ecotoxicology and Environmental Safety,* 148**,** 805-812.

AHMED, V., VERMA, M. K., GUPTA, S., MANDHAN, V. & CHAUHAN, N. S. 2018. Metagenomic profiling of soil microbes to mine salt stress tolerance genes. *Frontiers in Microbiology,* 9**,** 159.

AI, C., LIANG, G., SUN, J., WANG, X. & ZHOU, W. 2012. Responses of extracellular enzyme activities and microbial community in both the rhizosphere and bulk soil to long-term fertilization practices in a fluvo-aquic soil. *Geoderma,* 173**,** 330-338.

AIMEN, H., KHAN, A. S. & KANWAL, N. 2018. Methanotrophs: the natural way to tackle greenhouse effect. *Journal of Bioremediation and Biodegradation,* 9**,** 432.

ALAWIYE, T. T. & BABALOLA, O. O. 2019. Bacterial diversity and community structure in typical plant rhizosphere. *Diversity,* 11**,** 1-11.

ALAWIYE, T. T. & BABALOLA, O. O. 2021. Metagenomic insight into the community structure and functional genes in the sunflower rhizosphere microbiome. *Agriculture,* 11**,** 167.

ALI, A., IMRAN GHANI, M., LI, Y., DING, H., MENG, H. & CHENG, Z. 2019. Hiseq base molecular characterization of soil microbial community, diversity structure and predictive functional profiling in continuous cucumber planted soil affected by diverse cropping systems in an intensive greenhouse region of northern China. *International Journal of Molecular Sciences,* 20**,** 2619.

ALI, M. A., NAVEED, M., MUSTAFA, A. & ABBAS, A. 2017. The good, the bad and the ugly of rhizosphere microbiome. *Probiotics and Plant Health.* Switzerland: *Springer*.

ALIBE, I. & INUWA, B. 2012. Physicochemical and anti-microbial properties of sunflower (*Helianthus annuus* L.) seed oil. *International Journal of Science and Technology,* 2**,** 151-154.

ALOISIO, J. M., PALMER, M. I., TUININGA, A. R. & LEWIS, J. D. 2019. Plant colonization of green roofs is affected by composition of established native plant communities. *Frontiers in Ecology and Evolution***,** 238.

ALORI, E. T. & BABALOLA, O. O. 2018. Microbial inoculants for improving crop quality and human health in Africa. *Frontiers in Microbiology,* 9**,** 1-12.

ALVES, L. D. F., WESTMANN, C. A., LOVATE, G. L., DE SIQUEIRA, G. M. V., BORELLI, T. C. & GUAZZARONI, M.-E. 2018. Metagenomic approaches for understanding new concepts in microbial science. *International Journal of Genomics,* 2018.

ALVIRA, P., TOMÁS-PEJÓ, E., BALLESTEROS, M. & NEGRO, M. 2010. Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis: a review. *Bioresource Technology,* 101**,** 4851-4861.

AMBROSINI, A., BENEDUZI, A., STEFANSKI, T., PINHEIRO, F. G., VARGAS, L. K. & PASSAGLIA, L. M. 2012. Screening of plant growth promoting rhizobacteria isolated from sunflower (*Helianthus annuus* L.). *Plant and Soil,* 356**,** 245-264.

AMBROSINI, A., SANT'ANNA, F. H., DE SOUZA, R., TADRA-SFEIR, M., FAORO, H., ALVARENGA, S. M., PEDROSA, F. O., SOUZA, E. M. & PASSAGLIA, L. M. 2015. Genome sequence of *Bacillus mycoides* B38V, a growth-promoting bacterium of sunflower. *Genome Announcement,* 3**,** e00245-15.

AMBROSINI, A., SANT'ANNA, F. H., HEINZMANN, J., DE CARVALHO FERNANDES, G., BACH, E. & PASSAGLIA, L. M. P. 2018. *Paenibacillus helianthi* sp. nov., a nitrogen-fixing species isolated from the rhizosphere of *Helianthus annuus* L. *Antonie van Leeuwenhoek,* 111**,** 2463-2471.

AMBROSINI, A., STEFANSKI, T., LISBOA, B., BENEDUZI, A., VARGAS, L. & PASSAGLIA, L. 2016. Diazotrophic *Bacilli* isolated from the sunflower rhizosphere and the potential of *Bacillus mycoides* B38V as biofertiliser. *Annals of Applied Biology,* 168**,** 93-110.

AMOO, A. E. & BABALOLA, O. O. 2019. Impact of land use on bacterial diversity and community structure in temperate pine and indigenous forest soils. *Diversity,* 11**,** 1-12.

AMOO, A. E., DELGADO-BAQUERIZO, M. & BABALOLA, O. O. 2021. Forest plantations reduce soil functioning in terrestrial ecosystems from South Africa. *Pedobiologia,* 89**,** 150757.

ANAYA, C. A. & HUBER-SANNWALD, E. 2015. Long-term soil organic carbon and nitrogen dynamics after conversion of tropical forest to traditional sugarcane agriculture in East Mexico. *Soil and Tillage Research,* 147**,** 20-29.

ANDRADE, G. F., PANIZ, F. P., MARTINS JR, A. C., ROCHA, B. A., DA SILVA LOBATO, A. K., RODRIGUES, J. L., CARDOSO-GUSTAVSON, P., MASUDA, H. P. & BATISTA, B. L. 2018. Agricultural use of Samarco's spilled mud assessed by rice cultivation: A promising residue use? *Chemosphere,* 193**,** 892-902.

ANGST, G., MESSINGER, J., GREINER, M., HÄUSLER, W., HERTEL, D., KIRFEL, K., KÖGEL-KNABNER, I., LEUSCHNER, C., RETHEMEYER, J. & MUELLER, C. W. 2018. Soil organic carbon stocks in topsoil and subsoil controlled by parent material, carbon input in the rhizosphere and microbial-derived compounds. *Soil Biology and Biochemistry,* 122**,** 19-30.

ANTAR, M., GOPAL, P., MSIMBIRA, L. A., NAAMALA, J., NAZARI, M., OVERBEEK, W., BACKER, R. & SMITH, D. L. 2020. Inter-organismal signaling in the rhizosphere. *Rhizosphere biology: Interactions between microbes and plants.* Singapore: Springer.

ARIF, M. S., SHAHZAD, S. M., RIAZ, M., YASMEEN, T., SHAHZAD, T., AKHTAR, M. J., BRAGAZZA, L. & BUTTLER, A. 2017. Nitrogen-enriched compost application combined with plant growth-promoting rhizobacteria (PGPR) improves seed quality and nutrient use efficiency of sunflower. *Journal of Plant Nutrition and Soil Science,* 180**,** 464-473.

ARTZI, L., BAYER, E. A. & MORAÏS, S. 2017. Cellulosomes: bacterial nanomachines for dismantling plant polysaccharides. *Nature Reviews Microbiology,* 15**,** 83.

ASILIAN, E., GHASEMI-FASAEI, R., RONAGHI, A., SEPEHRI, M. & NIAZI, A. 2019. Chemical-and microbial-enhanced phytoremediation of cadmium-contaminated calcareous soil by maize. *Toxicology and Industrial Health,* 35**,** 378-386.

AYANGBENRO, A. S. & BABALOLA, O. O. 2017. A new strategy for heavy metal polluted environments: a review of microbial biosorbents. *International Journal of Environmental Research and Public Health,* 14**,** 1-16.

BABALOLA, O. & AKINDOLIRE, A. 2011. Identification of native rhizobacteria peculiar to selected food crops in Mmabatho municipality of South Africa. *Biological Agriculture and Horticulture,* 27**,** 1-17.

BABALOLA, O. O. 2010. Beneficial bacteria of agricultural importance. *Biotechnology Letters,* 32**,** 1559-1570.

BABALOLA, O. O., ADELEKE, B. S. & AYANGBENRO, A. S. 2021a. 16S rRNA gene amplicon sequence data from sunflower endosphere bacterial community. *Data in Brief,* 39**,** 107636.

BABALOLA, O. O., ALAWIYE, T. T., LOPEZ, C. R. & AYANGBENRO, A. S. 2020. Shotgun metagenomic sequencing data of sunflower rhizosphere microbial community in South Africa. *Data in Brief***,** 1-4.

BABALOLA, O. O., FADIJI, A. E., ENAGBONMA, B. J., ALORI, E. T., AYILARA, M. S. & AYANGBENRO, A. S. 2020c. The Nexus Between Plant and Plant Microbiome: Revelation of the Networking Strategies. *Frontiers in Microbiology,* 11**,** 1-16.

BABALOLA, O. O., NWACHUKWU, B. C. & AYANGBENRO, A. S. 2021b. High-Throughput Sequencing Survey of Sunflower Soil. *Microbiology Resource Announcements,* 10**,** 1-3.

BABALOLA, O. O., NWACHUKWU, B. C. & AYANGBENRO, A. S. 2021c. High-throughput sequencing survey of sunflower soil. *Microbiology Resource Announcements,* 10**,** e01331-20.

BABALOLA, O. O., SANNI, A. I., ODHIAMBO, G. D. & TORTO, B. 2007. Plant growth-promoting rhizobacteria do not pose any deleterious effect on cowpea and detectable amounts of ethylene are produced. *World Journal of Microbiology and Biotechnology,* 23**,** 747-752.

BACKER, R., ROKEM, J. S., ILANGUMARAN, G., LAMONT, J., PRASLICKOVA, D., RICCI, E., SUBRAMANIAN, S. & SMITH, D. L. 2018. Plant growth-promoting rhizobacteria: context, mechanisms of action and roadmap to commercialization of biostimulants for sustainable agriculture. *Frontiers in Plant Science,* 9**,** 1-17.

BAGHAIE, A. H. & AGHILIZEFREEI, A. 2019. Neighbor presence of plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) can increase sorghum phytoremediation efficiency in a soil treated with Pb polluted cow manure. *Journal of Human, Environment and Health Promotion,* 5**,** 153-159.

BAGYARAJ, D. & ASHWIN, R. 2017. Can mycorrhizal fungi influence plant diversity and production in an ecosystem. *Microbes for Restoration of Degraded Ecosystems,* 13**,** 1-17.

BAIS, H. P., WEIR, T. L., PERRY, L. G., GILROY, S. & VIVANCO, J. M. 2006. The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology,* 57**,** 233-266.

BAKKER, M. G., MANTER, D. K., SHEFLIN, A. M., WEIR, T. L. & VIVANCO, J. M. 2012. Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant and Soil,* 360**,** 1-13.

BAO, Y., DOLFING, J., WANG, B., CHEN, R., HUANG, M., LI, Z., LIN, X. & FENG, Y. 2019. Bacterial communities involved directly or indirectly in the anaerobic degradation of cellulose. *Biology and Fertility of Soils,* 55**,** 201-211.

BAO, Y., FENG, Y., QIU, C., ZHANG, J., WANG, Y. & LIN, X. 2021. Organic matter-and temperature-driven deterministic assembly processes govern bacterial community composition and functionality during manure composting. *Waste Management,* 131**,** 31-40.

BARGAZ, A., LYAMLOULI, K., CHTOUKI, M., ZEROUAL, Y. & DHIBA, D. 2018. Soil microbial resources for improving fertilizers efficiency in an integrated plant nutrient management system. *Frontiers in microbiology,* 9**,** 1606.

BARKA, E. A., VATSA, P., SANCHEZ, L., GAVEAU-VAILLANT, N., JACQUARD, C., KLENK, H.-P., CLÉMENT, C., OUHDOUCH, Y. & VAN WEZEL, G. P. 2016. Taxonomy, physiology and natural products of Actinobacteria. *Microbiology and Molecular Biology Reviews,* 80**,** 1-43.

BARQUERO, M., PASTOR-BUIES, R., URBANO, B. & GONZÁLEZ-ANDRÉS, F. 2019. Challenges, regulations and future actions in biofertilizers in the european agriculture: from the lab to the field. *Microbial Probiotics for Agricultural Systems. Springer*.

BASHAN, Y., DE-BASHAN, L. E. & PRABHU, S. 2016. Superior polymeric formulations and emerging innovative products of bacterial inoculants for sustainable agriculture and the environment. *Agriculturally important microorganisms.* Spingerpore: *Springer*.

BASU, A., PRASAD, P., DAS, S.N., KALA, S., SAYYED, R.Z., REDDY, M.S. & ENSHASY, H.E. 2021. Plant Growth Promoting Rhizobacteria (PGPR) as Green Bioinoculants: Recent Developments, Constraints, and Prospects. *Sustainability, 13(*3): 1-20.BECKERS, B., DE BEECK, M. O., WEYENS, N., BOERJAN, W. & VANGRONSVELD, J. 2017a. Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome,* 5**,** 1-17.

BECKERS, B., DE BEECK, M. O., WEYENS, N., BOERJAN, W. & VANGRONSVELD, J. 2017b. Structural variability and niche differentiation in the rhizosphere and endosphere bacterial microbiome of field-grown poplar trees. *Microbiome,* 5**,** 25.

BEHERA, B., SETHI, B., MISHRA, R., DUTTA, S. & THATOI, H. 2017. Microbial cellulases–Diversity and biotechnology with reference to mangrove environment: A review. *Journal of Genetic Engineering and Biotechnology,* 15**,** 197-210.

BENITEZ, M.-S., OSBORNE, S. L. & LEHMAN, R. M. 2017. Previous crop and rotation history effects on maize seedling health and associated rhizosphere microbiome. *Scientific Reports,* 7**,** 1-13.

BENNETT, J. A., MAHERALI, H., REINHART, K. O., LEKBERG, Y., HART, M. M. & KLIRONOMOS, J. 2017. Plant-soil feedbacks and mycorrhizal type influence temperate forest population dynamics. *Science,* 355**,** 181-184.

BERDY, J. 2005. Bioactive microbial metabolites. *The Journal of Antibiotics,* 58**,** 1-26.

BERLANAS, C., BERBEGAL, M., ELENA, G., LAIDANI, M., CIBRIAIN, J. F., SAGÜES, A. & GRAMAJE, D. 2019a. The fungal and bacterial rhizosphere microbiome associated with grapevine rootstock genotypes in mature and young vineyards. *Frontiers in microbiology,* 10**,** 1142.

BERLANAS, C., BERBEGAL, M., ELENA, G., LAIDANI, M., CIBRIAIN, J. F., SAGÜES, A. & GRAMAJE, D. 2019b. The fungal and bacterial rhizosphere microbiome associated with grapevine rootstock genotypes in mature and young vineyards. *Frontiers in Microbiology,* 10**,** 1-16.

BEVIVINO, A. & DALMASTRI, C. 2017. Impact of agricultural land management on soil bacterial community: A case study in the mediterranean area. *In: Soil Biological Communities and Ecosystem Resilience.* New York, United States of America: *Springer*.

BOLGER, A. M., LOHSE, M. & USADEL, B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics,* 30**,** 2114-2120.

BOOMER, S. M., LODGE, D. P. & DUTTON, B. E. 2002. Bacterial diversity studies using the 16S rRNA gene provide a powerful research-based curriculum for molecular biology laboratory. *Microbiology Education,* 3**,** 18-25.

BOZIC, D., PAVLOVIC, D., BREGOLA, V., DI LORETO, A., BOSI, S. & VRBNICANIN, S. 2015. Gene flow from herbicide-resistant sunflower hybrids to weedy sunflower. *Journal of Plant Diseases and Protection,* 122**,** 183-188.

BRADER, G., COMPANT, S., VESCIO, K., MITTER, B., TROGNITZ, F., MA, L.-J. & SESSITSCH, A. 2017. Ecology and genomic insights into plant-pathogenic and plant-nonpathogenic endophytes. *Annual Review of Phytopathology,* 55**,** 61-83.

BRAGA, R. M., DOURADO, M. N. & ARAÚJO, W. L. 2016. Microbial interactions: ecology in a molecular perspective. *Brazilian Journal of Microbiology,* 47**,** 86-98.

BRAY, R. H. & KURTZ, L. T. 1945. Determination of total, organic and available forms of phosphorus in soils. *Soil Science,* 59**,** 39-46.

BRETZEL, F., VANNUCCHI, F., BENVENUTI, S. & RUMBLE, H. 2017. Biodiversity of flora and fauna. *Rooftop Urban Agriculture.* Springer.

BRINK, C., POSTMA, A. & JACOBS, K. 2017. Rhizobial diversity and function in rooibos (*Aspalathus linearis*) and honeybush (*Cyclopia* spp.) plants: A review. *South African Journal of Botany,* 110**,** 80-86.

BUKHAT, S., IMRAN, A., JAVAID, S., SHAHID, M., MAJEED, A. & NAQQASH, T. 2020. Communication of plants with microbial world: Exploring the regulatory networks for PGPR mediated defense signaling. *Microbiological Research,* 238**,** 126486.

BULGARELLI, D., GARRIDO-OTER, R., MÜNCH, P. C., WEIMAN, A., DRÖGE, J., PAN, Y., MCHARDY, A. C. & SCHULZE-LEFERT, P. 2015. Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell Host and Microbe,* 17**,** 392-403.

BULGARELLI, D., ROTT, M., SCHLAEPPI, K., VAN THEMAAT, E. V. L., AHMADINEJAD, N., ASSENZA, F., RAUF, P., HUETTEL, B., REINHARDT, R. & SCHMELZER, E. 2012. Revealing structure and assembly cues for Arabidopsis root-inhabiting bacterial microbiota. *Nature,* 488**,** 91-95.

BUSBY, P. E., SOMAN, C., WAGNER, M. R., FRIESEN, M. L., KREMER, J., BENNETT, A., MORSY, M., EISEN, J. A., LEACH, J. E. & DANGL, J. L. 2017. Research priorities for harnessing plant microbiomes in sustainable agriculture. *Public Library of Science Biology,* 15**,** e2001793.

CÁCERES, R., MALIŃSKA, K. & MARFÀ, O. 2018. Nitrification within composting: A review. *Waste Management,* 72**,** 119-137.

CALVO, P., ZEBELO, S., MCNEAR, D., KLOEPPER, J. & FADAMIRO, H. 2019. Plant growth-promoting rhizobacteria induce changes in *Arabidopsis thaliana* gene expression of nitrate and ammonium uptake genes. *Journal of Plant Interactions,* 14**,** 224-231.

CAMPBELL, C. D., CAMERON, C. M., BASTIAS, B. A., CHEN, C. & CAIRNEY, J. W. 2008. Long term repeated burning in a wet sclerophyll forest reduces fungal and bacterial biomass and responses to carbon substrates. *Soil Biology and Biochemistry,* 40**,** 2246-2252.

CANIZO, B. V., AGOSTINI, E., OLLER, A. L. W., DOTTO, G. L., VEGA, I. A. & ESCUDERO, L. B. 2019. Removal of crystal violet from natural water and effluents through biosorption on bacterial biomass isolated from rhizospheric soil. *Water, Air and Soil Pollution,* 230**,** 210.

CAPORASO, J. G., KUCZYNSKI, J., STOMBAUGH, J., BITTINGER, K., BUSHMAN, F. D., COSTELLO, E. K., FIERER, N., PEÑA, A. G., GOODRICH, J. K. & GORDON, J. I. 2010. QIIME allows analysis of high-throughput community sequencing data. *Nature Methods,* 7**,** 335-336.

CARRELL, A. A. & FRANK, C. 2015. Bacterial endophyte communities in the foliage of coast redwood and giant sequoia. *Frontiers in Microbiology,* 6**,** 1-11.

CARRIÓN, V. J., PEREZ-JARAMILLO, J., CORDOVEZ, V., TRACANNA, V., DE HOLLANDER, M., RUIZ-BUCK, D., MENDES, L. W., VAN IJCKEN, W. F., GOMEZ-EXPOSITO, R. & ELSAYED, S. S. 2019. Pathogen-induced activation of disease-suppressive functions in the endophytic root microbiome. *Science,* 366**,** 606-612.

CASSAN, F. & DIAZ-ZORITA. 2016. *Azospirillum* sp. in current agriculture: From the laboratory to the field. *Soil Biology and Biochemistry,* 103, 117-130.CASTELLE, C. J. & BANFIELD, J. F. 2018. Major new microbial groups expand diversity and alter our understanding of the tree of life. *Cell,* 172**,** 1181-1197.

CHADWICK, D., WEI, J., YAN'AN, T., GUANGHUI, Y., QIRONG, S. & QING, C. 2015. Improving manure nutrient management towards sustainable agricultural intensification in China. *Agriculture, Ecosystems & Environment,* 209**,** 34-46.

CHANG, Y.-C., REDDY, M. V., UMEMOTO, H., KONDO, S. & CHOI, D. 2020. Biodegradation of alkylphenols by rhizosphere microorganisms isolated from the roots of Hosta undulata. *Journal of Environmental Chemical Engineering,* 8**,** 103771.

CHAPARRO, J. M., BADRI, D. V. & VIVANCO, J. M. 2014. Rhizosphere microbiome assemblage is affected by plant development. *The ISME journal,* 8**,** 790-803.

CHATURVEDI, S., CHANDRA, R. & RAI, V. 2006. Isolation and characterization of Phragmites australis (L.) rhizosphere bacteria from contaminated site for bioremediation of colored distillery effluent. *Ecological Engineering,* 27**,** 202-207.

CHAUDHARY, T., DIXIT, M., GERA, R., SHUKLA, A. K., PRAKASH, A., GUPTA, G. & SHUKLA, P. 2020. Techniques for improving formulations of bioinoculants. *3 Biotechology,* 10**,** 1-9.

