Diversity and functionality of rhizobacteria associated with different growth stages of maize plants under field conditions

AA Adedeji

orcid.org / 0000-0001-9141-1953

Thesis accepted for the degree Doctor of Philosophy in Science with Biology at the North-West University

Promoter: Prof OO Babalola

Graduation: May 2021

Student number: 31686346

DECLARATION

I, the undersigned, Atilade Adedayo ADEDEJI, declare that this thesis submitted in article format to the North-West University for the degree of Doctor of Philosophy in Biology in the Faculty of Natural and Agricultural Sciences, School of Environmental and Health Sciences, is my original work except for the citations and that this work has not been submitted at any other University in part or entirety for the award of any degree.

Name: **Atilade Adedayo ADEDEJI**

Signature……

Date……

Supervisor: **Prof. Olubukola Oluranti BABALOLA**

Signature……

Date……

DEDICATION

This work is dedicated to God alone, the giver of life, the Author and the finisher of my faith.

ACKNOWLEDGMENTS

The success of this study is not without the unwavering support of God and some very special and impactful persons. I am therefore wholeheartedly grateful to God for life, sustenance and wisdom.

I wish to extend special thanks to my supervisor, Prof. Olubukola Oluranti Babalola who gave me unlimited audience, guidance and support throughout this study even while I was on the exchange visit abroad. You are indeed a true advisor. Your expertise motivated me to learn excellence and your shining example in terms of discipline, knowledge and hard work has been inspiring. I am truly grateful for all your efforts.

I would like to thank the North-West University for the postgraduate bursaries and also the School of Environmental and Biological Science, Rutgers University, USA for the SEBS fellowship which funded this research.

I am grateful to my other supervisors and mentors at Rutgers University, USA, Profs Max Haggblom and Lee Kerkhof. Thank you for extending your arms of love to me throughout my stay in your laboratory. I am grateful for your immense academic input and time. Your constructive criticisms and intellectual contributions were milestones in the completion of this thesis.

Also, my eternal appreciation goes to my parents, Mr. and Mrs. Moronkeji Adedeji. Thank you for always inspiring and believing in me. Your commitment, support and prayers for me are immensely appreciated. Your wisdom has been invaluable to me and I cannot thank you enough. Your prayers kept me going. To my brothers, Akinade and Adebola Adedeji, thank you for your unconditional support and patience.

I also thank my uncle and aunt, Prof and Prof (Mrs) A. Adedeji, for supporting me spiritually. Much appreciation also goes to the Members of Dynamic Christian Assembly, OAU, Ile-Ife, Nigeria, thank you all for the heartfelt love.

I am indebted to Mr. and Mrs. Joel Amoo for taking good care of me in the process of this thesis. You made the USA a home from home. Thank you for the massive outpouring of love and care. I am deeply grateful. Also a very big thanks to my dear friends Fasanmi Joshua, Liu Huaquin, Ghandi Mohammed, Bob Dowden, Seda Mirzoyan and Lucas Mendes. Without you guys, I might not have had the courage to complete this work.

I also acknowledge the Deans, Faculty of Natural and Agricultural Sciences and Head of Department, Microbiology Department, North-West University, especially Prof. Eno Ebenso, Prof. Helen Drummond and Dr. Hazel Mophandu. Thank you for being there at all times.

To Dr. Amaoo, Dr. Omena, Dr. Ayanshina, Usman, Bob, Fiyin, Funmi, and my other colleagues and friends at NorthWest University, South Africa, Rutgers University, USA and Obafemi Awolowo University, Ile-Ife, Nigeria. I couldn't have come this far without you. Thank you my wife. Oshaji Esther Oluwaseun, you have been a valuable part of me, thank you for the love.

God bless you all.

TABLE OF CONTENT

LIST OF TABLES

LIST OF FIGURES

GENERAL ABSTRACT

The near root region of plants (rhizosphere) is a complex terrain where bacteria communities play significant roles in ecological system functions. The rhizosphere is capable of both directly and indirectly influencing the composition, diversity, and productivity of plant communities, thus, the belowground community has been suggested as an indicator of aboveground plant health and productivity. As a consequence, deeper knowledge underlying the dynamics and determinants of soil bacteria communities is critical for the comprehension of processes influencing or impacting soil fertility and agricultural sustainability. In our study, we used the new oxford nanopore sequencing technology (MinION) to analyze raw DNA samples recovered from the rhizospheric soil of maize plants at two growth stages (flowering and senescence) and bulk soil of the North-West University, Agricultural farmland, Mmabatho, Mafikeng, South Africa, and comparatively analyzed the functional diversities of both the rhizospheric soil and bulk soil of the bacteria communities. We hypothesized that bacteria communities around the root of maize are impacted by both growth stages and physicochemical properties of the soil. Our study revealed significant differences in taxonomic structures at the different growth stages and that taxonomic distributions were predominantly impacted by selected physicochemical parameters during the flowering stages. The predominating influential elements of soil properties (i.e pH, N, P, K) and precise shift of particular taxa provide insights into the agricultural practices. Therfore, it could be inferred that fertilization and agronomical practices cause changes in these elements and impact bacterial diversity. Cultural techniques may underestimate bacteria diversity but metagenomics, using whole-genome sequencing allows the estimation of the bacterial community more explicitly as well as the characterization of functions altogether. Our study revealed a distinctive selection at both taxonomic and functional profiles operating in

the assemblage of the maize rhizosphere community. Of the over 2 million reads, the result showed that *Proteobacteria* and *Firmicutes* were most prevalent (>40%). At the genus level, dominant rhizosphere genera (*Chlorasidobacterium, Candidatus, Flavisoli bacter Gaiella, Bacillus, Pseudomonas, Flavobacterium*, etc.) displayed different patterns of temporal changes in the rhizosphere as opposed to the bulk soil. Moreover, we observed unique genera, in particular, Plant-Growth Promoting Rhizobacteria (PGPR) such as *Bacillus*, *Pseudomonas, Psychrobacter, Nonomuraea, Thiobacillus* and *Bradyrhizobium* etc. Regarding functional profiles, data obtained showed significant differences in subsystems such as nitrogen fixation, carbohydrates metabolism, and metabolism of aromatic compounds. possible reason being high organic substances in the root region and increased prevalence of certain genera with high pesticide degradability, sequences of the adenylate cyclase (cAMP) pathway, which confer stability on bacteria community, among others. On the other hand, bulk soil had more sequences relating to dormancy and motility, sporulation, and stress response when compared with bulk soil. Nevertheless, the diversity and abundance of the taxa viewed does not correspond with functional traits identified, which could indicate some level of bacterial redundancy. Our study broadens our understanding of the assemblage, composition and function of the maize rhizosphere bacteria community in general, and has express implications in agricultural sustainability.

Keywords: Metagenomics, Next generation gene sequensing, Exudates, Bacteria, Plant-Microbe interactions

DISSEMINATION OF RESEARCH RESULTS AND LIST OF PUBLICATIONS

A. Presentation (Conference proceeding) at the 50th Symposium of School of Environmental and Biological Science, Rutgers University, New Brunswick, United State of America

Chapter 2: Sustainable Agriculture in Africa: Plant growth-promoting rhizobacteria (PGPR) to the rescue

This chapter has been published in Elsevier- Scientific African

DOI*:* https://doi.org/10.1016/j.sciaf.2020.e00492

Authors: Atilade Adedeji, Max M Haggblom and Olubukola Oluranti Babalola.

Candidate's contributions: Managed the literature searches, analyzed data and wrote the first draft of the manuscript.

Chapter 3: Rhizosphere: A complex determinant of soil microbial community.

This chapter has been published in Analele Universităţii din Oradea, Fascicula Biologie.

DOI:https://www.bioresearch.ro/2020-1/071-081-AUOFB.27.1.2020-ADEDEJI.A.A.-Rhizosphere.pdf

Authors: Atilade Adedayo Adedeji and Olubukola Oluranti Babalola.

Candidate's contributions: Managed the literature searches, analyzed data and wrote the first draft of the manuscript.

Chapter 4: Ecology, functions and applications of plant microbial community

This chapter is under review for publication in Plant Biology (Wileys).

Authors: Atilade Adedeji Adedayo, Oluwadamilare Ajagbe, Olubukola Ojo, Haniel Nkadi, Omolara Ibrahim, Huaquin Liu, Mohammed Usman, Lucas Mendes, Olubukola Oluranti Babalola

Candidate's contributions: Managed the literature searches, analyzed data and wrote the first draft of the manuscript.

Chapter 5: Secondary metabolites as plant defensive strategy: A large role for small molecules in near root

This chapter has been published in Springer-Planta.

DOI: https://doi.org/10.1007/s00425-020-03468-1

Authors: Atilade Adedayo Adedeji and Olubukola Oluranti Babalola.

Candidate's contributions: Managed the literature searches, analyzed data and wrote the first draft of the manuscript.

Chapter 6: Bacterial community profiling of maize plant rhizosphere at different growth stages in South African farmland as assessed by oxford nanopore sequencing (MinION)

This chapter is under consideration for publication in BMC Plant Biology.

Authors: Atilade Adedayo Adedeji, Lee Kerkhorf, Max M Haggblom and Olubukola Oluranti Babalola.

Candidate's contributions: Performed the research, analyzed data, contributed new methods/models and wrote the first draft of the manuscript.

Chapter 7: **Profiling functional diversity of maize plant rhizosphere as revealed by shortgun metagenomics**

This chapter is under consideration for publication in Springer - Symbiosis

Authors: Atilade Adedayo Adedeji, Lee Kerkhorf, Max M Haggblom and Olubukola Oluranti Babalola.

Candidate's contributions: Performed the research, analyzed data, contributed new methods/models and wrote the first draft of the manuscript.

LIST OF ABBREVIATIONS

Abbreviation	Full Meaning
ABA	Abscisic acid
ACC deaminase	1-aminocyclopropane-1-carboxylic acid deaminase
ACC deaminase	1-aminocyclopane-1-carboxylate (ACC) deaminase
AGPs	Arabinogalactan proteins
AM	Arbuscular mychorrizal
AMPO	2-amino-7-methoxy-3H-phenoxazin-3-one
APO	2-amino-3H-phenoxazin-3-one
ARDRA	Amplified rDNA restriction analysis
ARISA	Automated Ribosomal Intergenic Spacer Analysis
BOA	2-benzoxazolin-2(3H)-one
BXs	Benzoxazinoids
CHI	chalcone isomerase
CLPP	Community-level physiological profiling
CPR	Candidate Phyla Radiation
DAPG	2,4-diacetylphloroglucinol
DGGE	Denaturing gradient gel electrophoresis
DIBOA	4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one
DIMBOA	2,4-dihydroxy7-methoxy-2H-1,4-benzoxazine-3(4H)-

	one
DMAPP	Dimethylallyl diphosphate
DMDS	Dimethyl disulfide
DMNT	(E)-4,8- dimethyl-1,3,7-nonatriene
DMS	Dimethyl sulfide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
EPS	Exopolysaccharides
HBOA	2-hydroxy-1,4-benzoxazin-3-one
HDMBOA	2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one
HMBOA	2-hydroxy-7-methoxy-1,4-benzoxazin-3-one
IPP	Isopentenyl diphosphate
LH-PCR	Length Heterogeneity-Polymerase Chain Reaction
LOCs	Lipo-chitooligosaccharides
MBOA	6-methoxybenzoxazolin-2-one
NAFDAC	National Agency for Foods and Drugs Administration and Control
NESREA	National Environmental Standards and Regulations Enforcement Agency
N-fixation	Nitrogen fixation
NGS	Next-generation sequencing
NQs	Naphthoquinones
NSPRI	Nigeria Stored Products Research Institute
PCR	Polymerase chain reaction

PGP	Plant growth-promoting
PGPB	Plant growth-promoting bacteria
PGPR	Plant growth-promoting rhizobacteria
PHB	Polyhydroxybutyrate
PLFA	Phospholipid fatty acids
P-solubilization	Phosphorus solubilization
PSY	Phytoene synthase
ROS	Reactive oxygen species
SLB	Signature lipid biomarkers
SSCP	Single-strand conformation polymorphism analysis
TGGE	Temperature Gradient Gel Electrophoresis
TMA	Trimethylamine
TMV	Tobacco mosaic virus
T-RFLP	Terminal restriction fragment length polymorphism

CHAPTER ONE

GENERAL INTRODUCTION

For several decades, reports have shown that understanding the roles of plant-microbe relationships is not only crucial for plant growth and development but also ecosystem conservation and sustainable agriculture (Philippot et al., 2013). Interest was originally placed on understanding the aboveground ecosystem processes, which roles were better known. However, belowground studies have hinted that processes underneath might have as significant a role to play in ecosystem functioning and plant productivity (Philippot et al., 2013). Consequently, Further insight into the near root (rhizosphere) processes might reveal more knowledge.

The rhizosphere is the near root region, it is a unique ecological niche and critical interface that promotes exchange of materials or substances between plants and their close environment. In this region, microbial diversity is impacted by both the physical and chemical characteristics of the rhizosphere, which are commonly determined by the traits or attributes of the host plants. Plant roots release chemical substances (commonly known as exudates) in the form of organic compounds. These substances influence the bacterial population and when compared to bulk soils (i.e soils without vegetation) noticeable differences are observed (Tkacz et al., 2020). In the near root microbiome, the bacterial population could initiate positive interplay with the root and so prove fundamental for agricultural sustainability, as vital functions such as nutrient acquisition, growth stimulation, and biocontrol have been linked to the activities of these bacterial communities (Babalola et al., 2002; Mendes et al., 2018)

The functions and attributes of the rhizosphere vary, specific plants have been shown to have distinct near root properties (Eisenhauer et al., 2017). For example, Zhou et al. (2019) highlighted some noticeable differences among legume plants while contrasting nutrient acquisition and root exudation. It was reported that legumes release more amino acids and sugar when compared to grassroots. On the other hand, grass required more nutrients than legumes (Ghosh et al., 2009). Furthermore, Zhou et al. (2017) pinpoint that plants with identical taxonomic groups have got similar near root properties (such as quality of exudate, available nutrient and root biomass) and that these attributes may differ in others. While clear differences are seen within plant species belonging to other taxonomic groups, it is suggestive that the bacterial population in the near root region will be significantly impacted and driven by these variances. Admittedly, Ladygina and Hedlund (2010) reported distinct bacteria diversity in the rhizosphere of *Lotus corniculatus* when examined against *Holcus lanatus*. Zhou et al. (2017) also reported a similar outcome. Nevertheless, primary successions and soil rhizosphere effects are well documented as key drivers of bacterial diversity (Alawiye and Babalola, 2019), although interactions under varied developmental stages of specific plants have rarely been investigated.

The developmental stages of plants are one of the key determinants of soil bacterial community distribution. (Hannula et al., 2019). At different stages, chemical substances released in the root are distinct and they vary according to the growth stage. Physicochemical properties of the soil are being impacted during these stages, such that there are consequential effects on the rhizobacteria community. Considering the vital role the near root bacteria community plays in sustainable agriculture, it is necessary to elucidate how plant species select a bacteria community at different stages and bacteria response to these significant alterations.

In South Africa, maize is a staple food and globally maize is seen as one of the most important economic crops. Several countries including South Africa generate a large amount of income annually from its sale. Maize possesses distinct phenotypic and molecular diversity (Gore et al., 2009) and is easily influenced by variations in genetic conditions (Peiffer et al., 2013). Besides, considering that they are commonly cultivated in a monoculture system, they are often viewed as a strong determinant of ecological shifts for cohabiting species. However, studies on the diversity and functionality of the bacteria population are often limited. To date, reports of mature plants have been limited to screen houses only, growth in field conditions remains poorly elucidated. Additionally, the impact of the different stages of growth in these plants on the soil bacterial community are still unknown.

Understanding taxonomic, genomic and functional properties are vital for the management of sustainable agriculture. In this research, to ascertain to which level maize plant species select the distinct near root bacterial community, we hypothesized that the physicochemical attributes of the farm and growth stages (flowering and senescence) of maize plants will have significant impact on the dynamics and functions of the bacterial community in the maize rhizosphere. We utilized the new Oxford nanopore sequencing technology (MinION) in our community analysis investigation.

1.2 Problem statement

Soil habitats contain an ample population of bacteria species, which make up a large portion of the earth's biological diversity (Vitorino and Bessa, 2018a). These bacteria mediate processes that sustain soil functions. They exercise varying effects on crop growth and development, mobilization and transformation of nutrients in biochemical cycles and soil productivity. Considering just a gram of soil, there are myriads of bacteria species which could potentially

play important roles in soil fertility and health. However, less has been known of the entire community population structure and functionality of these bacteria species as they cannot be easily cultured in the laboratory. In recent times, technological advancements in microbial ecology have increased our appreciation and understanding of the phylogeny of these species. Nothwisthanding, interactions within several plant species, bacteria and variable environmental conditions remain poorly understood. Moreover, the majority of published work was carried out in regulated greenhouses. Consequently, replicability on the field could be challenging. Also, molecular understanding of the diversity and functional roles of rhizobacteria in the soil is very limited, and such understanding is critical to the maintenance of good soil health and increased crop production, such that it can ultimately form a base for incorporation into plant breeding. This conspicuous knowledge gap informed our decision to investigate the diversity and functionality of bacteria in the near root of maize plants under field conditions using metagenomics tools. We chose maize, a common staple food in South Africa with a huge economical impact.

1.3 Research aims and objectives

The specific objectives of this study were to:

1. Investigate the physicochemical properties of soils in of the maize farmland to determine microbial community restructuring
2. Determine the relative diversity and taxonomic abundance of bacterial communities in the maize farmland at two different growth stages.
3. Measure the functional diversity of soil bacterial communities across the soil sampling points

CHAPTER TWO

SUSTAINABLE AGRICULTURE IN AFRICA: PLANT GROWTH-PROMOTING RHIZOBACTERIA (PGPR) TO THE RESCUE

Abstract

The continued increase in the world's population is expected to make global food availability one of the major concerns of our future. Nowhere are the concerns greater than in Africa. With a poor economy, the continent is expected to be the home of the majority of people in the world in the next few years. Today, agriculture is faced with critical challenges of land degradation, reduced productivity, and susceptibility towards abiotic and biotic stresses. To fight this threat, it is crucial to increase agricultural productivity within the next few decades. Given this, agricultural systems that are more sustainable and eco-friendly are being adopted. Recent insight reveals that plant growth-promoting bacteria, especially those residing in the root region (rhizosphere), may play a role in helping to maintain a sustainable agricultural practice. Issues arising from the use of plant growth-promoting rhizobacteria (PGPR) are discussed alongside recent trends in plant microbiome study. It is envisioned that PGPR will take over the place of synthetic compounds in agriculture, horticulture, silviculture, and approaches in the cleaning up of environmental pollutants. In this review, the roles of PGPR, utilization challenges in Africa and possible solutions are discussed.

2.1 Introduction

Considering aggregate global trends and outlooks for many years to come, sustainable development efforts towards agriculture appear to be making an insufficient difference, most especially in the developing world where agriculture is the key driver of the economy. The world population continues to increase, and according to projections, by 2030 will reach 8.5 billion, and would further rise to 9.7 billion by 2050 and 11 billion by 2100 (DeSA, 2013). Of all the continents, Africa is the fastest-growing, with its population predicted to rise to 2.4 billion by 2050 (Bergaglio, 2017). For instance, Nigeria, Africa's most populous country and sixth in the world with an approximate population of 215 million is expected to exceed 330 million and edge the United States to become the third most populous country in the world (Basten et al., 2013). According to projections, Africa will be harboring more than 50% of the addition to the global population between the present day and 2050. This prediction epitomizes the agricultural challenges Africa is expected to face as there will be immense pressure on the quantity and quality of food available and also on how to conserve natural resources.

Presently, Africa is plagued with serious challenges in feeding its population, having relapsed from being a major exporter of agricultural products to becoming a net importer over the last three decades. As of today, it is estimated that one of every four persons in Africa lacks sufficient food to sustain proper and healthy living. The population of undernourished people has also increased to almost 300 million (FAO, 2015). To meet the food demand, many farms in Africa now use chemical fertilizers and pesticides uncontrollably, a practice that has led to degradation of land and biodiversity loss (Jacobsen and Hjelmsø, 2014). Although many countries in Africa rely on agriculture as the key driver of economic growth and development (Gindling and Newhouse, 2012), several of these such as Nigeria, Cote d'Ivoire, Ghana, South

Africa and Senegal are unfortunately losing crops equaling to at least US$10 billion annually due to land degradation, ecosystem disruption and pest infestation (Lal, 2013). The causes and effects of land degradation are multiple and interactive. They are closely linked to different attributes which include soil erosion and nutrient depletion, decreasing quality and quantity of available water, and loss of vegetative cover and biological diversity, all these have effects on crop productivity and susceptibility towards biotic and non-biotic stresses.

Given that increased food production is crucial for the growth, development and sustainability of poor economies, several technological inventions have been developed. Implementation of such agricultural technologies should be at the center of policy interest in Africa, as the growth of the agricultural sector in Africa will need to rely on improved and eco-friendly technologies such as novel disease-resistant species, climatic adjusted seeds, and up-to-date management practices. However, the adoption is seldom rapid, which may be due to fear of cost, reliability and long-term consequences. About these challenges, a purported strategy could be centered on the utilization of earth microbes for sustainable and robust crop production without having long term consequences on the ecosystem.

Soil microbes play important roles (microbial ecosystem services) in agriculture, essentially by promoting health and nutrient availability to plants, as well as improving the quality of the soil (Lugtenberg, 2015). A unique group of microorganisms that confer great benefits to plant and are involved in mutualistic interactions in the soil near root are known as plant growth-promoting rhizobacteria (PGPR) (Igiehon et al., 2019). PGPR promotes the growth of plants by utilizing varied mechanisms and assuring the accessibility of essential macro and micro-nutrients to the plant without adverse environmental consequences. Many PGPR can withstand unfavorable environmental conditions such as lack of water, salt stress, weed infestation, lack of nutrients and

heavy metal pollution. PGPR have not only gained much importance for their ability to stimulate key biological functions in the soil, but free-living microorganisms also control the yield of plants through the breakdown of, and competition for required nutrients for the productivity of the plant (Kumari and Kumar, 2018). Hence, we examine the roles of PGPR in sustainable agriculture with a particular focus on Africa.

2.2 Microbes: The readily available resources

Soil is the typical physical covering of the earth's surface. It represents the interface of three distinct material states (solid, liquid and gas) and is often seen as the base of all terrestrial ecological systems (Aislabie et al., 2013). The soil biota is a heterogeneous mix of microorganisms such as bacteria, archaea and fungi interacting with one another and their environment. The microbial community plays an immense role in contributing to basic functions. One gram of soil can contain up to 10^{10} bacterial cells and almost 200 m of living fungal hyphae which are actively involved in organic matter transformation, the release of nutrients, humification, breaking down of pollutants and maintenance of soil structure (Claire Horner-Devine et al., 2003). A large proportion of these microbes which are of agricultural significance are found in the plant rhizosphere with aptitude to increase plant growth through various mechanisms (Babalola, 2010). Plants exude a considerable fraction of the carbon that they fix by photosynthesis through their roots, and soil microbes make use of this exuded carbon as a food source. (See examples of exudates in Table 2.1) Also, nutrient availability in plants often relies on the communications among these microbes in the root region using these exudates, consequently, creating an interlink among plants and microorganisms.

Table 2.1: List of compounds in root exudates of different plant species.

Organic acids	Lactic acid, pyruvic acid, glycolic acid, piscidic acid, glutaric acid, malonic acid, citric acid, oxalic acid, malic acid, fumaric acid, succinic acid, acetic acid, butyric acid, valeric acid, formic acid, aconitic acid, tetronic acid, aldonic acid, erythronic acid
Amino acids	a-Alanine, b-alanine, cystine, glutamate, glycine, isoleucine, leucine, lysine, methionine, serine, ornithine, asparagines, aspartate, cystein, histidine, arginine, homoserine, phenylalanine, c-Aminobutyric acid, a-Aminoadipic acid, threonine, proline, valine, tryptophan
Sugars	rhamnose, arabinose, desoxyribose, glucose, fructose, galactose, ribose, xylose, oligosaccharides, raffinose, maltose
Vitamins	Pantothenate, riboflavin, biotin, thiamin, niacin
Purines/nucleosides	Cytidine, uridine, adenine, guanine
Inorganic ions and gaseous molecules	HCO_3^-, OH^-, H^+ CO_2, H_2
Enzymes	Acid/alkaline-phosphatase, invertase, amylase, protease

Source: (Adedeji et al., 2020)

2.2.1 Microbes and organic farming

Farmers in advanced countries are going back to the use of natural products and materials in farming practices (Siddique et al., 2014) and recently as knowledge and awareness of healthy living is increasing in the less developed regions such as Africa and Asia, people are becoming more careful about what they feed on. Although the continuous increase in the consumption of organic produce can conceivably improve the environment, well-being and sustainability of agricultural systems, organic farming cannot entirely meet the rapidly increasing demand for food.