CHAUDHARY, T. & SHUKLA, P. 2019. Bioinoculants for bioremediation applications and disease resistance: innovative perspectives. *Indian Journal of Microbiology,* 59**,** 129-136.

CHAUHAN, H., BAGYARAJ, D., SELVAKUMAR, G. & SUNDARAM, S. 2015. Novel plant growth promoting rhizobacteria—Prospects and potential. *Applied Soil Ecology,* 95**,** 38-53.

CHÁVEZ-ROMERO, Y., NAVARRO-NOYA, Y. E., REYNOSO-MARTÍNEZ, S. C., SARRIA-GUZMÁN, Y., GOVAERTS, B., VERHULST, N., DENDOOVEN, L. & LUNA-GUIDO, M.

2016. 16S metagenomics reveals changes in the soil bacterial community driven by soil organic C, N-fertilizer and tillage-crop residue management. *Soil and Tillage Research,* 159**,** 1-8.

CHEN, L., WANG, T., ZHAO, M. & ZHANG, W. 2019a. Ethylene-responsive miRNAs in roots of *Medicago truncatula* identified by high-throughput sequencing at the whole genome level. *The Model Legume Medicago truncatula,* 27**,** 777-784.

CHEN, S., QI, G., LUO, T., ZHANG, H., JIANG, Q., WANG, R. & ZHAO, X. 2018. Continuous-cropping tobacco caused variance of chemical properties and structure of bacterial network in soils. *Land Degradation & Development,* 29**,** 4106-4120.

CHEN, S., WAGHMODE, T. R., SUN, R., KURAMAE, E. E., HU, C. & LIU, B. 2019b. Root-associated microbiomes of wheat under the combined effect of plant development and nitrogen fertilization. *Microbiome,* 7**,** 1-13.

CHEN, W.C., KO, C.H., SU, Y.S., LAI, W.-A. & SHEN, F.T. 2021. Metabolic potential and community structure of bacteria in an organic tea plantation. *Applied Soil Ecology*, 157**,** 103762.

CHEN, Y., WANG, W., ZHOU, D., JING, T., LI, K., ZHAO, Y., TANG, W., QI, D., ZHANG, M., ZANG, X., LUO, Y. & XIE, J. 2020. Biodegradation of lignocellulosic agricultural residues by a newly isolated *Fictibacillus* sp. YS-26 improving carbon metabolic properties and functional diversity of the rhizosphere microbial community. *Bioresource Technology,* 310**,** 123381.

CHENNIAPPAN, C., NARAYANASAMY, M., DANIEL, G., RAMARAJ, G., PONNUSAMY, P., SEKAR, J. & RAMALINGAM, P. V. 2019. Biocontrol efficiency of native plant growth promoting rhizobacteria against rhizome rot disease of turmeric. *Biological Control,* 129**,** 55-64.

CHUKWUNEME, C. F., AYANGBENRO, A. S., BABALOLA, O. O. & KUTU, F. R. 2021. Functional diversity of microbial communities in two contrasting maize rhizosphere soils. *Rhizosphere,* 17**,** 100282.

CLARKE, K. & GREEN, R. 1988. Statistical design and analysis for a'biological effects' study. *Marine Ecology Progress Series***,** 213-226.

COLOMBO, F., MACDONALD, C. A., JEFFRIES, T. C., POWELL, J. R. & SINGH, B. K. 2016. Impact of forest management practices on soil bacterial diversity and consequences for soil processes. *Soil Biology and Biochemistry,* 94**,** 200-210.

Colombo, F., Paolo, D., Cominelli, E., Sparvoli, F., Nielsen, E. & Pilu, R. 2020. MRP transporters and low phytic acid mutants in major crops: Main pleiotropic effects and future perspectives. *Frontiers in Plant Science*, 11, p.1301.

CORDERO, J., DE FREITAS, J. R. & GERMIDA, J. J. 2020. Bacterial microbiome associated with the rhizosphere and root interior of crops in Saskatchewan, Canada. *Canadian Journal of Microbiology,* 66**,** 71-85.

CORREA-GARCÍA, S., RHEAULT, K., TREMBLAY, J., SÉGUIN, A. & YERGEAU, E. 2020. Soil characteristics constrain the response of microbial communities and associated hydrocarbon degradation genes during phytoremediation. *Applied and Environmental Microbiology*.

COSTA, O. Y., PIJL, A. & KURAMAE, E. E. 2020. Dynamics of active potential bacterial and fungal interactions in the assimilation of acidobacterial EPS in soil. *Soil Biology and Biochemistry,* 148**,** 107916.

COUËDEL, A., ALLETTO, L. & JUSTES, É. 2018. Crucifer-legume cover crop mixtures provide effective sulphate catch crop and sulphur green manure services. *Plant and Soil,* 426**,** 61-76.

CRAFT, C., SENECA, E. & BROOME, S. 1991. Loss on ignition and Kjeldahl digestion for estimating organic carbon and total nitrogen in estuarine marsh soils: calibration with dry combustion. *Estuaries,* 14**,** 175-179.

CRAWFORD, K. M. & KNIGHT, T. M. 2017. Competition overwhelms the positive plant–soil feedback generated by an invasive plant. *Oecologia,* 183**,** 211-220.

CREAMER, R., STONE, D., BERRY, P. & KUIPER, I. 2016. Measuring respiration profiles of soil microbial communities across Europe using MicroResp™ method. *Applied Soil Ecology*, 97**,** 36-43.

CREGGER, M., VEACH, A., YANG, Z., CROUCH, M., VILGALYS, R., TUSKAN, G. & SCHADT, C. 2018. The Populus holobiont: dissecting the effects of plant niches and genotype on the microbiome. *Microbiome,* 6**,** 1-14.

CUI, Y., BING, H., FANG, L., WU, Y., YU, J., SHEN, G., JIANG, M., WANG, X. & ZHANG, X. 2019. Diversity patterns of the rhizosphere and bulk soil microbial communities along an altitudinal gradient in an alpine ecosystem of the eastern Tibetan Plateau. *Geoderma,* 338**,** 118-127.

DARYANTO, S., WANG, L. & JACINTHE, P.-A. 2015. Global synthesis of drought effects on food legume production. *PloS One,* 10**,** e0127401.

DAVAMANI, V., PARAMESWARI, E. & ARULMANI, S. 2020. Mitigation of methane gas emissions in flooded paddy soil through the utilization of methanotrophs. *Science of The Total Environment,* 726**,** 138570.

DE LUNA, L. Z., STUBBS, T. L., KENNEDY, A. C. & KREMER, R. J. 2005. Deleterious bacteria in the rhizosphere. *roots and soil management: interactions between roots and the soil,* 48**,** 233-261.

DEKE, A. L., ADUGNA, W. T. & FITE, A. T. 2016. Soil physic-chemical properties in termite mounds and adjacent control soil in Miyo and Yabello Districts of Borana Zone, Southern Ethiopia. *American Journal of Agriculture and Forestry,* 4**,** 69-74.

DELAVAUX, C. S., SMITH-RAMESH, L. M. & KUEBBING, S. E. 2017. Beyond nutrients: a meta-analysis of the diverse effects of arbuscular mycorrhizal fungi on plants and soils. *Ecology,* 98**,** 2111-2119.

DELMONT, T. O., EREN, A. M., MACCARIO, L., PRESTAT, E., ESEN, Ö. C., PELLETIER, E., LE PASLIER, D., SIMONET, P. & VOGEL, T. M. 2015. Reconstructing rare soil microbial genomes using in situ enrichments and metagenomics. *Frontiers in Microbiology,* 6**,** 1-15.

DENG, H., GE, L., XU, T., ZHANG, M., WANG, X., ZHANG, Y. & PENG, H. 2011. Analysis of the Metabolic Utilization of Carbon Sources and Potential Functional Diversity of the Bacterial Community in Lab-Scale Horizontal Subsurface-Flow Constructed Wetlands. *Journal of environmental quality,* 40**,** 1730-1736.

DENG, S., WIPF, H. M.-L., PIERROZ, G., RAAB, T. K., KHANNA, R. & COLEMAN-DERR, D. 2019. A plant growth-promoting microbial soil amendment dynamically alters the strawberry root bacterial microbiome. *Scientific Reports,* 9**,** 1-15.

DENNIS, P. G., MILLER, A. J. & HIRSCH, P. R. 2010. Are root exudates more important than other sources of rhizodeposits in structuring rhizosphere bacterial communities? *FEMS Microbiology Ecology,* 72**,** 313-327.

DHALIWAL, S., NARESH, R., MANDAL, A., SINGH, R. & DHALIWAL, M. 2019. Dynamics and transformations of micronutrients in agricultural soils as influenced by organic matter build-up: A review. *Environmental and Sustainability Indicators,* 1**,** 100007.

DI SALVO, L. P., FERRANDO, L., FERNÁNDEZ-SCAVINO, A. & DE SALAMONE, I. E. G. 2018. Microorganisms reveal what plants do not: wheat growth and rhizosphere microbial communities after *Azospirillum brasilense* inoculation and nitrogen fertilization under field conditions. *Plant and Soil,* 424**,** 405-417.

DIMKPA, C., MERTEN, D., SVATOŠ, A., BÜCHEL, G. & KOTHE, E. 2009a. Siderophores mediate reduced and increased uptake of cadmium by Streptomyces tendae F4 and sunflower (Helianthus annuus), respectively. *Journal of Applied Microbiology,* 107**,** 1687-1696.

DIMKPA, C., WEINAND, T. & ASCH, F. 2009b. Plant–rhizobacteria interactions alleviate abiotic stress conditions. *Plant, Cell and Environment,* 32**,** 1682-1694.

DINSDALE, E., EDWARDS, R., HALL, D., ANGLY, F., BREITBART, M., BRULC, J., FURLAN, M. & DESNUES, C. 2008. Functional metagenomic profiling of nine biomes. *Nature,* 452**,** 629-32.

DOBROVOLSKAYA, T., GOLOVCHENKO, A., LYSAK, L. & YURCHENKO, E. 2020. Taxonomic structure of bacterial communities of rhizospheric soil under bogs' plants. *Moscow University Soil Science Bulletin,* 75**,** 93-100.

DONOVAN, P. D., GONZALEZ, G., HIGGINS, D. G., BUTLER, G. & ITO, K. 2018. Identification of fungi in shotgun metagenomics datasets. *Public Library of Science One,* 13**,** 1-16.

DOS SANTOS, J. J. & MARANHO, L. T. 2018. Rhizospheric microorganisms as a solution for the recovery of soils contaminated by petroleum: A review. *Journal of Environmental Management,* 210**,** 104-113.

DOU, T.Y., CHEN, J., LIU, W.J. & WANG, L. 2020a. Isolation and subunit structure of the xylanosome complex produced by Actinotalea fermentans JCM9966. *Biotechnology Letters,* 42**,** 143-149.

DOU, T.Y., LIU, W.J. & CHEN, J. 2020b. Isolation, substrate specificity and subunit characterization of the Xylanosomes Produced by *Oerskovia turbata* JCM 3160. *Current Microbiology***,** 1-7.

DRÖGE, J., GREGOR, I. & MCHARDY, A. C. 2015. Taxator-tk: precise taxonomic assignment of metagenomes by fast approximation of evolutionary neighborhoods. *Bioinformatics,* 31**,** 817-824.

DUŽÍ, B., FRANTÁL, B. & ROJO, M. S. 2017. The geography of urban agriculture: New trends and challenges. *Moravian Geographical Reports,* 25**,** 130-138.

DUŽÍ, B., TÓTH, A., BIHUŇOVÁ, M. & STOJANOV, R. 2014. Challenges of urban agriculture: highlights on the Czech and Slovak Republic specifics. *Current Challenges of Central Europe: Society and Environment***,** 82-107.

EARLE, S. G., WU, C.-H., CHARLESWORTH, J., STOESSER, N., GORDON, N. C., WALKER, T. M., SPENCER, C. C., IQBAL, Z., CLIFTON, D. A. & HOPKINS, K. L. 2016. Identifying lineage effects when controlling for population structure improves power in bacterial association studies. *Nature Microbiology,* 1**,** 1-8.

EDGAR, R. 2010. Usearch. Lawrence Berkeley National Lab.(LBNL), Berkeley, CA (United States).

EDGAR, R. C., HAAS, B. J., CLEMENTE, J. C., QUINCE, C. & KNIGHT, R. 2011. UCHIME improves sensitivity and speed of chimera detection. *Bioinformatics,* 27**,** 2194-2200.

EDWARDS, J., JOHNSON, C., SANTOS-MEDELLÍN, C., LURIE, E., PODISHETTY, N. K., BHATNAGAR, S., EISEN, J. A. & SUNDARESAN, V. 2015. Structure, variation and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences,* 112**,** E911-E920.

EL-FOULY, M. M., FAWZI, A., ABOU EL-NOUR, E., ZEIDAN, M. & FIRGANY, A. 2015. Impact of long-term intensive cropping under continuous tillage and unbalanced use of fertilizers on soil nutrient contents in a small holding village. *African Journal of Agricultural Research,* 10**,** 4850-4857.

ELMAGZOB, A. A. H., IBRAHIM, M. M. & ZHANG, G.-F. 2019. Seasonal Diversity of endophytic bacteria associated with *Cinnamomum camphora* (L.) Presl. *Diversity,* 11**,** 1-15.

EMMS, D. M. & KELLY, S. 2019. OrthoFinder: phylogenetic orthology inference for comparative genomics. *Genome Biology,* 20**,** 1-14.

ENAGBONMA, B. J., AJILOGBA, C. F. & BABALOLA, O. O. 2020. Metagenomic profiling of bacterial diversity and community structure in termite mounds and surrounding soils. *Archives of Microbiology,* 202**,** 2697-2709.

ENAGBONMA, B. J. & BABALOLA, O. O. 2019. Potentials of termite mound soil bacteria in ecosystem engineering for sustainable agriculture. *Annals of Microbiology,* 69**,** 211-219.

ENAGBONMA, B. J. & BABALOLA, O. O. 2020. Unveiling plant-beneficial function as seen in bacteria genes from termite mound soil. *Journal of Soil Science and Plant Nutrition,* 20**,** 421-430.

ENEBE, M. C. & BABALOLA, O. O. 2018. The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: a survival strategy. *Applied Microbiology and Biotechnology,* 102**,** 7821-7835.

ENEBE, M. C. & BABALOLA, O. O. 2021. Soil fertilization affects the abundance and distribution of carbon and nitrogen cycling genes in the maize rhizosphere. *AMB Express,* 11**,** 1-10.

ESMAEILZADEH, J. & AHANGAR, A. G. 2014. Influence of soil organic matter content on soil physical, chemical and biological properties. *International Journal of Plant, Animal and Environmental Sciences,* 4**,** 244-252.

ETESAMI, H. & MAHESHWARI, D. K. 2018. Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: action mechanisms and future prospects. *Ecotoxicology and Environmental Safety,* 156**,** 225-246.

FABIAŃSKA, I., GERLACH, N., ALMARIO, J. & BUCHER, M. 2019. Plant-mediated effects of soil phosphorus on the root-associated fungal microbiota in *Arabidopsis thaliana*. *New Phytologist,* 221**,** 2123-2137.

FADIJI, A. E. & BABALOLA, O. O. 2020. Metagenomics methods for the study of plant-associated microbial communities: A review. *Journal of Microbiological Methods,* 170**,** 1-13.

FAN, K., WEISENHORN, P., GILBERT, J. A., SHI, Y., BAI, Y. & CHU, H. 2018. Soil pH correlates with the co-occurrence and assemblage process of diazotrophic communities in rhizosphere and bulk soils of wheat fields. *Soil Biology and Biochemistry,* 121**,** 185-192.

FAROUK, F., EL SHIMY, R., ABDEL-MOTALEB, A., ESSAM, S. & AZZAZY, H. M. 2020. Detection of Acinetobacter baumannii in fresh produce using modified magnetic nanoparticles and PCR. *Analytical Biochemistry,* 609**,** 113890.

FERNÁNDEZ-LUQUEÑO, F., LÓPEZ-VALDEZ, F., MIRANDA-ARÁMBULA, M., ROSAS-MORALES, M., PARIONA, N. & ESPINOZA-ZAPATA, R. 2014. An introduction to the sunflower crop. *Book: Sunflowers: Growth and Development, Environmental Influences and Pests/Diseases***,** 1-18.

FILIPPIDOU, S., JUNIER, T., WUNDERLIN, T., LO, C.-C., LI, P.-E., CHAIN, P. S. & JUNIER, P. 2015. Under-detection of endospore-forming Firmicutes in metagenomic data. *Computational and Structural Biotechnology Journal,* 13**,** 299-306.

FILIPPIDOU, S., WUNDERLIN, T., JUNIER, T., JEANNERET, N., DORADOR, C., MOLINA, V., JOHNSON, D. R. & JUNIER, P. 2016. A combination of extreme environmental conditions favor the prevalence of endospore-forming firmicutes. *Frontiers in Microbiology,* 7**,** 1707.

FINZI, A. C., ABRAMOFF, R. Z., SPILLER, K. S., BRZOSTEK, E. R., DARBY, B. A., KRAMER, M. A. & PHILLIPS, R. P. 2015. Rhizosphere processes are quantitatively important components of terrestrial carbon and nutrient cycles. *Global Change Biology,* 21**,** 2082-2094.

FOLEY, J. A., DEFRIES, R., ASNER, G. P., BARFORD, C., BONAN, G., CARPENTER, S. R., CHAPIN, F. S., COE, M. T., DAILY, G. C. & GIBBS, H. K. 2005. Global consequences of land use. *Science,* 309**,** 570-574.

FONSECA-GARCÍA, C., COLEMAN-DERR, D., GARRIDO, E., VISEL, A., TRINGE, S. G. & PARTIDA-MARTÍNEZ, L. P. 2016. The cacti microbiome: interplay between habitat-filtering and host-specificity. *Frontiers in Microbiology,* 7**,** 1-16.

FUJII, K., SHIBATA, M., KITAJIMA, K., ICHIE, T., KITAYAMA, K. & TURNER, B. L. 2018. Plant–soil interactions maintain biodiversity and functions of tropical forest ecosystems. *Ecological Research,* 33**,** 149-160.

FUKAMI, J., NOGUEIRA, M. A., ARAUJO, R. S. & HUNGRIA, M. 2016. Accessing inoculation methods of maize and wheat with *Azospirillum brasilense*. *Annals of Microbiology Express,* 6**,** 1-13.

GARCIA-MAZCORRO, J. F., KAWAS, J. R. & MARROQUIN-CARDONA, A. G. 2019. Descriptive bacterial and fungal characterization of propolis using ultra-high-throughput marker gene sequencing. *Insects,* 10**,** 402.

GARCÍA-SALAMANCA, A., MOLINA-HENARES, M. A., VAN DILLEWIJN, P., SOLANO, J., PIZARRO-TOBÍAS, P., ROCA, A., DUQUE, E. & RAMOS, J. L. 2013. Bacterial diversity in the rhizosphere of maize and the surrounding carbonate-rich bulk soil. *Microbial Biotechnology,* 6**,** 36-44.

GARRIDO-OTER, R., NAKANO, R. T., DOMBROWSKI, N., MA, K.-W., TEAM, T. A., MCHARDY, A. C. & SCHULZE-LEFERT, P. 2018. Modular traits of the rhizobiales root microbiota and their evolutionary relationship with symbiotic rhizobia. *Cell Host and Microbe,* 24**,** 155-167. e5.

GASMI, A., PEANA, M., ARSHAD, M., BUTNARIU, M., MENZEL, A. & BJØRKLUND, G. 2021. Krebs cycle: activators, inhibitors and their roles in the modulation of carcinogenesis. *Archives of Toxicology***,** 1-18.

GASTAL, F., LEMAIRE, G., DURAND, J.-L. & LOUARN, G. 2015. Quantifying crop responses to nitrogen and avenues to improve nitrogen-use efficiency. *Crop Physiology.* Elsevier.

GENTSCH, N., BOY, J., BATALLA, J. D. K., HEUERMANN, D., VON WIRÉN, N., SCHWENEKER, D., FEUERSTEIN, U., GROß, J., BAUER, B. & REINHOLD-HUREK, B. 2020. Catch crop diversity increases rhizosphere carbon input and soil microbial biomass. *Biology and Fertility of soils*, 56**,** 943-957.

GHORI, Z., IFTIKHAR, H., BHATTI, M. F., SHARMA, I., KAZI, A. G. & AHMAD, P. 2016. Phytoextraction: the use of plants to remove heavy metals from soil. *Plant metal interaction.* United States of America: *Elsevier*.

GHOSH, S., AHMAD, R., GAUTAM, V. K. & KHARE, S. K. 2018. Cholesterol-oxidase-magnetic nanobioconjugates for the production of 4-cholesten-3-one and 4-cholesten-3, 7-dione. *Bioresource Technology*, 254**,** 91-96.

GIANOULIS, T. A., RAES, J., PATEL, P. V., BJORNSON, R., KORBEL, J. O., LETUNIC, I., YAMADA, T., PACCANARO, A., JENSEN, L. J. & SNYDER, M. 2009. Quantifying environmental adaptation of metabolic pathways in metagenomics. *Proceedings of the National Academy of Sciences*, 106**,** 1374-1379.

GKARMIRI, K., MAHMOOD, S., EKBLAD, A., ALSTRÖM, S., HÖGBERG, N. & FINLAY, R. 2017a. Identifying the active microbiome associated with roots and rhizosphere soil of oilseed rape. *Applied and Environmental Microbiology*, 83**,** e01938-17.