Several studies are ongoing in providing effectual use of alternative means to advance productivity via biological means rather than chemicals. Interactions between plants and beneficial microbes are a promising alternative to enhancing crop yields while sustaining the system. Although commonly in Africa, manures (plant and animal sources) are used as an alternative means to chemical fertilizers, microorganisms present a cheap and efficient option (Brígido et al., 2019; Goudjal et al., 2014; López et al., 2019). Microbes ensure increased crop productivity by their active roles in photosynthesis and N-fixation, P solubilization, production of several growth factors (such as hormones, vitamins, and enzymes), enhancing tolerance to drought, and controlling plant disease-causing organisms inhabiting the soil. They also play defining roles in several plant growth stimulating processes and microbial associations such as photocooperation, symbiosis, commensalism, amensalism, mutualism, etc. have been identified to improve crop production, consequently forming the base for plant-growth-promoting role of microbes (Figure 2.1)

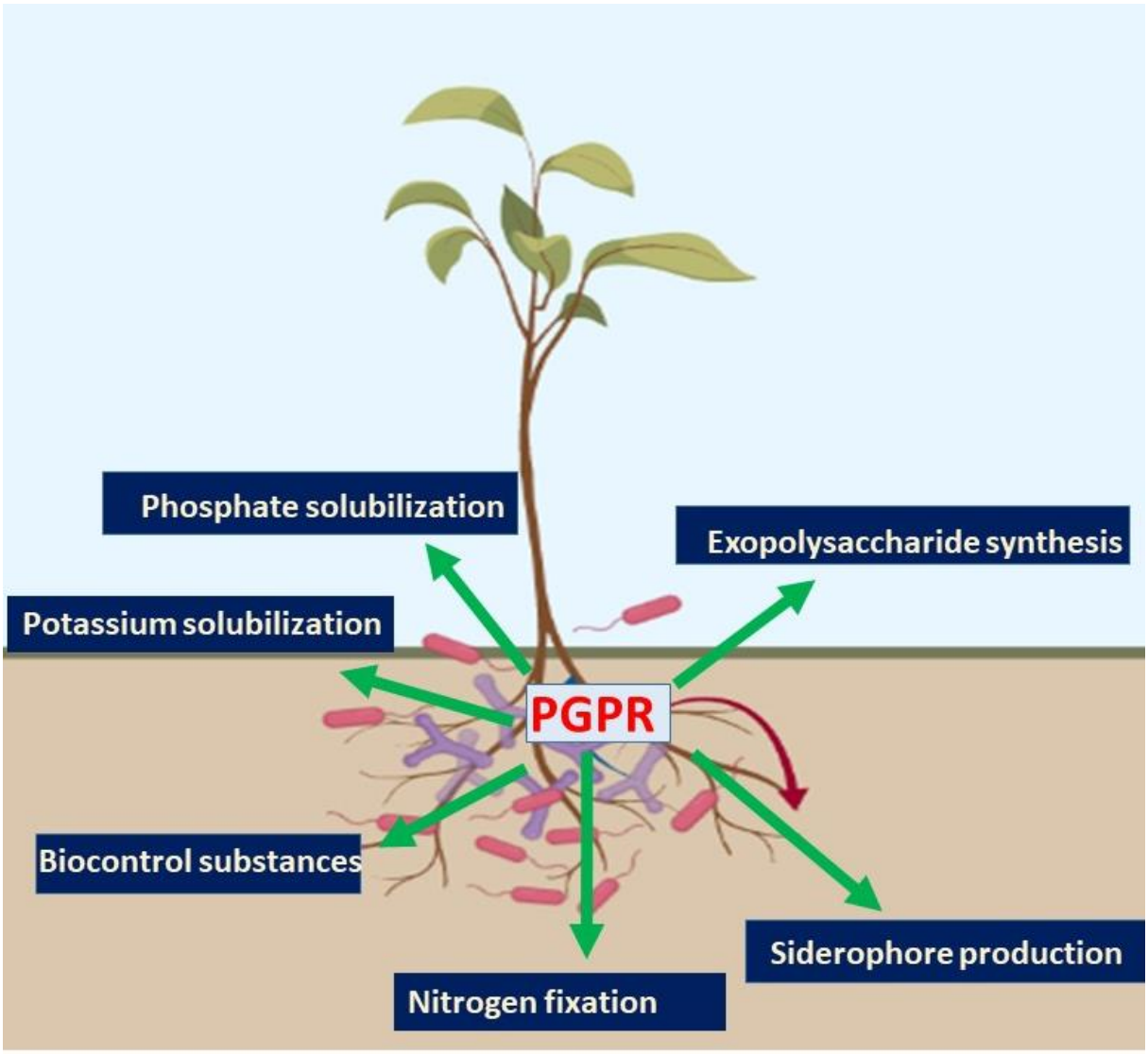


Figure 2.1: Plant growth-promoting role of rhizobacteria

2.3 PGPR-based solutions for agricultural sustainability

The rhizosphere can be viewed as the interface between plants and soil, from which organic substances and signaling compounds move into subsoil zones, enabling long-term mineralization processes as part of the ecological and biogeochemical disequilibrium (Gocke et al., 2017). It is a region consisting of several groups of microorganisms, including plant growth-promoting rhizobacteria. PGPR can confer a positive influence on the plant upon introduction, thereby displaying great competitive skills over the resident rhizospheric communities. These bacteria

are nonpathogenic; they strongly colonize roots and are found on the surfaces of the roots of plant. They improve yields and aid plants in adapting to various stresses as well as enhancing overall plant growth (Siddique et al., 2014). In the rhizosphere, plants commonly allow symbiotic microbes to thrive by providing organic compounds via their exudates. These exudates create a selective environment for beneficial microbes (Lucas et al., 2014). Furthermore, PGPR mediates plant growth by changing the whole microbial community in the near root region via the synthesis of several substances (Table 2.1). Organic substances secreted in the roots as exudates promote the establishment of PGPR in the rhizosphere. These exudates consist of distinct organic compounds including amino acids, proteins, sugars and signal peptides (Uren, 2007). The exudates hold nutritional requirements for soil bacterial growth and metabolic function

2.3.1 Mechanisms utilized by PGPR

An increasing number of bacterial species have been reported to show plant growth-promoting traits (Igiehon et al., 2019). The reason for this could be attributed to the diverse studies comprising a broader range of plant species, the vital role of the near root region as an important ecological unit in how the biosphere function, breakthroughs in bacterial taxonomy through metagenomics and advancement in knowledge of the various mechanisms of action of PGPR (Beneduzi et al., 2012). The mechanisms of PGPR functions can be split into direct and indirect ones **(**See Figure 2.2) (Table 2.2**).** A more comprehensive review of the mechanisms used by PGPR have already been published by Olanrewaju et al. (2017).

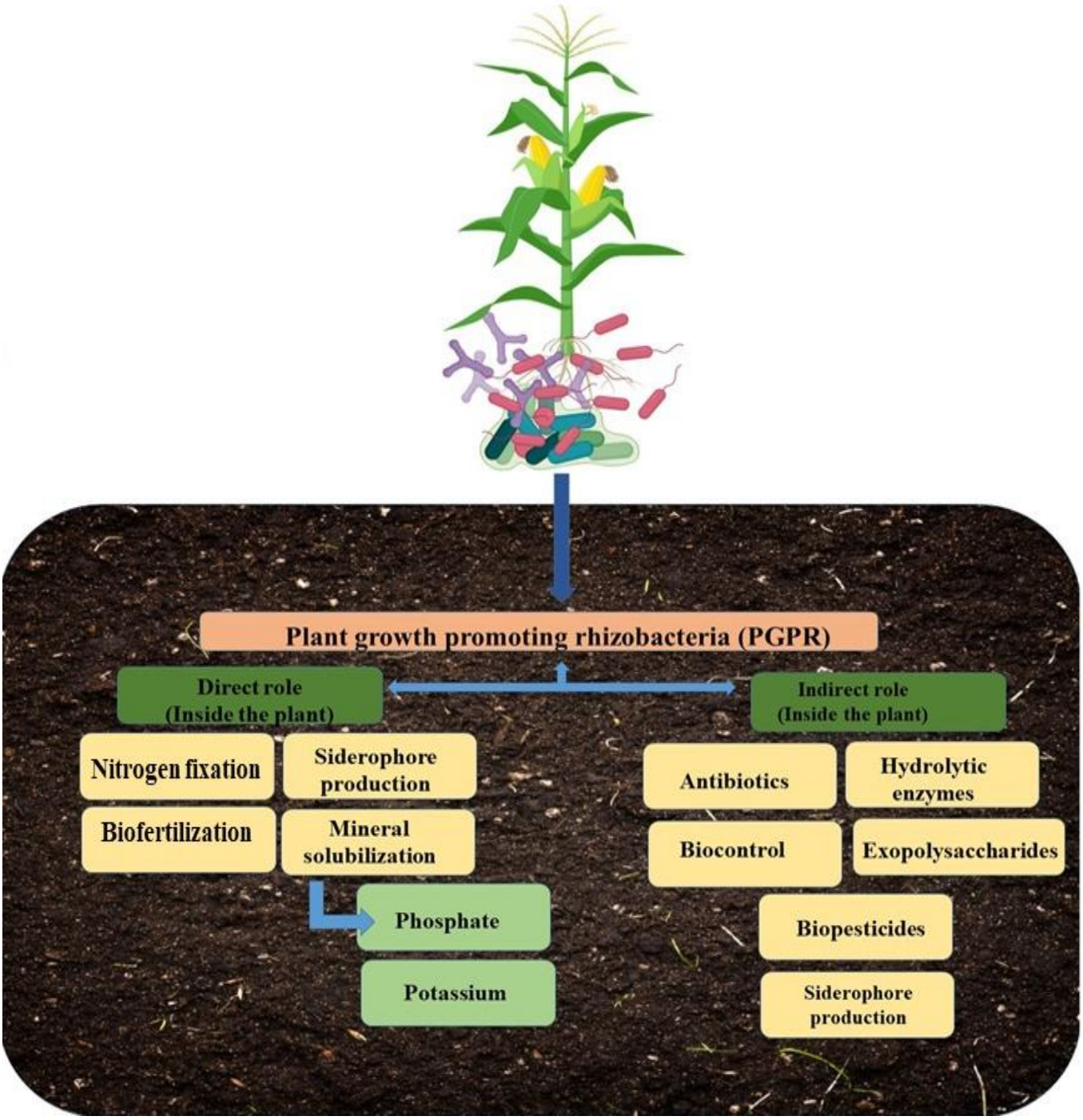


Figure 2.2: Illustration of the influence of PGPR on plant growth and metabolism

Table 2.2: Reported growth-promoting substances secreted by specific PGPR.

PGPR	Plant growth-promoting traits	References
Pseudomonas putida	Indole-3-acetic acid, siderophores, hydrogen cyanide, ammonia, exo-polysaccharides, phosphate solubilization	(Ahemad and Khan, 2012)
Rhizobium sp. (pea)	Siderophores, hydrogen cyanide, ammonia, exo-polysaccharides, Indole-3-acetic acid	(Ahemad and Khan, 2012)
Mesorhizobium sp.	Indole-3-acetic acid, siderophores, hydrogen cyanide, exo-polysaccharides, ammonia,	(Ahmad et al., 2015)
Acinetobacter spp.	Indole-3-acetic acid, phosphate solubilization, siderophores	(Rokhbakhsh-Zamin et al., 2011)
Rhizobium sp. (lentil)	Indole-3-acetic acid, siderophores, hydrogen cyanide, ammonia, exo-polysaccharides	(Babu et al., 2015)
Pseudomonas sp. A3R3	Indole-3-acetic acid, siderophores	(Asad et al., 2019)
Psychrobacter sp. SRS8	Heavy metal mobilization	(Manoj et al., 2020)
Bradyrhizobium sp.	Indole-3-acetic acid, siderophores, exo-polysaccharides, hydrogen cyanide, ammonia.	(Meena et al., 2017)
Pseudomonas aeruginosa 4EA	Siderophores	(Gaonkar and Bhosle, 2013)
Bacillus sp. PSB10	Indole-3-acetic acid, siderophores, hydrogen cyanide, ammonia	[(Ahemad and Kibret, 2014)

2.3.2 Application of PGPR presents great opportunity in sustainable agriculture

The use of microbial inoculants has been widely examined and the application of advantageous microbes has been employed to advance agricultural systems (Babalola, 2010; Kour et al., 2020), leading to enhanced crop growth, and reduction of plant pests and disease-causing organisms (Kumar and Ashraf, 2017). The natural abilities of plants to fight diseases have been enhanced by specific well-maintained inoculants which have the potential to be an alternative biocontrol (González-Chang et al., 2016). Likewise, biofertilizers, comprising live microbes implemented in plants, seed, or even soil, are widely encouraged in nonchemical agriculture as an alternative to artificial chemical fertilizers. These fertilizers boost plant nutrition by utilizing the native capacity of microbes to break down, solubilize and mobilize nutrients (Sattar et al., 2019), consequently reducing the cost and use of regular fertilizer (Singh and Gupta, 2018). The use of the aforementioned microbial-based crop improvements is vastly increasing in most developed countries (Timmusk et al., 2017), providing an encouraging option to conventional agricultural methods, most commonly in regions where agriculture is key to the economy. Harnessing this strategy, it could be inferred that Africa should greatly benefit from biotechnological advancement. However, despite the great opportunities the technology affords, its acceptance comes with a lot of limitations. Africa is still at a crossroads, with poor economic growth and education proving to be a big obstacle to positive scientific interventions. It is challenging to convince an African peasant farmer about the long-term advantages of the use of microbial inoculants. Furthermore, except for governmental financial funding and commitment, local farmers may be reluctant to embrace a technology whose gain and effects are seen only after long term use (Table 2.3) (Igiehon and Babalola, 2018)]

Table 2.3: Examples of plant growth-promoting rhizobacteria and their impacts.

PGPR	Plant	Conditions	Plant growth-promoting traits	Results of addition of bacteria to plants	Reference
Pseudomonas putida, *Azospirilium*, *Azotobacter*	Artichoke (*Cynara scolymus*)	*In vitro*	Indole-3-acetic acid, siderophores, hydrogen cyanide, ammonia, exo-polysaccharides, phosphate solubilization	Notable development in radicle and length and weight of shoot, seedling vigority index, and an evident reduction in germination time	(Jahanian et al., 2012)
Pseudomonas sp. PS1	Greengram (*Vigna radiata* (L.) wilczek)	Pots	Indole-3-acetic acid, siderophores, hydrogen cyanide, ammonia, exo-polysaccharides	Notably enhanced dry weight and nodule numbers of the plant, total chlorophyll content, root nitrogen, shoot nitrogen, root phosphorus, shoot phosphorus, seed robustness and protein	(Ahemad and Khan, 2012)
Bradyrhizobium MRM6	Greengram (*Vigna radiata* (L.) wilczek)	Pots	Indole-3-acetic acid, siderophores, hydrogen cyanide, ammonia, exo-polysaccharides	Improved growth factor at different concentrations of herbicides	(Ahemad and Khan, 2012)
Pseudomonas sp. A3R3	*Alyssum serpyllifolium*, *Brassica juncea*	Pots	Indole-3-acetic acid, siderophores	Improved considerably the biomass (*B. juncea*) and Nitrogen content (*A. serpyllifolium*) in plants grown in Nitrogen-stressed soil	(Babu et al., 2015)
Pseudomonas sp. S	Soybean, wheat	Fields	Heavy metal mobilization	Notably improved activities of the soil enzyme, total productivity, and assimilation of nutrients	(Sharma et al., 2011)
Psychrobacter sp. SRS8	*Ricinus communis*, *Helianthus annuus*	Pots		Accelerated the growth of plants and Nitrogen accumulation in both plant species with enhanced plant biomass, chlorophyll, and protein content	(Ma et al., 2011)
Rhizobium strain MRP1	Pea (*Pisum sativum*)	Pots	Indole-3-acetic acid, siderophores, hydrogen cyanide, ammonia, P-solubilization	Improved the growth, symbiotic properties, amount of Nitrogen and Phosphorus nutrients in plant organs, seed yield and seed protein of pea plants	(Ahemad and Khan, 2011)

2.3.4 PGPR ameliorate stress conditions in plants

In Africa, like every other part of the world today, agricultural systems are regularly hit by various biotic and abiotic stresses which usually alter the crop yield, robustness and abundance. Every year up to 30-50% of agricultural losses is attributed to these stresses (Kumar et al., 2018). These stresses can either be inherent or human-induced. The most common of the abiotic stresses are salinity, drought, temperature, and the accumulation of heavy metals (Table 2.4). These conditions have extended effects on structure, morphology, physiology, biochemistry and even control of genetic expressions in plants, consequently having significant effects on soil microbial diversity, fertility of the soil and competition for nutrient resources (Chodak et al., 2015). PGPR can improve plant growth and development in both natural and stressed conditions and the utilization of efficient microorganisms could help enhance and improve sustainable agriculture and ecological stability.

The mechanisms that plants utilize to tolerate stress are intricate and multifaceted. Various biochemical and molecular mechanisms are utilized by microorganisms to increase plant growth. PGPR promotes plant growth by regulating hormones and the availability of nutrients in plants, synthesizing plant growth regulators and inducing resistance against plant disease-causing organisms (Spence and Bais, 2015). Also, PGPR produces specific metabolites that control plant pathogens in the near root region. For example, rhizobitoxine enhances the growth of plants and development in stressed situations by repressing the production of ethylene (Kumar et al., 2009). Besides, to enhancing survival in difficult conditions, specific bacteria have sigma factors that modulate the expression of genes in plants (Gupta et al., 2013). Furthermore, *Pseudomonas putida* MTCC5279 ameliorate drought stress in chickpea (*Cicer arietinum*) plants by changing the integrity of the membrane, the accumulation of proline, glycine betaine and also altering the

movement of reactive oxygen species (ROS). These positive responses to stress were identified to be induced by bacteria causing differential gene expression associated with ethylene biosynthesis, salicylic acid, jasmonate, transcription activation, transcription factors expressed in abiotic stress states (Tiwari et al., 2019). Utilization of thuricin 17 synthesized by *Bacillus thuringiensis* NEB17 to *Glycine max* in a water-scarce environment causes changes in the root structure and noddle biomass and also the total nitrogen content (Prudent et al., 2015). It was viewed that PGPR could also help plants tolerate flood stress. *Oryza sativa* seedlings introduced with a 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase producing strain of *P. fluorescens* REN1 had enhanced root properties, an attribute that consequently helps plants survive in a regularly flooded environment (Etesami and Maheshwari, 2018). Salt stress impacts can be lessened by ACC deaminase. Pea plants introduced with *Variovorax paradoxus* 5C-2, which synthesize ACC deaminase, were shown to increase electron transport, rate of photosynthesis and balanced ion homeostasis by increasing K^+ flux to shoots and Na^+ removal on roots, reducing resistance of the stomata and balancing the pressure in the xylem by increasing the biomass under salt stress conditions of about at 70 and 130 mM NaCl (Wang et al., 2013). PGPR producing ACC improved ability of okra, a common crop in Africa, to tolerate salts, improved activities of antioxidant enzymes and upregulated reactive oxygen species (ROS) pathway genes (Habib et al., 2015). *Zea mays* seedlings treated with *Bacillus amyloliquefaciens* SQR9, improved tolerance to salt stress and chlorophyll content when matched with native plant. Further assessments revealed that the mechanisms were linked to improved total soluble sugar content resulting in reduced destruction of the cell, enhanced peroxidase/catalase activity and glutathione content for randomly moving ROS, and decreased Na^+ levels in the plant (Chen et al., 2016). Modern molecular techniques are also now allowing a more detailed exploration of

the mode of action of the interactions between plants and microorganisms in stress-induced tolerance. It is vital to note that improving tolerance levels of plants through common breeding systems could take a long period and much capital. Acceptability of genetic engineering is still confronted by ethical and social concerns. Nevertheless, the functions of PGPR to ameliorate stress in agriculture could still gain ground with good orientation.

Table 2.4: Plant growth-promoting rhizobacteria (PGPR)-ameliorating agriculture stresses.

Host Plant	PGPR	Stress	Combined effect on plant health	Study
Elaeis guineensis	*Pseudomonas aeruginosa*	Biotic	lowered epidemic rates of diseases in young seedlings pre-inoculated with both AMF and PGPR	(Sundram et al., 2015)
Avena sativa	*Acinetobacter* sp.	Petroleum	Improved antioxidant levels in leaves, heightened the removal of oil and restoration processes of the soil	(Xun et al., 2015)
Pisum sativum	*Arthrobacter protophormiae*	Salt	Improved the health of the plant, decreased proline content and lipid peroxidation, enhanced pigment activity	(Barnawal et al., 2014)
Carica papaya	*Pseudomonas* sp.	Biotic	Controlled diseases as well as the establishment of diseases causing organisms in seedlings	(Hernández-Montiel et al., 2013)
Triticum aestivum	*Azospirillum* sp.	Salt	Increased fresh and dry weights of the plants, photosynthetic pigments, and accumulation of proline	(Zarea et al., 2012)
Oryza sativa	*Azospirillum brasilense*	Drought	Enhanced the conductance of the stomata, photosynthesis, shoots fresh weight, and vigor of the plant	(Ruíz-Sánchez et al., 2011)
Trifolium repens	*Brevibacillus brevis*	Heavy metal	Decreased acquisition of metals, enhanced shoot and root plant biomass, increased nodulation	(Vivas et al., 2006)

2.3.5 PGPR as biofertilizers

Currently, the vast amount of agricultural chemical inputs such as artificial fertilizers, herbicides, fungicides, and insecticides, leads to high costs and increased environmental pollution. The immediate consequences of the pollution caused by these agrochemicals are commonly seen in groundwater and in the production of crops that are contaminated by the heavy metals present in the agricultural soils. Heavy metals have been identified to have public health significance as they can be transferred to humans, consequently resulting in major health issues such as cancer (Vahidinia et al., 2019). Besides the medical consequences, other outcomes such as alterations in the natural ecological nutrient cycling and community of biological organisms have been commonly identified (Karuppiah and Rajaram, 2011). Considering these damages, other research paths are being explored globally to overcome these problems, and biofertilizers present a useful alternative.

The use of biofertilizers is gradually gaining impetus for maintenance of soil health, reduction of pollutants in the environment, and a decrease in the utilization of chemicals in agriculture (Shahid et al., 2016). Examples of useful bacteria commonly used as biofertilizers for different crops are listed in Table 2.5. These microbes promote plant nutrition by aiding uptake of nutrients and also promoting nutrient availability in the root region via various methods, such as nitrogen fixation, solubilizing mineral nutrients, mineralizing organic compounds and production of phytohormones (Rasouli-Sadaghiani et al., 2014). They are utilized to increase the growth of crops and productivity when used complementarily or as alternatives for artificial fertilizers. For instance, a study aimed to characterize PGPR from maize roots in five agricultural and ecological regions of central and northern Benin, showed that different *Bacillus* strains *(B. thuringiensis,* and *B. circulans B. polymyxa, B. pantothenticus,*), three *Pseudomonas* species (*P.*

cichorii, *P. syringae* and *P. putida*), and *Serratia marcescens* were being used with positive effects as biofertilizers in different fields. Ammonia and hydrogen cyanide were all produced by these strains to possibly indicate the roles they play as biological fertilizers in increasing yield (Agbodjato et al., 2018).

Table 2.5: Plant growth-promoting rhizobacteria (PGPR) as bio-fertilizer and their plant growth promotion (PGP) activity

Plant	PGPR strain	PGPR activity	Reference
Withania somnifera	*Alcaligenes faecalis* sub sp. *faecalis* str. S8	Phosphate solubilization Indole-3- acetic acid	(Abdallah et al., 2016)
Ocimum sanctum	*Achromobacter xylosoxidans* Fd2 *Herbaspirillum seropedicae* Oci9, *Ochrobactrum rhizosphaerae* Oci13	Indole-3- acetic acid, Siderophore	(Barnawal et al., 2014)
Ocimum sanctum	*Serratia ureilytica* Bac5	Siderophore, ACC deaminase Phosphate solubilization	(Barnawal et al., 2014)
Capparis spinose	*Pseudomonas stutzeri* CSP03 *Bacillus subtilis* TTP02, *Pseudomonas putida* PHP03	Indole-3-acetic acid, N_2 fixation, Phosphate solubilization	(El-Sayed et al., 2014)
Curcuma longa L.	*Azotobacter chroococcum* CL13	Indole-3-acetic acid, Phosphate solubilization Siderophore	(Kumar et al., 2016)
Moringa peregrine	*Bacillus subtilis* LK14	Phosphate solubilization Indole-3- acetic acid,	(Latif Khan et al., 2016)
Asphodelus sp.	*Paenibacillus durus BR 30*	Indole-3-acetic acid, N_2 fixation, Phosphorus solubilization	(Navarro-Noya et al., 2012)
Juniperus sp.	*Paenibacillus borealis BR 32*	Indole-3-acetic acid, N_2 fixation, Phosphate solubilization	(Navarro-Noya et al., 2012)
Haplopappus sp.	*Azospirillum lipoferum KYR F6*	Indole-3- acetic acid, N_2 fixation, Phosphate solubilization	(Navarro-Noya et al., 2012)

2.3.6 PGPR in nitrogen fixation

Nitrogen is important for the continued existence of every form of life. Of all the nutrients obligatory for the growth, productivity and development of plants, it is the most imperative. However, even with about 78% N_2 in the atmosphere, it is not readily available to plants. Nevertheless, through two mechanisms (symbiotic and non-symbiotic), PGPR can fix this atmospheric nitrogen and make it accessible for the use of plants. *Rhizobium, Enterobacter, Sinorhizobium, Gluconacetobacter, Bradyrhizobium* and *Mesorhizobium* with leguminous plants, *Frankia* with non-leguminous trees and shrubs have all been identified to carry out this nitrogen-fixing activity to good effect (Bhattacharjee and Dey, 2014).

Farmers generally apply more than 100 kg of N per hectare (Deaker et al., 2004), whereas the use efficiency is generally below 40%, meaning that most applied fertilizer either wash out or is lost to the atmosphere. Biological nitrogen fixation (BNF) could fix between 100 and 290 million tons of N_2/year and provide plants with N without negative implications to terrestrial ecosystems (Boyer et al., 2004). Cyanobacteria in symbiotic association render 7–80 kg N_2 /ha/year, free-living 15 kg N_2 /ha/year and associative bacteria 36 kg N_2 /ha/year (Mishra et al., 2016). It has been noted, however, that cereal products may take up to 30% of their N from PGPR when enriched in combination with phosphorus and potassium also as with microelements (Mmbaga et al., 2014). For more than 120 years, rhizobial inoculants have been utilized in the propagation of legume plants as bio-fertilizers (Marks et al., 2013), and many African countries now use rhizobial inoculants for nitrogen uptake. In Kenya, due to its economic advantages,

rhizobia have been extensively utilized as an alternative to artificial fertilizers that are commonly employed in the production of legumes (Ouma, 2016).

Nitrogen fixation requires the presence of specific structural genes which include nitrogenase (*nif*). These genes are implicated in the activation of the iron-molybdenum, Fe protein, biosynthesis of cofactors, electron donation and regulatory genes needed for the functioning of specific biological proteins. In diazotrophic microbes, *nif* genes are commonly observed in a cluster of around 20–24 kb with seven operons encoding 20 variant proteins. Genetic approaches to improve nitrogen fixation have proven difficult due to the complexity of this system. Some researchers concluded that, once *nif* genes were identified and described, there would be possibilities to improve nitrogen fixation abilities through genetic engineering. Others, however, debated whether genetically engineered plants have the potential to fix nitrogen. These notions still appear to be somewhat naïve. Notwithstanding the divergent opinions, the two approaches still tend to the plant growth stimulation and maintaining the N level in agricultural soil.