GKARMIRI, K., MAHMOOD, S., EKBLAD, A., ALSTRÖM, S., HÖGBERG, N. & FINLAY, R. 2017b. Identifying the active microbiome associated with roots and rhizosphere soil of oilseed rape. *Applied and Environmental Microbiology,* 83**,** 1-14.

GOEL, R., SUYAL, D. C., DASH, B. & SONI, R. 2017. *Soil Metagenomics: A tool for sustainable agriculture*. *In: Mining of Microbial Wealth and MetaGenomics.* New York City, United States of America: *Springer*.

GOMES, E. A., LANA, U. G., QUENSEN, J. F., DE SOUSA, S. M., OLIVEIRA, C. A., GUO, J., GUIMARÃES, L. J. & TIEDJE, J. M. 2018. Root-associated microbiome of maize genotypes with contrasting phosphorus use efficiency. *Phytobiomes,* 2**,** 129-137.

GÓMEZ EXPÓSITO, R., DE BRUIJN, I., POSTMA, J. & RAAIJMAKERS, J. M. 2017a. Current insights into the role of rhizosphere bacteria in disease suppressive soils. *Frontiers in Microbiology,* 8**,** 2529.

GÓMEZ EXPÓSITO, R., DE BRUIJN, I., POSTMA, J. & RAAIJMAKERS, J. M. 2017b. Current insights into the role of rhizosphere bacteria in disease suppressive soils. *Frontiers in Microbiology,* 8**,** 1-12.

GOODWIN, S., MCPHERSON, J. D. & MCCOMBIE, W. R. 2016. Coming of age: ten years of next-generation sequencing technologies. *Nature Reviews Genetics,* 17**,** 333.

GOPALAKRISHNAN, S., SATHYA, A., VIJAYABHARATHI, R., VARSHNEY, R. K., GOWDA, C. L. & KRISHNAMURTHY, L. 2015. Plant growth promoting rhizobia: challenges and opportunities. *3 Biotech,* 5**,** 355-377.

GRAFTON, R. Q., DAUGBJERG, C. & QURESHI, M. E. 2015. Towards food security by 2050. *Food Security,* 7**,** 179-183.

GRANJOU, C. & PHILLIPS, C. 2019. Living and labouring soils: metagenomic ecology and a new agricultural revolution? *BioSocieties,* 14**,** 393-415.

GRAY, D. A., DUGAR, G., GAMBA, P., STRAHL, H., JONKER, M. J. & HAMOEN, L. W. 2019. Extreme slow growth as alternative strategy to survive deep starvation in bacteria. *Nature communications,* 10**,** 1-12.

GROBELAK, A., KOKOT, P., HUTCHISON, D., GROSSER, A. & KACPRZAK, M. 2018. Plant growth-promoting rhizobacteria as an alternative to mineral fertilizers in assisted bioremediation-sustainable land and waste management. *Journal of Environmental Management,* 227**,** 1-9.

GUERRA, A. B., OLIVEIRA, J. S., SILVA-PORTELA, R. C., ARAÚJO, W., CARLOS, A. C., VASCONCELOS, A. T. R., FREITAS, A. T., DOMINGOS, Y. S., DE FARIAS, M. F. & FERNANDES, G. J. T. 2018. Metagenome enrichment approach used for selection of oil-degrading bacteria consortia for drill cutting residue bioremediation. *Environmental Pollution,* 235**,** 869-880.

GUNINA, A. & KUZYAKOV, Y. 2015. Sugars in soil and sweets for microorganisms: review of origin, content, composition and fate. *Soil Biology and Biochemistry,* 90**,** 87-100.

GUPTA, N., VATS, S. & BHARGAVA, P. 2018. Sustainable agriculture: role of metagenomics and metabolomics in exploring the soil microbiota. *In: Silico Approach for Sustainable Agriculture.* New York City, United States of America: *Springer*.

GUTIERREZ BOEM, F. H., RUBIO, G. & BARBERO, D. 2011. Soil phosphorus extracted by Bray 1 and Mehlich 3 soil tests as affected by the soil/solution ratio in Mollisols. *Communications in soil science and plant analysis,* 42**,** 220-230.

HACQUARD, S. & SCHADT, C. W. 2015. Towards a holistic understanding of the beneficial interactions across the Populus microbiome. *New Phytologist,* 205**,** 1-7.

HAMEL, C., HANSON, K., SELLES, F., CRUZ, A. F., LEMKE, R., MCCONKEY, B. & ZENTNER, R. 2006. Seasonal and long-term resource-related variations in soil microbial communities in wheat-based rotations of the Canadian prairie. *Soil Biology and Biochemistry,* 38**,** 2104-2116.

HAMID, Y., TANG, L., SOHAIL, M. I., CAO, X., HUSSAIN, B., AZIZ, M. Z., USMAN, M., HE, Z.L. & YANG, X. 2019. An explanation of soil amendments to reduce cadmium phytoavailability and transfer to food chain. *Science of The Total Environment,* 660**,** 80-96.

HAMMER, Ø., HARPER, D. A. & RYAN, P. D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica,* 4**,** 1-9.

HANDELSMAN, J. 2004. Metagenomics: application of genomics to uncultured microorganisms. *Microbiology and Molecular Biology Reviews,* 68**,** 669-685.

HANDELSMAN, J., RONDON, M. R., BRADY, S. F., CLARDY, J. & GOODMAN, R. M. 1998. Molecular biological access to the chemistry of unknown soil microbes: a new frontier for natural products. *Chemistry and Biology,* 5**,** R245-R249.

HANNAM, K., QUIDEAU, S. & KISHCHUK, B. 2006. Forest floor microbial communities in relation to stand composition and timber harvesting in northern Alberta. *Soil Biology and Biochemistry,* 38**,** 2565-2575.

HANSON, C. A., FUHRMAN, J. A., HORNER-DEVINE, M. C. & MARTINY, J. B. 2012. Beyond biogeographic patterns: processes shaping the microbial landscape. *Nature Reviews Microbiology,* 10**,** 497-506.

HARUN, R., DANQUAH, M. K. & FORDE, G. M. 2010. Microalgal biomass as a fermentation feedstock for bioethanol production. *Journal of Chemical Technology and Biotechnology,* 85**,** 199-203.

HASSAN, W., BASHIR, S., ALI, F., IJAZ, M., HUSSAIN, M. & DAVID, J. 2016. Role of ACC-deaminase and/or nitrogen fixing rhizobacteria in growth promotion of wheat (*Triticum aestivum* L.) under cadmium pollution. *Environmental Earth Sciences,* 75**,** 267.

HASSEN, A.I. 2009. Efficacy of rhizobacteria for growth promotion and biocontrol of *Pythium ultimum* and *Fusarium oxysporum* on sorghum in Ethiopia and South Africa. University of Pretoria, South Africa.HE, J., XU, Z. & HUGHES, J. 2006. Molecular bacterial diversity of a forest soil under residue management regimes in subtropical Australia. *FEMS Microbiology Ecology,* 55**,** 38-47.

HEAD, I., SAUNDERS, J. & PICKUP, R. W. 1998. Microbial evolution, diversity and ecology: a decade of ribosomal RNA analysis of uncultivated microorganisms. *Microbial Ecology,* 35**,** 1-21.

HOLLISTER, E. B., BROOKS, J. P. & GENTRY, T. J. 2015. Bioinformation and'omic approaches for characterization of environmental microorganisms. *Environmental Microbiology.* Elsevier.

HRISTEVA, T. H. & DENEV, I. D. 2017. Changes at the rhizosphere microbiota of the sunflower–*Orobanche cumana* Wallr pathosystem. *International Journal of Current Microbiology and Applied Science,* 6**,** 733-746.

HU, A., JU, F., HOU, L., LI, J., YANG, X., WANG, H., MULLA, S. I., SUN, Q., BÜRGMANN, H. & YU, C. P. 2017. Strong impact of anthropogenic contamination on the co-occurrence patterns of a riverine microbial community. *Environmental Microbiology,* 19**,** 4993-5009.

HU, L., ROBERT, C. A., CADOT, S., ZHANG, X., YE, M., LI, B., MANZO, D., CHERVET, N., STEINGER, T. & VAN DER HEIJDEN, M. G. 2018. Root exudate metabolites drive plant-soil feedbacks on growth and defense by shaping the rhizosphere microbiota. *Nature Communications,* 9**,** 1-13.

HU, Z.Y., WANG, Y.Z., IM, W.T., WANG, S.Y., ZHAO, G.P., ZHENG, H.J. & QUAN, Z.X. 2014. The first complete genome sequence of the class *Fimbriimonadia* in the phylum *Armatimonadetes*. *PLoS One,* 9**,** e100794.

HUANG, X.F., CHAPARRO, J. M., REARDON, K. F., ZHANG, R., SHEN, Q. & VIVANCO, J. M. 2014. Rhizosphere interactions: root exudates, microbes and microbial communities. *Botany,* 92**,** 267-275.

HUGENHOLTZ, P., GOEBEL, B. M. & PACE, N. R. 1998. Impact of culture-independent studies on the emerging phylogenetic view of bacterial diversity. *Journal of Bacteriology,* 180**,** 4765-4774.

HWANG, K.S., KIM, H. U., CHARUSANTI, P., PALSSON, B. Ø. & LEE, S. Y. 2014. Systems biology and biotechnology of Streptomyces species for the production of secondary metabolites. *Biotechnology Advances,* 32**,** 255-268.

HYEON, J. E., YOU, S. K., KANG, D. H., RYU, S.H., KIM, M., LEE, S.S. & HAN, S. O. 2014. Enzymatic degradation of lignocellulosic biomass by continuous process using laccase and cellulases with the aid of scaffoldin for ethanol production. *Process Biochemistry,* 49**,** 1266-1273.

ICHIHASHI, Y., DATE, Y., SHINO, A., SHIMIZU, T., SHIBATA, A., KUMAISHI, K., FUNAHASHI, F., WAKAYAMA, K., YAMAZAKI, K. & UMEZAWA, A. 2020. Multi-omics analysis on an agroecosystem reveals the significant role of organic nitrogen to increase agricultural crop yield. *Proceedings of the National Academy of Sciences,* 117**,** 14552-14560.

IGIEHON, N. O. & BABALOLA, O. O. 2018a. Below-ground-above-ground plant-microbial interactions: focusing on soybean, rhizobacteria and mycorrhizal fungi. *The Open Microbiology Journal,* 12**,** 261.

IGIEHON, N. O. & BABALOLA, O. O. 2018b. Rhizosphere microbiome modulators: contributions of nitrogen fixing bacteria towards sustainable agriculture. *International Journal of Environmental Research and Public Health,* 15**,** 1-25.

IGIEHON, N. O. & BABALOLA, O. O. 2019. Fungal bio-sorption potential of chromium in Norkrans liquid medium by shake flask technique. *Journal of Basic Microbiology,* 59**,** 62-73.

IGIEHON, N. O., BABALOLA, O. O. & AREMU, B. R. 2019. Genomic insights into plant growth promoting rhizobia capable of enhancing soybean germination under drought stress. *BioMedCentral Microbiology,* 19**,** 1-22.

IGIEHON, N. O., BABALOLA, O. O., CHESETO, X. & TORTO, B. 2020. Effects of rhizobia and arbuscular mycorrhizal fungi on yield, size distribution and fatty acid of soybean seeds grown under drought stress. *Microbiological Research,* 242**,** 1-16.

IGIEHON, O. N. 2015. Bioremediation potentials of Heterobasidion annosum 13.12 B and Resinicium bicolor in diesel oil contaminated soil microcosms. *Journal of Applied Sciences and Environmental Management,* 19**,** 513-519.

ITELIMA, J., BANG, W., ONYIMBA, I. & OJ, E. 2018. A review: biofertilizer; a key player in enhancing soil fertility and crop productivity. *Journal Microbiology and Biotechnology Reports,* 2**,** 22-28.

JACOBY, R., PEUKERT, M., SUCCURRO, A., KOPRIVOVA, A. & KOPRIVA, S. 2017. The role of soil microorganisms in plant mineral nutrition—current knowledge and future directions. *Frontiers in Plant Science,* 8**,** 1617.

JADHAV, R. K. & ANIL, L. 2020. Attempt of Costus arbuscular mycorrhizal inoculants association with leguminous foliage whey on chlorophyll of sunflower (*Helianthus annus* L.) plants. *Journal of Plant Stress Physiology,* 6**,** 18-23.

JANSSON, J. K. & HOFMOCKEL, K. S. 2018. The soil microbiome—from metagenomics to metaphenomics. *Current Opinion in Microbiology,* 43**,** 162-168.

JEYANTHI, V. & KANIMOZHI, S. 2018. Plant growth promoting rhizobacteria (PGPR)–prospective and mechanisms: A review. *Journal of Pure and Applied Microbiology,* 12**,** 733-749.JIANG, L., SONG, M., YANG, L., ZHANG, D., SUN, Y., SHEN, Z., LUO, C. & ZHANG, G. 2016. Exploring the influence of environmental factors on bacterial communities within the rhizosphere of the Cu-tolerant plant, *Elsholtzia splendens*. *Scientific Reports,* 6**,** 36302.

JIANG, Z., LIU, Y., YANG, J., BROOKES, P. C. & GUNINA, A. 2021. Rhizosphere priming regulates soil organic carbon and nitrogen mineralization: The significance of abiotic mechanisms. *Geoderma,* 385**,** 114877.

JIAO, S., LI, Q., ZAI, X., GAO, X., WEI, G. & CHEN, W. 2019. Complexity of bacterial communities within the rhizospheres of legumes drives phenanthrene degradation. *Geoderma,* 353**,** 1-10.

JIMÉNEZ, J. A., NOVINSCAK, A. & FILION, M. 2020. Inoculation with the plant-growth-promoting rhizobacterium Pseudomonas fluorescens LBUM677 impacts the rhizosphere microbiome of three oilseed crops. *Frontiers in microbiology,* 11**,** 2534.

JOHNSON, J. S., SPAKOWICZ, D. J., HONG, B.-Y., PETERSEN, L. M., DEMKOWICZ, P., CHEN, L., LEOPOLD, S. R., HANSON, B. M., AGRESTA, H. O. & GERSTEIN, M. 2019. Evaluation of 16S rRNA gene sequencing for species and strain-level microbiome analysis. *Nature communications,* 10**,** 1-11.

JONES, D. L., HILL, P., SMITH, A., FARRELL, M., GE, T., BANNING, N. & MURPHY, D. 2018. Role of substrate supply on microbial carbon use efficiency and its role in interpreting soil microbial community-level physiological profiles (CLPP). *Soil Biology and Biochemistry,* 123**,** 1-6.

JU, W., JIN, X., LIU, L., SHEN, G., ZHAO, W., DUAN, C. & FANG, L. 2020. Rhizobacteria inoculation benefits nutrient availability for phytostabilization in copper contaminated soil: drivers from bacterial community structures in rhizosphere. *Applied Soil Ecology,* 150**,** 1-13.

JÜNEMANN, S., KLEINBÖLTING, N., JAENICKE, S., HENKE, C., HASSA, J., NELKNER, J., STOLZE, Y., ALBAUM, S. P., SCHLÜTER, A. & GOESMANN, A. 2017. Bioinformatics for Next Generation Sequencing based metagenomics and the application to biogas research. *Journal of Biotechnology,* 261**,** 10-23.

KALAIMURUGAN, D., BALAMURALIKRISHNAN, B., DURAIRAJ, K., VASUDHEVAN, P., SHIVAKUMAR, M., KAUL, T., CHANG, S., RAVINDRAN, B. & VENKATESAN, S. 2020. Isolation and characterization of heavy-metal-resistant bacteria and their applications in environmental bioremediation. *International Journal of Environmental Science and Technology,* 17**,** 1455-1462.

KAMUTANDO, C. N., VIKRAM, S., KAMGAN-NKUEKAM, G., MAKHALANYANE, T. P., GREVE, M., LE ROUX, J. J., RICHARDSON, D. M., COWAN, D. A. & VALVERDE, A. 2019.

The functional potential of the rhizospheric microbiome of an invasive tree species, *Acacia dealbata*. *Microbial Ecology,* 77**,** 191-200.

KANG, D., YU, T., XU, D., ZENG, Z., DING, A., ZHANG, M., SHAN, S., ZHANG, W. & ZHENG, P. 2019. The anammox process at typical feast-famine states: Reactor performance, sludge activity and microbial community. *Chemical Engineering Journal,* 370**,** 110-119.

KARI, A., NAGYMÁTÉ, Z., ROMSICS, C., VAJNA, B., KUTASI, J., PUSPÁN, I., KÁRPÁTI, É., KOVÁCS, R. & MÁRIALIGETI, K. 2019. Monitoring of soil microbial inoculants and their impact on maize (*Zea mays* L.) rhizosphere using T-RFLP molecular fingerprint method. *Applied Soil Ecology,* 138**,** 233-244.

KERKHOF, L. J., DILLON, K. P., HÄGGBLOM, M. M. & MCGUINNESS, L. R. 2017. Profiling bacterial communities by MinION sequencing of ribosomal operons. *Microbiome,* 5**,** 1-11.

KESWANI, C., DILNASHIN, H., BIRLA, H. & SINGH, S. P. 2019. Regulatory barriers to agricultural research commercialization: A case study of biopesticides in India. *Rhizosphere,* 11**,** 1-4.

KETTLER, T., DORAN, J. W. & GILBERT, T. 2001. Simplified method for soil particle-size determination to accompany soil-quality analyses. *Soil Science Society of America Journal,* 65**,** 849-852.

KHAN, A. R., PERVEZ, M. T., BABAR, M. E., NAVEED, N. & SHOAIB, M. 2018a. A comprehensive study of de novo genome assemblers: current challenges and future prospective. *Evolutionary Bioinformatics,* 14**,** 1176934318758650.

KHAN, N., ZANDI, P., ALI, S., MEHMOOD, A., ADNAN SHAHID, M. & YANG, J. 2018b. Impact of salicylic acid and plant growth promoting rhizobacteria on the drought tolerance and phytoremediation potential of *Helianthus annus*. *Frontiers in Microbiology,* 9**,** 2507.

KHOMTCHOUK, B. B., HENNESSY, J. R. & WAHLESTEDT, C. 2017. shinyheatmap: Ultra fast low memory heatmap web interface for big data genomics. *Public Library of Science One,* 12**,** 1-9.

KIELAK, A. M., BARRETO, C. C., KOWALCHUK, G. A., VAN VEEN, J. A. & KURAMAE, E. E. 2016. The ecology of Acidobacteria: moving beyond genes and genomes. *Frontiers in Microbiology,* 7**,** 744.

KIELAK, A. M., CASTELLANE, T. C., CAMPANHARO, J. C., COLNAGO, L. A., COSTA, O. Y., DA SILVA, M. L. C., VAN VEEN, J. A., LEMOS, E. G. & KURAMAE, E. E. 2017. Characterization of novel Acidobacteria exopolysaccharides with potential industrial and ecological applications. *Scientific Reports,* 7**,** 1-11.

KIM, C., WANG, X., LEE, T.-H., JAKOB, K., LEE, G.-J. & PATERSON, A. H. 2014. Comparative analysis of *Miscanthus* and *Saccharum* reveals a shared whole-genome duplication but different evolutionary fates. *The Plant Cell,* 26**,** 2420-2429.

KLIMEK, B., CHODAK, M., JAŹWA, M. & NIKLIŃSKA, M. 2016. Functional diversity of soil microbial communities in boreal and temperate Scots pine forests. *European Journal of Forest Research,* 135**,** 731-742.

KLOEPPER, J. & SCHROTH, M. 1981. Relationship of in vitro antibiosis of plant growth-promoting rhizobacteria to plant growth and the displacement of root microflora. *Phytopathology,* 71**,** 1020-1024.

KOSTYUCHENKO, N., LYAKH, V. & SOROKA, A. 2021. The state of soil microbiotes during sunflower growing with an herbicide of imidazolinone group. *Helia,* 44**,** 181-190.

KOTROCZÓ, Z., VERES, Z., FEKETE, I., KRAKOMPERGER, Z., TÓTH, J. A., LAJTHA, K. & TÓTHMÉRÉSZ, B. 2014. Soil enzyme activity in response to long-term organic matter manipulation. *Soil Biology and Biochemistry,* 70**,** 237-243.

KOUR, D., RANA, K. L., YADAV, N., YADAV, A. N., KUMAR, A., MEENA, V. S., SINGH, B., CHAUHAN, V. S., DHALIWAL, H. S. & SAXENA, A. K. 2019. Rhizospheric microbiomes: biodiversity, mechanisms of plant growth promotion and biotechnological applications for sustainable agriculture. *Plant Growth Promoting Rhizobacteria for Agricultural Sustainability. Springer*.

KSIAZEK, K., TONIETTO, R. & ASCHER, J. S. 2014. Ten bee species new to green roofs in the Chicago area. *The Great Lakes Entomologist,* 47**,** 13.

KUAN, K. B., OTHMAN, R., RAHIM, K. A. & SHAMSUDDIN, Z. H. 2016. Plant growth-promoting rhizobacteria inoculation to enhance vegetative growth, nitrogen fixation and nitrogen remobilisation of maize under greenhouse conditions. *Public Library of Science One,* 11**,** 1-19.

KULMATISKI, A., BEARD, K. H., NORTON, J. M., HEAVILIN, J. E., FORERO, L. E. & GRENZER, J. 2017. Live long and prosper: plant–soil feedback, lifespan and landscape abundance covary. *Ecology,* 98**,** 3063-3073.

KUMAR AWASTHI, M., RAVINDRAN, B., SARSAIYA, S., CHEN, H., WAINAINA, S., SINGH, E., LIU, T., KUMAR, S., PANDEY, A. & SINGH, L. 2020. Metagenomics for taxonomy profiling: tools and approaches. *Bioengineered,* 11**,** 356-374.

KUMAR BEHERA, S., SINGH, D. & SWAROOP DWIVEDI, B. 2009. Changes in fractions of iron, manganese, copper and zinc in soil under continuous cropping for more than three decades. *Communications in Soil Science and Plant Analysis,* 40**,** 1380-1407.

KUMAR, P. & DUBEY, K. K. 2019. Citric acid cycle regulation: Back bone for secondary metabolite production. *New and Future Developments in Microbial Biotechnology and Bioengineering.* Elsevier.

KUMAR, P. & FULEKAR, M. H. 2018. Research article rhizosphere bioremediation of heavy metals (copper and lead) by *Cenchrus ciliaris*. *Journal of Environmental Science,* 12**,** 166-176.

KUMAR, P., THAKUR, S., DHINGRA, G., SINGH, A., PAL, M. K., HARSHVARDHAN, K., DUBEY, R. & MAHESHWARI, D. 2018a. Inoculation of siderophore producing rhizobacteria and their consortium for growth enhancement of wheat plant. *Biocatalysis and Agricultural Biotechnology,* 15**,** 264-269.

KUMAR, S., STECHER, G., LI, M., KNYAZ, C. & TAMURA, K. 2018b. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution,* 35**,** 1547.

KUMARI, B., MALLICK, M., SOLANKI, M. K., SOLANKI, A. C., HORA, A. & GUO, W. 2019. Plant growth promoting rhizobacteria (PGPR): modern prospects for sustainable agriculture. *Plant Health Under Biotic Stress.* Switzerland: Springer.

.

KUZYAKOV, Y. & BLAGODATSKAYA, E. 2015. Microbial hotspots and hot moments in soil: concept & review. *Soil Biology and Biochemistry,* 83**,** 184-199.

LAGOS, L., MARUYAMA, F., NANNIPIERI, P., MORA, M., OGRAM, A. & JORQUERA, M. 2015. Current overview on the study of bacteria in the rhizosphere by modern molecular techniques: a mini–review. *Journal of Soil Science and Plant Nutrition,* 15**,** 504-523.

LAMBERS, H., RAVEN, J. A., SHAVER, G. R. & SMITH, S. E. 2008. Plant nutrient-acquisition strategies change with soil age. *Trends in Ecology & Evolution,* 23**,** 95-103.

LANG, M., BEI, S., LI, X., KUYPER, T. W. & ZHANG, J. 2019. Rhizoplane bacteria and plant species co-determine phosphorus-mediated microbial legacy effect. *Frontiers in Microbiology,* 10.

LANGE, M., EISENHAUER, N., SIERRA, C. A., BESSLER, H., ENGELS, C., GRIFFITHS, R. I., MELLADO-VÁZQUEZ, P. G., MALIK, A. A., ROY, J. & SCHEU, S. 2015. Plant diversity increases soil microbial activity and soil carbon storage. *Nature Communications,* 6**,** 1-8.

LANGILLE, M. G., ZANEVELD, J., CAPORASO, J. G., MCDONALD, D., KNIGHTS, D., REYES, J. A., CLEMENTE, J. C., BURKEPILE, D. E., THURBER, R. L. V. & KNIGHT, R. 2013. Predictive functional profiling of microbial communities using 16S rRNA marker gene sequences. *Nature Biotechnology,* 31**,** 814-821.

LASA, A. V., MAŠÍNOVÁ, T., BALDRIAN, P. & FERNÁNDEZ-LÓPEZ, M. 2019. Bacteria from the endosphere and rhizosphere of Quercus spp. use mainly cell wall-associated enzymes to decompose organic matter. *PloS One,* 14**,** e0214422.

LAWSON, C. E., WU, S., BHATTACHARJEE, A. S., HAMILTON, J. J., MCMAHON, K. D., GOEL, R. & NOGUERA, D. R. 2017. Metabolic network analysis reveals microbial community interactions in anammox granules. *Nature Communications,* 8**,** 1-12.

LECKIE, S., PRESCOTT, C., GRAYSTON, S. J., NEUFELD, J. & MOHN, W. W. 2004. Characterization of humus microbial communities in adjacent forest types that differ in nitrogen availability. *Microbial Ecology,* 48**,** 29-40.

LEE, S. A., KIM, Y., KIM, J. M., CHU, B., JOA, J.H., SANG, M. K., SONG, J. & WEON, H.-Y. 2019. A preliminary examination of bacterial, archaeal and fungal communities inhabiting different rhizocompartments of tomato plants under real-world environments. *Scientific Reports,* 9**,** 1-15.

LEMAIRE, G., DE FACCIO CARVALHO, P. C., KRONBERG, S. & RECOUS, S. 2018. *Agroecosystem Diversity: Reconciling Contemporary Agriculture and Environmental Quality*, *Academic Press*.

LEMAIRE, G. & GASTAL, F. 2016. Improved estimation of nitrogen uptake in grasslands using the nitrogen dilution curve (Reyes et al. 2015), 35: 1561–1570. *Agronomy for Sustainable Development,* 36**,** 46-47.

LETTICE, E. P. 2018. The rhizosphere: measuring the zone of interaction. *Annual Plant Reviews Online,* 2**,** 219-236.

LEVY, M. A. & LUBELL, M. N. 2018. Innovation, cooperation and the structure of three regional sustainable agriculture networks in California. *Regional Environmental Change,* 18**,** 1235-1246.

LI, F., CHEN, L., ZHANG, J., YIN, J. & HUANG, S. 2017a. Bacterial community structure after long-term organic and inorganic fertilization reveals important associations between soil nutrients and specific taxa involved in nutrient transformations. *Frontiers in Microbiology,* 8**,** 187.

LI, H. 2018. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics,* 34**,** 3094-3100.

LI, H., SU, J.-Q., YANG, X.-R. & ZHU, Y.-G. 2019a. Distinct rhizosphere effect on active and total bacterial communities in paddy soils. *Science of the Total Environment,* 649**,** 422-430.

LI, J., DONG, L., LIU, Y., WU, J., WANG, J., SHANGGUAN, Z. & DENG, L. 2022. Soil organic carbon variation determined by biogeographic patterns of microbial carbon and nutrient limitations across a 3, 000-km humidity gradient in China. *Catena,* 209**,** 105849.

LI, X., JOUSSET, A., DE BOER, W., CARRIÓN, V. J., ZHANG, T., WANG, X. & KURAMAE, E. E. 2019b. Legacy of land use history determines reprogramming of plant physiology by soil microbiome. *The ISME Journal,* 13**,** 738-751.

LI, Y., LIU, X., HAO, T. & CHEN, S. 2017b. Colonization and maize growth promotion induced by phosphate solubilizing bacterial isolates. *International Journal of Molecular Sciences,* 18**,** 1253.

LI, Y., WANG, C., WANG, T., LIU, Y., JIA, S., GAO, Y. & LIU, S. 2020. Effects of different fertilizer treatments on rhizosphere soil microbiome composition and functions. *Land,* 9**,** 329.

LINDGREEN, S., ADAIR, K. L. & GARDNER, P. P. 2016. An evaluation of the accuracy and speed of metagenome analysis tools. *Scientific Reports,* 6**,** 19233.

LINDSAY, E. A., COLLOFF, M. J., GIBB, N. L. & WAKELIN, S. A. 2010. The abundance of microbial functional genes in grassy woodlands is influenced more by soil nutrient enrichment than by recent weed invasion or livestock exclusion. *Applied and Environmental Microbiology,* 76**,** 5547-5555.

LINDSTRÖM, K. & MOUSAVI, S. A. 2019. Effectiveness of nitrogen fixation in rhizobia. *Microbial Biotechnology,* 0(0)**,** 1-22.

Ling, N., Zhang, W., Wang, D., Mao, J., Huang, Q., Guo, S. & Shen, Q. 2013. Root exudates from grafted-root watermelon showed a certain contribution in inhibiting *Fusarium oxysporum* f. sp. niveum. *PLoS One*. 20;8(5):e63383.

Liu, N., Zhou, B., Zhao, X., Lu, B., Li, Y. & Hao, J. 2009. Grafting eggplant onto tomato rootstock to suppress Verticillium dahliae infection: the effect of root exudates. Horticultural Science. 1;44(7):2058-62.

LIU, H., SUN, J., CHANG, J.S. & SHUKLA, P. 2018. Engineering microbes for direct fermentation of cellulose to bioethanol. *Critical Reviews in Biotechnology,* 38**,** 1089-1105.

LIU, P., WANG, X.H., LI, J.G., QIN, W., XIAO, C.Z., ZHAO, X., JIANG, H.X., SUI, J.K., SA, R.B. & WANG, W.Y. 2015. Pyrosequencing reveals fungal communities in the rhizosphere of Xinjiang jujube. *Biological and Medical Research International,* 2015**,** 1-8.

LLADÓ, S. & BALDRIAN, P. 2017a. Community-level physiological profiling analyses show potential to identify the copiotrophic bacteria present in soil environments. *Public Library of Science One,* 12**,** 1-15.

LLADÓ, S. & BALDRIAN, P. 2017b. Community-level physiological profiling analyses show potential to identify the copiotrophic bacteria present in soil environments. *PLoS One,* 12**,** e0171638.

LONG, X.E., YAO, H., HUANG, Y., WEI, W. & ZHU, Y.G. 2018. Phosphate levels influence the utilisation of rice rhizodeposition carbon and the phosphate-solubilising microbial community in a paddy soil. *Soil Biology and Biochemistry,* 118**,** 103-114.

LÓPEZ-LÓPEZ, O., E CERDAN, M. & I GONZALEZ SISO, M. 2014. New extremophilic lipases and esterases from metagenomics. *Current Protein and Peptide Science,* 15**,** 445-455.

LOUCA, S., DOEBELI, M. & PARFREY, L. W. 2018. Correcting for 16S rRNA gene copy numbers in microbiome surveys remains an unsolved problem. *Microbiome,* 6**,** 1-12.

LOVELL, S. T. & TAYLOR, J. R. 2013. Supplying urban ecosystem services through multifunctional green infrastructure in the United States. *Landscape Ecology,* 28**,** 1447-1463.

LU, G.H., HUA, X.M., LIANG, L., WEN, Z.L., DU, M.H., MENG, F.F., PANG, Y.J., QI, J.L., TANG, C.Y. & YANG, Y.H. 2018. Identification of major rhizobacterial taxa affected by a glyphosate-tolerant soybean line via shotgun metagenomic approach. *Genes,* 9**,** 214.

LU, Y., ZHANG, E., HONG, M., YIN, X., CAI, H., YUAN, L., YUAN, F., LI, L., ZHAO, K. & LAN, X. 2020. Analysis of endophytic and rhizosphere bacterial diversity and function in the endangered plant *Paeonia ludlowii*. *Archives of Microbiology,* 202**,** 1717-1728.

LUCAS, M., BALBÍN-SUÁREZ, A., SMALLA, K. & VETTERLEIN, D. 2018. Root growth, function and rhizosphere microbiome analyses show local rather than systemic effects in apple plant response to replant disease soil. *PLoS One,* 13**,** e0204922.

LUDWIG-MÜLLER, J. 2015. Plants and endophytes: equal partners in secondary metabolite production? *Biotechnology Letters,* 37**,** 1325-1334.

LUGTENBERG, B. 2015. Life of microbes in the rhizosphere. *In: Principles of plant-microbe interactions.* Switzerland: *Springer*.

LUNDBERG, D. S., LEBEIS, S. L., PAREDES, S. H., YOURSTONE, S., GEHRING, J., MALFATTI, S., TREMBLAY, J., ENGELBREKTSON, A., KUNIN, V. & DEL RIO, T. G. 2012. Defining the core *Arabidopsis thaliana* root microbiome. *Nature,* 488**,** 86-90.

LYU, M., LI, X., XIE, J., HOMYAK, P. M., UKONMAANAHO, L., YANG, Z., LIU, X., RUAN, C. & YANG, Y. 2019. Root–microbial interaction accelerates soil nitrogen depletion but not soil carbon after increasing litter inputs to a coniferous forest. *Plant and Soil,* 444**,** 153-164.

MA, C., LO, P. K., XU, J., LI, M., JIANG, Z., LI, G., ZHU, Q., LI, X., LEONG, S. Y. & LI, Q. 2020. Molecular mechanisms underlying lignocellulose degradation and antibiotic resistance

genes removal revealed via metagenomics analysis during different agricultural wastes composting. *Bioresource Technology,* 314**,** 123731.

MACIVOR, J. S. & KSIAZEK, K. 2015. Invertebrates on green roofs. *Green roof ecosystems.* Springer.

MACOUZET, M. 2016. Critical aspects in the conception and production of microbial plant biostimulants. *Probiotic Intelligentsia* 5**,** 29-38.

MAHMOUD, M., ZYWICKI, M., TWARDOWSKI, T. & KARLOWSKI, W. M. 2019. Efficiency of PacBio long read correction by 2nd generation Illumina sequencing. *Genomics,* 111**,** 43-49.

MAIDAK, B. L., LARSEN, N., MCCAUGHEY, M. J., OVERBEEK, R., OLSEN, G. J., FOGEL, K., BLANDY, J. & WOESE, C. R. 1994. The ribosomal database project. *Nucleic Acids Research,* 22**,** 3485-3487.

MAILLARD, F., LEDUC, V., BACH, C., DE MORAES GONÇALVES, J. L., ANDROTE, F. D., SAINT-ANDRÉ, L., LACLAU, J.-P., BUÉE, M. & ROBIN, A. 2019. Microbial enzymatic activities and community-level physiological profiles (CLPP) in subsoil layers are altered by harvest residue management practices in a tropical *Eucalyptus grandis* plantation. *Microbial Ecology,* 78**,** 528-533.

MAJEED, A., ABBASI, M. K., HAMEED, S., IMRAN, A., NAQQASH, T. & HANIF, M. K. 2015a. Isolation and characterization of sunflower associated bacterial strain with broad spectrum plant growth promoting traits. *Microbiological Research,* 13**,** 110-123.

MAJEED, A., ABBASI, M. K., HAMEED, S., IMRAN, A., NAQQASH, T. & HANIF, M. K. 2018a. Isolation and characterization of sunflower associated bacterial strain with broad spectrum plant growth promoting traits. *International Journal of Biosciences,* 13**,** 110-123.

MAJEED, A., ABBASI, M. K., HAMEED, S., IMRAN, A. & RAHIM, N. 2015b. Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Frontiers in Microbiology,* 6**,** 198.

MAJEED, A., ABBASI, M. K., HAMEED, S., YASMIN, S., HANIF, M. K., NAQQASH, T. & IMRAN, A. 2018b. *Pseudomonas* sp. AF-54 containing multiple plant beneficial traits acts as growth enhancer of *Helianthus annuus* L. under reduced fertilizer input. *Microbiological Research,* 216**,** 56-69.

MAJEED, A., MUHAMMAD, Z. & AHMAD, H. 2018c. Plant growth promoting bacteria: role in soil improvement, abiotic and biotic stress management of crops. *Plant Cell Reports,* 37**,** 1599-1609.

MAKONDE, H. M., MWIRICHIA, R., OSIEMO, Z., BOGA, H. I. & KLENK, H.P. 2015. 454 Pyrosequencing-based assessment of bacterial diversity and community structure in termite guts, mounds and surrounding soils. *Springer Plus,* 4**,** 1-11.

MANIVANH, L., PIERRET, A., RATTANAVONG, S., KOUNNAVONGSA, O., BUISSON, Y., ELLIOTT, I., MAEGHT, J.-L., XAYYATHIP, K., SILISOUK, J. & VONGSOUVATH, M. 2017. *Burkholderia pseudomallei* in a lowland rice paddy: seasonal changes and influence of soil depth and physico-chemical properties. *Scientific Reports,* 7**,** 1-11.

MANJULA, A. & NARSIMHA, G. XCYPF: A flexible and extensible framework for agricultural crop yield prediction. 2015 Institute of Electrical and Electronic Engineers 9th International Conference on Intelligent Systems and Control (ISCO), 2015. *Institute of Electrical and Electronic Engineers,* 1-5.

MAQUIA, I. S., FARELEIRA, P., VIDEIRA E CASTRO, I., BRITO, D. R., SOARES, R., CHAÚQUE, A., FERREIRA-PINTO, M. M., LUMINI, E., BERRUTI, A. & RIBEIRO, N. S. 2020. Mining the microbiome of key species from african savanna woodlands: Potential for soil health improvement and plant growth promotion. *Microorganisms,* 8**,** 1291.

MARCHESI, J. R. & RAVEL, J. 2015. The vocabulary of microbiome research: a proposal. *Springer*.

MARKOWITZ, V. M., CHEN, I.-M. A., CHU, K., SZETO, E., PALANIAPPAN, K., GRECHKIN, Y., RATNER, A., JACOB, B., PATI, A. & HUNTEMANN, M. 2012. IMG/M: the integrated metagenome data management and comparative analysis system. *Nucleic acids research,* 40**,** D123-D129.

MAROPOLA, M. K. A., RAMOND, J.-B. & TRINDADE, M. 2015. Impact of metagenomic DNA extraction procedures on the identifiable endophytic bacterial diversity in Sorghum bicolor (*L. Moench*). *Journal of Microbiological Methods,* 112**,** 104-117.

MARTELLACCI, L., QUARANTA, G., PATINI, R., ISOLA, G., GALLENZI, P. & MASUCCI, L. 2019. A Literature Review of Metagenomics and Culturomics of the Peri-implant Microbiome: Current Evidence and Future Perspectives. *Materials,* 12**,** 3010.

MARTIN, M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet. Journal,* 17**,** 10-12.

MARTÍNEZ-PORCHAS, M. & VARGAS-ALBORES, F. 2017. Microbial metagenomics in aquaculture: a potential tool for a deeper insight into the activity. *Reviews in Aquaculture,* 9**,** 42-56.

MASEKO, I., BELETSE, Y. G., NOGEMANE, N., DU PLOOY, C. P., MUSIMWA, T. R. & MABHAUDHI, T. 2017. Productivity of non-heading Chinese cabbage (*Brassica rapa* subsp. chinensis) under different agronomic management factors. *South African Journal of Plant and Soil,* 34**,** 275-282.

MATHEWS, S. L., PAWLAK, J. & GRUNDEN, A. M. 2015. Bacterial biodegradation and bioconversion of industrial lignocellulosic streams. *Applied Microbiology and Biotechnology,* 99**,** 2939-2954.

MATTHEWS, A., PIERCE, S., HIPPERSON, H. & RAYMOND, B. 2019. Rhizobacterial community assembly patterns vary between crop species. *Frontiers in Microbiology,* 10**,** 581.

MAYER, Z., CSORBAINÉ, A. G., JUHÁSZ, Á., OMBÓDI, A., PÁPAI, A., NÉMETHNÉ, B. K. & POSTA, K. 2021. Impact of Soil-Applied Microbial Inoculant and Fertilizer on Fungal and Bacterial Communities in the Rhizosphere of *Robinia* sp. and *Populus* sp. Plantations. *Forests,* 12**,** 1218.

MCGUIRE, K. L., PAYNE, S. G., ORAZI, G. & PALMER, M. I. 2015. Bacteria and fungi in green roof ecosystems. *Green Roof Ecosystems.* Springer.

MEDVEDEV, P., GEORGIOU, K., MYERS, G. & BRUDNO, M. Computability of models for sequence assembly. International workshop on algorithms in bioinformatics, 2007. Springer, 289-301.

MEENA, V. S., MAURYA, B. & VERMA, J. P. 2014. Does a rhizospheric microorganism enhance K+ availability in agricultural soils? *Microbiological Research,* 169**,** 337-347.

MEYER, F., PAARMANN, D., D'SOUZA, M., OLSON, R., GLASS, E. M., KUBAL, M., PACZIAN, T., RODRIGUEZ, A., STEVENS, R. & WILKE, A. 2008a. The metagenomics RAST server–a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics,* 9**,** 1-8.

MEYER, W., BIEDERMANN, K., GUBO, M., HAMMER, L. & HEINZ, K. 2008b. Surface structure of polar Co3O4 (111) films grown epitaxially on Ir (100)-(1× 1). *Journal of Physics: Condensed Matter,* 20**,** 265011.

MILLER, R. N. 2018. Quantitative assessment of bacterial and fungal degradation of glucose and cellulose. *The Research Repository- West Virginia University***,** 1-42.

MISHRA, J., SINGH, R. & ARORA, N. K. 2017. Plant growth-promoting microbes: diverse roles in agriculture and environmental sustainability. *Probiotics and Plant Health.* Springer.

MOHAMMADI, K., HEIDARI, G., KHALESRO, S. & SOHRABI, Y. 2011. Soil management, microorganisms and organic matter interactions: A review. *African Journal of Biotechnology,* 10**,** 19840-19849.

MOHAMMED, A. F., OLOYEDE, A. R. & ODESEYE, A. O. 2020. Biological control of bacterial wilt of tomato caused by *Ralstonia solanacearum* using *Pseudomonas* species isolated from the rhizosphere of tomato plants. *Archives of Phytopathology and Plant Protection,* 53**,** 1-16.