2.3.7 PGPR in phosphate solubilization

After nitrogen, the essentiality of phosphorus surpasses every other element. Phosphorus is generally high in the soil (typically between 400 and 1,200 mg kg^{-1} of soil) but insoluble and consequently unavailable to support the growth of plants. Unavailable phosphorus can either be in the form of inorganic minerals such as apatite, or organic forms such as inositol phosphate, phosphomonesters and phosphotriesters (Rizvi et al., 2014). An inadequate or short supply of P usually restrains plant growth. Hence, phosphate solubilizing ability in PGPR is crucial. PGPR directly solubilizes and mineralize inorganic phosphorus or aid the flow of organic phosphorus via microbial turnover and/or enhancing the root system (Richardson and Simpson, 2011). Organic acids are released by bacteria which in turn reduce the pH in the root region,

consequently releasing trapped forms of phosphate like $Ca_3(PO4)_2$ in calcareous soils. Other than supplying the available cumulated phosphate (through solubilization), phosphorus biofertilizers further aid in improving the ability of organismal N_2-fixation and make Zn, Fe etc. available, via the synthesis of some plant growth-promoting compounds. It could be inferred that phosphate solubilizing bacteria provide a biotechnological key in sustainable agriculture, especially in phosphorus-deficient soils.

2.3.8 PGPR in potassium solubilization

The third most significant macronutrient is potassium. The soluble potassium concentration is generally minimal in the soil and around 90% of the total potassium exists either in insoluble rocks or silicate minerals (Han and Lee, 2006). Potassium deficiency is becoming one of the principal concerns in crop production. When potassium is deficient, the development of plant roots is poor. The plant generally grows moderately, produces tiny seeds, and yields drop below expectations. This highlights the quest to find supplementary sources of potassium for uptake by plants and to manage the state of potassium in soils for maintaining crop productivity (Kumar and Ashraf, 2017).

PGPR can solubilize potassium through the synthesis of organic acids (Han and Lee, 2006). Potassium solubilizing PGPR such as *Acidithiobacillus ferrooxidans, Burkholderia* sp., *Bacillus mucilaginosus, Bacillus edaphicus, Paenibacillus* sp. and *Pseudomonas* have been described to free potassium in forms that are available from minerals in soils that bear potassium (Liu et al., 2016). Consequently, introducing potassium solubilizing PGPR as biological fertilizer for crop enhancement can decrease the employment of agrochemicals and encourage ecologically friendly strategies for crop production.

2.3.9 Siderophore production by PGPR

The fourth most abundant element in this sphere is iron. As a micronutrient, Fe is crucial to the existence and survival of nearly all organisms. However, absorption by both bacteria and plants is not easy. Fe^{+3} is the dominant form on earth; this form, however, is only slightly soluble, so that the quantity of Fe obtainable for absorption by living organisms is very minimal. Plants and microbes require an adequate amount of iron, which is of greater concern in the root region where there is stiff competition from plant and microbes for iron (Hider and Kong, 2010). To withstand such limited supply and also to make iron available to plants in an iron-deficient environment, PGPR secretes low-molecular-weight siderophores (~400–1500 Da), and molecules having an increased inclination for Fe^{+3} ($KKaa$ between 1023 and 1052). Siderophores can be grouped into three principal categories based on their functional groups which are hydroxamates, catecholates and carboxylates. Notable advantages of siderophores produced by PGPR on growth of plants have been highlighted in various studies (Braud et al., 2006; Mandal and Kotasthane, 2014). For instance: (i) some experiments using radio-labeled ferric-siderophores as the basis of iron show that plants can uptake the labeled iron (Rasouli-Sadaghiani et al., 2014); (ii) mung bean plants, introduced along with the siderophore producing *Pseudomonas* strain GRP3 and cultivated in iron-deficient soils demonstrated reduction in chlorotic symptoms and an improved level of chlorophyll compared to uninoculated plants (Sharma et al., 2003); (iii) the Fe-pyoverdine complex produced by *P. fluorescens* C7 was taken up by *Arabidopsis thaliana* plants, resulting in enhanced iron concentrations in the tissues of the plant (Robin et al., 2008). In environmentally stressed situations such as heavy metal pollution, the supply of iron is of great importance. In conditions like these, siderophores mitigate the stresses inflicted on plants by the high amount of heavy metals (Braud et al., 2006).

2.3.10 PGPR as bioformulations for plant growth promotion

For several years, microorganisms have been used to enhance the growth of plants, but the noticeable outcomes on plant growth were not ascribed to the inoculation of these microbes. For more than 100 years since the discovery of *Rhizobium*, rhizobial inoculants have been commercialized and produced in large amounts in most developed countries (Deaker et al., 2004). In African countries and many other developing countries, however, the story is completely different. Most farms are yet to apply such technology, owing largely to improper education and very poor economies. Surprisingly, many practical studies and reports have been performed in regions such as Nigeria (Omomowo et al., 2009), South Africa (Igiehon and Babalola, 2017), Benin (Agbodjato et al., 2018), and Egypt (Mohamed et al., 2019). Bioformulation involves an alternative mix of microbes that may be partially or wholly used as a substitute for chemical fertilization or pesticides. Microbes present an eco-friendly sustainable strategy for improving crop productivity and wellness. They are biologically active substances utilized in agricultural plots. Bioformulants are incorporated in economically viable carrier materials which may contain one or more valuable bacteria strains capable of survival under strenuous conditions. These bacteria are able to survive, maintaining a population sufficient to support growth-stimulating responses on plants (Singh and Gupta, 2018). It should be highlighted that during inoculation, parameters such as plant types, soil types and microbiome should be considered, as there are chances of competition between inoculants and the resident microbiota, which may consequently lead to reduced population of inoculants. Such inconsistencies were highlighted in a comparative study carried out involving greenhouse experiments and field trials (Glick, 2018). The native community of the soil presents a key obstacle where inocula sometimes strive to find a vacant ecological role in the soil. When this

happens, the newly introduced inoculum will have to strive with the well-adapted normal microbiota and endure the risk of predation by soil microfauna. Invariably, one of the key reasons for the formulation of inoculants is to afford a more fitting microenvironment in combination with physical protection for a long-lasting duration. Bioformulants have great advantages compared to chemical fertilizers. When the microbe is selected appropriately, there are limited risks of environmental degradation and potential hazards to human health. The application is safe and effective in small quantities. Its activity is more targeted and survival can last up to a year. Decomposition is very fast and these bacteria can be applied or used alongside conventional pest management systems (Berg and Smalla, 2009). However, dosage and inoculum size should be monitored to get an effective result. Hence, PGPR offers a likely sustainable and environmentally friendly strategy.

2.3.11 PGPR for biocontrol

Approximately 40% of the possible global crop yield is damaged by diseases even before harvest, and an additional 20% is damaged after harvest (Mesterházy et al., 2020). It is even more worrisome that approximately 125,000–130,000 metric tons of chemicals are being utilized to kill pests every year in Nigeria (Asogwa and Dongo, 2009). Although there are increasing efforts to reduce pesticide application by instituting regulatory bodies such as National Environmental Standards and Regulations Enforcement Agency (NESREA), National Agency for Foods and Drugs Control (NAFDAC) and Nigeria Stored Products Research Institute (NSPRI), implementation and enforcement of prescribed management systems and legislative will are still lacking (Nnedinma, 2016). PGPR offers a possible solution. Globally, around 1400 biopesticide products can be found on the market (Mishra et al., 2016) and the figure is rising daily (Arora, 2015a). According to Kaur et al, biocontrol can be viewed as the use of microbes

that reduce the occurrence and frequency of plant diseases (Kaur et al., 2019). These microbes give the benefit of increased selectivity and decreased toxicity when compared with conventional chemical pesticides. PGPR autochthonous to soil and the rhizosphere region performs a vital role in controlling phytopathogens as they can contain or repress a varied range of fungal bacterial, nematode diseases and even viral diseases. In other parts of the world, the utilization of PGPR as biocontrol is increasing. Nonetheless, meaningful control of phytopathogens has been described mainly in laboratory and greenhouse experiments, as field experiments have been largely inconsistent.

The useful outcomes of microbial inoculants on plants include the elimination of phytopathogens (Babalola and Glick, 2012) and inhibiting the multiplication of phytopathogens (mostly fungi). Several inhibitory substances have been recognized. These include compounds such as phenazine, pyoluteorin, oomycin A, pyrrolnitrin, tensin, tropolone, and cyclic lipopeptides produced by species of *Pseudomonas* (Loper and Gross, 2007) and zwittermicin A, oligomycin A, kanosamine, 2,4-diacetylphloroglucinol (DAPG), amphisin, and xanthobaccin produced by *Bacillus*, *Streptomyces*, and *Stenotrophomonas* sp. (Wang et al., 2013). Of these different antibiotics, specificity and activity have been extensively studied, and a few of these biocontrol strains are now available commercially. A common issue, however, is the need for the continuous and persistent application of these strains. Some plant pathogens may become resistant to some specific antibiotics. As a preventive measure, some scientists have used hydrogen cyanide producing strains as the sole or augmented biocontrol strains (Devi et al., 2007). This strategy is efficient since hydrogen cyanide individually may lack significant activity to act as biocontrol. Nevertheless, it seems to act mutually with bacterial encoded an antibiotics gene.

Production of siderophores provides an added competitive edge to plant root colonizers. Siderophore producers thrive by the proliferation of disease-fighting organisms through sequestration of Fe^{3+} around the rhizosphere. This consequently makes nutrients readily inaccessible for PGPR. Bacterial siderophores vary in their iron sequestration abilities and they commonly deny disease-causing fungi vital nutrients/elements since fungal siderophores lack strong affinity. In some cases, PGPR further draw Fe from heterologous siderophores synthesized by co-residents, consequently depriving them of the essential element. For instance, *Pseudomonas* sp. possesses the ability to make use of siderophores secreted by different bacteria and fungi species to promote iron availability within their habitat (Ahmed and Holmström, 2014). Strains of *P. fluorescens* along with *P. putida* have been identified to secrete high yields of hydroxamate-type siderophore in an altered succinic acid medium. Other soil microbes also, including *Azotobacter vinelandii* and *B. cereus* produce these substances and can be utilized as competent plant growth-promoting substances to increase crop yield (Singh et al., 2015). *Bacillus megaterium* from the root region also has the capacity to produce siderophores and therefore aids in the improvement of plant growth and reduces the intensity of disease (Chakraborty et al., 2006). Distinct strains of the *P. fluorescens* have lately been harnessed as seed inoculants on crop plants to stimulate growth and escalate productivity of several crops (O'Callaghan, 2016).

Plant growth has been reported to be enhanced by utilizing the antagonistic potential of rhizoplane fungi and bacteria (Beneduzi et al., 2012), however, it has been difficult to ascertain the characteristics of the antagonistic activity. PGPR demonstrates their growth-promoting capacity by competing with resident microflora for available iron. These mechanisms are crucial for the functioning of antagonistic bacteria through induced resistance, and with resistance-

inducing and antagonistic PGPR, new inoculants can be formulated which can serve as an ecologically friendly approach to reducing plant diseases and increasing crop productivity (Beneduzi et al., 2012).

It is unclear however, to what extent competition between disease-causers and non-pathogens (PGPR) can restrict the incidence and severity of diseases. For instance, ample non-pathogenic soil bacteria quickly thrive and establish themselves along plant surfaces and in the process they utilize large amounts of the available nutrients. This consequently makes it difficult for disease-causers to survive.

Furthermore, specific bacteria have been identified to produce a broad array of multifunctional polysaccharides, such as structural polysaccharides, intracellular polysaccharides and extracellular polysaccharides. Exopolysaccharides (EPS) are involved in the development of biofilms and as supplements, they aid in trapping the free phosphorus from the insoluble in soils. They also aid the distribution of required nutrients to the plant for adequate growth and development. These EPS also shield plants from invasion by non-native disease-causing microbes (Qurashi and Sabri, 2012). Commonly, most PGPR produces antifungal substances (lysing enzymes or hydrogen cyanides) which stifle the survival of diseases causing fungi species. This presents an alternative strategy in the management of numerous diseases caused by fungal pathogens. Nevertheless, biocontrol agents still lack a suitable delivery system.

2.4 Challenges and possible solutions

Even though the technology of PGPR bio-inoculants points to an encouraging future for agriculture in Africa, some major bottlenecks need to be addressed. Notwithstanding the many benefits, the widespread use and acceptance are restricted by some of these identified challenges. The work in the laboratory and greenhouse experiment does not yet translate well to the field.

Scientific reports rely mainly on selected PGPR mechanisms, consequently, isolates that exhibit insignificant or minimal growth promoting attributes may possess another mode of action to promote the growth of plants but these mechanisms are not generally known and are less well-understood. Hence, advantageous strains that utilize these modes are rejected and considered as a poor performer following the traditional PGPR screening methods.

Most of the successes recorded for the use of inoculants have been in the laboratory or small scale, however, there are still few reports on the successes of these techniques in large scale. This may be due to the large amount that is needed for the peak functioning of the inoculants (Kabaluk et al., 2010). PGPR are also highly specific and differ from artificial chemicals that are broad-spectrum products. Actions of PGPR are mostly directed towards a targeted organism. Therefore, quality and efficiency can be inconsistent under field conditions (Timmusk et al., 2017). Commercially, the use of microorganisms as inoculants does not appear to be economically viable considering the low shelf life of the inoculum and high root region colonization which may also be influenced negatively due to competition with native residents of the region. Challenges are also encountered in keeping viable microorganisms incorporated in microbial inoculant formulations. Viable microorganisms could depend on treatment methods and temperature of storage. Prolonged survival of PGPR at room temperature is suggested for PGPR to be a part of mainstream agriculture. However, the total cost of seed viability maintenance and microorganism during storage is not economically viable (O'Callaghan, 2016).

The utilization of some PGPR as biocontrol agents may pose a health risk as some microbial agents have been documented to be toxic and disease-causing to non-target organisms (Kabaluk et al., 2010). In addition, fumigation with broad-spectrum pesticides is quite common in Africa most especially when high-value crops are planted. This eventually alters the microbial

community structure of the soil and extensively impacts plant-microbe interaction that helps plants with acquisition and mobilization of nutrients (Dangi et al., 2017). All these, pose challenges to the use and acceptability of PGPR. Furthermore, plants can select or alter their microbial community to keep helpful colonizers that are resident in their tissues (Hardoim et al., 2012). This ability is usually controlled by host immunity and exudates of the root or native endophytic microbial community resident in the plant tissues.

Inoculants that cannot thrive or cannot overcome competition are at risk of elimination. The success, however, relies on the strength of these microbes to withstand unfavorable situations and the ability to out-compete native microbes and overcome plant specificity. Also, the consequences of inoculants may not be advantageous. Ecological succession after the introduction of PGPR may eliminate or displace more beneficial host-adapted microorganisms (Qiu et al., 2019). Inoculants may also contain some likely diseases causing organisms, which may further repress crop productivity (Deacon and Berry, 1993). The employment of foreign species has inherent threat as meddling with ecological integrity, whereby native communities could be exposed to new species (Traveset and Richardson, 2014), with hidden outcomes for ecological system functionality.

2.4.1 Enrichment of near roots with PGPR inoculants

Several factors are considered and seen to affect the performance of PGPR in the field and so outcomes can vary. Usually, root regions of plants are colonized by microbes from the seed and soil, which could be based on attributes such as carbon, nitrogen, organic matter content, water availability and pH as well as the biogeographic structure which includes seasons and the type of soil (Kristin and Miranda, 2013). Consequently, it is essential to uncover approaches for efficient inoculation techniques, such that PGPR can gain the edge in colonization efficiency over others.

One technique is the use of biochar (charchoal produced by the pyrolysis of biomass) which can enhance the growth and continuous existence of microorganisms in the soil and is a good agent for soil amendment with the ability to enhance soil fertility and consequently increase crop yields. Biochar alters soil fertility characteristics that consequently influence the tolerance and existence of microorganisms in the soil (attributes such as organic matter content, pH, cation exchange capacity, nutrient and water retentions and oxygen tension), all of which have significant consequences to the growth of PGPR over predatory fungi (Becker et al., 2012).

2.4.2 Collaborative partnership between private-public stakeholders for increased knowledge and improved training

The use, development and acceptability of microbes in Africa for agricultural sustainability require time and several laborious processes. As part of the developmental procedures, policymakers, industries, research and educational institutions should work together collaboratively and these approaches should be seen as a crucial part of governmental policies. Biotechnology agencies should further engage scholars to carry out scientific projects and make the results, discussions more readily available to the general public by engaging social media platforms. Commercialization of knowledge and discoveries should be encouraged among Universities such as in the more developed countries where relationships between corporations, industries and academia are common. Also, as this area advances, there will be increasing demand for mentors who specialize in this sector and University research projects should be encouraged. This will bring economic perspective via research activities and impart it to the public.

2.5 Future Perspective: Plant Microbiome Studies

It is well established that native microbes residing in the below-ground region play fundamental roles in increasing the survival of plants (Dupuy et al., 2018). Considering the advancement of recent technologies to insert wanted traits into bacterial inoculants, an innovative approach could be the modification of plant-associated microbial community *in-situ* to enhance the productivity of plants (Mueller and Sachs, 2015). The bulk of environmental microorganisms are difficult to culture, indicating that only a very limited number of conceivably advantageous microbes can be isolated, engineered and used in agricultural systems.

The community of microorganisms in diverse crops such as maize, sugarcane and rice has been extensively characterized, with numerous core microbial taxa identified in the roots of specific plant species (Peiffer et al., 2013), with variations in structure implying the plant health. Reports have highlighted the significance of these central microbes to orchestrating the prevalence of other beneficial microbes (Shade and Handelsman, 2012). To achieve this, core microbiomes can be obtained in a couple of ways: transmission and recruitment. For example plant to seed transfer could be embraced, seeds of plants contain a vast number of microbes, with a notable amount that could be carried from the parent plant (Gundel et al., 2010). During plant germination, signals and exudates could be harnessed as they form vital determinants for the recruitment of microbes.

Considering the strong interplay between the core microbiome and associated plants, the inherent ability of plant species to influence the microbial community can be utilized for the selection of beneficial organisms. Microbial community interactions are both physical and chemical. Their identities and relationship can also be known through network analysis (Bender et al., 2016), possibly, by aiding the means of engineering significant constituents of the microbial community

in-situ. Admittedly, network maps can pinpoint central microbial community and their related members display distinct functions. These microorganisms can be marked for isolation and whole-genome sequencing to distinguish their functional competency. Consequently, recognizing central microbes and their impact on plant microbiomes and health are key for developing vital host–microbe-microbe interplays. The native microbiota of plants can be modified *in-situ* by artificially using typically existing ecological processes that utilize biochemical and molecular-based tools.

2.6 Conclusions

The use of PGPR as a key element of agricultural practice is a technology that has come to stay and the use of these techniques are already being embraced in many developed countries. In Africa, however, there is still room for growth. In advanced countries, where the cost of artificial chemicals is relatively high, the utilization of PGPR holds a major role in the development of nonchemical agriculture systems. Understanding their potential impacts on environmental restoration is also encouraging. This collectively helps to obtain the goal of sustainable development. Nevertheless, broadening the use of PGPR in Africa will require that key highlighted issues to be addressed. Firstly, educating the public about the advantages of PGPR is necessary for public acceptance and their use in large scale agriculture. Public mysticism is much directed toward the misconception that bacteria are mostly pathogenic. This misinformation must change before the populace embraces the use and introduction of PGPR into the environment on a large scale. Secondly, transfer of technology from laboratory and greenhouse applications to field experiments to commercialized scale will involve novel strategies for the development, storage, delivery, formulation and use of these microbes. Thirdly, it is crucial to have a detailed understanding of the mechanism of growth stimulation as PGPR are likely to be non-transformed

bacterial strains for certain positive traits. As researchers might genetically engineer more effective strains, regulations and policies should also be put in place to prevent present and future hazards.

CHAPTER THREE

RHIZOSPHERE: A COMPLEX DETERMINANT OF SOIL MICROBIAL COMMUNITY

Abstract

Several attempts have been made by researchers to evaluate the abundance and distribution of microorganisms in the soil following the first discovery and publication of the estimated number of prokaryotes that could be occupying the soil. Many described this information based on the relatedness of the community structure to the functions of the ecosystem. It was revealed that the amount and heterogeneity of microbial species inhabiting the soil are significant for the continued sustenance of plant growth and development, as a broad assortment of microbes are involved in vital soil functions. Current studies further explain the roles of the rhizosphere in defining the arrangement and composition of the soil microbes, the ability of plants to specifically shape their microbial community, and the interplay between plants and soil in shaping their community. Furthermore, the bulk of soil microbes are yet to be cultured and their functions still largely unknown. With the advent of molecular biology, there is a growing concern about the possible effects of difficult-to-culture microbial species in soil environments and the contributing factors to their dynamics. This review consequently deploys both old and recent molecular tools in describing these variables and introduces metagenomics as a modern tool to unravel the dynamics and community functional potential focusing on up-to-date data in describing them.

3.1 Introduction

Soil microbial community describes one of the largest and most beneficial reservoirs of biodiversity on earth (Vitorino and Bessa, 2018b). Members of this community hold

meaningful interactions with plants present in the soil as the individual microbial populations are necessary for biological processes that contribute to plant performance and productivity. Microbes mediate processes that sustain soil functions. They exercise varying effects on crop growth and development, mobilization and transformation of nutrients in biochemical cycles and soil productivity (Roger-Estrade et al., 2010). Microorganisms in the soil also contribute substantially to plant health and development by preventing attachment or adherence of pathogenic species to plant parts, inhibiting pathogen spread and proliferation, inducing systemic resistance thereby improving plant growth (Babalola, 2010). They also provide plants with nutrients (Smith and Smith, 2011), increase the plants' tolerance to drought (Enebe and Babalola, 2018) and even protect plants against herbivores (Rasmann et al., 2017).

The bacterial population deduced to be present in one gram of soil may approach 10^{10}–10^{11} cells (Raynaud and Nunan, 2014) and fungal hyphae can be estimated to be 200 m/cm^3 (Leake, 2004). The abundance, richness and composition of these microbes are subjective. They are sensitive to modifications which may be influenced by various biotic and abiotic factors (Zhao et al., 2020) such that, in changing environments, minute shifts in soil microbial composition may drive notable changes in health, growth and how nutrients are transformed in the plant-soil system (Bragazza et al., 2015), plant developments via either beneficial or deleterious interactions that influence root and shoot development, nutrient demand, growth and resistance to biotic and abiotic stresses (Enebe and Babalola, 2018).

The implied diversity and dynamic composition of the soil microbiota also bear a direct relation to soil function, structure and aggregation. Considering the dynamics of these microbes, it was argued that the effects of the physiological activities of plants should be

taken into account as a more important factor than any other non-living factor that influences the soil microbiome. This is due to the resultant consequences of the activities of the wide varieties of organisms present in the ecological system of the soil. It was deduced that the existence of plants does not only have direct effects on the inhabitation of soil microbes but also influences the abiotic determinants that shape their growth and distribution indirectly. A different study reported that the properties of the soil and the physiographic determinants are the paramount components when defining the composition, structure and abundance of the soil microbial communities; and in return, these soil microorganisms can have vital consequences on the development of soil aggregates (Bronick and Lal, 2005). Thus, as the importance of soil microbiota cannot be underestimated for the long-term sustainability of agricultural systems, a quantitative description of soil microbial structure as influenced by the region in which they both coexist is of great significance.

Over the years, many approaches employed in studying microbial diversity have shown several limitations. Soil microbial consortia have been challenging to be fully described largely due to the extensive diversity of their phenotypes, genotypes and crypticity (McPherson et al., 2018). Currently, less than 1% of this diversity could still be cultivable by traditional methods (Vitorino and Bessa, 2018a). Nevertheless, the discovery and use of new microbial identification methods are increasingly gaining scientific reputation and correcting the perspective of microbial ecology. The composition, structure and function of microbial consortia can now be estimated through metagenomics. Metagenomic methods offer the plausibility to evaluate the overall heterogeneity directly by circumventing the constraints posed by cultivation-based techniques. Several researchers have applied

metagenomics in the study of a range of different soil environments (Jiménez et al., 2015). The use of metagenomics has increased greatly and has advanced knowledge of microbial ecology; however, we have to be cautious of the biases involved.

3.2 Soil: a unique environment

The soil environment is very intricate. The soil is fundamental and irreplaceable; it represents a diverse, highly heterogeneous environment and provides several key functions to the ecosystems (García-Orenes et al., 2013). Soil is formed by an aggregation of geological parent matter, glacial and geomorphological antiquity, the presence and actions of biological species, specific cultural or anthropogenic history and disturbance regimes. The different elements of the solid fractions that make up the soil (sand, clay, silt and organic matter) represent an innumerable assortment of microhabitats. The soil as a habitat for several organisms is consequently open to different conditions which may be ramified into abiotic, biotic and nutritional requirements over the micrometer scale. The exact characteristics of a habitat housing a community of organisms is determined by a complex interplay of geology, climate and vegetation (see Fig 3.1). Therefore, one can hypothesize that in a "stable" system, a specific microhabitat is filled with organisms that have the best capacity to find a role and become stabilized. These organisms together form the key catalysts of the biochemical processes in soil ecosystems. Therefore, microhabitat and organismal biospheres determine the microbial processes in soil, diversities and species richness (Ritz, 2005; Roesch et al., 2007). The totality of the fresh weight of organisms below temperate grasslands can be more than 45 tonnes per hectare, matching or exceeding the above-ground biomass. Of these, bacterial species are the most abundant as their abundance is about ten times that of archaeal species. Fungi, nonetheless, also occupy a

significant niche and they often contribute the most to the total microbial biomass in soil ecosystems (Aislabie et al., 2013). However, the soil structure, heterogeneity and discontinuous system, disparity in nutrient abundance and differences in energy sources cause microbial populations to occupy very distinct microhabitats. Soils as microhabitats are seemingly dynamic and change over time as the measures of the environments rely solely on the size of the organisms present. Even in cases where the usable space is unrestricted in the soil, these microorganisms still occupy spaces that are favorable to their existence and may represent a minute proportion (usually not up to 5% of the entire space). Another unique distinguishing feature of the soil as a microhabitat is the ability possessed by the solid phase to accumulate essential organic molecular compounds and growth factors which include proteins and nucleic acids. The amount and activities of these biological molecules generally influence the actions and occurrence of extracellular enzymes accumulated in clay minerals or trapped within humic molecules as they sustain enzyme activity, protect them against proteolysis as well as thermic and pH denaturation (Nannipieri et al., 2002). Deoxyribonucleic acid (DNA) molecules bound to particles of sand and clay and humic molecules are commonly safeguarded from degradation by nucleases, but can still be picked up by competent bacterial cells in a bioprocess known as transformation. The buildup of organic syntheses by soil colloids slows down microbial activity and could affect the community structure.