MOHAPATRA, M., YADAV, R., RAJPUT, V., DHARNE, M. S. & RASTOGI, G. 2021. Metagenomic analysis reveals genetic insights on biogeochemical cycling, xenobiotic degradation and stress resistance in mudflat microbiome. *Journal of Environmental Management,* 292**,** 112738.

MOK, H.-F., WILLIAMSON, V. G., GROVE, J. R., BURRY, K., BARKER, S. F. & HAMILTON, A. J. 2014. Strawberry fields forever? Urban agriculture in developed countries: a review. *Agronomy for sustainable development,* 34**,** 21-43.

MOLEFE, R. R., AMOO, A. E. & BABALOLA, O. O. 2021. Metagenomic insights into the bacterial community structure and functional potentials in the rhizosphere soil of maize plants. *Journal of Plant Interactions,* 16**,** 258-269.

MOOD, S. H., GOLFESHAN, A. H., TABATABAEI, M., JOUZANI, G. S., NAJAFI, G. H., GHOLAMI, M. & ARDJMAND, M. 2013. Lignocellulosic biomass to bioethanol, a comprehensive review with a focus on pretreatment. *Renewable and Sustainable Energy Reviews,* 27**,** 77-93.

MORA-RUIZ, M. D. R., ALEJANDRE-COLOMO, C., LEDGER, T., GONZÁLEZ, B., ORFILA, A. & ROSSELLÓ-MÓRA, R. 2018. Non-halophilic endophytes associated with the euhalophyte *Arthrocnemum macrostachyum* and their plant growth promoting activity potential. *Federation of European Microbiologicals Microbiology Letters,* 365**,** 1-11.

MORETTI, L. G., CRUSCIOL, C. A., KURAMAE, E. E., BOSSOLANI, J. W., MOREIRA, A., COSTA, N. R., ALVES, C. J., PASCOALOTO, I. M., RONDINA, A. B. & HUNGRIA, M. 2020. Effects of growth-promoting bacteria on soybean root activity, plant development and yield. *Agronomy Journal,* 112**,** 1-11.

MORETTI, L. G., LAZARINI, E., BOSSOLANI, J. W., PARENTE, T. L., CAIONI, S., ARAUJO, R. S. & HUNGRIA, M. 2018. Can additional inoculations increase soybean nodulation and grain yield? *Agronomy Journal,* 110**,** 1-7.

MORGAN JR, H. M., BU, Q., LIANG, J., LIU, Y., MAO, H., SHI, A., LEI, H. & RUAN, R. 2017. A review of catalytic microwave pyrolysis of lignocellulosic biomass for value-added fuel and chemicals. *Bioresource Technology,* 230**,** 112-121.

MOSCATELLI, M. C., SECONDI, L., MARABOTTINI, R., PAPP, R., STAZI, S., MANIA, E. & MARINARI, S. 2018. Assessment of soil microbial functional diversity: land use and soil properties affect CLPP-MicroResp and enzymes responses. *Pedobiologia,* 66**,** 36-42.

MOSIMANN, C., OBERHÄNSLI, T., ZIEGLER, D., NASSAL, D., KANDELER, E., BOLLER, T., MÄDER, P. & THONAR, C. 2017. Tracing of two *Pseudomonas* strains in the root and rhizoplane of maize, as related to their plant growth-promoting effect in contrasting soils. *Frontiers in Microbiology,* 7**,** 1-14.

MOURATO, M. P., MOREIRA, I. N., LEITÃO, I., PINTO, F. R., SALES, J. R. & MARTINS, L. L. 2015. Effect of heavy metals in plants of the genus *Brassica*. *International Journal of Molecular Sciences,* 16**,** 17975-17998.

MUKHTAR, S., MEHNAZ, S., MIRZA, M. S. & MALIK, K. A. 2019. Isolation and characterization of bacteria associated with the rhizosphere of halophytes (*Salsola stocksii* and

Atriplex amnicola) for production of hydrolytic enzymes. *Brazilian Journal of Microbiology*, 50**,** 85-97.

MULLER, E. E., FAUST, K., WIDDER, S., HEROLD, M., ARBAS, S. M. & WILMES, P. 2018. Using metabolic networks to resolve ecological properties of microbiomes. *Current Opinion in Systems Biology,* 8**,** 73-80.

MULLER, M. H., LATREILLE, M. & TOLLON, C. 2011. The origin and evolution of a recent agricultural weed: population genetic diversity of weedy populations of sunflower (Helianthus annuus L.) in Spain and France. *Evolutionary Applications,* 4**,** 499-514.

MURPHY, C. J., BAGGS, E. M., MORLEY, N., WALL, D. P. & PATERSON, E. 2015. Rhizosphere priming can promote mobilisation of N-rich compounds from soil organic matter. *Soil Biology and Biochemistry,* 81**,** 236-243.

MWAJITA, M. R., MURAGE, H., TANI, A. & KAHANGI, E. M. 2013. Evaluation of rhizosphere, rhizoplane and phyllosphere bacteria and fungi isolated from rice in Kenya for plant growth promoters. *SpringerPlus,* 2**,** 606.

NAUMOFF, D. G. & DEDYSH, S. N. 2012. Lateral gene transfer between the Bacteroidetes and Acidobacteria: the case of α-L-rhamnosidases. *FEBS letters,* 586**,** 3843-3851.

NAVEED, M., MITTER, B., REICHENAUER, T. G., WIECZOREK, K. & SESSITSCH, A. 2014. Increased drought stress resilience of maize through endophytic colonization by

Burkholderia phytofirmans PsJN and Enterobacter sp. FD17. *Environmental and Experimental Botany,* 97**,** 30-39.

NAVEED, M., BROWN, L., RAFFAN, A., GEORGE, T. S., BENGOUGH, A. G., ROOSE, T., SINCLAIR, I., KOEBERNICK, N., COOPER, L. & HACKETT, C. A. 2017. Plant exudates may stabilize or weaken soil depending on species, origin and time. *European Journal of Soil Science,* 68**,** 806-816.

NAYLOR, D. & COLEMAN-DERR, D. 2018. Drought stress and root-associated bacterial communities. *Frontiers in Plant Science,* 8**,** 1-16.

NEHLS, T., JIANG, Y., DENNEHY, C., ZHAN, X., BEESLEY, L., LOHRBERG, F., LICKA, L., SCAZZOSI, L. & TIMPE, A. 2015. From waste to value: urban agriculture enables cycling of resources in cities. *Urban Agriculture Europe***,** 170-173.

NELSON, D. W., SOMMERS, L. E. & SPARKS, D. 1996. Methods of soil analysis. Part 3. Chemical methods. *Soil Science Society of America Book Series,* 5**,** 961-1010.

NEWMAN, D. J. & CRAGG, G. M. 2016. Natural products as sources of new drugs from 1981 to 2014. *Journal of Natural Products,* 79**,** 629-661.

NGARA, T. R. & ZHANG, H. 2018. Recent advances in function-based metagenomic screening. *Genomics, Proteomics and Bioinformatics,* 16**,** 405-415.

NKUEKAM, G. K., COWAN, D. A. & VALVERDE, A. 2018. Arable agriculture changes soil microbial communities in the South African grassland biome. *South African Journal of Science,* 114**,** 1-7.

NOVAK, B. J. 2018. De-extinction. *Genes,* 9**,** 548.

NUCCIO, E. E., STARR, E., KARAOZ, U., BRODIE, E. L., ZHOU, J., TRINGE, S. G., MALMSTROM, R. R., WOYKE, T., BANFIELD, J. F. & FIRESTONE, M. K. 2020. Niche differentiation is spatially and temporally regulated in the rhizosphere. *The ISME Journal,* 14**,** 999-1014.

NWACHUKWU, B. C., AYANGBENRO, A. S. & BABALOLA, O. O. 2021. Elucidating the rhizosphere associated bacteria for environmental sustainability. *Agriculture,* 11**,** 1-18.

NWACHUKWU, B. C. & BABALOLA, O. O. 2021. Perspectives for sustainable agriculture from the microbiome in plant rhizosphere. *Plant Biotechnology Reports,* 15**,** 1-20.

O'CONNOR, S. E. 2015. Engineering of secondary metabolism. *Annual review of genetics,* 49**,** 71-94.

O'BRIEN, P. A., WEBSTER, N. S., MILLER, D. J. & BOURNE, D. G. 2019. Host-microbe coevolution: applying evidence from model systems to complex marine invertebrate holobionts. *MBio,* 10**,** e02241-18.

OAK, U. S., AMRITA KUMAR, VINAY 2019. *Microbial intervention in agriculture and environment,* New York City, United States of America, *Springer*.

OBERHOLSTER, T., VIKRAM, S., COWAN, D. & VALVERDE, A. 2018. Key microbial taxa in the rhizosphere of sorghum and sunflower grown in crop rotation. *Science of The Total Environment,* 624**,** 1-10.

ODELADE, K. A. & BABALOLA, O. O. 2019. Bacteria, fungi and archaea domains in rhizospheric soil and their effects in enhancing agricultural productivity. *International Journal of Environmental Research and Public Health,* 16**,** 3873.

OFFRE, P., SPANG, A. & SCHLEPER, C. 2013. Archaea in biogeochemical cycles. *Annual Review of Microbiology,* 67**,** 437-457.

OLANREWAJU, O. S., AYANGBENRO, A. S., GLICK, B. R. & BABALOLA, O. O. 2019. Plant health: feedback effect of root exudates-rhizobiome interactions. *Applied Microbiology and Biotechnology,* 103**,** 1155-1166.

OLIVEROS, J. 2017. 2007–2015. Venny. An interactive tool for comparing lists with Venn's diagrams.

ORLIKOWSKA, T., NOWAK, K. & REED, B. 2017. Bacteria in the plant tissue culture environment. *Plant Cell, Tissue and Organ Culture (PCTOC),* 128**,** 487-508.

OULAS, A., PAVLOUDI, C., POLYMENAKOU, P., PAVLOPOULOS, G. A., PAPANIKOLAOU, N., KOTOULAS, G., ARVANITIDIS, C. & ILIOPOULOS, L. 2015. Metagenomics: tools and insights for analyzing next-generation sequencing data derived from biodiversity studies. *Bioinformatics and Biology Insights,* 9**,** 75-88.

PALANSOORIYA, K. N., SHAHEEN, S. M., CHEN, S. S., TSANG, D. C., HASHIMOTO, Y., HOU, D., BOLAN, N. S., RINKLEBE, J. & OK, Y. S. 2020. Soil amendments for immobilization of potentially toxic elements in contaminated soils: a critical review. *Environment International,* 134**,** 1-29.

PANDEY, R., CHAVAN, P., WALOKAR, N., SHARMA, N., TRIPATHI, V. & KHETMALAS, M. 2013. Pseudomonas stutzeri RP1: a versatile plant growth promoting endorhizospheric bacteria inhabiting sunflower (*Helianthus annus*). *J. Biotechnol,* 8**,** 48-55.

PARFENIUK, A., TUROVNIK, Y., BEZNOSKO, I., HAVRYLIUK, L., GORGAN, T., TYMOSHENKO, L. & GENTOSH, D. 2021. Mycobiome of sunflower rhizosphere in organic farming. *Ukrainian Journal of Ecology,* 11**,** 149-154.

PARNELL, J. J., BERKA, R., YOUNG, H. A., STURINO, J. M., KANG, Y., BARNHART, D. & DILEO, M. V. 2016. From the lab to the farm: an industrial perspective of plant beneficial microorganisms. *Frontiers in Plant Science,* 7**,** 1-12.

PATEL, V., SHARMA, A., LAL, R., AL-DHABI, N. A. & MADAMWAR, D. 2016. Response and resilience of soil microbial communities inhabiting in edible oil stress/contamination from industrial estates. *BioMed Central Microbiology,* 16**,** 50.

PEIFFER, J. A., SPOR, A., KOREN, O., JIN, Z., TRINGE, S. G., DANGL, J. L., BUCKLER, E. S. & LEY, R. E. 2013. Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences,* 110**,** 1-6.

PELAGIO-FLORES, R., ESPARZA-REYNOSO, S., GARNICA-VERGARA, A., LÓPEZ-BUCIO, J. & HERRERA-ESTRELLA, A. 2017. *Trichoderma*-induced acidification is an early trigger for changes in Arabidopsis root growth and determines fungal phytostimulation. *Frontiers in Plant Science,* 8**,** 1-13.

PENG, W., LI, X., SONG, J., JIANG, W., LIU, Y. & FAN, W. 2018. Bioremediation of cadmium- and zinc-contaminated soil using Rhodobacter sphaeroides. *Chemosphere,* 197**,** 33-41.

PERUJO, N., ROMANÍ, A. & MARTÍN-FERNÁNDEZ, J. 2020. Microbial community-level physiological profiles: Considering whole data set and integrating dynamics of colour development. *Ecological Indicators,* 117**,** 1-9.

PHILIPPOT, L., RAAIJMAKERS, J. M., LEMANCEAU, P. & VAN DER PUTTEN, W. H. 2013a. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology,* 11**,** 789-799.

PHILIPPOT, L., RAAIJMAKERS, J. M., LEMANCEAU, P. & VAN DER PUTTEN, W. H. 2013b. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology,* 11**,** 1-12.

PHOUR, M., SEHRAWAT, A., SINDHU, S. S. & GLICK, B. R. 2020. Interkingdom signaling in plant-rhizomicrobiome interactions for sustainable agriculture. *Microbiological Research,* 241**,** 1-19.

PII, Y., MIMMO, T., TOMASI, N., TERZANO, R., CESCO, S. & CRECCHIO, C. 2015. Microbial interactions in the rhizosphere: beneficial influences of plant growth-promoting rhizobacteria on nutrient acquisition process. A review. *Biology and Fertility of Soils,* 51**,** 403-415.

PINU, F. R., VILLAS-BOAS, S. G. & AGGIO, R. 2017. Analysis of intracellular metabolites from microorganisms: quenching and extraction protocols. *Metabolites,* 7**,** 53.

PLAKIAS, A. C. 2016. The farm on the roof: What Brooklyn Grange taught us about entrepreneurship, community and growing a sustainable business, Penguin.

PÖLLING, B., MERGENTHALER, M. & LORLEBERG, W. 2016. Professional urban agriculture and its characteristic business models in Metropolis Ruhr, Germany. *Land use policy,* 58**,** 366-379.

PÖLLING, B., SROKA, W. & MERGENTHALER, M. 2017. Success of urban farming's city-adjustments and business models—Findings from a survey among farmers in Ruhr Metropolis, Germany. *Land Use Policy,* 69**,** 372-385.

PRABHA, R., SINGH, D. P., GUPTA, S., GUPTA, V. K., EL-ENSHASY, H. A. & VERMA, M. K. 2019. Rhizosphere metagenomics of *Paspalum scrobiculatum* l.(kodo millet) reveals rhizobiome multifunctionalities. *Microorganisms,* 7**,** 608.

PRASAD, M., SRINIVASAN, R., CHAUDHARY, M., CHOUDHARY, M. & JAT, L. K. 2019a. Plant Growth Promoting Rhizobacteria (PGPR) for sustainable agriculture: Perspectives and challenges. *In: Plant Growth Promoting Rhizobacteria Amelioration in Sustainable Agriculture.* United Kingdom: *Elsevier*.

PRASAD, R. K., CHATTERJEE, S., MAZUMDER, P. B., GUPTA, S. K., SHARMA, S., VAIRALE, M. G., DATTA, S., DWIVEDI, S. K. & GUPTA, D. K. 2019b. Bioethanol production from waste lignocelluloses: A review on microbial degradation potential. *Chemosphere,* 231**,** 588-606.

PREECE, C. & PENUELAS, J. 2016. Rhizodeposition under drought and consequences for soil communities and ecosystem resilience. *Plant and Soil,* 409**,** 1-17.

PRIYA, G., LAU, N.-S., FURUSAWA, G., DINESH, B., FOONG, S. Y. & AMIRUL, A.-A. A. 2018. Metagenomic insights into the phylogenetic and functional profiles of soil microbiome from a managed mangrove in Malaysia. *Agri Gene,* 9**,** 5-15.

PROSSER, J. I. 2015. Dispersing misconceptions and identifying opportunities for the use of'omics' in soil microbial ecology. *Nature Reviews Microbiology,* 13**,** 439-446.

PURWATI, R. D. A. & HERWATI, A. 2016. Evaluation of quantitative and qualitative morphological characters of sunflower (*Helianthus annuus*) germplasm. *Biodiversitas Journal of Biological Diversity,* 17**,** 461-465.

QU, Z., LIU, B., MA, Y. & SUN, H. 2020. Differences in bacterial community structure and potential functions among Eucalyptus plantations with different ages and species of trees. *Applied Soil Ecology,* 149**,** 103515.

RABIEE, M., SAYYARI, E. & MIRARAB, S. 2019. Multi-allele species reconstruction using ASTRAL. *Molecular Phylogenetics and Evolution,* 130**,** 286-296.

RAI, M., INGLE, A. P., PANDIT, R., PARALIKAR, P., BISWAS, J. K. & DA SILVA, S. S. 2019. Emerging role of nanobiocatalysts in hydrolysis of lignocellulosic biomass leading to sustainable bioethanol production. *Catalysis Reviews,* 61**,** 1-26.

RASCOVAN, N., CARBONETTO, B., PERRIG, D., DÍAZ, M., CANCIANI, W., ABALO, M., ALLOATI, J., GONZÁLEZ-ANTA, G. & VAZQUEZ, M. P. 2016. Integrated analysis of root microbiomes of soybean and wheat from agricultural fields. *Scientific Reports,* 6**,** 28084.

RASMANN, S. & TURLINGS, T. C. J. 2016. Root signals that mediate mutualistic interactions in the rhizosphere. *Current Opinion in Plant Biology,* 32**,** 62-68.

RAYMANN, K. 2014. *Reconstructing the evolutionary relationships between Archaea and Eukaryotes: a phylogenomic approach.* Université Pierre et Marie Curie-Paris VI.

RAZZAQ, A., SADIA, B., RAZA, A., KHALID HAMEED, M. & SALEEM, F. 2019. Metabolomics: A Way Forward for Crop Improvement. *Metabolites,* 9**,** 303.

REAVY, B., SWANSON, M. M., COCK, P. J., DAWSON, L., FREITAG, T. E., SINGH, B. K., TORRANCE, L., MUSHEGIAN, A. R. & TALIANSKY, M. 2015. Distinct circular single-stranded DNA viruses exist in different soil types. *Applied and environmental microbiology,* 81**,** 3934-3945.

REED, S. C., CLEVELAND, C. C. & TOWNSEND, A. R. 2011. Functional ecology of free-living nitrogen fixation: a contemporary perspective. *Annual Review of Ecology, Evolution and Systematics,* 42**,** 489-512.

REGUEIRA, M., RIAL, E., BLANCO, B., BOGO, B., ALDREY, A., CORREA, B., VARAS, E., SÁNCHEZ, C. & VIDAL, N. 2018. Micropropagation of axillary shoots of *Salix viminalis* using a temporary immersion system. *Trees*, 32: 61–71.

REN, H., HUANG, B., FERNÁNDEZ-GARCÍA, V., MIESEL, J., YAN, L. & LV, C. 2020. Biochar and rhizobacteria amendments improve several soil properties and bacterial diversity. *Microorganisms,* 8**,** 502.

RESENDE, M., JAKOBY, I., DOS SANTOS, L., SOARES, M., PEREIRA, F., SOUCHIE, E. & SILVA, F. 2014. Phosphate solubilization and phytohormone production by endophytic and rhizosphere *Trichoderma* isolates of *guanandi* (*Calophyllum brasiliense Cambess*). *African Journal of Microbiology Research,* 8**,** 2616-2623.

REVILLINI, D., GEHRING, C. A. & JOHNSON, N. C. 2016. The role of locally adapted mycorrhizas and rhizobacteria in plant–soil feedback systems. *Functional Ecology,* 30**,** 1086-1098.

ROMAN-PONCE, B., REZA-VÁZQUEZ, D. M., GUTIERREZ-PAREDES, S., MARÍA DE JESÚS, D., MALDONADO-HERNANDEZ, J., BAHENA-OSORIO, Y., ESTRADA-DE LOS SANTOS, P., WANG, E. T. & VÁSQUEZ-MURRIETA, M. S. 2017. Plant growth-promoting traits in rhizobacteria of heavy metal-resistant plants and their effects on *Brassica nigra* seed germination. *Pedosphere,* 27**,** 511-526.

ROMERA, F. J., GARCÍA, M. J., LUCENA, C., MARTÍNEZ-MEDINA, A., APARICIO, M. A., RAMOS, J., ALCÁNTARA, E., ANGULO, M. & PÉREZ-VICENTE, R. 2019. Induced systemic resistance (ISR) and Fe deficiency responses in dicot plants. *Frontiers in Plant Science,* 10**,** 287.

RUIZ-DUEÑAS, F. J. & MARTÍNEZ, Á. T. 2009. Microbial degradation of lignin: how a bulky recalcitrant polymer is efficiently recycled in nature and how we can take advantage of this. *Microbial Biotechnology,* 2**,** 164-177.

RUIZ, O. N., BROWN, L. M., RADWAN, O., BOWEN, L. L., GUNASEKERA, T. S., MUELLER, S. S., WEST, Z. J. & STRIEBICH, R. C. 2021. Metagenomic characterization reveals

complex association of soil hydrocarbon-degrading bacteria. *International Biodeterioration & Biodegradation,* 157**,** 105161.

RUSSO, A., ESCOBEDO, F. J., CIRELLA, G. T. & ZERBE, S. 2017. Edible green infrastructure: An approach and review of provisioning ecosystem services and disservices in urban environments. *Agriculture, Ecosystems & Environment,* 242**,** 53-66.

RYGAARD, A. M., THØGERSEN, M. S., NIELSEN, K. F., GRAM, L. & BENTZON-TILIA, M. 2017. Effects of gelling agent and extracellular signaling molecules on the culturability of marine bacteria. *Applied and Environmental Microbiology,* 83**,** e00243-17.

SABALE, S. N., SURYAWANSHI, P. P. & KRISHNARAJ, P. 2019. Soil metagenomics: Concepts and applications. *Metagenomics-basics, methods and applications.* London, United Kingdom: *IntechOpen*.

SALAS, M. E., LOZANO, M. J., LÓPEZ, J. L., DRAGHI, W. O., SERRANIA, J., TORRES TEJERIZO, G. A., ALBICORO, F. J., NILSSON, J. F., PISTORIO, M. & DEL PAPA, M. F. 2017. Specificity traits consistent with legume-rhizobia coevolution displayed by *Ensifer meliloti* rhizosphere colonization. *Environmental Microbiology,* 19**,** 3423-3438.

SALEEM, M., ASGHAR, H. N., ZAHIR, Z. A. & SHAHID, M. 2018. Impact of lead tolerant plant growth promoting rhizobacteria on growth, physiology, antioxidant activities, yield and lead content in sunflower in lead contaminated soil. *Chemosphere,* 195**,** 606-614.

SALEEM, M., LAW, A. D. & MOE, L. A. 2016. Nicotiana roots recruit rare rhizosphere taxa as major root-inhabiting microbes. *Microbial Ecology,* 71**,** 469-472.

SALEH, D., JARRY, J., RANI, M., ALIFERIS, K., SEGUIN, P. & JABAJI, S. H. 2019. Diversity, distribution and multi-functional attributes of bacterial communities associated with the rhizosphere and endosphere of timothy (*Phleum pratense* L.). *Journal of Applied Microbiology,* 127**,** 794-811.

SALEM, M. A., PEREZ DE SOUZA, L., SERAG, A., FERNIE, A. R., FARAG, M. A., EZZAT, S. M. & ALSEEKH, S. 2020. Metabolomics in the Context of Plant Natural Products Research: From Sample Preparation to Metabolite Analysis. *Metabolites,* 10**,** 37.

SANTAMARIA, M., FOSSO, B., LICCIULLI, F., BALECH, B., LARINI, I., GRILLO, G., DE CARO, G., LIUNI, S. & PESOLE, G. 2018. ITSoneDB: a comprehensive collection of eukaryotic ribosomal RNA Internal Transcribed Spacer 1 (ITS1) sequences. *Nucleic Acids Research,* 46**,** D127-D132.

SANTI, C., CERTINI, G. & D'ACQUI, L. P. 2006. Direct determination of organic carbon by dry combustion in soils with carbonates. *Communications in Soil Science and Plant Analysis,* 37**,** 1-8.

SANTOS, M. S., NOGUEIRA, M. A. & HUNGRIA, M. 2019. Microbial inoculants: reviewing the past, discussing the present and previewing an outstanding future for the use of beneficial bacteria in agriculture. *AMB Express,* 9**,** 1-12.

SARHAN, M. S., PATZ, S., HAMZA, M. A., YOUSSEF, H. H., MOURAD, E. F., FAYEZ, M., MURPHY, B., RUPPEL, S. & HEGAZI, N. A. 2018. G3 PhyloChip analysis confirms the promise of plant-based culture media for unlocking the composition and diversity of the maize root microbiome and for recovering unculturable candidate divisions/phyla. *Microbes and Environments,* 33**,** 1-9.

SASSE, J., MARTINOIA, E. & NORTHEN, T. 2018. Feed your friends: do plant exudates shape the root microbiome? *Trends in Plant Science,* 23**,** 25-41.

SCHLAEPPI, K. & BULGARELLI, D. 2015. The plant microbiome at work. *Molecular Plant-Microbe Interactions,* 28**,** 212-217.

SCHLATTER, D. C., HANSEN, J. C., SCHILLINGER, W. F., SULLIVAN, T. S. & PAULITZ, T. C. 2019. Common and unique rhizosphere microbial communities of wheat and canola in a semiarid Mediterranean environment. *Applied Soil Ecology,* 144**,** 170-181.

SCHLEMPER, T. R., LEITE, M. F., LUCHETA, A. R., SHIMELS, M., BOUWMEESTER, H. J., VAN VEEN, J. A. & KURAMAE, E. E. 2017. Rhizobacterial community structure differences among sorghum cultivars in different growth stages and soils. *Federation of European Microbiology Societies Microbiology Ecology,* 93**,** 1-11.

SCHMID, M. W., HAHL, T., VAN MOORSEL, S. J., WAGG, C., DE DEYN, G. B. & SCHMID, B. 2019. Feedbacks of plant identity and diversity on the diversity and community composition of

rhizosphere microbiomes from a long-term biodiversity experiment. *Molecular Ecology,* 28**,** 863-878.

SCHREITER, S., DING, G.C., HEUER, H., NEUMANN, G., SANDMANN, M., GROSCH, R., KROPF, S. & SMALLA, K. 2014. Effect of The Soil Type on The Microbiome in The Rhizosphere of Field-Grown Lettuce. *Frontiers in Microbiology,* 5**,** 1-13.

SCHWARZ, K., CUTTS, B. B., LONDON, J. K. & CADENASSO, M. L. 2016. Growing gardens in shrinking cities: A solution to the soil lead problem? *Sustainability,* 8**,** 141.

ŞEKER, M. G., ŞAH, I., KıRDÖK, E., EKINCI, H., ÇIFTÇI, Y. Ö. & AKKAYA, Ö. 2017. A hidden plant growth promoting bacterium isolated from in vitro cultures of fraser photinia (Photinia× fraseri). *International Journal of Agriculture and Biology,* 19**,** 1511-1519.

SERGAKI, C., LAGUNAS, B., LIDBURY, I., GIFFORD, M. L. & SCHÄFER, P. 2018. Challenges and approaches in microbiome research: from fundamental to applied. *Frontiers in Plant Science,* 9**,** 1-12.

SESSITSCH, A., HARDOIM, P., DÖRING, J., WEILHARTER, A., KRAUSE, A., WOYKE, T., MITTER, B., HAUBERG-LOTTE, L., FRIEDRICH, F. & RAHALKAR, M. 2012. Functional characteristics of an endophyte community colonizing rice roots as revealed by metagenomic analysis. *Molecular Plant-Microbe Interactions,* 25**,** 28-36.

SESSITSCH, A. & MITTER, B. 2015. 21st century agriculture: integration of plant microbiomes for improved crop production and food security. *Microbial Biotechnology,* 8**,** 32-33.

SHAHBAZ, M., KUZYAKOV, Y., SANAULLAH, M., HEITKAMP, F., ZELENEV, V., KUMAR, A. & BLAGODATSKAYA, E. 2017. Microbial decomposition of soil organic matter is mediated by quality and quantity of crop residues: mechanisms and thresholds. *Biology and Fertility of Soils,* 53**,** 287-301.

SHARMA, B., SARKAR, A., SINGH, P. & SINGH, R. 2017. Agricultural utilization of biosolids: A review on potential effects on soil and plant grown. Journal of Waste Management 64: 117–132.

SHARMA, H. K., XU, C. & QIN, W. 2019. Biological pretreatment of lignocellulosic biomass for biofuels and bioproducts: An overview. *Waste and Biomass Valorization,* 10**,** 235-251.

SHI, J.Y., YUAN, X.F., LIN, H.R., YANG, Y.Q. & LI, Z.Y. 2011a. Differences in soil properties and bacterial communities between the rhizosphere and bulk soil and among different production areas of the medicinal plant Fritillaria thunbergii. *International Journal of Molecular Sciences,* 12**,** 3770-3785.

SHI, Z.L., LIU, F.M. & YAO, S.H. 2011b. Adsorptive removal of phosphate from aqueous solutions using activated carbon loaded with Fe (III) oxide. *New Carbon Materials,* 26**,** 299-306.

SHRESTHA, B., POKHREL, K., PAUDEL, N., POUDEL, S., SHABBIR, A. & ADKINS, S. 2019. Distribution of Parthenium hysterophorus and one of its biological control agents (Coleoptera: Zygogramma bicolorata) in Nepal. *Weed Research,* 59**,** 467-478.

SIEBER, C. M., PROBST, A. J., SHARRAR, A., THOMAS, B. C., HESS, M., TRINGE, S. G. & BANFIELD, J. F. 2018. Recovery of genomes from metagenomes via a dereplication, aggregation and scoring strategy. *Nature Microbiology,* 3**,** 836-843.

SILVA, C. O., VAZ, R. P. & FILHO, E. X. 2018. Bringing plant cell wall-degrading enzymes into the lignocellulosic biorefinery concept. *Biofuels, Bioproducts and Biorefining,* 12**,** 277-289.

SIMON ROJO, M., MORATALLA, A. Z., ALONSO, N. M. & JIMENEZ, V. H. 2014. Pathways towards the integration of periurban agrarian ecosystems into the spatial planning system. *Ecological Processes,* 3**,** 1-16.

SINGH, J., SHARMA, M., SINGH, S., BANO, R. & MAHAWAR, A. 2018. Effect of Organic and Inorganic Sources of NPK and Bio-fertilizer on Enhancement of Growth Attributes and Chlorophyll Content of Sweet Potato. *International Journal of Current Microbiology and Applied Science,* 7**,** 3659-3667.

SINGH, S. B., GOWTHAM, H., MURALI, M., HARIPRASAD, P., LAKSHMEESHA, T., MURTHY, K. N., AMRUTHESH, K. & NIRANJANA, S. 2019a. Plant growth promoting ability of ACC deaminase producing rhizobacteria native to Sunflower (*Helianthus annuus* L.). *Biocatalysis and Agricultural Biotechnology,* 18**,** 1-9.

SINGH, S. B., GOWTHAM, H., MURALI, M., HARIPRASAD, P., LAKSHMEESHA, T., MURTHY, K. N., AMRUTHESH, K. & NIRANJANA, S. 2019b. Plant growth promoting ability of ACC deaminase producing rhizobacteria native to Sunflower (*Helianthus annuus* L.). *Biocatalysis and Agricultural Biotechnology,* 18**,** 101089.

SINGH, S. K., SINGH, P. P., GUPTA, A., SINGH, A. K. & KESHRI, J. 2019c. Tolerance of heavy metal toxicity using PGPR strains of *Pseudomonas* species. *In: PGPR amelioration in sustainable agriculture.* United Kingdom: *Elsevier*.

SINGH, T., GANGIL, B., PATNAIK, A., BISWAS, D. & FEKETE, G. 2019d. Agriculture waste reinforced cornstarch-based biocomposites: effect of rice husk/walnut shell on physicomechanical, biodegradable and thermal properties. *Materials Research Express,* 6**,** 1-11.

SMITH-RAMESH, L. M. & REYNOLDS, H. L. 2017. The next frontier of plant–soil feedback research: unraveling context dependence across biotic and abiotic gradients. *Journal of Vegetation Science,* 28**,** 484-494.

SONG, C. W., PARK, J. M., CHUNG, S. C., LEE, S. Y. & SONG, H. 2019. Microbial production of 2,3-butanediol for industrial applications. *Journal of Industrial Microbiology and Biotechnology,* 46**,** 1583-1601.

SONI, R. & GOEL, R. 2011. nifH homologs from soil metagenome. *Ekologija*, 57.

SOUZA, R. D., AMBROSINI, A. & PASSAGLIA, L. M. 2015. Plant growth-promoting bacteria as inoculants in agricultural soils. *Genetics and Molecular Biology,* 38**,** 401-419.

SRIVASTAVIA, R. S., ANSHIKA 2017. Plant growth promoting rhizobacteria (PGPR) for sustainable agriculture. *International Journal of Agricultural Science and Research,* 7**,** 505-510.

STARR, E. P., SHI, S., BLAZEWICZ, S. J., PROBST, A. J., HERMAN, D. J., FIRESTONE, M. K. & BANFIELD, J. F. 2018. Stable isotope informed genome-resolved metagenomics reveals that *Saccharibacteria* utilize microbially-processed plant-derived carbon. *Microbiome,* 6**,** 1-12.

STRUECKER, J. & JOERGENSEN, R. G. 2015. Microorganisms and their substrate utilization patterns in topsoil and subsoil layers of two silt loams, differing in soil organic C accumulation due to colluvial processes. *Soil Biology and Biochemistry,* 91**,** 310-317.

SUÁREZ, J. & STENCEL, A. 2020. A part-dependent account of biological individuality: Why holobionts are individuals and ecosystems simultaneously. *Biological Reviews,* 95**,** 1308-1324.

SUN, J., ZOU, L., LI, W., YANG, J., WANG, Y., XIA, Q. & PENG, M. 2018. Rhizosphere soil properties and banana Fusarium wilt suppression influenced by combined chemical and organic fertilizations. *Agriculture, Ecosystems & Environment,* 254**,** 60-68.

SUN, K. 2020. Ktrim: an extra-fast and accurate adapter-and quality-trimmer for sequencing data. *Bioinformatics,* 36**,** 3561-3562.

SUSILOWATI, D. N., SUDIANA, I., MUBARIK, N., AGATIS, J. & CAMPUS, D. 2015. Species and functional diversity of rhizobacteria of rice plant in the coastal soils of Indonesia. *Indonesian Journal of Agricultural Science,* 16**,** 39- 50.

SUYAL, D. C., YADAV, A., SHOUCHE, Y. & GOEL, R. 2015. Bacterial diversity and community structure of Western Indian Himalayan red kidney bean (Phaseolus vulgaris) rhizosphere as revealed by 16S rRNA gene sequences. *Biologia,* 70**,** 305-313.

TAKAGI, H. & OHTSU, I. 2016. L-Cysteine metabolism and fermentation in microorganisms. *Amino acid fermentation***,** 129-151.

TAMAMES, J. & PUENTE-SÁNCHEZ, F. 2019. SqueezeMeta, a highly portable, fully automatic metagenomic analysis pipeline. *Frontiers in Microbiology,* 9**,** 3349.

TAN, B., NG, C. M., NSHIMYIMANA, J. P., LOH, L.-L., GIN, K. Y.-H. & THOMPSON, J. R. 2015. Next-generation sequencing (NGS) for assessment of microbial water quality: current progress, challenges and future opportunities. *Frontiers in Microbiology,* 6**,** 1027.

TAN, W., WANG, J., BAI, W., QI, J. & CHEN, W. 2020. Soil bacterial diversity correlates with precipitation and soil pH in long-term maize cropping systems. *Scientific Reports,* 10**,** 1-12.

TANG, Y., ZHANG, X., LI, D., WANG, H., CHEN, F., FU, X., FANG, X., SUN, X. & YU, G. 2016. Impacts of nitrogen and phosphorus additions on the abundance and community structure of

ammonia oxidizers and denitrifying bacteria in Chinese fir plantations. *Soil Biology and Biochemistry,* 103**,** 284-293.

TANGAHU, B. V., SHEIKH ABDULLAH, S. R., BASRI, H., IDRIS, M., ANUAR, N. & MUKHLISIN, M. 2011. A review on heavy metals (As, Pb and Hg) uptake by plants through phytoremediation. *International Journal of Chemical Engineering,* 2011.

TASSI, E., GIORGETTI, L., MORELLI, E., PERALTA-VIDEA, J., GARDEA-TORRESDEY, J. & BARBAFIERI, M. 2017. Physiological and biochemical responses of sunflower (*Helianthus annuus* L.) exposed to nano-CeO2 and excess boron: modulation of boron phytotoxicity. *Plant Physiology and Biochemistry,* 110**,** 50-58.

TAUBERT, M., STÄHLY, J., KOLB, S. & KÜSEL, K. 2019. Divergent microbial communities in groundwater and overlying soils exhibit functional redundancy for plant-polysaccharide degradation. *Public Library of Science One,* 14**,** e0212937.

TESTE, F. P., KARDOL, P., TURNER, B. L., WARDLE, D. A., ZEMUNIK, G., RENTON, M. & LALIBERTÉ, E. 2017. Plant-soil feedback and the maintenance of diversity in Mediterranean-climate shrublands. *Science,* 355**,** 173-176.

THOKCHOM, E., THAKURIA, D., KALITA, M. C., SHARMA, C. K. & TALUKDAR, N. C. 2017. Root colonization by host-specific rhizobacteria alters indigenous root endophyte and rhizosphere soil bacterial communities and promotes the growth of mandarin orange. *European Journal of Soil Biology,* 79**,** 48-56.

THOMAS, F., HEHEMANN, J.H., REBUFFET, E., CZJZEK, M. & MICHEL, G. 2011. Environmental and gut Bacteroidetes: the food connection. *Frontiers in microbiology,* 2**,** 93.

TIAN, T., REVERDY, A., SHE, Q., SUN, B. & CHAI, Y. 2020. The role of rhizodeposits in shaping rhizomicrobiome. *Environmental Microbiology Reports,* 12**,** 160-172.

TIEMANN, L., GRANDY, A., ATKINSON, E., MARIN-SPIOTTA, E. & MCDANIEL, M. 2015. Crop rotational diversity enhances belowground communities and functions in an agroecosystem. *Ecology Letters,* 18**,** 761-771.

TIMMUSK, S., BEHERS, L., MUTHONI, J., MURAYA, A. & ARONSSON, A.C. 2017. Perspectives and challenges of microbial application for crop improvement. *Frontiers in Plant Science,* 8**,** 49.

TIRRY, N., JOUTEY, N. T., SAYEL, H., KOUCHOU, A., BAHAFID, W., ASRI, M. & EL GHACHTOULI, N. 2018. Screening of plant growth promoting traits in heavy metals resistant bacteria: prospects in phytoremediation. *Journal of Genetic Engineering and Biotechnology,* 16**,** 613-619.

TIWARI, S. & LATA, C. 2018. Heavy metal stress, signaling and tolerance due to plant-associated microbes: an overview. *Frontiers in Plant Science,* 9**,** 1-12.

TOYAMA, T., FURUKAWA, T., MAEDA, N., INOUE, D., SEI, K., MORI, K., KIKUCHI, S. & IKE, M. 2011. Accelerated biodegradation of pyrene and benzo [a] pyrene in the Phragmites australis rhizosphere by bacteria–root exudate interactions. *Water research,* 45**,** 1629-1638.

TRIVEDI, P., MATTUPALLI, C., EVERSOLE, K. & LEACH, J. E. 2021. Enabling sustainable agriculture through understanding and enhancement of microbiomes. *New Phytologist,* 230**,** 2129-2147.

TSENG, S.C., LIANG, C.M., CHIA, T. & TON, S.S. 2021. Changes in the composition of the soil bacterial community in heavy metal-contaminated farmland. *International Journal of Environmental Research and Public Health,* 18**,** 8661.

TURNER, T. R., JAMES, E. K. & POOLE, P. S. 2013. The plant microbiome. *Genome Biology,* 14**,** 1-10.

TYLER, H. L. 2021. Shifts in bacterial community in response to conservation management practices within a soybean production system. *Biology and Fertility of Soils,* 57**,** 575-586.

UDVARDI, M. & POOLE, P. S. 2013. Transport and metabolism in legume-rhizobia symbioses. *Annual Review of Plant Biology,* 64**,** 1-7.

UMADEVI, P., ANANDARAJ, M., SRIVASTAV, V. & BENJAMIN, S. 2018. *Trichoderma harzianum* MTCC 5179 impacts the population and functional dynamics of microbial community

in the rhizosphere of black pepper (*Piper nigrum* L.). *Brazilian Journal of Microbiology,* 49**,** 463-470.

URANA, R., DAHIYA, A., SHARMA, P. & SINGH, N. 2019. Effects of plant growth promoting rhizobacteria on phytoremediation of phenanthrene contaminated sodic soil. *Polycyclic Aromatic Compounds,* 12**,** 1-10.

VALENTINE, A. J., BENEDITO, V. A. & KANG, Y. 2018. Legume nitrogen fixation and soil abiotic stress: from physiology to genomics and beyond. *Annual Plant Reviews Online,* 42**,** 207-248.

VALLIAMMAI, M. G., GOPAL, N. O. & ANANDHAM, R. 2020. Elucidation of microbial diversity and lignocellulolytic enzymes for the degradation of lignocellulosic biomass in the forest soils of Eastern and Western Ghats of Tamil Nadu, India. *Biofuels, Bioproducts and Biorefining***,** 1-14.

VAN DER HEIJDEN, M. G. & SCHLAEPPI, K. 2015. Root surface as a frontier for plant microbiome research. *Proceedings of the National Academy of Sciences,* 112**,** 2299-2300.

VAN NIFTRIK, L., GEERTS, W. J., VAN DONSELAAR, E. G., HUMBEL, B. M., WEBB, R. I., FUERST, J. A., VERKLEIJ, A. J., JETTEN, M. S. & STROUS, M. 2008. Linking ultrastructure and function in four genera of anaerobic ammonium-oxidizing bacteria: cell plan, glycogen storage and localization of cytochrome c proteins. *Journal of Bacteriology,* 190**,** 708-717.

VAN VEENHUIZEN, R. & DANSO, G. 2007. *Profitability and sustainability of urban and periurban agriculture*, Food & Agriculture Organisation.