In the soil, the breakdown of soil organic matter is impacted by resident microbes via enzymes that catalyze reactions needed for life processes, the formation of organic matter and soil structure. Enzymes usually produced, accumulated and inactivated have great effects on nutrient cycling processes and consequent microbial diversity, such that soil

enzyme activities can be an indicator of biochemical processes in the soil and possible alterations in the soil management. Soil enzyme activity can be used to indicate the intensity of certain biochemical processes. Soil enzyme activity can be used as a unique integrative biological indicator of the intensity of certain biochemical processes, underlying soil evaluations due to the close relationship of soil enzymes with soil biology and the rapid response to changes in soil management. Thus, a good understanding of the microhabitat is essential for improved crop productivity and soil health.

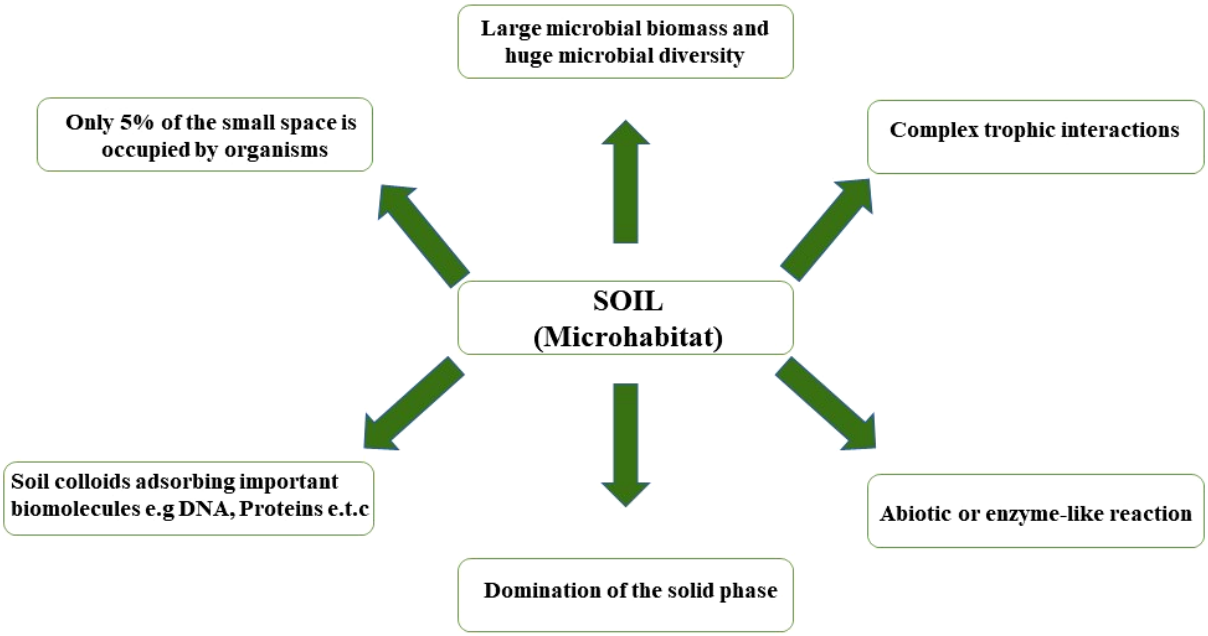


Figure 3.1. Selected characteristics of the soil as a microhabitat

3.3 The rhizosphere effects: the root of the matter

The region of soil unearthed in the range of about 2 mm in length from the root surface is referred to as the rhizosphere. The rhizosphere is a chemically complex zone having a changing microbiome (Haldar and Sengupta, 2015; Viebahn et al., 2005). Usually, the rhizosphere comprises the plant roots and the neighboring soil, more often this region is seen to include

rhizoplanes which are attachment of microbial biofilms. This is a widespread definition coined more than one hundred years ago by Hiltner (1904) and later modified by Pinton to be the precincts of the soil that are under the control of the root and the tissues of the roots colonized by microbes (Pinton et al., 2007). In this region, a strong relationship exists between soil biota and aboveground vegetation. This significantly changes both the physical and chemical characteristics of the soil and further goes on to modify the community of microbes in the near root region.

The characteristics of the soil close to plant roots can be transformed by a series of processes that take place during the phase of growth. These processes in turn modify the near root microbiota. During the growth period, exudates with low molecular mass [e.g, sugars, amino acids and organic acids], polymerized sugar [that is, mucilage], root border cells and dead root cap cells are released. These rhizodeposits are utilized as sources of carbon and energy by soil microbes and they account for an estimated 25% of the carbon allotted to the roots of cereals and grasses (Jones et al., 2019a). These deposits in the near root regions also comprise secondary metabolites, which may include anti-microbial substances, nematicides and flavonoids (Oldroyd, 2013), usually associated with the establishment of symbiosis or pest and pathogen resistance. The soil pH, another vital determinant of the soil microbial structure, could rise or drop by up to two units in the root region due to the ions that are released and uptaken. Uptake of water and respiration in the root affects the soil oxygen pressure, thereby impacting microbial respiration. Also, as chelators such as phytosiderophores and sequestered metallic micronutrients are released, they have significant effects on nutrient availability around the root region. However, untangling the influence of these drivers is complex, as the ways of influence are interconnected. For instance, the measure and manner of influence of roots on the features of soil could vary

depending on the type of soil, the species of plants and the feedback response of the root region microbial occupants. In addition, the characterization of the near root community could be questioned by several changes of properties of the soil along the region of the root as it relates to the age and physiological state of the plant.

3.4 Key players in soil microbial distribution

3.4.1 Root exudates

Generally, plant root exudates are metabolic responses of the plants and they mediate interactions in both the roots of plants and the microbes in the near regions (Chaparro et al., 2013). Comparing one plant to another, the type, chemical constituents and amount of these exudates differ and could directly or indirectly impact the corresponding composition and abundance of near root microbes. Consequently, this shapes the rhizosphere to be a 'hotspot' microhabitat where there is increasing microbial interaction, abundance and exchange of genetic materials.

Plant roots release about 10 to 250 mg C/g, which is an estimated 5%–21% of the photosynthetically fixed carbon by plants and is exuded most commonly as amino acids, soluble sugars, or secondary metabolites (Badri et al., 2013). The rates of exudation of these substances differ widely among species and environmental conditions, this influence changes in soil parameters and feedback to affect the growth of plants and microbial consortia (Rúa et al., 2016). Mostly these carbon sources are supplied by plants to the microbes after breakdown return in the form of minerals (Larsen et al., 2015). Consequently, the released materials [minerals] create unique environments for the microorganisms and alter the input of nutrients in the soil. The resource-altered environment then creates substantial effects on the configuration of soil bacterial communities (Ramirez et al., 2010), in this root region, microorganisms usually give a

unique response to the minerals released. that plants release different root exudates, it could easily be inferred that the difference in the compositions of root exudates will most likely select distinct rhizosphere communities (McPherson et al., 2018). Also, the specific metabolites secreted within the root region can arouse increased responses in many soil microbes. As an example, flavonoids from plants can be an attractant not just for symbionts like *Bradyrhizobium ejaponicum*, but may also be for disease-causing organisms like *Phytophthora sojae (Cesco et al., 2010)*. Similarly, flavonoids also enhance plant-fungal relationships in the germination of spores and branching of hyphae. In addition, they influence quorum sensing. Likewise, constitutive secondary defensive metabolic substances, which include pyrrolizidine alkaloids, can modify the near root microbial environment by promoting tolerant or resistant microbes, or in some other circumstances, microbes that break down these substances.

3.4.2 Antimicrobial substances

In the rhizosphere, a nutrimental rich environment, plants and microbes interact and exchange nutrients that may not even be directly available. The microbes in the near root are involved in key functions such as promoting the growth and development of the plant, nutrient acquisition, yields, disease and insect resistance mechanisms while the photosynthetic produce from plants is used as both a substrate and energy source for rhizosphere microbial support (Mendes et al., 2011). In this regard, the plants do not only offer these nutrients for these microbes, but some species of plants also hold some distinct antimicrobial metabolites present in their plant root secretions which could ward off some susceptible species of microorganism. Such plants are employed in herbal medicine. For instance, chamomile, thyme and eucalyptus, and other related secondary metabolic products of such plants (Hu et al., 2016) affect underground diversity. Interestingly, some of these antimicrobial inducing plants can also hold significant consequences

on the communities of the soil microbes. Furthermore, in the near root, microbial community interactions can also be impacted by substances produced by other microbes. For instance, a study by Jones et al. (2019b) highlighted that *Streptomyces* growth around the root was favored by interplays with the yeast *Saccharomyces cerevisiae* via the emission of trimethylamine (TMA), a volatile substance which increases the pH around the root. It was identified that the TMA synthesized considerably modified the root region and distinctively decreases the availability of iron, this consequently impacted the viability and structure of resident organisms

3.4.3 Signaling and interconnections of the plant microbiome

The connection existing among plants and millions of microbes entails great communication (Smalla et al., 2001). Quite a number of signatures encoding communications through quorum sensing and different signaling molecules have been identified in metagenomes of microbes in close association with plants (Bragina et al., 2012). Nevertheless, the mechanism involved in the interaction of this community to bring about a structured microbiome is still not properly understood. Volatile organic substances are liable for 'microbial small talk' but can also act as long-distance messengers for communication with the plant host (Bragina et al., 2012).

3.5 Soil and plant types interplay to shape microbial community

The composition of microorganisms in the soil mediates vital processes in the soil that could affect plant growth and development (Jacoby et al., 2017). For instance, microbes in the soil stimulate nutrient cycling and enhance the availability of nutrients to plants (Baker et al., 2018). Some specific groups of microorganisms can fix nitrogen (Boyd and Peters, 2013) and make nutrients available to plants (Shakeel et al., 2015), which consequently extend to affect the global nutrient cycles. Furthermore, microbes in close association with the root can also modify specific plant characteristics such as its ability to protect against diseases (Santhanam et al., 2015), root

architecture (Zhou et al., 2016), and the ability of the plant to withstand water-scarce conditions. Commonly, the mechanism utilized involves translocation, mineralization and mobilization of soil P, K and Fe through the production of phytohormone (cytokinins, gibberellins and auxins), together with antimicrobial substances to protect the crops against diseases.

Recent advances in molecular biology which allow the study of the genetic material directly obtained from the soil have further afforded scholars opportunities to examine a much wider spectrum of microbes resident in the near root region. In an experiment using PCR-denaturing gradient gel electrophoresis [DGGE] to investigate the 16S rRNA gene fragments, it was first reported that the composition of bacteria species in the near root region is usually influenced by multiple interplays which involve the type of soil, species of plants and the region occupied by the root when they investigated three plant species (Grape, chickpea and Sudan grass) planted in three Californian soils (sandy, loamy and clay) (Marschner et al., 2001). Other similar studies indicated that either the species of plants or types of soil are usually the most considerable determinants when examining the community construction of the near root microbial community. The extent to which the rhizosphere will be plant-dependent, and whether the resultant effect is promoted when the same crop is grown for two continuous years, was further identified in another investigation. It was observed in the second year that potato, strawberry, and oilseed rape were planted. that the plant-dependence changed in the relative bacterial compositions (Smith and Smith, 2011). This was not limited to species of plants alone, as cultivar can also alter the structure of the near root microbe (İnceoğlu et al., 2012). However, the interplay between plant and soil types and the structure of the rhizosphere microbiota is a more elaborate subject and exceeds the scope of this review.

3.6 Changes in microbial diversity during plant developmental stages

Plant species possess different kinds of root architectural patterns, metabolism and growth strategies that influence the microbial quality and diversity of soil (Weir et al., 2010). Current data also show that the numerous actions of microorganisms and their corresponding abundance can depend on the plant species (Clairmont et al., 2019; Yu et al., 2019). Furthermore, the balance of the microbial community varies in certain periods owing to the differing and dynamic root exudates which could vary during the life processes and how the plant responds as season changes (Li et al., 2019). Similarities among species of plants revealed that there are clear observable differences among plant rhizosphere communities when evaluating the community structure and function at specific periods of time along with their growth phase, with the biggest changes observed in young plants (İnceoğlu et al., 2012; Smalla et al., 2001). Furthermore, a work conducted on the influences of cultivars and growth of plants on the rhizosphere community composition revealed that cultivars had a near root effect on the bacteria community and the stages of growth modified the beta-proteobacterial communities greatly (İnceoğlu et al., 2012). It is indicative that the community of microorganisms inhabiting the rhizosphere of a plant is not constant but changes over time with the same plant type.

3.7 Specific plants, specific microbial community

Commonly, rhizosphere microbial communities have less diversity than those of the bulk soil (Hein et al., 2008). Out of the prevailing population of microorganisms inhabiting the bulk soil, the root of the plant creates an environment suitable for the survival and thriving of specific microorganisms in the rhizosphere. The plant roots usually do not do this alone but rather collectively with some other significant drivers which include the genotype of the plants and the soil type (Garbeva et al., 2008). From earlier reports, a mere relationship between the different compositions of the bacterial community and plants were initially documented (Germida et al.,

1998). However, subsequent discoveries showed there was more to the relationship. Viebahn et al. (2005) observed that the microbial consortia in the rhizospheres of individual plant species occupying a particular soil were also usually different. It was inferred that considering the significance of mutualistic/ parasitic interplay existing between plants and microorganisms in microbial food webs, a robust influence of a particular species of plant on soil fungal and bacterial community composition can be expected. Also, an extensively studied association between rhizobia–legume interactions further pinpoints the singular effects of plants on microbial diversity and its precision (Thrall et al., 2011).

When exudates are released in the roots, they encourage relationships connecting specific microbes and plant species (el Zahar Haichar et al., 2008; Singh et al., 2014). This interplay could alter the composition of microbial consortia in the root in a manner that favors specific plants (Broeckling et al., 2008). Badri et al. (2013) observed that a mutant Arabidopsis ABC transporter that synthesizes chemical compounds (phenolics) better than sugars in relation to the wild type gave notable modifications in the native community of microbes in the soil. The resulting modifications in root exudate synthesis were observed to favor beneficial bacterial communities which included plant growth promoting rhizobacteria, microorganisms that fix nitrogen and metal remediators. In a similar experiment, Micallef et al. (2009) also noted that Arabidopsis ecotypes did not only exude specific sets of substances but that the changes in the root exudates allowed near root bacterial communities which may be favorable for the existence of the plant. Furthermore, benzoxazinoids released in moderate quantities from the root of some cereal plants were identified to influence the survival of rhizosphere microorganisms. In maize (*Zea mays*), there is a natural antimicrobial substance [benzoxazinoid] called 2,4-dihydroxy7-methoxy-2H-1,4-benzoxazine-3(4H)-one (DIMBOA). Nacke et al. (2016) observed that

Pseudomonas putida KT2440 does not just tolerate DIMBOA but the compound also chemotactically attracts them. However, in the roots of a mutant species of the maize, KT2440 was notably not present as much as in the wild-type plants, indicating that DIMBOA particularly allows this plant beneficial bacterium. This suggests that these microorganisms were specifically enriched in the soil to further protect them from disease-causing organisms. Badri et al. further highlighted that by adding a specific mix of native chemicals obtained from Arabidopsis, root exudates created a different near root community of microorganisms that appear to possess the trait to break down atrazine or included more mutualistic microbes (Badri et al., 2013). Also, in a purely isolated exudate of seeds, young plants and rootlets of tomato (*Lycopersicon esculentum*), cucumber (*Cucumis sativus*) and sweet pepper (*Capsicum annuum*), the most common constituent was organic acids. The strength possessed by strains of rhizobacterial to survive in vitro on citric acid as the only source of carbon seemed to correspond to their potentiality to colonize the root. See Table 3.1 for specific plants identified with specific microbial community.

Table 3.1. Specific Bacterial phyla dominating rhizosphere and assemblages

Host Species	**Dominating Phyla**
Cultivated rice [*Oryza sativa*] [c]	*Actinobacteria, Proteobacteria,*
Cultivated potato [*Solanum tuberosum*][a]	*Actinobacteria, Proteobacteria*
Oak [*Quercus* spp.]	*Acidobacteria, Actinobacteria Proteobacteria*
Poplar [*Populus deltoides*]	*Acidobacteria Proteobacteria*
Cultivated potato [*Solanum tuberosum*]a	*Actinobacteria, Bacteroidetes Firmicutes, Proteobacteria*
Thale cress [*Arabidopsis thaliana*]	*Acidobacteria Actinobacteria Bacteroidetes Proteobacteria*
Wild oat [*Avena fatua*][a]	*Actinobacteria, Firmicutes Proteobacteria*
Cultivated maize [*Zea mays]*[b]	*Proteobacteria*
Sugar beet [*Beta vulgaris*][a]	*Actinobacteria, Firmicutes Proteobacteria*

[a]data obtained with phylochip. [b]data obtained with a specific system-designed 16S rRNA gene microarray. [c]data obtained from whole-metagenome shotgun and 16S rRNA gene clone library

Table culled from Mendes et al. (2011)

3.8 Plant species composition alter soil microbial community

A molecular-based experiment by Wardle et al. documented that in a field, when specific plants were removed from an assortment, the removed plant had a noticeable impact on the community of microorganisms; nevertheless, there was no observable difference in the total biomass of bacterial and fungal species (Weir et al., 2010). To their disappointment, when they tried to aim at a more pronounced shift in the soil microbial diversity, they only identified mere temporary root-induced influences. Furthermore, throughput shotgun sequencing employed in a study of soil microbial consortia in close relationship to antarctic vascular plants carried out by Molina-Montenegro et al. (2018) in a view to studying how microorganisms influence changes in plants under unfavorable conditions showed that bacterial species had a soaring relative richness in the sites (98%), which was far more than Archaea (0.22%) and Eukaryota (1.77%). Among the

bacterial phyla, *Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria* and *Firmicutes* were the most abundant, constituting almost 85% of the sequences in the near root soil samples . These identified phyla have often been reported to abound in other soil samples with specific plants (Imchen et al., 2017). They also make up an essential root microbiome where they play a pivotal role in promoting the growth of plants due to their ability to acquire nutrients and tolerate unfavorable conditions (Chen et al., 2017). A conceivable explanation for the role and observed relative abundance of these phyla is that specific plant composition in a specific habitat could shape the root region by selectively favoring specific species across these sites (Bakker et al., 2013; Mahoney et al., 2017). Baker et al. (2018) mentioned that the species richness of nearby plants caused a major alteration in the structure of the *Streptomyces* spp. of neighboring vegetal species. As plant richness increased, the community of *Streptomyces* decreased and there were observable increases and relatedness in the new community. The more distinct the community of plants, the more diverse the composition of root exudates found, and this consequently influences the diversity of microorganisms inhabiting the region.

3.9 Notable methodological approaches in the study of soil microbial communities

Taxonomic and methodological limitations have to an extent hindered the study of species and genetic diversity in microbial communities. Over the years, the methodologies employed in the investigation of the rhizosphere have been rooted deeply in the use of several culture-based procedures and molecular techniques. Some culture media were composed in a bid to heighten the recovery and isolation of several groups of organisms within soil microbial communities. Scientific developments further birthed the introduction of a biolog-based method for the direct examination and study of the potential activities of soil microbial communities, commonly referred to as community-level physiological profiling (CLPP). However, a fundamental

challenge associated with many conventional physiological and biochemical approaches was their dependence on the study of phenotypic expressions (e.g., enzymes, respiration, and catabolic potential), and despite the demonstration of metabolic activities, many microbial populations are yet unculturable under laboratory conditions. Furthermore, the resulting metabolic fingerprints seem to be a less-accurate, weak, or false representation of the in-situ functional diversity in a typical consortium of microbes (Singh et al., 2014). Besides, as a result of weak gene expression following the test conditions, using biochemical test methods resulted in fairly common negative results. Several procedures have been identified to surmount this challenge. These approaches include the use of signature lipid biomarkers (SLB) which include phospholipid fatty acids (PLFA), nucleic acid technologies (molecular biology) such as amplified rDNA restriction analysis (ARDRA), Denaturing Gradient Gel Electrophoresis/ Temperature Gradient Gel Electrophoresis (DGGE/TGGE), terminal restriction fragment length polymorphism, ribosomal intergenic spacer length polymorphism. Although these PCR-based techniques are in source reproducible and robust, they are predisposed to possible bents. The benefits and drawbacks of different techniques are summarised in Table 3.2.

Table 3.2: Common techniques adopted in the investigation of soil microbial communities before metagenomics

Method	Advantages	Weaknesses
DGGE/ TGGE	Renders full sequences that can be subjected to additional analysis	Gel-to-gel variation PCR primer design (GC clamp) only short sequences < 400 base pair (bp) can be analyzed using TGGE
SSCP	Presents full sequences that can be subject to further analysis Technically simple gel preparation Variant folding of single-strand molecules	Complicated DNA preparation (two purification steps) Only short sequences < 200 bp can be analyzed
T-RFLP	Technically simple High discrimination power	Loss of some variability (sequences not cleaved or cleaved near to primer)
LH-PCR/ ARISA	Technically simple	Low discrimination power
Microarrays	No bias due to PCR	Detects only sequences corresponding to probes Detection limit lower than in PCR-based methods
PLFA analysis	Can cover whole communities across kingdoms Quantitative description of the community	Low taxonomic separation limited to community composition analysis

Source: Garbeva et al. (2008)

Abbreviations: DGGE/ TGGE- Denaturing Gradient Gel Electrophoresis/ Temperature Gradient Gel Electrophoresis; SSCP- Single-strand conformation polymorphism analysis, T-RFLP- Terminal Restriction Length Polymorphism, LH-PCR/ ARISA- Length Heterogeneity-Polymerase Chain Reaction/ Automated Ribosomal Intergenic Spacer Analysis, PLFA-phospholipid fatty acids

3.10 Metagenomics: the new way of seeing the soil

The use and advancement of metagenomic tools in the study of soil microbial consortia offer a new way of thinking and system-level perspective of microbial diversity. In lieu of analyzing just one organism or single function, this approach explores the whole consortium of genes in a community, allowing the building of a framework of genes and functions on which to establish systems about community structure and function. Metagenomics involves the genomic

investigation of microbial communities (Vitorino and Bessa, 2018a). This approach entails the direct isolation of DNA from an environmental sample [water, soil, gut], and then analyses the DNA sample afterward, such that it further unveils the diversity concealed within environmental samples. Metagenomics has a high power of genomic analysis, such that when the 16S and 18S rRNA are sequenced, the regions of microbe resident in the natural samples otherwise permits a straightforward classification of genera, circumventing the stress to isolate and culture individual microbial species. Nonetheless, the complexity linked with metagenomic DNA results from its build-up as it is a composition of genomes from several distinct organisms. This could consequently result in a challenging analysis and relatively intricate approach.

However, with this improvement and popularization of metagenomics, a tremendous amount of research on the heterogeneity of microbial consortia has been conducted and more is in progress (Mašínová et al., 2017; Nacke et al., 2016). The metagenomic approach has also been efficiently utilized by several researchers in recent times to advance comprehensively their description of the taxonomic and functional diversity of soil microorganisms (Jiang et al., 2018). One of such reported developments was (Nacke et al., 2016). who showed that about 10% of environmental microbial sequences could be lost from classical PCR-based Small Subunit ribosomal RNA gene surveys, which often include members of the Candidate Phyla Radiation (CPR) and also uncharacterized Archaea. This report underscores previous approaches and further provides fruitful avenues for describing additional phylogenetic lineages. Furthermore, the arrival of next-generation sequencing (NGS) now allows scholars to investigate large sequences of a specific genome. The arrival of NGS is increasingly changing sequencing technology and the landscape of metagenomics. Still, these unexplored microbial niches are in great need in premises where metagenomics tools are being utilized to unveil the hidden potential of such valuable

environments. For instance, a recent study in Portugal by Romão et al. (2017) used NGS in combination with cultivation-based approaches to study the community of fungi and prokaryotes for the occurrence of potential disease-causing organisms in beach sands in Portugal. The study highlighted that cultivation-based fungal enumeration showed low and variable concentrations of the species targeted (yeasts and dermatophytes) (Rúa et al., 2016). This also showed that the population was inadequately represented in the community when analyzed by NGS targeting the ITS1 region. Conversely, NGS showed that uncultivable *Purpureocillium liliacinum* was present among the complete fungal community. It was also reported that cultivable fecal indicator bacterial concentrations were moderate during the investigation and were not similar to the communities marked by NGS. This further buttresses the importance of metagenomics in the understanding of the biochemical functions of uncultivable microorganisms and their interplay within their environments. Nevertheless, it is believed that metagenomics is still underutilized as this breakthrough in microbial ecology holds a great promise for tapping the rich genetic resources, phylogenetic and functional diversity of microorganisms that appear difficult to culture.

3.10.1 Approaches to metagenomics

Metagenomics is divided into two main approaches, which are geared at numerous parts of the local microbial community associated with the soil habitat. In the first technique, which is also identified as ‘sequence-driven metagenomics’, the DNA obtained from the soil is sequenced and analyzed with bioinformatics and computational tools. The metagenomic sequences are then subjected to comparison with sequences present in an open and accessible database such as Genebank. The genes are then assembled in groups of much-related function, and the natures of proteins that conduct those functions. The construction of a metagenome library involves

successive steps which include: [1] Recovery of whole DNA from an environmental sample; [2] shotgun cloning of random DNA fragments in a proper vector; and [3] reconstructing the clones into a host bacterium as well as screening for positive clones. Metagenome libraries built of small DNA fragments in the range of 2–3 kb render higher-grade coverage of the metagenome of an environment than those with larger fragments. Reports show that to recover the genomes from limited groups of microbial communities, not less than 1011 genomic clones will be required [30]. Small-insert DNA libraries are also important to select for phenotypes that are encoded by singular genes and for reconstructing the metagenomes for genotypic analysis. Large-fragment metagenomic libraries (100– 200 kb) are advantageous while reviewing multigene biochemical pathways. See Fig. 3.2 for construction and analysis of metagenomic libraries.