VANDENKOORNHUYSE, P., QUAISER, A., DUHAMEL, M., LE VAN, A. & DUFRESNE, A. 2015. *The importance of the microbiome of the plant holobiont*. *New Phytologist*, 206**,** 1196-1206.

VARGAS-ALBORES, F., MARTÍNEZ-CÓRDOVA, L. R., MARTÍNEZ-PORCHAS, M., CALDERÓN, K. & LAGO-LESTÓN, A. 2019. Functional metagenomics: a tool to gain knowledge for agronomic and veterinary sciences. *Biotechnology and Genetic Engineering Reviews*, 35**,** 69-91.

VEJAN, P., ABDULLAH, R., KHADIRAN, T., ISMAIL, S. & BOYCE, A.N. 2016. Role of plant growth promoting rhizobacteria in agricultural sustainability—a review. *Molecules, 21*(5):573.

VELÁZQUEZ-SEPÚLVEDA, I., OROZCO-MOSQUEDA, M., PRIETO-BARAJAS, C. & SANTOYO, G. 2012. Bacterial diversity associated with the rhizosphere of wheat plants (*Triticum aestivum*): Toward a metagenomic analysis. *Phyton*, 81**,** 81.

VELVIZHI, G., BALAKUMAR, K. & DHARANIDHARAN, S. 2019. Sequestering of CO2 to Value-Added Products through Various Biological Processes. *Next Generation Biomanufacturing Technologies.* ACS Publications.

VIESSER, J. A., SUGAI-GUERIOS, M. H., MALUCELLI, L. C., PINCERATI, M. R., KARP, S. G. & MARANHO, L. T. 2020. Petroleum-tolerant rhizospheric bacteria: Isolation, characterization and bioremediation potential. *Scientific Reports,* 10**,** 1-11.

VIVES-PERIS, V., DE OLLAS, C., GÓMEZ-CADENAS, A. & PÉREZ-CLEMENTE, R. M. 2020. Root exudates: from plant to rhizosphere and beyond. *Plant Cell Reports,* 39**,** 3-17.

WALAKLEY, A. & BLACK, C. 1934. Estimation of organic carbon by chromic acid titration method. *Soil Science,* 37**,** 29-38.

WALKLEY, A. & BLACK, I. A. 1934. An examination of the Degtjareff method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil Science,* 37**,** 1-10.

WAN, Y., HUANG, Q., WANG, Q., YU, Y., SU, D., QIAO, Y. & LI, H. 2020. Accumulation and bioavailability of heavy metals in an acid soil and their uptake by paddy rice under continuous application of chicken and swine manure. *Journal of Hazardous Materials,* 384**,** 121293.

WANG, A., REDINGTON, L., STEINMETZ, V. & LINDEMAN, D. 2011. The ADOPT model: Accelerating diffusion of proven technologies for older adults. *Ageing International,* 36**,** 29-45.

WANG, C.Y., ZHOU, X., GUO, D., ZHAO, J.H., YAN, L., FENG, G.Z., GAO, Q., YU, H. & ZHAO, L.-P. 2019a. Soil pH is the primary factor driving the distribution and function of microorganisms in farmland soils in northeastern China. *Annals of Microbiology,* 69**,** 1461-1473.

WANG, D., WANG, Y. & WU, F. 2012. Effect of different cultivation modes on cucumber growth and the numbers of culturable rhizosphere soil microorganisms. *Journal of Northeast Agricultural University,* 7**,** 95-99.

WANG, Q., GARRITY, G. M., TIEDJE, J. M. & COLE, J. R. 2007. Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. *Applied and Environmental Microbiology,* 73**,** 5261-5267.

WANG, S., LI, T., ZHENG, Z. & CHEN, H. Y. 2019b. Soil aggregate-associated bacterial metabolic activity and community structure in different aged tea plantations. *Science of the Total environment,* 654**,** 1023-1032.

WANG, X., BEI, Q., YANG, W., ZHANG, H., HAO, J., QIAN, L., FENG, Y. & XIE, Z. 2020a. Unveiling of active diazotrophs in a flooded rice soil by combination of NanoSIMS and 15 N 2-DNA-stable isotope probing. *Biology and Fertility of Soils***,** 1-11.

WANG, X., DONG, G., LIU, X., ZHANG, S., LI, C., LU, X. & XIA, T. 2020b. Poly-γ-glutamic acid-producing bacteria reduced Cd uptake and effected the rhizosphere microbial communities of lettuce. *Journal of Hazardous Materials,* 398**,** 123146.

WANG, Z., ZHANG, X.X., HUANG, K., MIAO, Y., SHI, P., LIU, B., LONG, C. & LI, A. 2013. Metagenomic profiling of antibiotic resistance genes and mobile genetic elements in a tannery wastewater treatment plant. *PLoS One,* 8**,** e76079.

WARD, N. L., CHALLACOMBE, J. F., JANSSEN, P. H., HENRISSAT, B., COUTINHO, P. M., WU, M., XIE, G., HAFT, D. H., SAIT, M. & BADGER, J. 2009. Three genomes from the phylum Acidobacteria provide insight into the lifestyles of these microorganisms in soils. *Applied and Environmental Microbiology,* 75**,** 2046-2056.

WEBER, N., LIOU, D., DOMMER, J., MACMENAMIN, P., QUIÑONES, M., MISNER, I., OLER, A. J., WAN, J., KIM, L. & COAKLEY MCCARTHY, M. 2018. Nephele: a cloud platform for simplified, standardized and reproducible microbiome data analysis. *Bioinformatics,* 34**,** 1411-1413.

WEI, X., WANG, X., CAO, P., GAO, Z., CHEN, A. J. & HAN, J. 2020. Microbial community changes in the rhizosphere soil of healthy and rusty *Panax ginseng* and discovery of pivotal fungal genera associated with rusty roots. *BioMed Research International,* 2020**,** 1-14.

WEI, Y., ZHAO, X., SUN, J. & LIU, H. 2019. Fast repetition rate fluorometry (FRRF) derived phytoplankton primary productivity in the Bay of Bengal. *Frontiers in Microbiology,* 10**,** 1164.

WEN, X.-Y., DUBINSKY, E., YAO, W., RONG, Y. & FU, C. 2016. Wheat, maize and sunflower cropping systems selectively influence bacteria community structure and diversity in their and succeeding crop's rhizosphere. *Journal of Integrative Agriculture,* 15**,** 1892-1902.

WIECZOREK, A. S., SCHMIDT, O., CHATZINOTAS, A., VON BERGEN, M., GORISSEN, A. & KOLB, S. 2019. Ecological functions of agricultural soil bacteria and microeukaryotes in chitin degradation: a case study. *Frontiers in Microbiology,* 10.

WIERZBICKA-WOŚ, A., HENNEBERGER, R., BATISTA-GARCÍA, R. A., MARTÍNEZ-ÁVILA, L., JACKSON, S. A., KENNEDY, J. & DOBSON, A. D. W. 2019. Biochemical characterization of a novel monospecific endo-β-1,4-glucanase belonging to GH family 5 from a rhizosphere metagenomic library. *Frontiers in Microbiology,* 10**,** 1-19.

WILLIAMS, N. S., LUNDHOLM, J. & SCOTT MACIVOR, J. 2014. Do green roofs help urban biodiversity conservation? *Journal of Applied Ecology,* 51**,** 1643-1649.

WINAND, R., BOGAERTS, B., HOFFMAN, S., LEFEVRE, L., DELVOYE, M., VAN BRAEKEL, J., FU, Q., ROOSENS, N. H., DE KEERSMAECKER, S. C. & VANNESTE, K. 2019. Targeting the 16s rRNA gene for bacterial identification in complex mixed samples: Comparative evaluation of second (illumina) and third (oxford nanopore technologies) generation sequencing technologies. *International Journal of Molecular Sciences,* 21**,** 298.

WONGKIEW, S., POLPRASERT, C., KOOTTATEP, T., LIMPIYAKORN, T., SURENDRA, K. & KHANAL, S. K. 2022. Chicken manure-based bioponics: Effects of acetic acid supplementation on nitrogen and phosphorus recoveries and microbial communities. *Waste Management,* 137**,** 264-274.

WRIGHTON, K. C., THOMAS, B. C., SHARON, I., MILLER, C. S., CASTELLE, C. J., VERBERKMOES, N. C., WILKINS, M. J., HETTICH, R. L., LIPTON, M. S. & WILLIAMS, K. H. 2012. Fermentation, hydrogen and sulfur metabolism in multiple uncultivated bacterial phyla. *Science,* 337**,** 1661-1665.

WU, L., CHEN, J., XIAO, Z., ZHU, X., WANG, J., WU, H., WU, Y., ZHANG, Z. & LIN, W. 2018a. Barcoded pyrosequencing reveals a shift in the bacterial community in the rhizosphere and rhizoplane of *Rehmannia glutinosa* under consecutive monoculture. *International Journal of Molecular Sciences,* 19**,** 850.

WU, L., WANG, J., WU, H., CHEN, J., XIAO, Z., QIN, X., ZHANG, Z. & LIN, W. 2018b. Comparative metagenomic analysis of rhizosphere microbial community composition and functional potentials under *Rehmannia glutinosa* consecutive monoculture. *International Journal of Molecular Sciences,* 19**,** 2394.

XIAO, X.Y., WANG, M.W., ZHU, H.W., GUO, Z.H., HAN, X.Q. & ZENG, P. 2017. Response of soil microbial activities and microbial community structure to *vanadium* stress. *Ecotoxicology and Environmental Safety,* 142**,** 200-206.

XIN, F., CHEN, T., JIANG, Y., DONG, W., ZHANG, W., ZHANG, M., WU, H., MA, J. & JIANG, M. 2017. Strategies for improved isopropanol–butanol production by a *Clostridium* strain from glucose and hemicellulose through consolidated bioprocessing. *Biotechnology for Biofuels,* 10**,** 118.

XIONG, Q., HU, J., WEI, H., ZHANG, H. & ZHU, J. 2021. Relationship between plant roots, rhizosphere microorganisms and nitrogen and its special focus on rice. *Agriculture*, 11**,** 234.

XU, L., NAYLOR, D., DONG, Z., SIMMONS, T., PIERROZ, G., HIXSON, K. K., KIM, Y.M., ZINK, E. M., ENGBRECHT, K. M. & WANG, Y. 2018. Drought delays development of the

sorghum root microbiome and enriches for monoderm bacteria. *Proceedings of the National Academy of Sciences,* 115**,** E4284-E4293.

XU, S., XING, Y., LIU, S., HAO, X., CHEN, W. & HUANG, Q. 2020a. Characterization of Cd2+ biosorption by *Pseudomonas* sp. strain 375, a novel biosorbent isolated from soil polluted with heavy metals in Southern China. *Chemosphere,* 240**,** 124893.

XU, Y., DU, A., WANG, Z., ZHU, W., LI, C. & WU, L. 2020b. Effects of different rotation periods of Eucalyptus plantations on soil physiochemical properties, enzyme activities, microbial biomass and microbial community structure and diversity. *Forest Ecology and Management,* 456**,** 117683.

XU, Y., GE, Y., SONG, J. & RENSING, C. 2019. Assembly of root-associated microbial community of typical rice cultivars in different soil types. *Biology and Fertility of Soils,* 56**,** 1-12.

YADAV, A. N., VERMA, P., KUMAR, S., KUMAR, V., KUMAR, M., SUGITHA, T. C. K., SINGH, B. P., SAXENA, A. K. & DHALIWAL, H. S. 2018. Actinobacteria from rhizosphere: molecular diversity, distributions and potential biotechnological applications. *New and Future Developments in Microbial Biotechnology and Bioengineering.* Elsevier.

YADAV, A. N., VERMA, P., SINGH, B., CHAUHAN, V. S., SUMAN, A. & SAXENA, A. K. 2017. Plant growth promoting bacteria: biodiversity and multifunctional attributes for sustainable agriculture. *Advanced Biotechnology and Microbiology,* 5**,** 1-16.

YAN, Y., KURAMAE, E. E., DE HOLLANDER, M., KLINKHAMER, P. G. & VAN VEEN, J. A. 2017a. Functional traits dominate the diversity-related selection of bacterial communities in the rhizosphere. *The International Society for Microbial Ecology Journal,* 11**,** 56-66.

YAN, Y., KURAMAE, E. E., DE HOLLANDER, M., KLINKHAMER, P. G. & VAN VEEN, J. A. 2017b. Functional traits dominate the diversity-related selection of bacterial communities in the rhizosphere. *The ISME Journal,* 11**,** 56-66.

YANG, J., KLOEPPER, J. W. & RYU, C.-M. 2009. Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science,* 14**,** 1-4.

YEE, M. O., KIM, P., LI, Y., SINGH, A. K., NORTHEN, T. R. & CHAKRABORTY, R. 2021. Specialized Plant Growth Chamber Designs to Study Complex Rhizosphere Interactions. *Frontiers in Microbiology,* 12**,** 507.

YIN, H., ZHANG, X., LI, X., HE, Z., LIANG, Y., GUO, X., HU, Q., XIAO, Y., CONG, J. & MA, L. 2014. Whole-genome sequencing reveals novel insights into sulfur oxidation in the extremophile *Acidithiobacillus thiooxidans*. *BMC Microbiology,* 14**,** 1-14.

YOUNG, A. D. & GILLUNG, J. P. 2020. Phylogenomics—principles, opportunities and pitfalls of big-data phylogenetics. *Systematic Entomology,* 45**,** 225-247.

YOUSEIF, S. H., ABD EL-MEGEED, F. H. & SALEH, S. A. 2017. Improvement of faba bean yield using rhizobium/agrobacterium inoculant in low-fertility sandy soil. *Agronomy,* 7**,** 1-12.

YU, J., BLOM, J., GLAESER, S., JAENICKE, S., JUHRE, T., RUPP, O., SCHWENGERS, O., SPÄNIG, S. & GOESMANN, A. 2017. A review of bioinformatics platforms for comparative genomics. Recent developments of the EDGAR 2.0 platform and its utility for taxonomic and phylogenetic studies. *Journal of Biotechnology*, 261**,** 2-9.

YU, X., HU, X., PENG, Y., WU, Z., ZHANG, Q., LI, Z., SHI, C. & DU, K. 2019. Amplicon sequencing reveals different microbial communities in living poplar wetwood and sapwood. *Trees,* 33**,** 851-865.

YUAN, J., YUAN, Y., ZHU, Y. & CAO, L. 2018. Effects of different fertilizers on methane emissions and methanogenic community structures in paddy rhizosphere soil. *Science of The Total Environment,* 627**,** 770-781.

YURGEL, S. N., NEARING, J. T., DOUGLAS, G. M. & LANGILLE, M. G. I. 2019. Metagenomic functional shifts to plant induced environmental changes. *Frontiers in Microbiology,* 10, 1618.

ZARIK, L., MEDDICH, A., HIJRI, M., HAFIDI, M., OUHAMMOU, A., OUAHMANE, L., DUPONNOIS, R. & BOUMEZZOUGH, A. 2016. Use of arbuscular mycorrhizal fungi to improve the drought tolerance of *Cupressus atlantica* G. *Comptes Rendus Biologies,* 339**,** 185-196.

ZARRAONAINDIA, I., OWENS, S. M., WEISENHORN, P., WEST, K., HAMPTON-MARCELL, J., LAX, S., BOKULICH, N. A., MILLS, D. A., MARTIN, G. & TAGHAVI, S.

2015. The soil microbiome influences grapevine-associated microbiota. *America Society of Microbioloy- MBio,* 6**,** e02527-14.

ZENG, M., ZHONG, Y., CAI, S. & DIAO, Y. 2018. Deciphering the bacterial composition in the rhizosphere of *Baphicacanthus cusia* (NeeS) Bremek. *Scientific Reports,* 8**,** 1-11.

ZHANG, B.H., HONG, J.P., ZHANG, Q., JIN, D.S. & GAO, C.H. 2020. Contrast in soil microbial metabolic functional diversity to fertilization and crop rotation under rhizosphere and non-rhizosphere in the coal gangue landfill reclamation area of Loess Hills. *PLoS One,* 15**,** e0229341.

ZHANG, L.N., WANG, D.C., HU, Q., DAI, X.Q., XIE, Y.S., LI, Q., LIU, H.M. & GUO, J.H. 2019a. Consortium of plant growth-promoting rhizobacteria strains suppresses sweet pepper disease by altering the rhizosphere microbiota. *Frontiers in Microbiology,* 10**,** 1668.

ZHANG, L., CHEN, F., ZENG, Z., XU, M., SUN, F., YANG, L., BI, X., LIN, Y., GAO, Y. & HAO, H. 2021. Advances in Metagenomics and Its Application in Environmental Microorganisms. *Frontiers in Microbiology,* 12**,** 766364-766364.

ZHANG, R., VIVANCO, J. M. & SHEN, Q. 2017. The unseen rhizosphere root–soil–microbe interactions for crop production. *Current Opinion in Microbiology,* 37**,** 8-14.

ZHANG, X.Y., JIA, X.M., ZHANG, R., ZHU, Z.L., LIU, B., GAO, L.Y. & WANG, Y.X. 2019b. Metabolic analysis in *Malus halliana* leaves in response to iron deficiency. *Scientia Horticulturae,* 258**,** 108792.

ZHANG, X., NIU, J., LIANG, Y., LIU, X. & YIN, H. 2016. Metagenome-scale analysis yields insights into the structure and function of microbial communities in a copper bioleaching heap. *BMC genetics,* 17**,** 1-12.

ZHANG, Y., GAO, X., WANG, S., ZHU, C., LI, R. & SHEN, Q. 2018. Application of *Bacillus velezensis* NJAU-Z9 enhanced plant growth associated with efficient rhizospheric colonization monitored by qPCR with primers designed from the whole genome sequence. *Current Microbiology,* 75**,** 1574-1583.

ZHANG, XY., JIA XM., ZHANG, R., ZHU, ZL., LIU, B., GAO, LY., WANG, YX. 2019. Metabolic analysis in *Malus halliana* leaves in response to iron deficiency. *Scientia Horticulturae*, 258:108792.

ZHAO, L., ZHANG, H., KOHNEN, M., PRASAD, K., GU, L. & REDDY, A. S. 2019. Analysis of transcriptome and epitranscriptome in plants using PacBio Iso-Seq and nanopore-based direct RNA sequencing. *Frontiers in Genetics,* 10**,** 253.

Zhao, L. & Zhang, Y.Q. 2015. Effects of phosphate solubilization and phytohormone production of *Trichoderma asperellum* Q1 on promoting cucumber growth under salt stress. *Journal of Integrative Agriculture*, 14, 1588–1597.

ZHAO, L., WANG, Y. & KONG, S. 2020. Effects of *Trichoderma asperellum* and its siderophores on endogenous auxin in *Arabidopsis thaliana* under iron-deficiency stress. *International Microbiology*, 23, 501–509.

ZHONG, Y., YANG, Y., LIU, P., XU, R., RENSING, C., FU, X. & LIAO, H. 2019. Genotype and rhizobium inoculation modulate the assembly of soybean rhizobacterial communities. *Plant, Cell and Environment*, 42**,** 2028-2044.

ZHOU, C., HEAL, K., TIGABU, M., XIA, L., HU, H., YIN, D. & MA, X. 2020a. Biochar addition to forest plantation soil enhances phosphorus availability and soil bacterial community diversity. *Forest Ecology and Management*, 455**,** 117635.

ZHOU, J., JIANG, X., ZHOU, B., ZHAO, B., MA, M., GUAN, D., LI, J., CHEN, S., CAO, F. & SHEN, D. 2016. Thirty four years of nitrogen fertilization decreases fungal diversity and alters fungal community composition in black soil in northeast China. *Soil Biology and Biochemistry*, 95**,** 135-143.

ZHOU, Q., SU, X. & NING, K. 2014. Assessment of quality control approaches for metagenomic data analysis. *Scientific Reports*, 4**,** 1-11.

ZHOU, Z., DING, G., YU, M., GAO, G. & WANG, G. 2020b. Diversity and Structural Variability of Bacterial Microbial Communities in Rhizocompartments of Desert Leguminous Plants. *Bio - archive***,** 1-47.

ZHU, Y. 2016. International trade and food security: Conceptual discussion, WTO and the case of China. *China Agricultural Economic Review,* 8.

ZHU, Z., GE, T., LIU, S., HU, Y., YE, R., XIAO, M., TONG, C., KUZYAKOV, Y. & WU, J. 2018. Rice rhizodeposits affect organic matter priming in paddy soil: the role of N fertilization and plant growth for enzyme activities, CO2 and CH4 emissions. *Soil Biology and Biochemistry,* 116**,** 369-377.

ZWETSLOOT, M. J., UCROS, J. M., WICKINGS, K., WILHELM, R. C., SPARKS, J., BUCKLEY, D. H. & BAUERLE, T. L. 2020. Prevalent root-derived phenolics drive shifts in microbial community composition and prime decomposition in forest soil. *Soil Biology and Biochemistry,* 145**,** 1-13.

APPENDICES

Table S9.1: Agricultural history of Ditsobottla and Kraaipan farms.