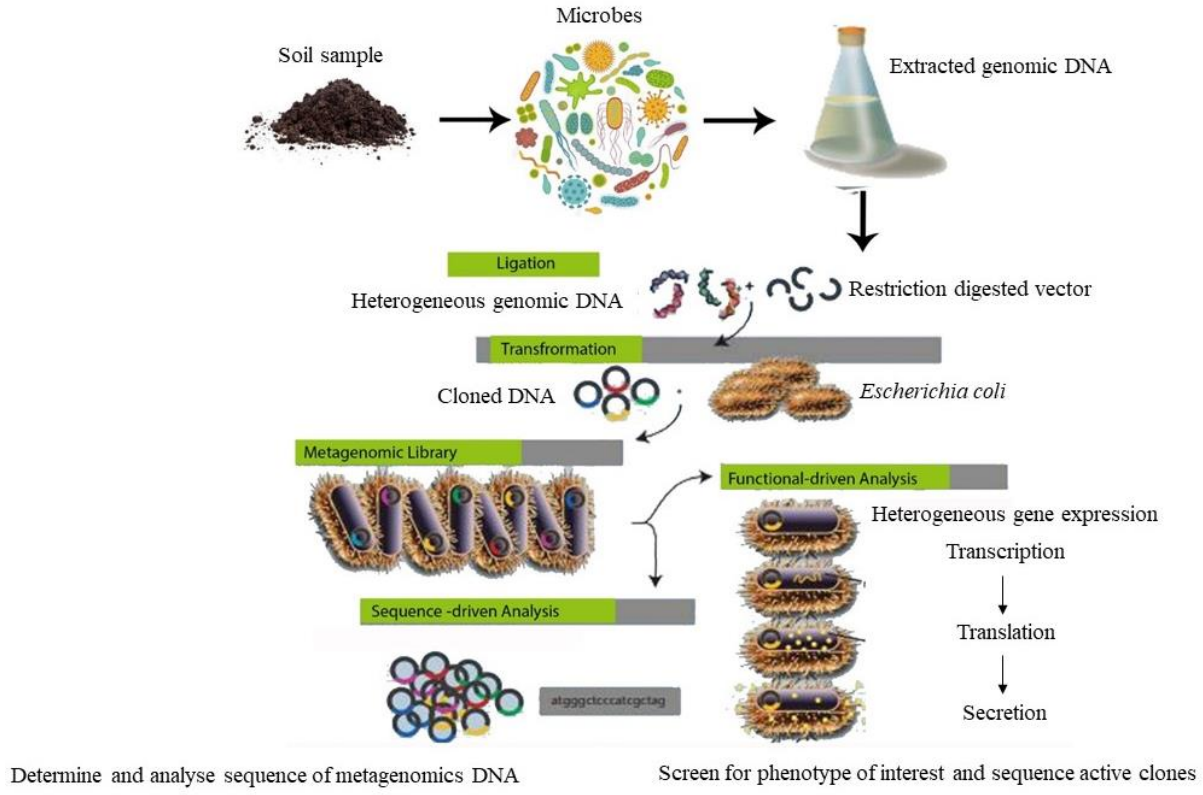


Figure 3.2. Construction and analysis of metagenomic libraries.

In the other method termed ‘function-driven metagenomics’, the isolated DNA from the soil is also obtained and filled into an alternate host as a storage technique, but instead of proceeding to a sequencing step, the captured fragments of DNA will be screened, or ‘cloned’, for a specific function. It is required that the surrogate host is devoid of this function so that acquisition of the function by the host following the metagenomic DNA expression can solely be said to be a function of the presence of the metagenomic DNA. In function-driven metagenomic investigations, libraries are screened based on a preferred and distinctive phenotypic expression on a specific medium. This approach was used in a study by Tringe et al. who performed compositional and functional comparisons of microbial communities from two nutrient-poor and two nutrient-enriched environments (Tringe et al., 2005). The major concern of the approach was centered on gene function rather than genome composition, thus overcoming limitations

experienced when assembling genome from complex environments. Researchers, however, demonstrated that gene function and structure differed in nutrient-limited as compared to nutrient-abundant environments. Functional metagenomics can consequently be viewed as a reliable explorative tool for the identification and characterization of new genes (Nacke et al., 2011), metabolic traits, bioactive compounds (Craig et al., 2010), or pathways (Illeghems et al., 2015) from yet to be cultured soil microorganisms.

3.10.2 Limitations and way Forward

Studies have shown that the two approaches have been very effective in appraising the diversity of function of the microbial world. Nonetheless, both methods still possess their benefits and weaknesses. The sequence driven approach, on the one hand, is still confined by existing information. For instance, if metagenomic gene information is not an identified function collected in the databases, then limited information can be extracted about the gene sequences. However, one way of solving such challenges confronted by soil microbial ecologists is to drive the generation of a wide catalog of all microbial consortia members and functions for at least a reference soil. This comprehensive reference dataset would cast more light and be a pool of the yet unknown structure of a soil microbial species frequency distribution. This could also possibly be a prospective reference for evaluating community composition shifts across soil landscapes. The function-driven analysis, on the other hand, can define genes that have not been identified to anything earlier examined as genes are distinguished by their displayed function instead of sequence. However, the shortcoming is that the common genes from organisms in wild communities are not shown simply by the selected surrogate host. Furthermore, a very weak level or no expression of the preponderance environmental genes could also be an issue. In another instance, enhanced gene expression can be obtained by inputting metagenomic DNA into

several supplementary alternate hosts such as *Streptomyces, Bacillus, Pseudomonas, and Agrobacterium*. Thus, regarding the inadequate ability of *E. coli* to express genes from different taxonomic groups of organisms, additional shuttle vectors with extended host range are required.

3.11 Conclusion and future perspective

From this review, we have been able to demonstrate the inherent ability of plants and soil types in shaping their microbial community. Furthermore, it was suggested that the composition of microorganisms can be altered solely or synergistically by the types of plants or/and soil. In some cases, microbiomes are suitably formed by specific plants based on their metabolic and physiological responses or shaped to complement the beneficial effects they confer. Microbial diversity and balance are key for healthy plants. Traditional knowledge and current perceptions form a clearer picture of how the composition could go a long way to determine the ability of the plants to resist other disease-causing organisms. New insights further showed how notable microbial diversity can play key roles as antagonistic phytopathogens. Even though plant microbial diversity depends on these factors, the secondary metabolites which originate from plants often trigger the arrangement of species compositions and should be considered in future screening strategies. Usually, microbes associated with vegetal create a network that can be influenced by soil and plant types. These network models the soil and plant microbiomes. However, it is still left to reason that those plants that modify their microbiota in a manner that is profitable to their reproductive success and survival will be favored during evolutionary selection. Meanwhile, it is important to highlight that the factors affecting microbial diversity in the soil are not limited to the points discussed (Wardle et al., 1999) (see Fig 3.3) and microbial structures are not solely influenced by these factors but also by their functions. Also, by the close relationship with microorganisms from the same soil environment, plants at times can easily

reach a better fitness advantage than if they are in relationship with microorganisms from other soil environments. New developments in investigating and understanding the diversity of microorganisms are wrought with taxonomic and methodological deficiencies.

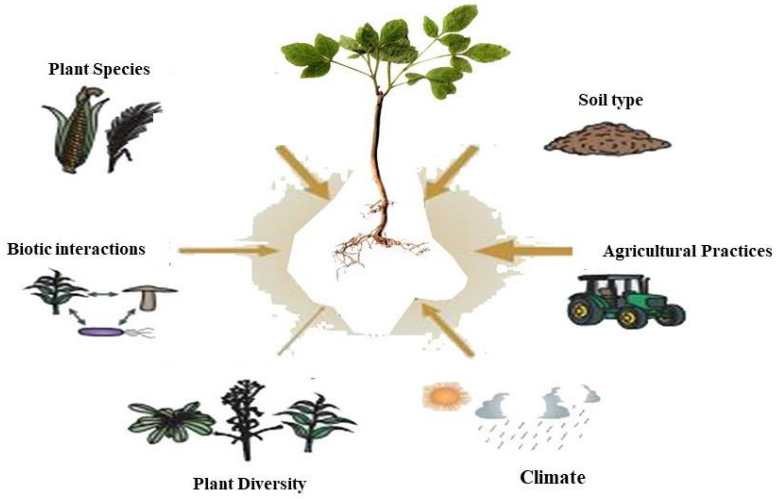


Figure 3.3. Major determinants of soil microbial community structure

CHAPTER FOUR

ECOLOGY, ROLES AND APPLICATIONS OF MICROBIAL COMMUNITY IN PLANTS

Abstract

Plants have evolved with several identifiable microbial involvements in their growth and health. Now, there are more studies on the microbial community composition, dynamics, and functional abilities. Considering the immense prospect of identified functional potentials of the soil microbiome and the need for sustainable agriculture, there is a necessity for the development and practical use of microbial innovations. In our study, we discuss the combined utilization of microbial consortia under field conditions for improved crop productivity. We discuss the appropriate knowledge-driven choice of the microbial community as well as proper delivery techniques and formulations. Also, we considered the influence of farm practices or plant genotypes on the functioning of the soil microbial community. In conclusion, distinctive ways of using new inoculants and their applications are highlighted. This review highlights the significance and functionalities of the bacteria plant community, its challenges and its concepts.

4.1 Introduction

For the past few decades, outcomes have profoundly shown the complexity of microbial communities linked with diverse plants and distinct plant organs (Jones et al., 2019a; Reinhold-Hurek et al., 2015). The plant holobiont consists of a microbial component commonly known as the plant microbiome in the root region, phyllosphere, and endosphere. This community plays an essential role in supporting the health and growth of plants (Brader et al., 2017). Uncovering the usefulness of association that exists between microbes and plants can prompt a superior comprehension of the plant as a meta-life form and how the interaction with their microbial partners can be of benefit to plants (Hacquard, 2016). These days, development in demography,

changes in climate and growing demand for sustainable production are posing threats to crop production. To tackle these threats, microorganisms are now considered as a potential alternative for biofertilizers or biopesticides in agricultural practices over chemical products which have adverse effects on the soil (Adedeji et al., 2020). In the past years, several studies were designed to address the concept of bioinoculants (Ibiene et al., 2012; Mia et al., 2005). While the success achieved has been limited, there is an increased understanding of the plant microbiome as more information regarding the living and non-living stresses, environmental factors, and plant genotype have been gathered, thereby increasing the possibility of obtaining suitable viable microbial prospects for inoculation in a designated area (Mitter et al., 2016). The plant microbiome encompasses a variety of organisms such as bacteria, fungi, and archaea. Given the abundance of accessible knowledge about bacteria and the attention from the industrial sector, this study will focus on bacteria as a unit of plant microbiota, the functionalities, threats and theories regarding the application of plant-associated bacteria.

4.2 Abundance, diversity and functional potential of plant microbiota

Plants obtain their microbial partners actively from ambient microbial repositories like the rhizosphere (near-root region), the phyllosphere (leaf surface relating to the exterior environment), the spermosphere (exterior of grown seed), the anthosphere (outer environment of flowers), and the external fruit environment referred to as the carposphere (Hardoim et al., 2015). The root microbial community is obtained from the soil layer, i.e. horizontal transfer, and comprises extremely diverse microorganisms, largely populated by Planctomycetes, Verrucomicrobia, Acidobacteria, Bacteroidetes, Actinobacteria and Proteobacteria (Urich et al., 2008). However, vertical transfer of bacteria can occur via seeds. Additionally, plant seeds serve as a vital source of microbes that proliferate in developing plants' roots (Wassermann et al.,

2019). Through their root system and other parts, plants provide distinctive eco-friendly habitats for the soil microbiome that inhabit the rhizosphere, roots and some parts above ground level (Igiehon and Babalola, 2018). The rhizosphere under the close influence of plant roots is regarded as the main area of microbial activity and this conforms as a complex environment (Alawiye and Babalola, 2019). Lately, Donn et al. (2015) revealed alterations induced by the roots in the bacterial population at the near-root region of wheat and discovered a ten-times increase in abundance of copiotrophs, oligotrophs, *Pseudomonas,* and *Actinobacteria* at the near-root in comparison with the bulk soil. Further, they affirmed that with time, the near-root region and rhizoplane communities were changed while the bulk soil population was consistent. Similarly, the rhizosphere of *Brachypodium distachyon*, a wheat model, was found to be populated by *Burkholderiales*, *Xanthomonadales,* and *Sphingobacteriales*, and in contrast, the order *Bacillales* inhabited the bulk soil (Kawasaki et al., 2016). The composition of the microbial community around the root is known to be affected by compounds exuded from the root, which include fatty acids, amino and organic acids, sterols, phenolics, nucleotides, plant growth regulators, and putrescine. This is referred to as the rhizosphere effect (Baudoin et al., 2003; Hartmann et al., 2008). For example, the root-associated microbial composition of the maize plant was altered by benzoxazinoids (BXs), which are a group of protective exudates released by the roots, and most affected microorganisms belong to the Actinobacteria and Proteobacteria groups (Hu et al., 2018). Zhalnina et al. (2018) worked on the rhizosphere of *Avena barbata*, whereby they studied the fundamental factors influencing the bacterial community and discovered that the root exudation chemistry combined with the bacterial preference for certain substrates induce assemblage of bacterial communities in the rhizosphere. Many rhizosphere bacterial taxonomic groups, particularly those belonging to the genus *Pseudoxanthomonas*, have

been reported by Fitzpatrick et al. (2018) to have importantly varied abundance across 30 angiosperm plant species. Altogether, the composition of the rhizosphere microbiota is influenced by the diverse plant species and genotypes and this all depends on the nature and composition of the compounds exuded by the root.

The root endosphere is also colonized by a wide variety of bacterial endophytes. The processes through which bacterial endophytes enter the tissues of the root include passive mechanisms, cracks in the root, growth points of lateral roots and the active mechanism (Omomowo and Babalola, 2019). Endophytes' ability to populate and plant resource allocation are factors that determine the transmission and population of endophytes in plants. Bacteria belonging to diverse taxonomic groups can enter into root tissues. To illustrate this, grapevine roots were often found to be populated by diverse bacteria phyla such as Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes, Planctomycetes, Chloroflexi, Gemmatimonatedes, Verrucomicrobia, and Bacteroidetes (Samad et al., 2017b) while in rice roots, Rhizobiaceae, Bradyrhizobiaceae, Comamonadaceae and Streptomycetaceae were found to be the most populating families (Edwards et al., 2015).

4.3 Above-ground plant microbiota

Though there are significant differences in the ecology of bacteria found in the endosphere and phyllosphere, plant tissues found above the soil level such as the vegetative foliar segments, floral parts, and leaves give rise to environments that are specialized for endophyte and epiphyte diversities. Nearly all endophytes spread in a structured fashion through the xylem to discrete areas of the plant such as the stem, leaves, and fruits (Compant et al., 2010). However, they can have access to the plant tissues through the flowers and leaves that are the aerial parts of the plant (Compant et al., 2011). This depends on the allocation of the plant source, as several

topmost plant compartments host distinct endophytic communities. Records have shown that phyllosphere bacteria are often derived from the soil area and are regulated by the plant and the conditions of the environment, with the latter having a more serious effect (Zarraonaindia et al., 2015). As a result, the endosphere and phyllosphere contain diverse microbial taxa. For example, the evaluation of the microbial composition of the phyllosphere of grapevine based on its structure showed that *Pseudomonas, Bacillus, Sphingomonas, Curtobacterium, Methylobacterium, Enterobacter, Citrobacter, Acinetobac*ter, *Pantoea, Erwinia,* and *Frigoribacterium* are the predominant genera (Zarraonaindia et al., 2015), while the assessment of grape berries' endophytes showed genera such as *Ralstonia, Pseudomonas, Burkholderia, Staphylococcus, Propionibacterium, Mesorhizobium, Dyella,* and *Bacillus* to be more populated (Campisano et al., 2014). Wallace et al. (2018) found methylobacteria and sphingomonads to be the main groups of microorganisms in their recent study on the maize leaf microbiota across 300 different maize lines. Besides, they reported that the microbiota of the phyllosphere was majorly influenced by the environmental parameters. Numerous investigations have revealed *Pseudomonas* as the most plentiful genus found in apple, grapefruit, almond, pumpkin flowers, and tobacco (Wallace et al., 2018). Similarly, Steven et al. (2018) found *Pseudomonas* and Enterobacteriaceae to be the most populated groups in apple flowers. It has also been addressed and discovered that seed-associated bacteria comprise essentially Proteobacteria, Actinobacteria, Bacteroidetes, and Firmicutes (Rodríguez et al., 2018). There is a specific relation that exists between the seed microbiota, soil microbiota, and the microbiota of flowers and fruits (Compant et al., 2019). The soil, air, and seeds of plants contribute majorly to the plants' microbiota above ground level and the organisms adjust to life inside or outside the tissues of the plant where variables like the soil, environment and farm management influence the diversity of the

community. Host and compartmentalized arrangements show an effective relationship between plants and the microbiota of the soil level. The microbiota above the soil level and endophytes are strongly recognized for their potential to boost the growth of plants, reduce susceptibility to diseases, and mitigate stress tolerance (Stone et al., 2018).

4.4 Determinants of plant microbial compositions

The microbiota of any plant part is determined by a series of biotic and abiotic factors such as soil pH, salt concentration, soil structure, soil type, soil moisture, organic content, and exudates (Vos et al., 2013) and these play a major role for plant parts found below the ground. On the other hand, factors that influence the microbiota of plant parts above and below the ground level include extrinsic environmental factors like climate, presence of pathogens, and human activities (Hardoim et al., 2015). The plant genotype and species select microbes from the soil environment where the characterization and composition of the roots, secondary metabolites, and type of rhizodeposits perform a vital function in selecting members of the plant's microbial community (Reinhold-Hurek et al., 2015). Irrespective of the similarity in the soil ecosystem in which they grow, plant species select distinctly different microorganisms to make up the microbiota in the near root and root compartments (Kawasaki et al., 2016). Bulgarelli et al. (2015) examined the root microbiome of several barley accessions, using the 16S rRNA gene sequencing and shotgun metagenome technique, through which they discovered that the host innate immune system and root exudates essentially formed the structure of the microbial community. Additional host-related factors such as the age of plants, health, developmental stages, and fitness are recognized to induce the structure of the plant bacterial community through the induced systemic resistance of plants and the constituents of the root exudates (Aleklett et al., 2015).

4.5 Key and adjunct microbial community

The core plant microbiome is referred to as a close association between microorganisms and plant species or genotypes, regardless of soil and environmental factors (Toju et al., 2018). The plant's core microbiome is considered to contain fundamental microorganisms that are essential for the plant's fitness. By modification techniques of selection and supplementation, the microbiota has been confirmed to contain genes of vital function for the fitness of the plant and its microbiota (Lemanceau et al., 2017). On the other hand, microorganisms that occur less frequently and are present in lower amounts in sites are referred to as satellite taxa (Magurran and Henderson, 2003). The satellite taxa can further be described considering their geographical radius, habitat specificity and richness at local level (Jousset et al., 2017). The satellite taxa are frequently valued for driving fundamental functions in the ecosystem. A current study showed that microorganisms that occur in lower fractions are significant for decreasing harmful invasions into soil communities (Mallon et al., 2015). Also, the presence of these bacterial species in low fraction aids the development of antifungal volatile compounds that shield the plant from infectious agents present in the soil (Hol et al., 2015). They discovered that the shift of these soil microorganisms that exist in rare amounts can affect the productivity of plants negatively. Many investigations propose that the less-occurring soil microbes perform critical roles that might be irregular to their increased occurrence. In order to understand the different ecological explanations of the satellite to core dynamics, proper comprehension of the roles and resistant ability of plant microbiota against adverse environmental conditions is required.

4.6 Functions of the plant microbiome

The plant microbiome has members that include beneficial, neutral, or disease-causing organisms. Plant growth is improved by the plant growth-promoting bacteria (PGPB) through direct or indirect techniques. Phytohormones such as auxin, gibberellin, and cytokinin are

produced by PGPB, which affect the growth of plants by altering the concentrations of endogenous hormone associated with the plant. Similarly, an enzyme called 1-aminocyclopane-1-carboxylate (ACC) deaminase can be secreted by some PGPB to reduce plant stress hormones. Via synthesis of ACC deaminase, isolates of *Pseudomonas, Bacillus, and Arthrobacter* spp. have been found to improve plant growth. A variety of bacteria like *Pseudomonas*, *Pantoea,* and *Paraburkholderia* spp. in the roots of soybean and wheat were discovered. Igiehon and Babalola (2018) showed important properties of enhancing plant growth such as nitrogen fixation, phosphate solubilization, synthesis of ACC deaminase, and indole acetic acid, techniques for an increase in nutrient uptake, stress tolerance, and growth. The synthesis of phytotoxic compounds, proteins and phytohormones by some bacteria can result in plants showing disease symptoms. For instance, *Pseudomonas syringae* is a popular plant infectious agent that has a wide range of hosts such as tomato, olive, tobacco, and green bean. *Erwinia amylovora* is another example of a bacterium that has been implicated in fire blight disease of fruit trees. Many major diseases of crops such as potatoes and bananas are related to the species of *Xanthomonas, Xylella fastidiosa* and *Ralstonia solanacearum* (Mansfield et al., 2012). Plant disease austerity is multifactorial, as it depends on different factors that cumulatively influence the outcome of plant-infectious agent interactions. These factors include the number of infectious agents, how conducive the environment is, host susceptibility and living components such as the plant microbiota (Brader et al., 2017). Either by establishing a commensal relationship with pathogens or by modulating plant defense, plant-associated bacteria at the upper and lower ground level help to reduce the degree of susceptibility of plants to infections caused by pathogens (De Vrieze et al., 2018). Examples of bioinhibitory activities against invading pathogens and diseases include synthesis of antibiotics, production of inhibitory volatile compounds, lytic enzymes, and siderophores (Berg

and Koskella). By altering the plant hormone concentration, some bacteria shield plants from pathogens as this induces systemic resistance in plants. Pathogen pressure can be created and disease-suppressive soils can be developed through the constant use of agricultural soils that contain microbes that influence disease suppression (Durán et al., 2018). The role of some microorganisms belonging to taxonomic groups such as *Pseudomonas, Bacillus, Streptomyces, Paenibacillus, Enterobacter, Burkholderia*, *Pantoea,* and *Paraburkholderia* in the suppression of pathogens has been reported (Gómez Expósito et al., 2017). Three fundamental phyla belonging to Firmicutes, Actinobacteria, and Acidobacteria that regulate the continental attack of Fusarium wilt have recently been identified by Trivedi et al. (2017). The ability of *Paraburkholderia graminis* PHS1 to suppress diseases caused by fungal pathogens and the associated soil suppressiveness brought about by the production of sulfurous enzymes like cysteine desulfurase and dimethyl sulfoxide reductase has been reported by Carrión et al. (2018). The role of endosphere microbiota towards the suppression of the take-all disease (*Gaeumannomyces graminis*) has been reported by Durán et al. (2018).

4.7 Utilization and modulation of the plant microbial community - Microbial inoculation

The development of a single strain application is the first stage in the screening process of collected isolates for different plant growth-promoting characteristics in the laboratory. The determinants of screening assays for distinct microbial roles include nitrogen fixation, phosphate solubilization, or the synthesis of plant hormones, antibiotics, ACC deaminase, and siderophores. The bottom-line method entails testing the most viable strains in the greenhouse. The application of this method shows several bacteria isolates show great results in the laboratory and the greenhouse, but the contrary is seen on the field (Backer et al., 2018), where it fails to improve the suboptimal plant microbiome. For example, Hungria et al. (2010) discovered that during field

trials, *Azospirillum brasilense* strain Ab-V5 improved the yields of wheat grains and maize by 16 and 30% respectively. The effect of *Kosakonia radicincitans* formulations on maize was analyzed by other researchers in three different field plots. It revealed that the bacterial inoculation was profoundly effective in enhancing the grain yield as well as maize silage (Berger et al., 2018). In contrast to this, under field conditions, other investigations have observed no major impact with bacterial inoculation. For example, inoculation of *Azospirillum brasilense* has been observed to improve the growth of wheat and maize in the laboratory but has shown no notable effect on crop yield in field experiments (Fukami et al., 2016). *Trifolii* substantially enhanced the biomass of rice plants in the greenhouse but did not make a clear improvement in the biomass of plants and yield during farmland experiments (Kecskés et al., 2016).

Many reasons are conceivably responsible for the modest efficacy of the field application of microbial inoculants and the low reproducibility of the laboratory success. The high diversity and adequate adaptation of microorganisms in the environment under consideration are key factors that have to be considered while comparing with an introduced microorganism because this "foreign" microorganism being inoculated will not be able to contend adequately with the autochthonous microbiome. Nonetheless, the competitiveness of an inoculant strain is usually not the prerequisite for selection. The volume of cells introduced alongside the physiological activity also influences the ability of the inoculant strain to compete with resident flora (Samad et al., 2017a). Suitable formulations are crucial for successful application to ensure the deployment of specified cell doses as well as their durability. Another important element is the suitability of the strain to populate the respective species of plants, genotypes, or tissues, or to demonstrate the needed environmental function. For example, some biocontrol functions such as antagonistic activities will require a biocontrol strain to populate the same niches as the

pathogens and also exhibit antagonistic properties. Regulation of these activities may be difficult and depends on the interaction of the plant holobiont.

4.8 Applications of microbial consortia

The new paradigm to overcome lab to field obstacles is the application of microbial consortium (Parnell et al., 2016). The basis of this method involves the mixing of microorganisms with diverse characteristics, either supplementing each other to complement various techniques required for varied results which include improving plant growth and control of infectious agents by biological means. Strains with a similar mechanism of action but differing in their level of tolerance to different environmental stress or plant genotype can be included in microbial consortia. Several experiments on grapevine (Rolli et al., 2015), maize (Molina-Romero et al., 2017), Arabidopsis (Berendsen et al., 2018), tomato (Berg and Koskella, 2018), and potato (De Vrieze et al., 2018) have shown that microbial pairings, as opposed to single inoculants, possess the ability to enhance the influence of plant growth-promoting microorganism (PGPM). (Table 4.1).

Furthermore, bacteria that exhibit little or no PGP effects when combined as single inoculants can reveal PGP impact in a group, varying from the aggregate of three bacterial species living in a biofilm (Berendsen et al., 2018) to broad application of the microbial community (Berg and Koskella, 2018). Nonetheless, compared to single inoculants, some microbial consortia have been revealed to decrease PGP impacts (Rolli et al., 2015), which shows the need for ingenious knowledge-driven selection of consortia and strains. Hu et al. (2016) have implemented one fascinating and promising approach in which they presented an ecological framework that showed the direct relationship between the level of diversity and the degree of survival of *Pseudomonas* consortia being introduced. Moreover, an increase in the diversity of

Pseudomonas reduced the incidence of the pathogen *Ralstonia solanacearum* as a result of heightened competition for resources and interference with the pathogen's activity. The application of this theory is based on ecological community principles and it was also revealed by the author that an increase in the diversity of *Pseudomonas* consortia further increased the aggregation of plant biomass and significant incorporation of nutrients into plant tissue (Hu et al., 2017). The identity of the *Pseudomonas* strain is not as relevant as the effect of its diversity, which is linked with growth-promoting effects such as solubilization of phosphorus in vitro and a greater synthesis of siderophores and plant hormones. In some situations, it is important to consider how suitable the environmental factors (such as plant, soil type and climate) of a consortium source that is a PGP isolate are to the field conditions where the inoculant is to be employed. To illustrate, Actinobacteria are more prevalent in drought soils and *Azospirillum spp*. prefer to populate the rice cultivar from which they were initially isolated (Chamam et al., 2013). By utilizing the approach of matching the field of origin and the one to the applied, achievement of the establishment and the likelihood of finding bacteria that exhibit the desired PGP effect may be increased.

The identification of the biocontrol agent K84 effective against crown gall disease (New and Kerr, 1972) and the development of a group of six endophytes that prevents tobacco wilt disease (New and Kerr, 1972) are dynamic outcomes from the isolation of bacteria linked to infectious agents. Screening for asymptomatic plants that are open to non-living stresses also caused the discovery of bacteria that aid the resistance of plants to metals and organic pollutants and are therefore beneficial for biological remediation (Syranidou et al., 2016). In agreement with this, bacteria capable of nutrient-solubilization thrive more in poor conditions in which nutrients are limited (da Costa et al., 2014). In totality, relevant information on ecological behavior important

for field application can be gotten from the origin of an inoculant strain. The procedure for bottom-up selection; for the identification of prospects for enhancing plant growth, begins with collecting bacteria and investigating interaction dynamics in culture-dependent screenings (Armanhi et al., 2018). Characterization and selection of candidates in axenic culture is done by testing for bacterial resistance to stress conditions (temperature, desiccation, or toxic compounds) and plant growth-enhancing activities (Baldan et al., 2015). Traditional laboratory tests are partially substituted by the screening for underlining PGP genes (Lemanceau et al., 2017). While this has been successfully applied in numerous investigations as selection criteria (Syranidou et al., 2016), the effectiveness of plant growth-promoting bacteria does not explicitly refer to the abundance of molecular and genetic PGP-traits in bacteria (Cardinale et al., 2015). The use of PGP-traits identification in pure culture and their genomes relies on the mechanistic knowledge of a distinct trait.

Besides, the information obtained from laboratory screening may be inadequate. For example, one *Pseudomonas* strain that could inhibit the growth of *Phytophthora infestans* was suppressed by another *Pseudomonas* strain when grown in a co-culture and lost its inhibitory capability after co-inoculation (De Vrieze et al., 2018). However, bacteria can normally escape competition by populating micro-niches and compartments, thus reducing the benefit of in vitro bacterial relationships without plants. The possible permutations for PGP-consortia increase exponentially with the population of the starting set of potential PGP-bacteria. Moreover, PGP-effects in plants may be inconsistent due to several environmental variables (temperature, moisture, nutrients, soil content, etc.) which lead to variable trade-offs (Berg and Koskella, 2018). To handle the complex quantities of combinations, methods that utilize insufficient data (e.g. supply of nutrients in the growth medium, existence of a combination of bacteria) have been utilized to forecast the plant

phenotype (Herrera Paredes et al., 2018). Utilizing this theory that improves the choice of PGP-consortia is probable even without a full understanding of the mode of action and the relationships between members of the bacteria consortia. Also, synthetic biology seems to be a promising approach that is used to design microbial consortium with mechanisms, interactions and pathways that are desired.

The top-down strategies enable the investigation of microbiome features at a molecular level and to choose PGP-consortium candidates centered on the data obtained. This was accomplished by the direct detection of nucleic acid core and satellite microbiota in environmental samples established on single amplicon variants in high throughput nucleic acid sequence (Callahan et al., 2016), as mentioned above. The benefits of top-down methods include the pre-selection of candidates subjected to a practical stress scenario under practical field conditions whereas bottom-up screening strategies simulate field conditions in a relatively simple setting.

Table 4.1: Examples of selected bacterial consortia in plant ecology

Plant and growth conditions	Consortia/origin of bacteria	Stress	Consortia effect	References
Solanum lycopersicum cv. Moneymaker, growth chamber	*Pseudomonas psychrotolerans* SOGA_13, *Pseudomonas rhizosphaerae* SOGA_14 and 19, *Bacillus megaterium* SOGA_2, *Curtobacterium ceanosedimentum* SOGA_3, *Curtobacterium* sp. SOGA_6, *Masilla aurea* SOGA_7, *Pseudomonas coleopterorum* SOGA_5, 11 and 12, *Frigoribacterium faeni* SOGA_17, *Xanthomonas campestris* SOGA_20/pyllosphere of field-grown tomato plants	*Pseudomonas syringae* pv. Tomato	Lesser pathogen DNA copies on leaf disks	(Berg and Koskella, 2018)
	Combinations of *Pseudomonas* sp. R32, R47, R76, R84, S04, S19, S34, S35, S49/rhizosphere and phyllosphere of field-grown potatoes	*Phytophthora infestans*	Decreased developments of fungal sporangiophore	(De Vrieze et al., 2018)
Arabidopsis thaliana, growth chamber, non-sterile soil	*Stenotrophomas* sp. WCS2014-113, *Xanthomonas* sp. WCS2014-23, *Microbacterium* sp. WCS2014-259/ field soil with prevalent *Arabidopsis* plants	*Hyaloperonospora arabidopsidis*	Decreased spores of fungi and improved plant fresh weight	(Berendsen et al., 2018)
Lycopersicon esculentum cv. Jiangshu, greenhouse pots with soil	*Pseudomonas* spp. CHA0, PF5, Q2-87, Q8R1-96, 1M1-96, MVP1-4, F113, PHI1C2/pea, wheat, cotton, tomato, sugar beet, tobacco	*Ralstonia solanacearum*	Reduced disease impact and pathogen abundance	(Hu et al., 2016)
Blue maize CAP15-1 TLAX/greenhouse pots with vermiculite	*Acinetobacter* sp. EMMO2, *Pseudomonas putida* KT2440, *Sphingomonas* sp. OF178, *Azospirillum brasilense* Sp7	Desiccation	Increased shoot and root dry weight, plant height and plant radius	(Molina-Romero et al., 2017)
Capsicum annuum, *Vitis vinifera* cv. Barbera, growth chamber, greenhouse	*Sphinogobacterium* sp. S6, *Enterobacter* sp. S7, *Acinetobacter* sp. S2 and *Bacillus* sp. S4 and *Delftia* sp.	Drought	Increased fresh root, photosynthesis and aerial biomass	(Rolli et al., 2015)

4.8.1 Requirements for formulations and methods of delivery

Formulations are necessary to guarantee cell viability over a long period during storage and to provide adequate viable cell numbers for field-grown plants. However, there are no suitable formulations for several microbes, specifically Gram-negative bacteria (Berninger et al., 2018), and their viability is also restricted to how the bacteria can withstand low moisture (Köhl et al., 2011). PGP-effects can be enhanced by the use of formulations from various compounds. For example, experiments adding rhizobic-isolated lipo-chitooligosaccharides (LOCs) to formulations (Marks et al., 2015) or changing an inoculant's growth medium to increase the content of polyhydroxybutyrate (PHB) and exopolysaccharides (EPS) in the formulation (Oliveira et al., 2017), have improved PGP effects. While surfactants modify the size of the droplet and rheological properties, lessen drift, and enhance adherence to hydrophobic cuticular surfaces (Preininger et al., 2018), the mechanisms of bacterial adhesion remain unclear. The humid environment and nanoparticles are gotten from PGPB encapsulated by macrobeads, which enhance the adherence of PGP-bacteria to roots (Perez et al., 2018). Some field studies have shown generally that leaf, seed, and soil inoculation methods of the same PGP-bacteria successfully improved yield (Berger et al., 2018). There might be interference between seed inoculants and pesticides used for seed treatment but the establishment of the plant must first occur and building up of defenses while in mature plants, establishment requires suppression of the existing microbiome (Bulgarelli et al., 2015). New methods have been developed to add to the classical delivery approaches. Mitter et al. (2017) developed a seed microbiome modulation in which a spray inoculation of the flower was used to obtain the following generation seeds

endophytically populated by the inoculant strain and alteration of the seed microbiome. Alternative strategies have been shown to lead to enhanced production of microbial inoculants through efficient colonization of the germinated plant by the inoculant stress.

4.9 Impact of agricultural management on plant microbiota

Distinct plant microbiomes are linked to specific plant traits such as biomass production (Sugiyama et al., 2013), disease suppression (Mendes et al., 2011), flowering phenotype (Panke-Buisse et al., 2015), and growth response (Bainard et al., 2013). As a result, the outcomes of agricultural management or modulation of plant microbiota will have an impact on plant characteristics and performance (Fig 4.1). This is an option besides the inoculation of single or microbial consortia. Sustainable agricultural production can be achieved through the use of intercropping, organic approaches, crop diversification, and other cultural practices. Although limited data are available on practices such as fertilization, protection of biodiversity, little or no-tillage, and other practices that influence plant microbiome, low input farming systems have been shown to increase the density and diversity of microorganisms (Postma-Blaauw et al., 2010). Knowing how cultural practices affect plant microbiota can help in creating strategies to alter plant microbiota in the preferred direction (Fig. 4.1). For example, Campisano et al. (2014) noted that organic pest management can result in the formation of various soil and plant microbiomes linked to grapevine.

Likewise, Longa et al. (2017) have shown that varied agricultural management practices in viticulture (organic or biodynamic) induce varied microbiota. Green manure treatment specifically leads to significant variations as opposed to organic and bio-dynamic management practices.

Vineyards with decades of integrated organic or biodynamic management practices were examined. Significant reductions in the richness of bacterial species were recorded in soil under integrated management in contrast to organic management but there was similarity in the composition of the community to organic and biodynamic managed soils (Hendgen et al., 2018). In a long-term field experiment, Hartmann et al. (2015) further showed the influence of different agricultural management on soil microbiome for more than 20 years. Organic farming in contrast to traditionally-managed soils appears to improve the soil microbial abundance of grass-clover and winter wheat, but also reduced uniformity, decreased dispersion, and changed the soil microbial community structure.

Researchers have found that while organic fertilizers affect microorganisms responsible for the breakdown of complex organic compounds, the impact of pesticides on soil microbiota is less (Hartmann et al., 2015). Also, Hartman et al. (Hartman et al., 2018) recently revealed definite crop production effects on the community make-up of winter wheat roots and the soil. Researchers have shown that soil bacterial composition is essentially structured by root bacteria management type and tillage practices while fungal communities respond primarily to the type of management and other tillage-related effects. Several practices affect the microbial composition, with variations in bacteria and fungi component of the soil and roots. Approximately 10% of differences in microbiota could be described by the harvesting practices assessed (Hartman et al., 2018). Our knowledge of the relationships between the complexities of the microbial ecosystem and the circumstances of work has progressed. Nevertheless, the effects of agricultural management and other environmental conditions are extremely complex, and more information is needed to make explicit recommendations.

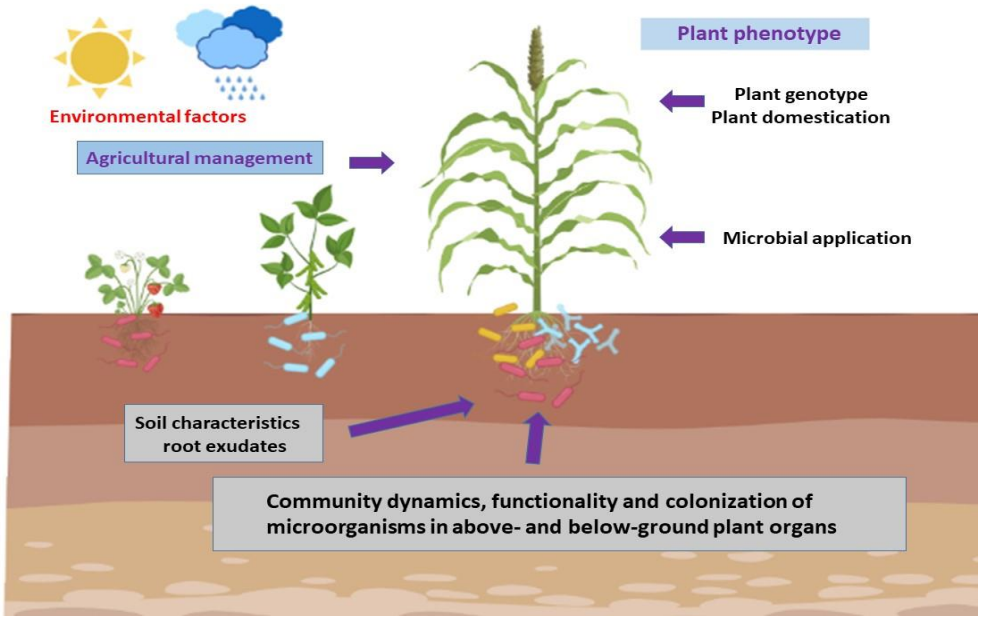


Figure 4.1 Plants selection for effective interaction with plant microbial community

4.10 Selection of plants for efficient interaction with plant microbiota

The selection of satisfactory plant microbiomes is yet to be included in crop breeding programs (Schlatter et al., 2017) (Fig. 4.1). Various plant genotypes interact individually with microbiota and excite a number of microbiome members by conferring tolerance and resistance to biotic and abiotic stress conditions or by supporting plant nutrition and growth (Jousset et al., 2017). Thus it is likely that plants capable of attracting useful microbiota will be designed or selected (Abhilash et al., 2012).

While there is insufficient knowledge as to how plant mechanisms and fundamental genes lead to the link with distinct microbiomes, or how each particular microbial activity is impacted, plant breeding programs have prioritized the selection of distinct and enhanced plants. In most cases, there was a decrease in genes of hybrid plants in contrast to wild-type plants (Gopal and Gupta, 2016).

Particular microbiota has emerged from wild plants, but this selection has been hindered by the selective breeding of significant crops (Gopal and Gupta, 2016). Selective breeding has led to a loss of genetic plant diversity, a loss of ability to relate to distinct microorganisms that promote plant growth, and a decrease in plant-linked microbial diversity (Pérez-Jaramillo et al., 2016). Considerations regarding linked microbiomes within the holobiont should be put in place as regards plant breeding to bestow added plant traits or to modify them. But this method is hindered by a lack of adequate knowledge of the functions of the microbiome, the techniques involved in plant-microbiome interactions and the lack of novel high-performance screening methods. Nonetheless, in the production of a new generation of microbe-enhanced plants, the breeding and selection of plants for their communication with beneficial micro-organisms is profoundly assuring.

CHAPTER FIVE

SECONDARY METABOLITES AS PLANT DEFENSIVE STRATEGY: A LARGE ROLE FOR SMALL MOLECULES IN THE NEAR ROOT REGION

Abstract

The roles of plant roots are not merely limited to the provision of mechanical support, nutrients and water, but also include more specific roles, such as the capacity to secrete diverse chemical substances. These metabolites are actively secreted in the near root and play specific and significant functions in plant defense and communication. In this review, we detail the various preventive roles of these powerful substances in the rhizosphere with a perspective as to how plants recruit microbes as a preventive measure against other pathogenic microbes, also, briefly about how the rhizosphere can repel insect pests, and how these chemical substances alter microbial dynamics and enhance symbiotic relationships. We also highlight the need for more research in this area to detail the mode of action and quantification of these compounds in the environment and their roles in some important biological processes in microorganisms and plants.

5.1 Introduction

The soil habitat is often seen as the most diverse ecological system on Earth (Amoo and Babalola, 2019), inhabited by bacteria, fungi, nematodes, arthropod herbivores and plants among many other biotas (Alawiye and Babalola, 2019). Organic matter is often limited in soils and this consequently results in increased competition and dynamics among the biota. To survive in the ecosystem, organisms produce variable signals to adapt and fit in rapidly changing ecological conditions. Plants interact with resident organisms by synthesizing chemical substances within the near root region. Also, plants use these metabolites to maintain soil health and other physical conditions, which allows them to withstand varied environmental stresses. Consequently, a view

of the near root region (rhizosphere) presents an important perspective on improving the biological control of phytopathogens.

The rhizosphere, often considered to be one of the most dynamic ecosystems on earth – is the soil region directly influenced by the interactions between plant roots and microorganisms. The composition and functional diversity of the rhizosphere is dependent on an array of biotic and abiotic factors (de Boer et al., 2019; Enebe and Babalola, 2018). These factors include plant species, soil types, root exudates, soil porosity, and anthropological activities (Fierer, 2017). An interplay exists between plants and soil (micro)organisms that are either advantageous or detrimental (Mommer et al., 2016). These complex interactions influence biogeochemical processes such as nutrient cycling and greenhouse gas emissions. In addition, these biological interactions dictate the types and amount of metabolites produced by the plant as these metabolites are produced in response to the activities within its immediate environment. The plant metabolites can shape the composition of the soil microbial community around the root region (Cotton et al., 2019). These secretions are termed exudates (primary metabolites and secondary metabolites). These exudates are composed of various low-molecular-weight molecules with characteristic chemical properties that form concentration gradients in the root region (Hu et al., 2018).

Metabolites synthesized by plants play crucial roles in a myriad of activities, from both good to bad. The plant rhizosphere has direct consequences on its ability to fight disease-causing organisms and make use of chemical substances produced in the root (Olanrewaju et al., 2019). Root exudates, comprised of allelochemicals, have been linked with signaling in the plant-microbe interplay and promote communication in the rhizosphere (el Zahar Haichar et al., 2014). Exudates with potential allelopathic characteristics can help plant phytobiome selection both

favorably and adversely (Sasse et al., 2018) by allowing plants to select for a rhizospheric community that could be advantageous or harmful to other microorganisms, a common biocontrol strategy often utilized by plants against pathogens (Ajilogba and Babalola, 2016). The composition of secondary metabolites can change over time and are mostly impacted by various cues from the rhizosphere (De Coninck et al., 2015).

Recent studies have increased our understanding of the interplay between plant roots, resident microbes and their secondary metabolites. However, the analysis of these compounds is a major challenge and could explain the somewhat limited number of investigations and reports on specific metabolites in the soil matrix. Moreover, the understanding is limited concerning the biocontrol functions of these compounds in the near root. In this review, we discuss secondary metabolites in the near root region with respect to how they protect plants and their responses in the rhizosphere, with focus on compounds for which mechanistic information is available.

5.2 Near root microbial environment and root exudates

Microorganisms are one of the very important biotic factors that determine growth and development of plants either for good or bad. Hence, they could be viewed as plant growth-promoting rhizobacteria and pathogens. Investigations have increased knowledge of our understanding of rhizosphere ecology. The microbial community in this region is impacted by root exudates which serve as vital carbon and energy sources (Yarzábal and Chica, 2019). Consequently, root exudates alter the near root plant-microbe interactions. Flavonoid compounds from exudates of legumes were reported by Phillips and Tsai (1992) to improve the growth rate of bacteria, migration towards specific plants and the induction of plant nodules. It is suggested that the key reason for plant-microbe interactions is for adaptability, survival and multiplication (Zilber-Rosenberg, 2013). Root exudates render nutrients to microbes and in return, the

community breaks down and solubilizes complex organic compounds, thus improving the soil organic content (Canarini et al., 2019). Meanwhile, the influence of exudates on the rhizosphere microbial community is well known, but there is still a paucity of information on the precise and chronic effects of these exudates on microbial establishment in the near root, the understanding of which could serve as the base for the development of specific biocontrol methods in the near root.

5.3 Classifications of rhizosphere metabolites

The rhizosphere is commonly viewed as the near root region of about 0-2mm in radius. It is the region of the root with notable chemical signaling transfer and metabolite secretion by both plants and interacting microorganisms. In this region, metabolites (exudates) are released by plants and in response, microbes release varied classes of compounds to interact mutually or pathogenically. Exudates secreted are usually classified based on their molecular weights (Huang et al., 2014). Root exudate composition is determined by varying factors which include pH, plant developmental stages, soil texture, plant species and other biotic factors (de Boer et al., 2019). Root exudates can also be grouped on their biochemical nature, such as proteins, carbohydrates, peptides and glycopeptides, although some of these, such as carbohydrates and proteins, are not found in most root exudates due to their quick degradation and uptake by microbial community in the near root (van Dam and Bouwmeester, 2016). Several findings related to carbohydrates and derivatives are available as influencers of mutualistic associations between legumes and fungi (Fang and Leger, 2010; Kiers et al., 2011). Amino acids also act as chemical attractants for plant growth-promoting rhizobacteria to the roots (Huang et al., 2014). Arabinogalactan proteins (AGPs) belonging to glycoprotein have been implicated to play a significant role in the

modification of the near root biotic environment by positively influencing beneficial microbes and repelling pathogens.

Though most secondary metabolites are genus and species-specific (Uarrota et al., 2011), major classes of secondary metabolites are terpenes (originating from acetyl-CoA and glycolytic intermediates), phenolics (with characteristic defensive properties, e.g. coumarin, lignin, etc.), sulfur-containing metabolites (e.g. phytoalexins, thionins, defensins etc.), and nitrogen-containing metabolites (alkaloids, cyanogenic glucosides etc.) (Pagare et al., 2015). Secondary metabolites play roles in defensive mechanisms, hormonal and signaling events, and regulatory pathways. Due to the noticeable disparity and variety of these secondary metabolites, their functions are prized by humans as fragrances, drugs, inhibitors, molecular tools for profiling transcripts and metabolites, stimulants, hallucinogens, poisons, enzymes, dyes, colorants, insecticides, etc. (Pagare et al., 2015). Their usefulness in plant survival and interactions are exploited by biotechnology for the production of specific and valuable biological products for mankind. Usually, allelopathic substances are found among varied chemical classes which include benzene derivatives, hydroxamic acids, phenolics and terpenes (Fig 5.1).

5.4 Root-derived specialized secondary metabolites in plant interactions

Metabolites play central roles in the interactions between and among plants and their microbiomes, a phenomenon commonly known as allelopathy. This can be either advantageous or detrimental but is commonly viewed as competition between plants and resident microbes (Sturz and Christie, 2003). Metabolites produced during this process can either be active or passive at both interspecific and intraspecific levels (Huang et al., 2014). Allelochemicals can also be found in tissues of plants such as bark, flowers, leaves and even fruits. However, the most prominent route for substances is via root exudations into soil (Canarini et al., 2019).

Plants synthesize metabolites through intricate biosynthetic and signaling pathways. These can be categorized as primary vs. secondary metabolites. Primary metabolites are deemed essential for functional metabolic pathways within the plant and are required by the plant at all times and under different environmental conditions. These metabolites include monomers and polymers of nucleic acids, sugars, amino acids and lipid derivatives (Pagare et al., 2015). Secondary metabolites on the other hand do not play direct roles in the functioning of the plant but rather influence its interactions with other members of the ecosystem – they determine the plant's fitness for the involved niche (Pagare et al., 2015). The variety of secondary metabolites within a particular plant species determines the complexity of its interactions with the biotic and abiotic components of its environment (Enebe and Babalola, 2018). Likewise, the abiotic conditions of the soil could impact the composition and functions of metabolites in the near root (de Boer et al., 2019). For instance pH, temperature, water activity and even texture have been reported to have great impacts by several researchers (Kramshøj et al., 2019; Raza et al., 2015; Wang et al., 2015).

5.4.1 Carotenoids

Carotenoids are yellow, orange, or red lipophilic organic pigments primarily composed of carotene and xanthophyll groups. They are abundant naturally occurring pigments on earth, second only to Chlorophyll a. They are mostly C_{40} terpenoids with photosynthetic, photomorphogenetic, photoprotective, and developmental functions (Nisar et al., 2015). Carotenoids are produced by pathways hinged on two isoprene isomers – Isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP). Production via the carotenoid pathway is regulated by phytoene synthase (PSY) – a rate-limiting enzyme in the biosynthesis of carotenoid (Nisar et al., 2015). Maize PSY3 isoform specifically expresses itself in the root

(Ruiz-Sola et al., 2014), and assists in a resultant carotenoid flux during abiotic stress conditions, for example, drought and saline stress (Nisar et al., 2015; Ruiz-Sola et al., 2014). This accumulation of carotenoids within the root aids the synthesis of abscisic acid (ABA) (Nisar et al., 2015) – a plant hormone that plays a role in adaptation to stress conditions – which is synthesized from the cleavage of carotenoid precursors e.g. xanthophylls by specific dioxygenases (Ruiz-Sola et al., 2014). Carotenoids have antioxidant properties and their synthesis by maize plants have assisted in meeting Vitamin A demands in human populations. Beta-carotene, beta-cryptoxanthin, alpha-carotene lutein and zeaxanthin are common examples of carotenoids produced by maize (Uarrota et al., 2011).

5.4.2 Flavonoids

These nutraceuticals consist of phenolic, antioxidant, and flavone derivatives which act as pigments, colorants, and preservatives. These secondary metabolites – derivatives of 2-phenyl-benzyl-gamma-pyrone (Mierziak et al., 2014) – are common in maize grain pericarps and germs (Atanasova-Penichon et al., 2016) and play beneficial roles such as in stress tolerance (D'Amelia et al., 2018a) and pollen germination (Falcone Ferreyra et al., 2012) Some plants have been identified to synthesize sesquiterpenes commonly known as strigolactone (Fig 4.1) as below-ground signals which prompt symbiotic relationships between the roots of plants and arbuscular mycorrhizal (AM) fungi of the Glomeromycota. Furthermore, these metabolites have been observed to influence plant-plant interactions, produce effects within mycorrhizal associations and regulate plant-microbe symbiotic interactions (Mierziak et al., 2014). Exudates are capable of inhibiting seed germination of surrounding plants, increasing the secretion of Reactive oxygen species (ROS) in the root systems of other plants (causing death), initiating symbiosis with microbial species within the rhizosphere, influencing hypersensitivities, modulating auxin

activity, etc. (Mierziak et al., 2014). Additionally, flavonoids have been observed to aid resistance to metal toxicity (e.g. aluminum) in maize plants (Falcone Ferreyra et al., 2012). Environmental stress to beneficial plants such as maize has an indirect negative impact on human societies. During biotic (e.g. herbivore and pathogen attack) and abiotic (e.g. drought and UV radiation) stress, maize plants produce free radicals such as Reactive Oxygen Species (ROS) which accumulate and educe debilitating effects on plant development (D'Amelia et al., 2018a). ROS adversely affect cell membrane components such as lipids and carbohydrates; DNA; and cellular proteins. Flavonoids assist antioxidant enzymes such as glutathione reductase, superoxide dismutase, catalase, etc. to scavenge these ROS and maintain them at a level that is not toxic to plant health (D'Amelia et al., 2018a).

Phenolics have been identified to be a key chemoattractant for plant growth-promoting rhizobacteria and defend against disease-causing organisms. Flavonoids have been seen in leguminous rhizobium as a regulatory factor for nodulation and initiation of symbiosis (Abdel-Lateif et al., 2012). Antioxidant properties of flavonoids have been linked to neuroprotective, vascular protective and anti-inflammatory activities as well as ameliorating cases of cancers in humans. Flavonoids from potatoes and maize are useful in alleviating breast and prostate cancers, respectively (D'Amelia et al., 2018a).