Agricultural practices	**Farm**	
	Ditsobottla sunflower farm	Kraaipan sunflower farm
Herbicides application before planting	Yes (Roundup: Glyphosate-based herbicide)	No
Herbicides application after planting	Yes (Metagan Gold)	No
Bio-fertilizer	Yes (Omina fertilizer)	No
Pesticides	Yes (Judo 50EC)	No
Fertilizer	Yes (NPK15:8:4)	Yes (NPK15:8:4)
Organic manure	No	No
Mechanized	Yes(Ploughing and Tillage)	Yes (Mowing, Ploughing, Tillage)

Table S9.2: Forward selection of soil physio-chemical components that best described difference in bacterial components between sunflower rhizosphere and bulk soils of Ditsobottla and Kraaipan farms.

Environmental variable	**Explains %**	**Contribution %**	**Pseudo-F**	***P***
pH	71.4	71.4	5.0	0.184
Total N (%)	20.0	20.0	2.3	0.218
OM (%)	8.6	8.6	<0.1	1.

Legend: N – nitrate, % - percentage, OM – organic matter, p – probability value

Table S9.3: Forward selection of soil carbon substrates that best described difference in bacterial components between sunflower rhizosphere and bulk soils of Ditsobottla and Kraaipan farms.

Carbon substrate	Explained %	Contribution %	Pseudo-F	**P**
Tryptophan	75.9	75.9	6.3	0.102
Galactose	16.7	16.7	2.2	0.286
Citric acid	7.5	7.5	<0.1	1.

Legend: % - percentage, p – probability value

Table S9.4: Forward selection of soil physio-chemical components that best described difference in carbon substrate utilization between sunflower rhizosphere and bulk soils of Ditsobottla and Kraaipan farms.

PC	Explain %	Contribution %	Pseudo-F	P-
OM (%)	51.4	51.4	2.1	0.052
pH (N/A)	32.5	32.5	2.0	0.212
Total N (%)	16.2	16.2	<0.1	1.000

Legend: PC – physiochemical components, N – nitrate, % - percentage, OM – organic matter, p – probability value

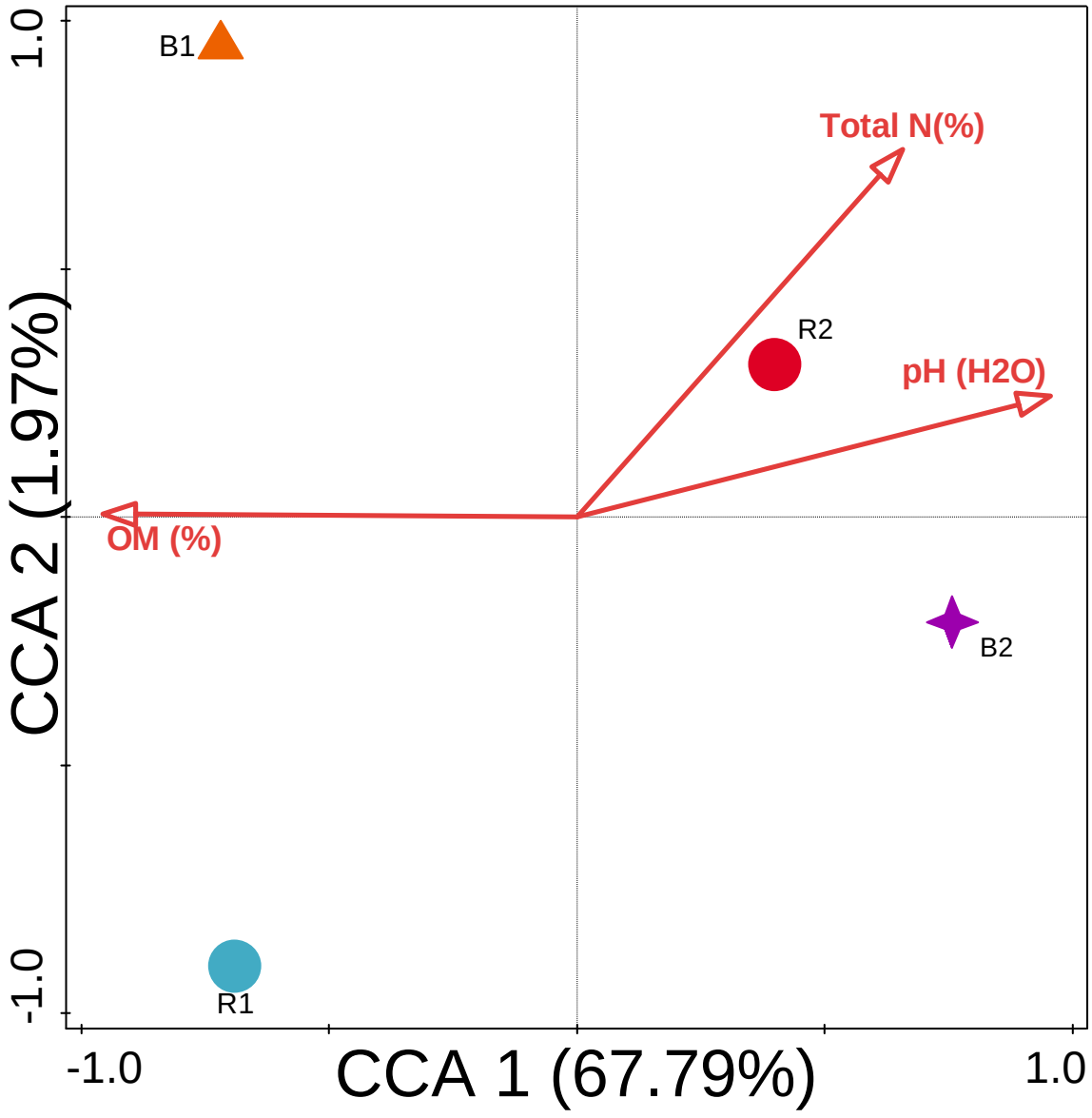

Figure S9.1: The influence of pH, total N (%) and OM (%) on sunflower rhizosphere and bulk soils obtained from Ditsobottla and Kraaipan farms using Canonical correspondence analysis. N – nitrate, % - percentage, OM – organic matter, R1- Ditsobottla rhizosphere soil, B1- Ditsobottla bulk soil, R2- Kraaipan rhizosphere soil, B2- Kraaipan bulk soil.

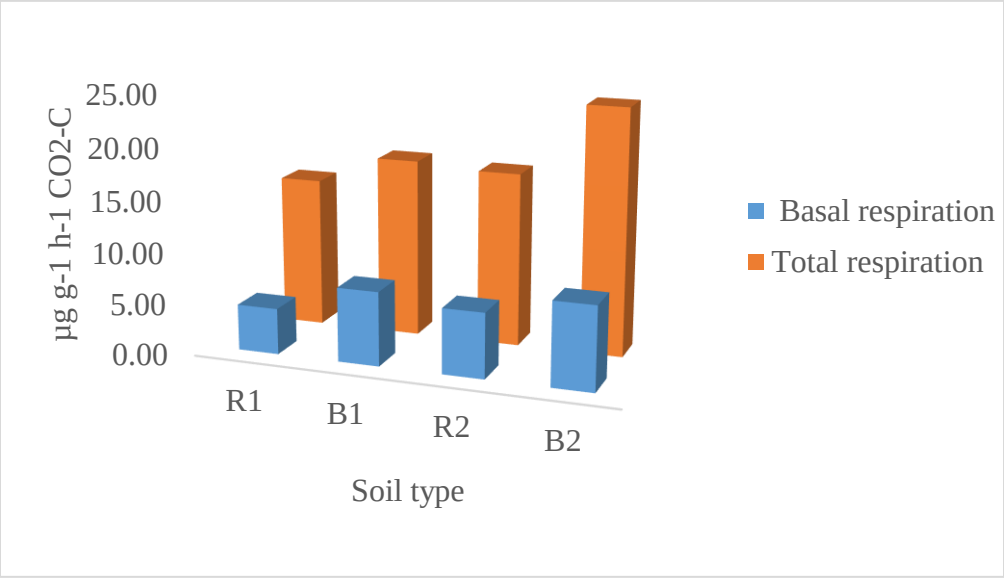

Figure S9.2: Effects of soil type on soil functional properties as measured for distilled water (basal respiration) and sum of the individual respiration rate of the 11 carbon substrates (total respiration) used in the CLPP assay. Number of replicates (n) = 2. R1= Ditsobottla rhizosphere soil, B1= Ditsobottla bulk soil, R2= Kraaipan rhizosphere soil, B2 = Kraaipan bulk soil.

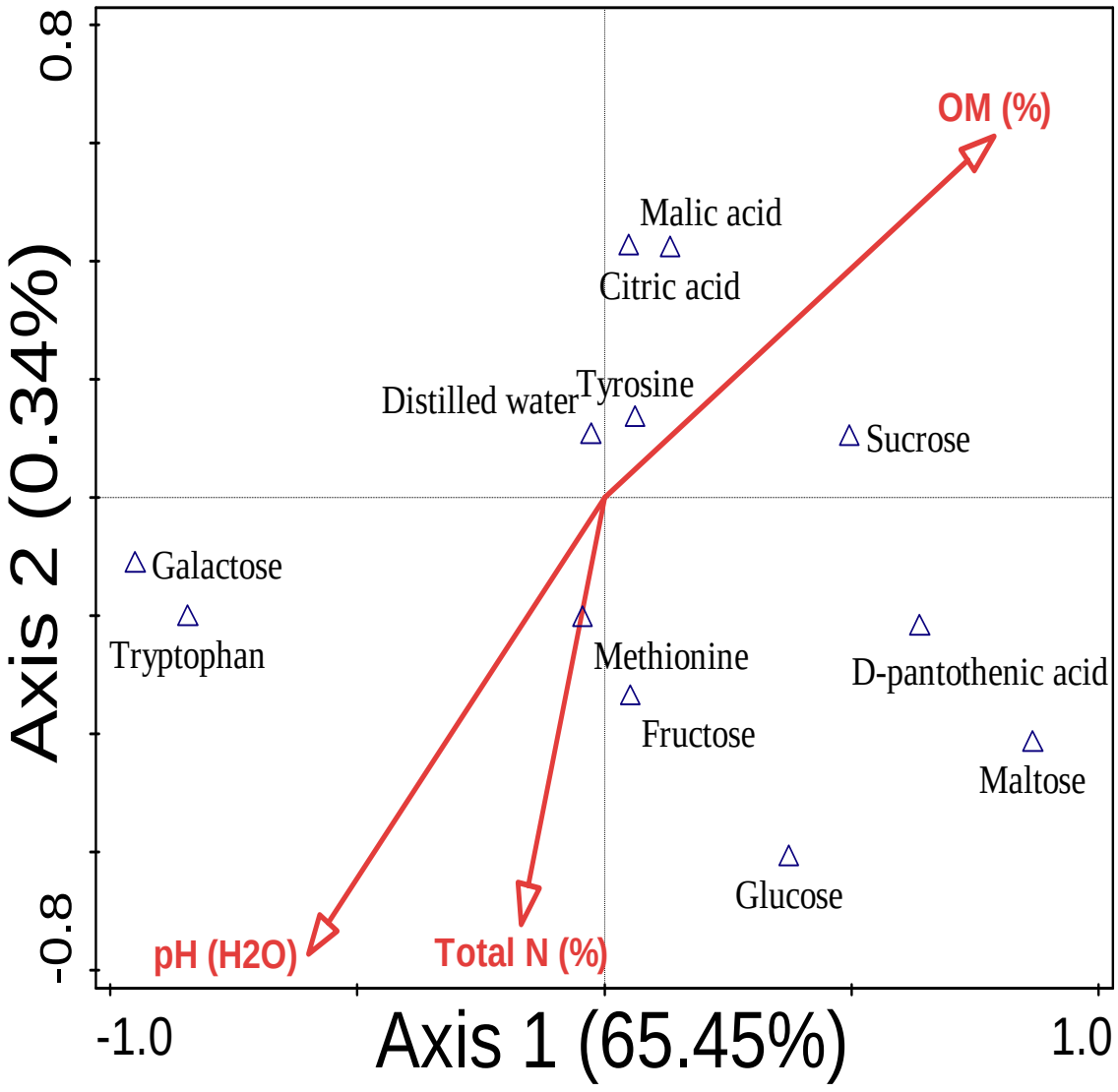

Figure S9.3: **The influence of pH, total N (%) and OM (%) on carbon substrate utilization by bacterial components of sunflower rhizosphere and bulk soils obtained from Ditsobottla and Kraaipan farms using canonical correspondence analysis.** N – nitrate, % - percentage, OM – organic matter

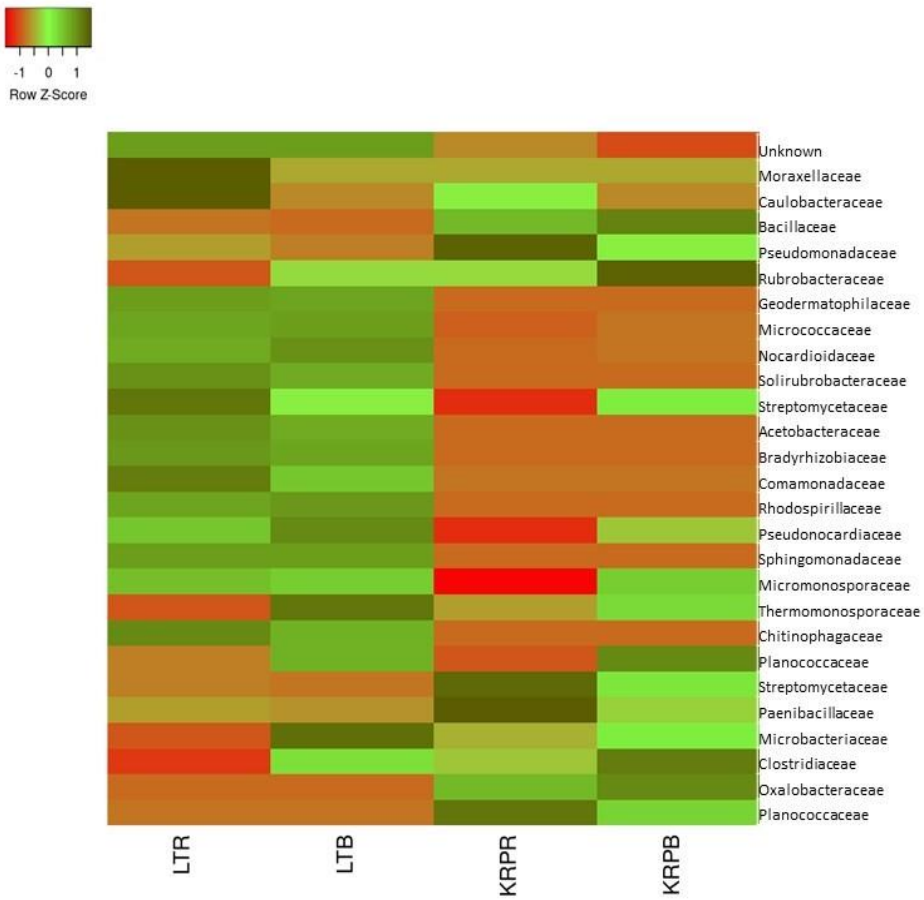

Figure S10.1: Taxonomic classification of the relative abundance of bacterial family from rhizosphere and bulk soils from Lichtenburg and Krayburg (LTR= Rhizosphere soils from Lichtenburg, LTB= Bulk soils from Lichtenburg, KRPR= Rhizosphere soils from Krayburg, KRPB= Bulk soils from Krayburg). The colour saturation gradient is shown as the scale bar based on the relative abundances; with a row z-score of the bacterial community transformed relative abundance.

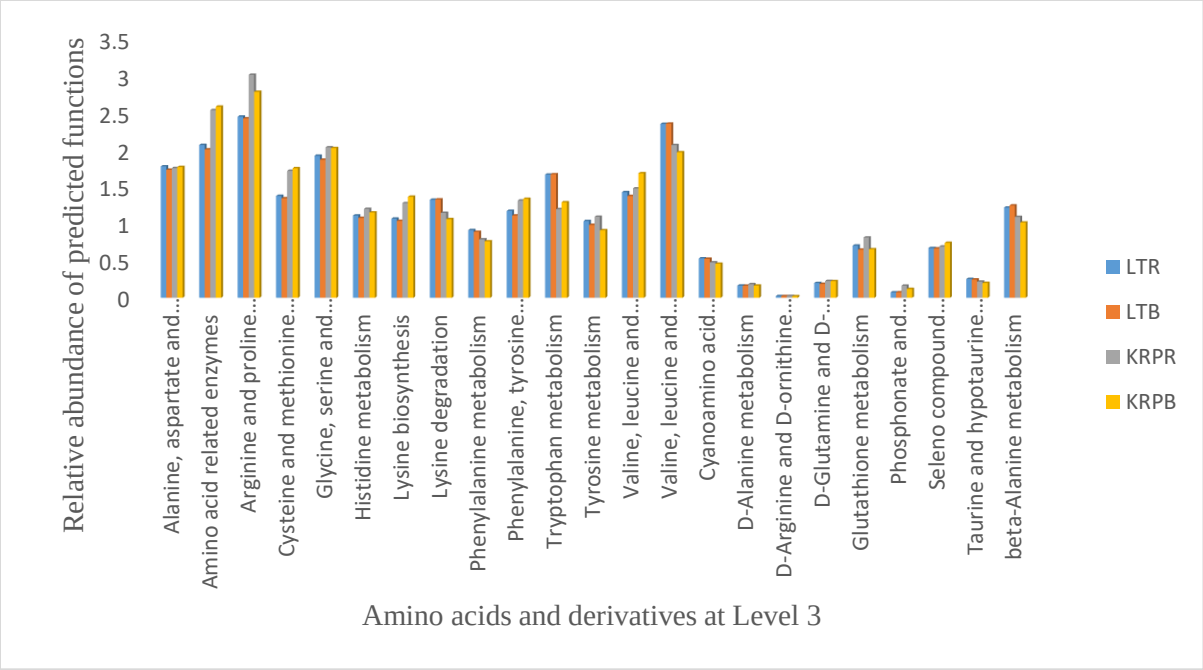

Figure S10.2: Predictive metabolic functional profile of the pathways of amino acids and derivatives of rhizosphere and bulk soils from Lichtenburg and Krayburg at level 3 classification. (LTR- rhizosphere soils from Lichtenburg, LTB- bulk soils from Lichtenburg, KRPR- rhizosphere soils from Krayburg, KRPB- bulk soils from Krayburg).

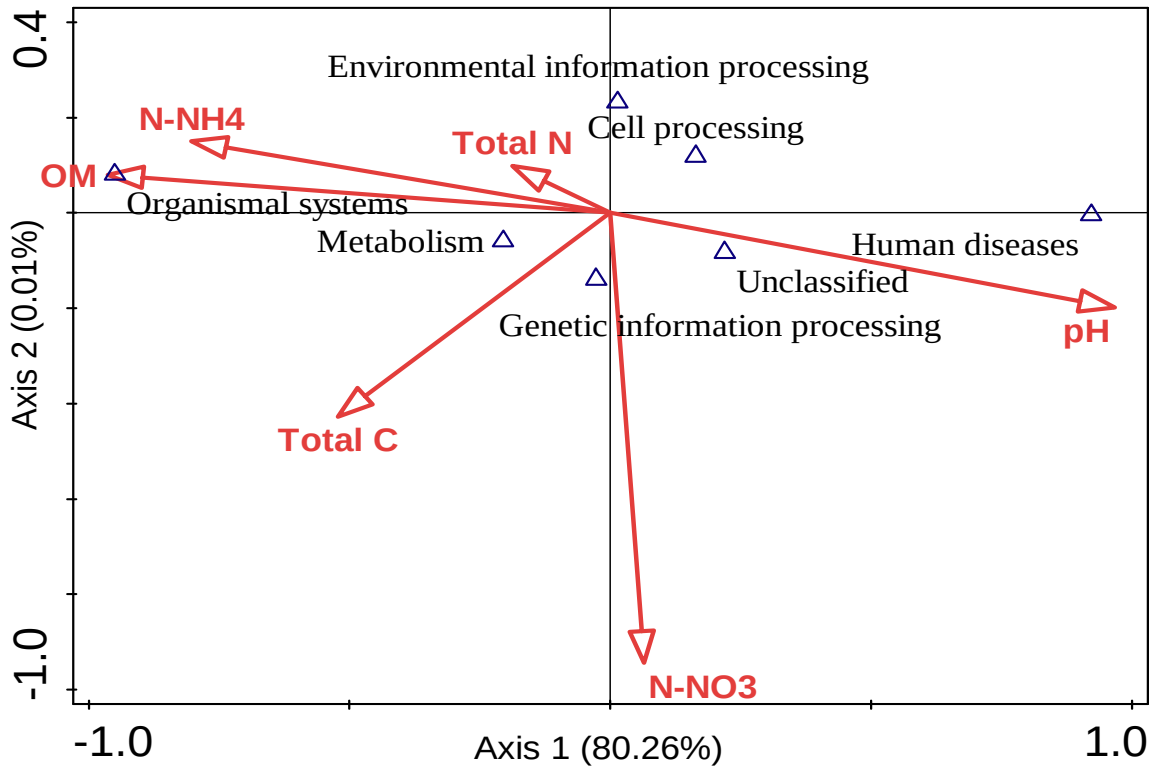

Figure S10.3: Principal Component Analysis (PCA) plot illustrating the influence of soil physical and chemical analysis on the distribution of predictive functional categories (Level 1) in rhizosphere and bulk soils from Lichtenburg and Krayburg. (OM= Organic matter, N-NH4= Ammonium, N-NO3= Nitrate, Total C= Total carbon, Total N= Total nitro