The activity of flavonoids against human diseases is attributed to their antioxidant properties – as they are strong scavengers of reactive oxygen and nitrogen species in the human body (D'Amelia et al., 2018b). With positive effects recorded in the health sector, flavonoids are of importance in the food industry and biotechnology. They increase shelf-life due to their antioxidant and antimicrobial properties by regulating lipid auto-oxidation thus preventing spoilage by food-borne pathogens (D'Amelia et al., 2018b). In comparison with other cereals such as rice, wheat

and oat, maize grains have been observed to contain the highest concentrations of these phenolic acids (Atanasova-Penichon et al., 2016). Common flavonoids in maize grains include flavonols – kaempferol and quercetin, flavones – luteolin and apigenin, and flavone glycosides – maysin, iso-orentin, and iso-vitexin (Atanasova-Penichon et al., 2016). The biosynthesis of flavonoids in plant tissues relies on the activity of enzymes in the phenylpropanoid pathway (Falcone Ferreyra et al., 2012). Chalcone synthase (CHS) mediates the condensation of p-coumaroyl-CoA with malonyl-CoA (in a ratio of 1:3) to produce chalcone which is subsequently isomerized to flavanone by chalcone isomerase (CHI) (Mierziak et al., 2014). Being the first participant of the flavonoid synthesis pathway, CHS directs the synthesis of flavones, flavonols, and anthocyanins (D'Amelia et al., 2018b) as the flavanone produced by CHI is utilized for the production of various flavonoid products such as anthocyanins, flavonols, aurones, flavanonols, isoflavones, flavones, etc. (Mierziak et al., 2014).

5.4.3 Terpenoids

Terpenoids (isoprenoids) include diterpenoids (kauralexin and dolabralexin groups) and sesquiterpenoinds (zealexins) (Block et al., 2019; Mafu et al., 2018). Directly implicated in plant growth and development, these molecules are essential for the phyto-physiology of maize plants. Diterpenoids are necessary for defense roles, pollination, allelopathy, repair responses to tissue damage, resistance to pests and infectious agents such as European corn borers (*Ostrunia nubilalis*), *Fusarium* spp., *Aspergillus* spp., *Rhizopus microspores, Cochliobolus heterostrophus,* and *Collectotrichum* spp. (Block et al., 2019; Zerbe, 2015). Maize terpenoids are antimicrobial, defend the plant against insects and in some intense cases of microbial or insect infestation, act as attractants for parasitoids and predatory insects (Block et al., 2019). Diterpenoids – zealexins and kauralexins – were initially thought to be restricted to defensive roles in the aerial parts of

maize; later studies show that defense pathways mediated by diterpenoids extend to belowground tissues, such as within and around root tissues (Mafu et al., 2018; Zerbe, 2015). Besides biotic defense, diterpenoids also play a part in the response of plants to environmental stress such as high salinity and drought just like carotenoids (Zerbe, 2015). Though kauralexins exude a negative effect on insects (biotic stress), they are also observed to accumulate in response to abiotic stress (Mafu *et al*., 2018).

5.4.4 Benzoxazinoids (1,4-benzoxazin-3(4H)-one derivatives, BXs) –

These substances were first reported in an experiment that demonstrated reduced growth of weed biomass in a rye (*Secale cereale* L.) field when matched with plots without rye (Barnes and Putnam, 1983). Consequently, DIBOA [4-dihydroxy-2H-1,4-benzoxazin-3(4H)-one] and its methoxylated analogue DIMBOA [2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)-one] were identified as the causative active substances (Macías et al., 2008). BXs are secondary metabolites classified as lactams, hydroxamic acids, and benzozazolinones (Mikic and Ahmad, 2018). Located in maize grains with higher concentrations in bran and germ (Atanasova-Penichon et al., 2016), they play a crucial role against insect herbivores for the survival of maize plants. The toxic nature of benzoxazinoid derivatives such as 2,4-dihydroxy-1,4-benzoxazin-3-one (DIBOA), 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), 2-hydroxy-1,4-benzoxazin-3-one (HBOA), 2-hydroxy-7-methoxy-1,4-benzoxazin-3-one (HMBOA), 2-hydroxy-4,7-dimethoxy-1,4-benzoxazin-3-one (HDMBOA), 6-methoxybenzoxazolin-2-one (MBOA), and 2-benzoxazolin-2(3H)-one. (BOA) are quite similar to DIBOA and its methyl derivative DIMBOA being the most extensively studied (Mikić and Ahmad, 2018). The BXs are defensive and allelopathic phytochemicals with antibacterial, antifungal, insecticidal, antifeedant and phytotoxic functions (Mikić and Ahmad, 2018); as major components of innate immunity and

disease resistance (Liu et al., 2019). Their modes of action and toxicity vary – BOA and MBOA are breakdown products of and are less toxic than DIBOA and DIMBOA, the phytotoxicity of HBOA and HMBOA are considerably lesser than DIBOA and DIMBOA etc. (Mikić and Ahmad, 2018). DIBOA and DIMBOA are very toxic indeed and hence stored in their inactive forms within cell vacuoles. Physical and chemical trauma by pathogens and pests results in the release of these BXs from the vacuoles which cause their activation by enzymes in the plastids (Mikić and Ahmad, 2018). Certain plants e.g. maize, wheat and rye, utilize these secondary metabolites as induced defenses against herbivore infestation (Qi et al., 2018). The production of BXs is triggered by elicitor molecules resulting from impulses from external events such as a pathogen or pest attack (Dafoe et al., 2013). Dolman (2014) reported that host defenses in maize were unregulated concomitant from the infection of *Sporisorium relianum* – which causes Head smut. The biosynthesis of Benzoxazinoids (BXs) via the BX biosynthesis pathway employs enzymes such as BX1 (acts on indole-3-glycerol phosphate to yield indole), cytochrome P450-dependent monooxygenases, UDP-glucosyltransferases, 2-oxoglutarate-dependent dioxygenases and O-methyltransferases (Qi et al., 2018). Stored as glucosides in the vacuoles of whole maize cells, these BXs are converted to unstable aglucones via hydrolytic leaf damage by herbivores (Qi et al., 2018). In maize plants, BXs occur in all plant tissues, with varying amounts in specific tissues – maize leaves and stems have a varying amount of BXs with response to growth and development while maize roots show a constant composition irrespective of plant growth and development. The overall BX concentrations also vary. Some BXs are more abundant in young maize plants while others are characteristically more associated with older maize plants (Mikić and Ahmad, 2018). BXs have been observed to defend the maize plant from insect herbivores such as European corn borer (*O. nubilalis*) and Asian corn borer (*O. furnacalis*) (Qi et al., 2018).

Its toxicity is due to its role in NADH oxidation of cell wall peroxidases; hydrogen peroxide accretion; lignification of the cell wall, inhibition of trypsin, cholinesterase, chymotrypsin proteases; decreased production of chlorophyll; alteration of lipid metabolism; disruption of biochemical activities during oxidative phosphorylation; and growth inhibition (Makowska-Grzyska et al., 2015). Microorganisms in the rhizosphere play a major role in the activation of BXs. Rhizosphere microbes breakdown BXs to yield more, antibacterial, antifungal and allelopathic products (Neal et al., 2012).

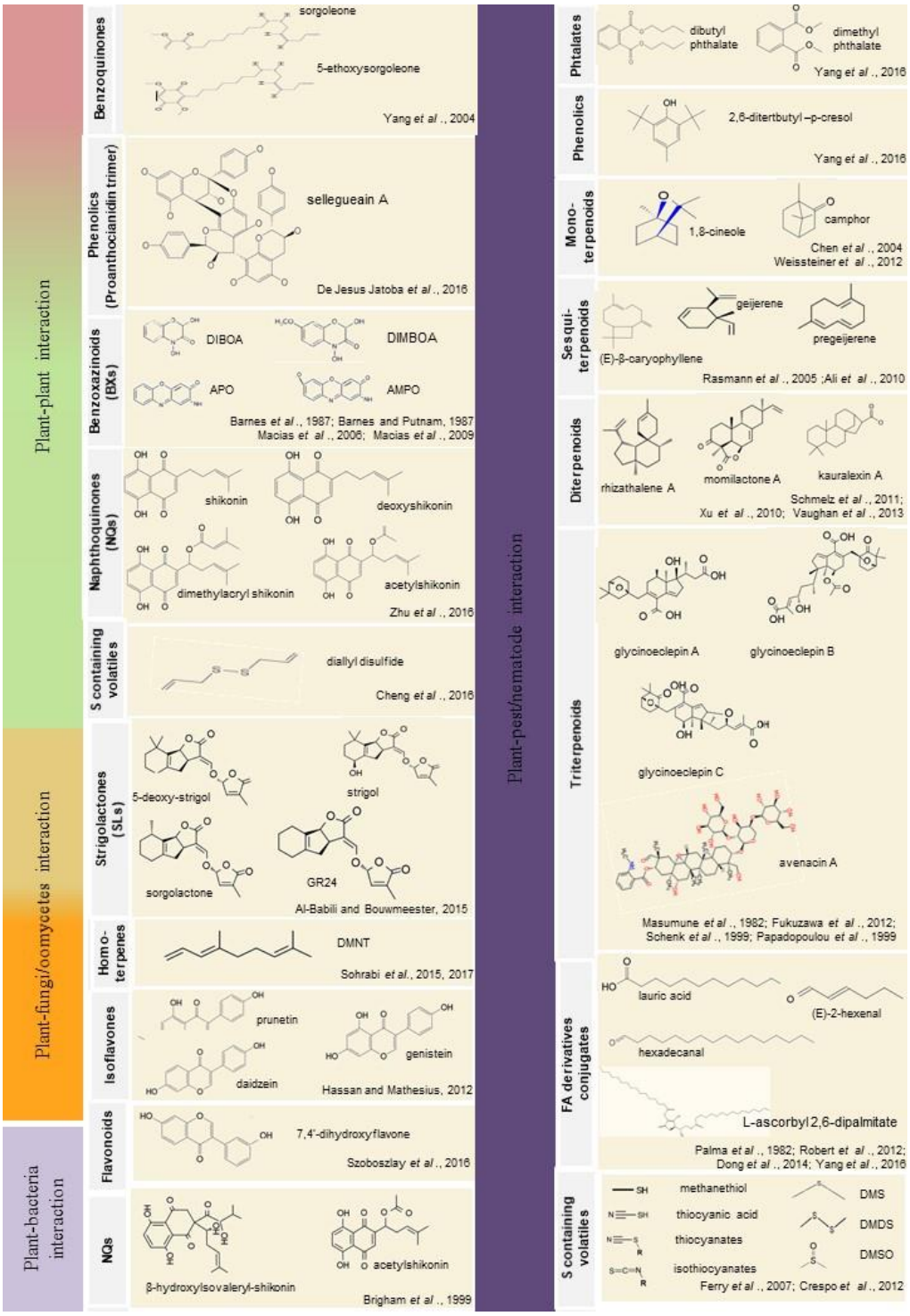


Figure 5.1: Specialized metabolite classes and representative compounds with functions in root-organism interactions. Strigolactones (SLs), fatty acids (FA), DIMBOA [2,4-dihydroxy-7-methoxy-2H-1,4-benzoxazin-3(4H)- one], APO [2-amino-3H-phenoxazin-3-one], AMPO [2-

amino-7-methoxy-3H-phenoxazin-3-one], Benzoxazinoids (BXs), naphthoquinones (NQs),DMNT [(E)-4,8- dimethyl-1,3,7-nonatriene], dimethyl sulfide (DMS), dimethyl disulfide (DMDS), dimethyl sulfoxide (DMSO) DIBOA [4-dihydroxy- 2H-1,4-benzoxazin-3(4H)-one],. Strigolactones are obtainable in both interactions between plant–plant and plant–fungi.(Barnes and Putnam, 1983; de Jesus Jatoba et al., 2016)

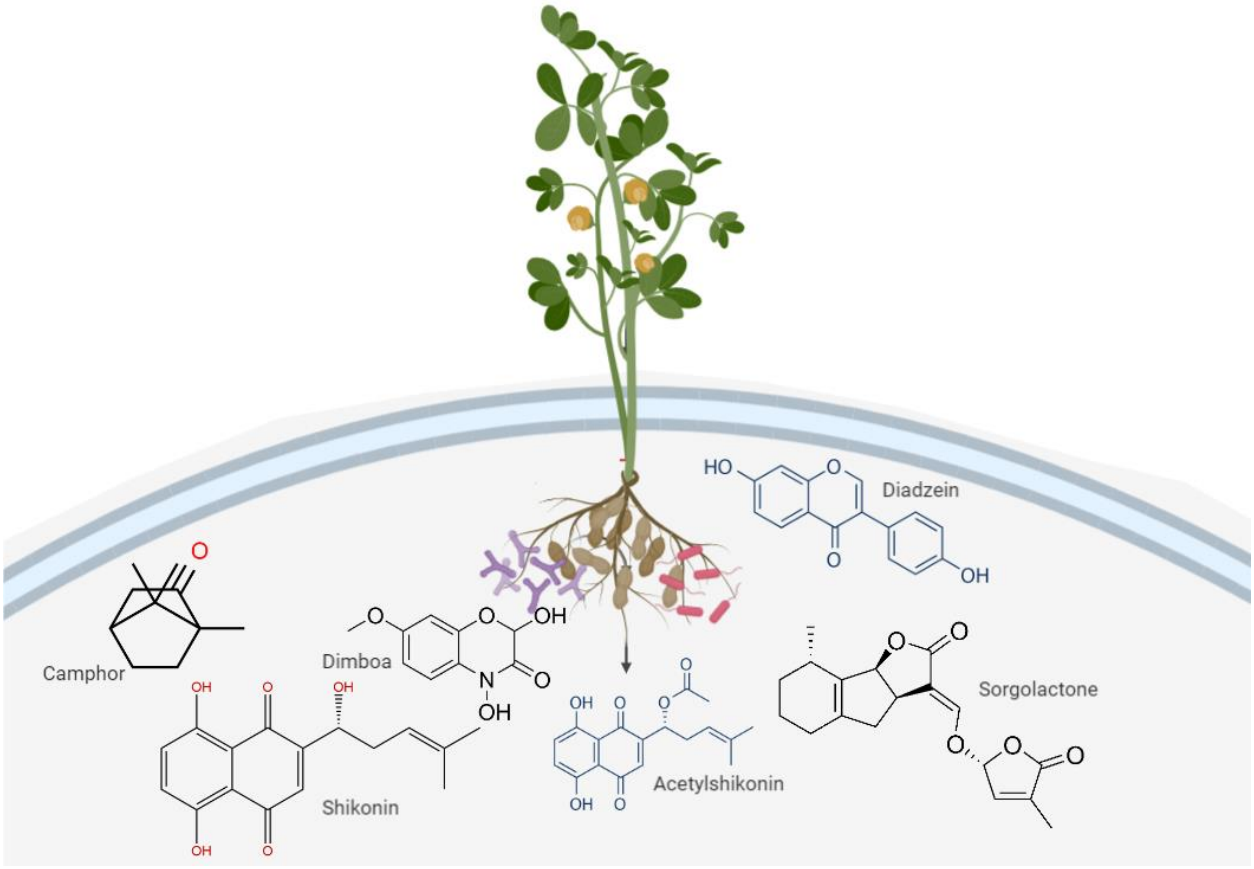


Figure 5.2 Secondary metabolites in plant interactions with pathogenic fungi

Due to adaptation and coevolution, plant and soil-borne fungi exhibit diverse relationships, from symbiotic, parasitic and pathogenic relationships. Many plants over the years have sustained beneficial relationships with arbuscular mycorrhizal fungi (AM). However, there is more to just

chemical communications in their relationship. Strigolactones are often considered as the most common metabolite in plant- fungi relationships. Akiyama et al. (2005) showed that the sesquiterpenes, 5-deoxy-strigol, sorgolactone and strigol retrieved from the exudates of *Lotus japonicas* caused an elongated hyphal branching in AM fungus. A comparable result was also seen in sorgolactone synthetic homologue (Liu et al., 2011). These plants, however, change responses to pathogens via the activation of integrates stress response. Asides from the mutual relationship that exists between plant and AM fungi, plant roots are often the target of soil fungal pathogens. Before colonization, hyphae of disease-causing fungi can detect chemical communication which signals their movement towards the plant host. For instance, *Fusarium oxysporum*, a plant pathogen growing towards tomato (*Solanum lycopersicum*) root, was identified to have been catalyzed by the actions of class III peroxidases (Pandey et al., 2017). Although the substrate of these enzymes was not fully identified, it was suggested that it was synthesized from the root. On the contrary, *F. oxysporum* chemotrophic sensing was identified in the experiment to be controlled by a unique for sex pheromone receptor-like protein, which was suggestive that the signal sensed by the fungi was from proteins also (Turra et al., 2015).

Quite a number of secondary metabolites have antifungal attributes (Ke et al., 2020; Kujur et al., 2020; Palla et al., 2018). Phenolics and flavonoids are both viewed to be a part of an extensive group of phytochemicals with high concentrations in fruits, skin, leaves and roots. These chemicals have shown some activity in plant defense against pigmentation and resistance to diseases (Table 5.1) (Zaynab et al., 2018). Phenolics impact cell permeability of microorganisms and deform the function and structures of the protein-membrane, consequently leading to the distraction of pH gradient, energy production, membrane-bounded enzymes and the use of substrates for energy generation (López-Gámez et al., 2020). Exudates with antimicrobial

properties such as saponins, α-tomatine (tomato saponin) stimulate monomeric G-protein pathways and phosphotyrosine kinase that fixes to cell membranes, such that it results in the rupture and release of the cell component (Khan et al., 2020).

Table 5.1: Examples of phenolic compound with anti-fungal activity.

Chemical	Fungus	Reference
Benzaldehyde	*Botrytis cinerea*	(Caruso et al., 2011)
Protocatechuic acid	*Colletotrichum circinans*	(Zaynab et al., 2018)
Salicylic acid	*Eutypa lata*	(Amborabé et al., 2002)
Vanillic acid	*Phytophthora infestans*	(de Vries et al., 2018)
Chlorogenic acid	*Fusarium oxysporum*	(Ling et al., 2013)
Naringin	*Penicillium digitatum*	(Ortuño et al., 2011)
Flavones	*Aspergillus*	(Wu et al., 2014)
Oleuropein	*Phytophthora*	(Báidez et al., 2006)
Nobiletin	*Phoma tracheiphila*	(Aboody and Mickymaray, 2020; de Lamo and Takken, 2020)

5.5 Secondary metabolites in plant interactions with bacteria

The rhizosphere serves as habitat for numerous bacterial taxa (Fu et al., 2017), and these community members have direct influence on the health of plants and their ability to fight phytopathogens. Secondary metabolite profiles and expression of genes that link with microbial colonization have been depicted to initiate significant alterations in the bacteria community immediately there is biological stress (Mendes et al., 2014; Reinhold-Hurek et al., 2015). The community structure of bacteria in the rhizosphere is controlled partially by specific traits or

factors exhibited by the host plant as well as soil dependent processes. Various root-derived metabolites are in some cases released to recruit specific bacterial taxa as defense during stress conditions. Moreover, the investigation on *Pseudomonas syringae*-infected Arabidopsis root exudates showed the defensive attributes of selected antibacterial compounds exuded by the root against select bacteria taxa. Specific metabolites were recovered from plants infected with pathogenic strains of bacteria and more metabolites were recovered from non-pathogenic infected plants (Pascale et al., 2020).

Studies on different plant species exudates have reported various classes of flavonoids e.g flavonol (quercetin), flavanones (liquiritigenin), isoflavones (daidzein) (Cesco et al., 2010). An experiment by Szoboszlay et al. (2016) showed that the application of flavonoid (7,40 - dihydroxyflavone (Figure 5.1)) recovered from a stressed *Medicago sativa* to a neutral soil caused the recruitment and increase in the relative abundance of *Acidobacteria, Gaiella, Nocardioidaceae*, and *Thermomonosporaceae* taxa, that are recognized to associate with plant roots. These findings pinpoint the significance of such exudates in the surroundings of near root regions especially during responses against pathogenic organisms. However, the precise mechanism of action was unknown.

Of the compounds with allopathy effects, naphthoquinones are important as they also show great influence on the soil microbial community (Smith, 2013). Acetyl-shikonin and β - hydroxyisovaleryl-shikonin (Figure 5.1) were prominent active substances identified in the hair roots of *Lithospermum erythrorhizon* (Tatsumi et al., 2016). These substances have also been implicated as chemoattractants for the beneficial bacterium *Pseudomonas putida* in plant roots. It is commonly suggested that plant exudates attract bacterial species that have the metabolic capacity to break down the released compounds. Metabolites can be used as carbon sources for

the catabolism of the monoterpenoid α -pinene by *P. fluorescens* and *Alcaligenes xylosoxidans* (Kleinheinz et al., 1999). Metabolites secreted in plant roots can chemoselect organisms that support or hinder the growth of bacterial pathogens, although how the relationship stimulates the release of the exudates via the promotion of immune responses remains to be known.

5.6 Secondary metabolites in plant interactions with viruses

Many plant secondary metabolic compounds such as alkaloids, phenolics and flavonoids have antiviral attributes. Commonly, alkaloids, due to their varied structures, exhibit biologically active substances that could impact the existence of resident organisms in the soil (Molinski, 1993). Several plants with antiviral properties have been recognized (Ahmad et al., 2015; Choudhary et al., 2020; Hassan et al., 2016). For instance, an antiviral alkaloid compound, 7-deoxytrans-dihydronarciclasine, from plantain lilies (*Hosta plantaginea*) has activity against Tobacco Mosaic Virus (Wang et al., 2007). Likewise, Bruceine-D from *Brassica javanica* inhibits the growth of potato virus and tobacco mosaic virus (Islam et al., 2018). Furthermore, quassinoids have been reported as active metabolites against TMV infections (Yan et al., 2010). Chen et al. (2009) also investigated the TMV traits of *Picarma quassioides* and identified the presence of β-carboline alkaloids

5.7 Secondary metabolites in plant interactions with insect pests

In the roots of plants, several endogenous secondary metabolites trigger behavioral changes. Townsend and Ebizuka (2010) showed that grass grubs could be hindered by isoflavonoids and other phytoalexins from legumes. The development of *Heteronychus arator* (African black beetle) larvae was also hindered by flavonoids, phaseolin, vestitol, genistein and medicarpin (Townsend and Ebizuka, 2010). Emitted metabolites from the roots contribute immensely to plants' defense systems against herbivores. For instance, report showed that *Arabidopsis* secreted

semi-volatile diterpenes against *Bradysia larvae*, an opportunistic root-pest (Vaughan et al., 2011). Myrosinases also hydrolyze precursors of glucoside to release substances that reshuffle into thiocyanates, volatile isothiocyanates and nitriles, these breakdown products have preventive effects against insect pests. A more comprehensive review of the activities of secondary metabolites in root- insect pest association has already been presented by Erb and Lu (2013). Remarkably, quite a limited number of reports of the negative impacts have been demonstrated in-situ. See Example in Table 5.2 for selected herbivorous plants.

Table 5.2: List of plant secondary metabolites against insects and their link to a specific category

Secondary	Metabolites Plants	Categories	Resistance against	Reference
Cyanogenic Glucosides	Plunatus	CNglcs	*Spodoptera eridania*	(Zagrobelny et al., 2008)
Terpenoids	Tobbaco	Transanethole and thymol, citronellal	*Spodoptera litura*	(Pavela, 2014)
Phenolics	Strawberry	Phenolics	*Tetranychus urticae*	(Afifi et al., 2010)
Cyanogenic Glucosides	Bitter almond plants	Amygdalin and prunasin	*Capnodis tenebronis*	(Sánchez-Pérez et al., 2008)
Phenolics	Willow plant	Phenolics	*Galerucella lineola*	Larsson *et al*, (1986)
Phenolics	Wheat	Phenolics	*Rhopalosiphum padi*	(Havlíčková et al., 1996)
Benzoic acid	Salix	Benzoic acid	*Operophtera brumata*	(Zaynab et al., 2018)
Cyanogenic Glucosides	*Trifolium repens*	Amygdalin and prunasin	*Hypera postica*	(Fürstenberg-Hägg et al., 2013)

Phenolics	Cotton	Gossypol	*Heliothis virescens, Heliothis zea*	(Jenkins et al., 1983)
Terpenoids	Citrus	Terpenoid Limonene	*Atta cephalotes*	(Bennett and Wallsgrove, 1994)
Alkaloids	Nightshade potato	Alkaloid demissine	*Leptinotarsa decemlineata*	(Sablon et al., 2013)
Benzoxazinoides	Gramineae	DIMBOA	*Ostrinia nubilalis*	(Friebe et al., 2012)

5.7 Future perspective

In this review, we have extrapolated the ability of plants to synthesize varied chemical compounds in their rhizosphere with the capacity to suppress disease-causing organisms and with recognized potential for their application in biocontrol strategies. There is however a gap in knowledge regarding our understanding of secondary metabolites and their specific functions in the near root region, more precisely the ones relating to allelopathic interactions, which includes a comprehensive understanding of the metabolite exudation processes and the identification of transporter proteins that are specific for exudation and transport mechanisms. Studies on the regulation of transporters by abiotic and biotic near-root signals will be of significance to better understand release rates and flux over time. Moreover, measurements of the exact concentrations of these chemical substances are still very much in need. This can be achieved by solid-phase root zone micro-extraction in selected rhizosphere points to quantify the spatial and temporal changes in exudation (Weidenhamer and Callaway, 2010). With such strategy, we might be able to estimate the total amount of exudate breakdown and movement in the soil. Additionally, mutants and transgenic plants with altered exudation should be encouraged in the study of effects of enhanced vs. suppressed secondary metabolite exudation on the rhizosphere. Characterization and quantification of metabolites by mass spectrometry can be used to monitor mutants with modified root exudation. Up to date tools are also required for such assessments. Metabolomics and proteomics investigations will assuredly improve our understanding of these metabolites. Furthermore, enormous gaps in knowledge still exist with respect to how some of these secondary metabolites act as allelopathic compounds. It will be important to know the molecular targets of these metabolites in plant species that could be hindered, consequently identifying the mode by which the activities of these beneficial metabolites can be preserved. It is yet to be determined if the exudates released are first transformed in the rhizosphere by some microbes before they become bioactive as allelochemicals.

CHAPTER SIX

BACTERIAL COMMUNITY PROFILING OF MAIZE PLANT RHIZOSPHERE AT DIFFERENT GROWTH STAGES IN SOUTH AFRICAN FARMLAND AS ASSESSED BY OXFORD NANOPORE SEQUENCING (MinION)

Abstract

The rhizosphere is the frontier between plant and soil, often characterized by varied attributes and conditions. These complexities of the rhizosphere as a consequence impact soil bacterial distribution, which in turn; affects plant health and growth. In this study, the influence of soil physicochemical properties, bacterial composition and the dynamics of maize plant rhizosphere at different growth stages under field conditions were examined utilizing the Oxford Nanopore Technology (MinION). Physiochemical characteristics of collected soil samples were determined using standard techniques. Raw DNA samples from bulk and rhizosphere soil samples of growing maize plants were collected at 35 (flowering) and 75 (senescence) days and probed for rRNA operon using the Minion. Physicochemical properties significantly influenced rhizobacteria composition and structure with key elements such as pH, NH_4, P and K as drivers more during the early stages of growth. Using the MinION, profiling of rRNA operons yielded 55,706 2-D sequences which were screened against 16S rRNA gene database (NCBI). The rhizospheric effect was more pronounced at the early stages as opposed to samples collected from mature maize plants. Significant differences in the bacterial communities of the two stages were observed. Members of *Proteobacteria* and *Firmicutes* were prevalent (> 40%). Dominant rhizosphere genera (*Chlorasidobacterium, Candidatus, Flavisoli bacter Gaiella, Bacillus, Pseudomonas, Flavobacterium,* etc.) displayed different patterns of temporal changes in the rhizosphere as opposed to the bulk soil. Moreover, unique genera in especial Plant-Growth Promoting Rhizobacteria (PGPR) such as *Bacillus, Pseudomonas, Psychrobacter, Nonomuraea, Thiobacillus* and *Bradyrhizobium,* etc., which were advantageous to plant, growth were viewed to be more abundant in the rhizosphere than bulk soil, displaying the importance of plants' ability to select the rhizobacteria community in variations between rhizobacteria and bulk soils. Also, we observed that

notable growth-related dynamic shifts in bacterial community structure were largely linked with phyla *Bacteroidetes, Proteobacteria* and *Actinobacteria* (mainly genera *Burkholderia*, *Flavisolibacter* and *Pseudomonas*), showing that the different growth stages influenced the bacterial community dynamics in maize rhizosphere.

6.1 Introduction

In South Africa and other parts of the world, maize is an economically important crop, possessing unique phenotypic and molecular diversity (Peiffer et al., 2013), and these diversities are considerably impacted by changes within the environment (Gore et al., 2009). Maize is extensively planted in monoculture systems, and can thus greatly impact both the biota and the physical properties of the soil ecosystem. Like most plants, organic compounds exuded from the near root region such as sugars, organic acids, aromatics, and enzymes promotes increased microbial biomass and metabolic activities around the root zones (Chauhan et al., 2011).

The interface between plant roots and the surrounding bulk soil is the rhizosphere, this region are considered to be adjascent to and influenced by plant roots for the continued sustainance of the plant, and are also viewed to be the most active and complex part of the dynamic soil environment (Odelade and Babalola, 2019). The extent of the rhizosphere depends on the characteristics of the plant and the morphology of the root system, and its properties change radially and longitudinally along the root (McNear Jr, 2013). The microbial community in this zone promotes plant growth by utilizing a wide variety of mechanisms which includes nitrogen fixation, organic matter mineralization, macro and micronutrient solubilization, and protecting plants from biotic and abiotic stress (Gopal and Gupta, 2016; Pii et al., 2015). As plants grow, the abundance and structure of the rhizospheric microbial community are shaped and structred by several factors that include the developmental stages of the plant. Over the years many of this factors have been well examined and published. But there has been little informatin regarding the influence of growth stages. As a consequence we considered it to be very essential to elucidate how plant species at different growth

stages shape the bacterial community in thus very important region. We believe that the analysis and proper investigation of the taxonomic and functional diversities can provide more insights into the relationship between plants and microorganisms.

As earlier mentioned, several prior investigations have shown various characteristics and the influence the maize plant types could impact on changes in the soil microbial community. For instance, reports by Lei et al. (2019) on the analysis of the rhizosphere microbiome across different maize genotypes utilizing 16S rRNA gene microarrays suggested that bacterial communities and diversity of the rhizosphere microbiome could be significantly influenced by plant genotypes. Cline and Zak (2015) reported plants-derived influence in the modifications and variations in bacteria communities in the rhizosphere. Du et al. (2018) highlighted that soil bacteria can respond to the functional groups of plants and consequently result in soil bacterial modification. Aira et al. (2010) also suggested that different genotypes of maize plants could influence the rhizobacteria community. These studies revealed that varied plant species could alter soil microbial community structure by causing a change in the profile of secreted metabolites and the interplay with other organisms (Eviner et al., 2006). However, to our knowledge, there has been no report on the influence of the varying growth stages.

Accumulating evidence suggests that analysis of the soil bacteria communities overtime across different terrains have commonly utilized several conventional cultivation methods (Joseph et al., 2003). But with the advent of newer community analysis technology and also considering that the importance of maize as a vital economic crop, one can easily infer that widespread investigations on the phylogeny of the microbiome have been conducted utilizing such technology which is based on several high throughput pyrosequencing techniques (Rastogi et al., 2013; Tenaillon et al., 2011) and some other additional traditional methods (Ferrari et al., 2005). Unfortunately, several reports have been limited by different bisases associated with techniques used, as culture-based techniques

cannot fully identify resident species and the 16S rRNA gene-based sequencing techniques could not determine up to generic and species levels.

Recent developments in high-throughput molecular biology methods have tremendoulsly increased our understanding of the soil microbiome in such significant manner than the previous decades. In recent times, using microbiome DNA or RNA-based analyses, such as amplicon sequencing and shotgun metagenomics, a large number of new phyla could be identified. Notwithstanding the astounding technological improvements in previous years, different technical biases in various techniques have been reported (Fadiji and Babalola, 2020). Third generation techniques are now beginning to offer remarkable advantages over the biases in the previous generations. For example, PCR bias due to clonal amplification for the detection of base incorporated signals, and the short reads that often require to be assembled by bioinformatic tools into the original length template have been eliminated (Schadt et al., 2010). Now, a single-molecule is sequenced instead of a clonally amplified template, such that longer reads are analyzed in a short space of time. A typical example is the nanopore sequencing technology, a third-generation sequencing program for direct analysis of single strands of DNA translocating nanoscale pores in a semiconductor membrane (Gracheva et al., 2006), which profiles microbiota utilizing tools that can be acquired at minimal cost and data analysis methods, the DNA molecule has got an adaptor cleaved to one end which interacts with a docking protein binding to the nanopore, reads are obtained as incorporated nucleotides pass through the nanopore. The electrical conductance of the nanopore (protein pore enclosed in a membrane) is interrupted as DNA flows through, such that signals are obtained and measured using the Markov models and Metrichor base-calling software. The nanopore provides longer reads and much potentially higher microbiome analysis. Although with more error rate than Illumina, accuracy has improved to up to 92 percent. Commonly, this platform does not need any chemical labels or intermediate PCR amplification. Also, the device is very handy and can fit into the pocket, consequently, analysis can be performed on-site, instead of moving samples to the

laboratory. Several metagenomic investigations have been described using the device (Hamner et al., 2019; Kerkhof et al., 2017; Piñar et al., 2020). Considering these, we decided to use the nanopore sequencing technology (MinION), a more affordable and portable tool to investigate soil bacterial community study of the maize plant rhizosphere at different growth stages on the field.

6.2 Method and Materials

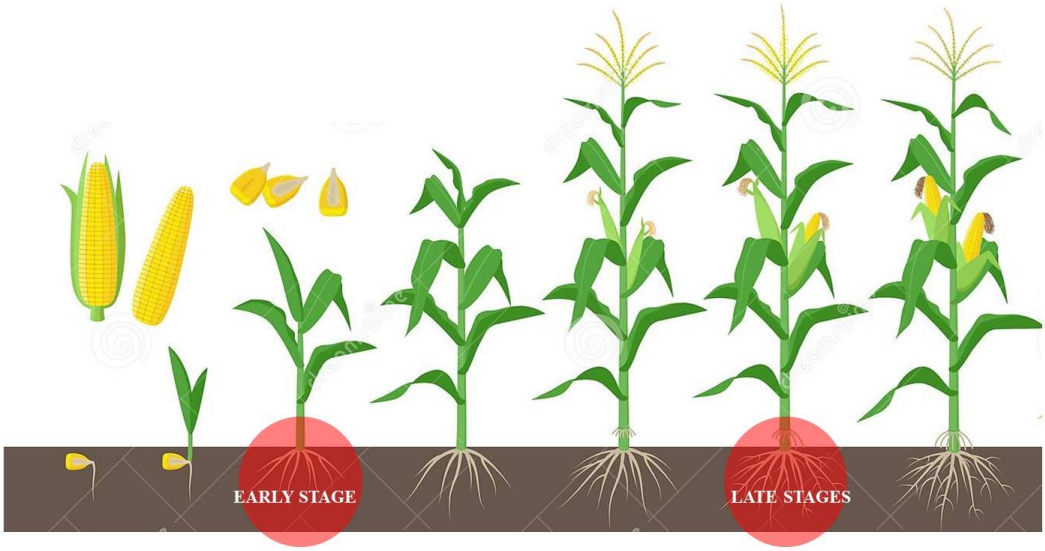


Figure 6.1: Stages of maize plant rhizosphere sample collection

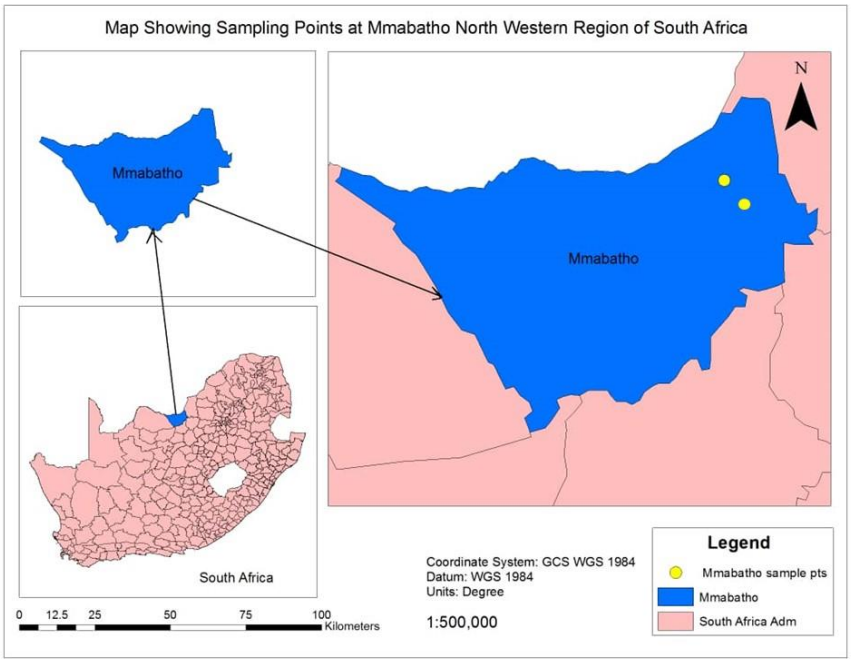


Figure 6.2: Sampling points for sample collection

6.2.1 Study area

Soil samples from the root of maize plants of the same species were obtained from selected farmland in the Mmabatho, North-Western province, South Africa (Figure 6.2). Farm was located in Mmbatho region, with a semi-arid climate with a yearly average rainfall of about 250 to 650 mm. The region is characterized by an inconsistent rainfall pattern that differs every year (Kabanda and Palamuleni, 2011). Farmers ascertained that planted seeds were disease-resistant with extensive adaptability to varied conditions. Maize plants were cultivated on farmland with similar field management.

We evaluated different maize lines within the farmland and pooled (Gomes et al., 2018) in a block design (randomized) with at least four replicates from each pooled rhizosphere sample. Each trial plot consisted of two roles, 3m long with 0.8m separating the rows and space of around 0.2 m in between the plants.

6.2.2 Sample collection

Bulk and rhizosphere soil was collected to determine the influence of the rhizosphere of the same maize plant species on bacterial community. Soil that adhered to the near root was retrieved by juddering the plant root vigorously to remove soil attached to the root from three different plants at separate points of the field, and mixed to form a composite sample. During the plant growth at stages shown in Figure 5.1, the rhizospheric soil samples were obtained at flowering and senescence stages. Samples were collected in duplicates (each consisting of composite soil samples). Bulk soil, i.e sample without vegetation, was collected as control within 0-10 cm depth. Samples were sieved (2-mm) and kept at −20 °C until further processing (Tatangelo et al., 2014).

6.2.3 Soil chemical and physical properties

Samples collected for physicochemical analysis were air-dried, mixed, ground, and sieved through a 2mm pore filter before further laboratory analysis. The soil pH was measured by mixing 2 g of fresh soil in 10 ml deionized water using a Jenway 3520 pH-meter (Cole-Parmer Instruments,

Staffordshire, UK). Soil organic carbon (SOC) was determined by the modified Walkley and Black wet digestion method (Heanes, 1984). Total carbon and nitrogen were measured using the dry combustion method as described by Santi et al. (2006). Soil nitrate was determined by the KCl extraction method. The modified Bray II method was used to determine the extractable total phosphorus (Otani and Ae, 1999). All readings were obtained in triplicates for statistical analysis.

6.2.4 DNA extraction and sequencing

Power soil DNA isolation kit, Power Lyszer (MoBIO Laboratories, Carlsbad, CA, USA) was used to extract total DNA from 0.5g (total humid weight) of soil according to the manufacturer's instructions. The DNA extraction was performed in duplicate for each collected soil sample.

6.2.5 Amplification of rRNA operons

Touchdown PCR was used for the amplification of ribosomal operon. 2 μL of extracted soil DNA at the different stages of growth, high-quality Taq polymerase (Bimake LLC, Houston, TX, USA), Modified forward 16S rRNA primers, 27 Forward primer (5′ TTT CTG TTG GTG CTG ATA TTG C-[barcode overhang for PCR labeling]-AGA GTT TGA TCC TGG CTC AG 3′) (Kerkhof et al., 2017) and modified 23S rRNA-2241Reverse primer (5′ ACT TGC CTG TCG CTC TAT CTT C-[barcode overhang for PCR labeling]-ACC GCC CCA GTH AAA CT 3′) were utilized as described (Ibironke et al., 2020). Briefly, initial denaturing temperature was set at 95^{o}C for 5 min, Double cycles for 20 sec at 95^{o}C for denaturation, primer annealing temperature was at 68^{o}C for 15 sec, extension was 72^{o}C for 75 sec. These were followed by 2 cycles of 66^{o}C, 64^{o}C, 62^{o}C for primer annealing, 22 cycles of denaturation, then 60^{o}C for primer annealing, extension at 72^{o}C for 90 sec and an ultimate extension at 72^{o}C for 5 min. After the 16th cycles consisting of 8 touch down and 8 standard cycles, 12 μL of amplification mix was withdrawn and kept at -80^{o}C. Next, amplification continued for up to 30 cycles, and PCR products were viewed using agarose gel electrophoresis. Purification of the 16-cycles PCR products was carried out and bead cleaned. Further, barcode amplification was carried out. Initial temperature of 95^{o}C for 5 min, then 30 cycles at 95^{o}C for 20

sec, 60°C for 15 sec and 72°C for 1.5 sec, these were followed by extension at 72°C for 5 min. rRNA amplicons of sample were viewed an measured by gel electrophoresis.

6.2.6 Library preparation and sequencing by MinION

For the MinION library preparation, 2D sequencing kit ((SQKLSK108-Oxford Nanopore; Oxford, UK). Two 12- Barcoded amplicons were pooled together in low binding tubes, end-repaired, dA-tailed as described by Kerkhof et al. (2017) with little modification. Blunt/ TA ligase master mix (NEB) was used for the ligation of oxford nanopore technology adaptor by adding 1 μL newly prepared ATP solution (~3 mg/mL) to expedite the process. Libraries were analyzed on R9.4 flow cells.

6.2.7 QA/QC on Geneious

Poretools was used to open the 2D reads and respective fasta format files were exported. Sequences files went through QA/QC examination by annotation in Geneious using pairs of each universal 16S rRNA primer sequences (27F, 343F, 518F, 907F, 1392F, and 1492F) (Kerkhof et al., 2017) and 23S rRNA primer sequences (129F, 473F, 820F, 1623F, 2069F, and 2758F) (Hunt et al., 2006). Sequences with 4 to 5 kb with minimum of two rRNA priming sites were kept for further analysis (~ 85% of the 2D sequences). Files were aligned in uniform direction and 16S rRNA sequences were extracted in Geneious.

6.2.8 OTU determination

Barcodes were screened against the NCBI 16S rRNA gene bacterial database using Discontinuous MegaBlast in Geneious. Top Blast results were reaped as .csv format and opened Microsoft Excel and grouped by best blast hit, computed the total number of OTUs and parse for sample comparison Kerkhof et al. (2017).

6.2.9 Statistical analysis

The community structure and its correlation with soil parameters were visualized using Canonical Correspondence Analysis (CCA). Before the analysis, Shapiro-Wilk and Levene tests were applied to check the data distribution and homoscedasticity, which indicated a non-normal distribution, revealing that the best-fit mathematical model for the data was CCA. Forward selection (FS) and the Monte Carlo permutation test were applied with 1000 random permutations to verify the significance of environmental parameters upon the microbial community structure. CCA plots were generated using Canoco 4.5 software (Biometrics, Wageningen, Netherlands). Permutational multivariate analysis of variance (PERMANOVA) was used to test whether sample categories harbored significantly different microbial community structures. Alpha diversity was calculated from a matrix of richness at the genus level using Shannon's index. PERMANOVA and alpha diversity indexes were calculated with the software PAST 3 (Hammer et al., 2001). To visualize the differential microbial community composition among treatments, we used the Statistical Analysis of Metagenome Profile software (STAMP) (Parks et al., 2014). The OTU table generated from the 16S rRNA gene profiling was used as input. The comparison was based on P-values calculated using the two-sided Welch's t-test and the correction was made using Benjamini-Hochberg false discovery rate (Benjamini and Hochberg, 1995). Lastly, Spearman’s rank correlation coefficients were calculated to explore the relationship between microbial groups and soil chemical parameters using the ‘multtest’ package in R, with the correction made using the Benjamini-Hochberg FDR.

6.3 Result

6.3.1 Soil physicochemical characteristics

Soil physicochemical properties of selected attributes were determined. These properties revealed the soil samples were either acidic or neutral, with a very high amount of potassium and phosphorus (See Table 6.1)**.** Considering the physicochemical properties of the soil, pH, potassium, phosphorus and ammonium were key elements influencing bacteria community alpha diversity (PERMOVA F= 2.64, P=0.009)(Fig 6.3). With an increased pH, ammonium, phosphorus and potassium, there is an

increase in abundance and diversity. The extent of the difference in these soil properties also significantly influenced the similarity of species composition across the two different growth stages, and the relationship (Fig 6.3). Spatial distance between the three soil samples tended to influence similarity of species composition in the soil communities.

Table 6.1: Mean value of selected physical and chemical attributes of the soil

Factor	ES 1	ES 2	ES 3	ES 4	LS 1	LS 2	LS 3	LS 4	Bulk1	Bulk 2
pH (Water)	6.79±0.02	7.04±0.01	6.38±0.04	6.64±0.01	6.45±0.18	6.78±0.12	6.45±0.74	7.04±0.26	6.40±0.22	6.5±0.11
S (mg/kg)	16.2±0.01	0±0.00	3.75±0.13	2.78±0.02	0±0.00	0.76±0.01	0±0.00	0±0.00	14.2±0.02	12.2±0.16
P (mg/kg)	24.29±0.12	19.75±0.01	26.1±0.32	40.39±0.72	26.52±0.02	23.57±0.01	26.52±0.01	19.75±0.21	15.6±0.77	19.0±0.89
TOC (%)	1.24±0.01	1.19±0.03	1.67±0.02	1.18±0.11	1.04±0.33	1.35±0.05	1.04±0.02	1.19±0.34	1.11±0.021	1.09±0.01
K (mg/kg)	338±2.12	430±0.03	165±0.02	338±0.03	254±0.01	184±0.02	254±0.04	430±0.02	220±0.04	180±0.03
N_NO_3 (mg/kg)	16.56±0.03	16.42±0.04	11.9±0.23	18.02±0.02	14.16±0.03	9.41±0.22	14.16±0.02	16.42±0.13	14.1±0.15	13.5±0.05
Org_C(%)	1.04±0.04	1.07±0.01	1.19±0.02	1.06±0.01	0.81±0.02	1.12±0.03	0.81±0.01	1.07±0.02	0.65±0.01	0.72±0.02
OM (%)	4.02±0.02	4.16±0.02	3.8±0.001	4.19±0.03	3.46±0.02	3.7±0.00	3.46±0.06	4.16±0.02	3.31±0.00	3.00±0.00
N_NH_4(mg/kg)	3.22±0.12	3.32±0.11	3.85±0.02	3.68±0.01	1.87±0.01	1.74±0.02	1.87±0.01	3.32±0.05	2.21±0.01	2.22±0.02

BS–Bulk soil; ES– Early stages 1; LS- Late Stages; S-Sulphur; P- Phosphorus; TOC–total organic C; K- Potassium; N_NO_3- Nitrare; Org C- Organic Carbon; OM- Organic matter; N_NH_4- Ammonium. Mean values for selected attributes of collected soil samples. Mean= (n= 3) ±Standard error of the means (SEM). Variables that were significantly ($P < 0.05$)

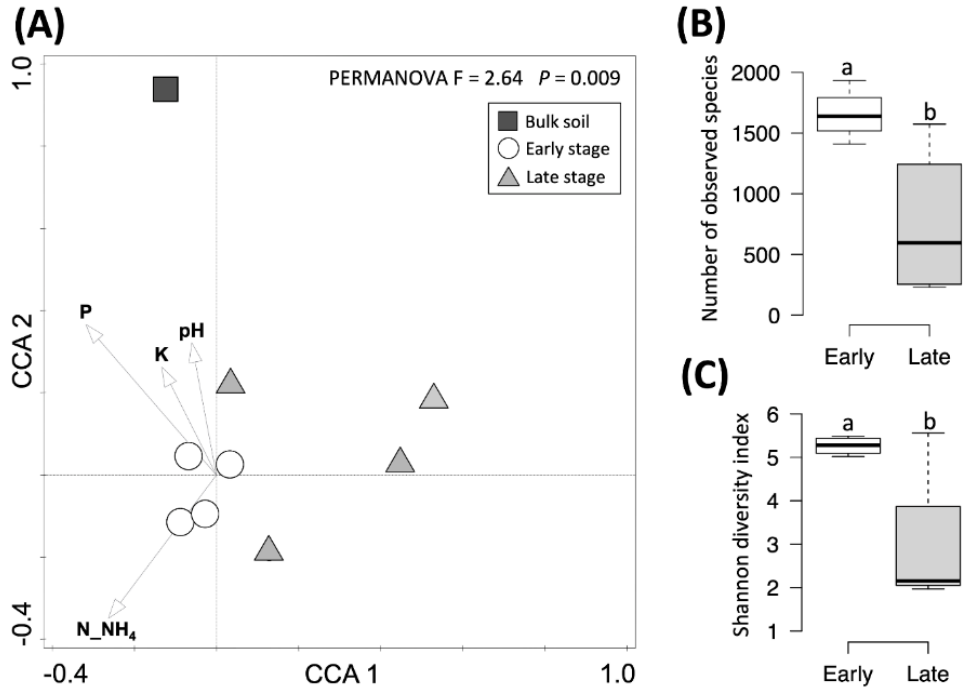


Figure 6.3. (A) Canonical correspondence analysis (CCA) of the microbial community pattern and soil physicochemical properties from bulk soil and rhizosphere of maize in the early and late stages of development. Arrows indicate the parameters with significant correlation ($P < 0.05$) with bacterial structure. (B) Taxonomic richness and (C) diversity based on genus level at 97% similarity of the 16S RNA gene sequencing. Error bars indicate the standard deviation of the duplicates. Different lower case letters refer to the significant differences between treatments based on Turkey's HSD test ($P < 0.05$).

6.3.2 Microbial community composition

The BLAST screening showed that the rhizosphere soils were dominated by 5 phyla: Proteobacteria (46% of the total sequence), Actinobacteria (29%), Firmicutes (21%), Acidobacteria (23%), Cyanobacteria (1%) and Gemmatimonadetes (1.17%). All other phyla were below 1% of relative abundance. As indicated in Figure 6.4C, there were some significant differences between the early

and late stages of growth, which are marked with asterisks. When samples were contrasted using STAMP software, the abundance of specific bacterial phyla showed shifts along the growth stages (Fig. 6.4A). Bulk soil presented lower abundance of Acidobacteria, Proteobacteria, Elusimicrobia, Armatimonadetes, on the other hand, in the rhizosphere samples, there was an increase in abundance of these phyla. Proteobacteria increased in the late stages, while Artimonades, Gemmatimonadetes and Elusimicrobia decreased in the late stages. Differential abundance is based on Welch's test with Benjamini-Hochberg correction ($P < 0.05$).

In lower taxonomic levels, the distribution of genera at each growth stage showed the relative abundance of *Bacillus, Pseudomonas, Candidatus, Solirubrobacter, Psychrobacter* as presented in Fig. 6.5 A-G. We also compared family compositions at different growth stages. In general, we observed that thirty-two bacterial families were differently distributed in the rhizosphere of maize (Fig. 6.4B). Early stages had a higher abundance of ten families, in particular *Burkholderiaceae, Conexibactraceae, Frankiaceae, Sporichthyaceae, Bradyrhizobiaceae, Gammatimonadeceae, Gaiellaceae*. Eight families belonging to Actinobacteria significantly changed. For example, Gaiellaceae increased in abundance significantly during the early stages and decreased during the late stages of growth.

6.3.3 Bacterial community structure and diversity

After bacterial community profiling 16S rRNA operon and quality trimming, gene sequencing generated reads were used for downstream analysis. CCA analysis was used to evaluate the bacterial community structure and relate to soil physicochemical properties (Figure 3A). Our results showed that the bacterial communities were different between the treatments, clustering the samples according to the site and plant developmental stage, i.e. Bulk soil, early rhizosphere, and late rhizosphere (PERMANOVA $F = 2.64$, $P = 0.009$). The explanatory variables account for 51.2% of the total variation, and the CCA followed by Monte Carlo analysis indicated that NH_4 (17.4%), K (12.5%), pH (11.1%), and P (10.2%) showed significant correlation with the general community

structure. We also observed that these parameters are more correlated to the samples from the early stage.

The pattern of richness and diversity measurements revealed a decreased diversity following plant development. As shown in figure 3B-C, we observed that there is a significant decrease in richness and diversity in the late stage.

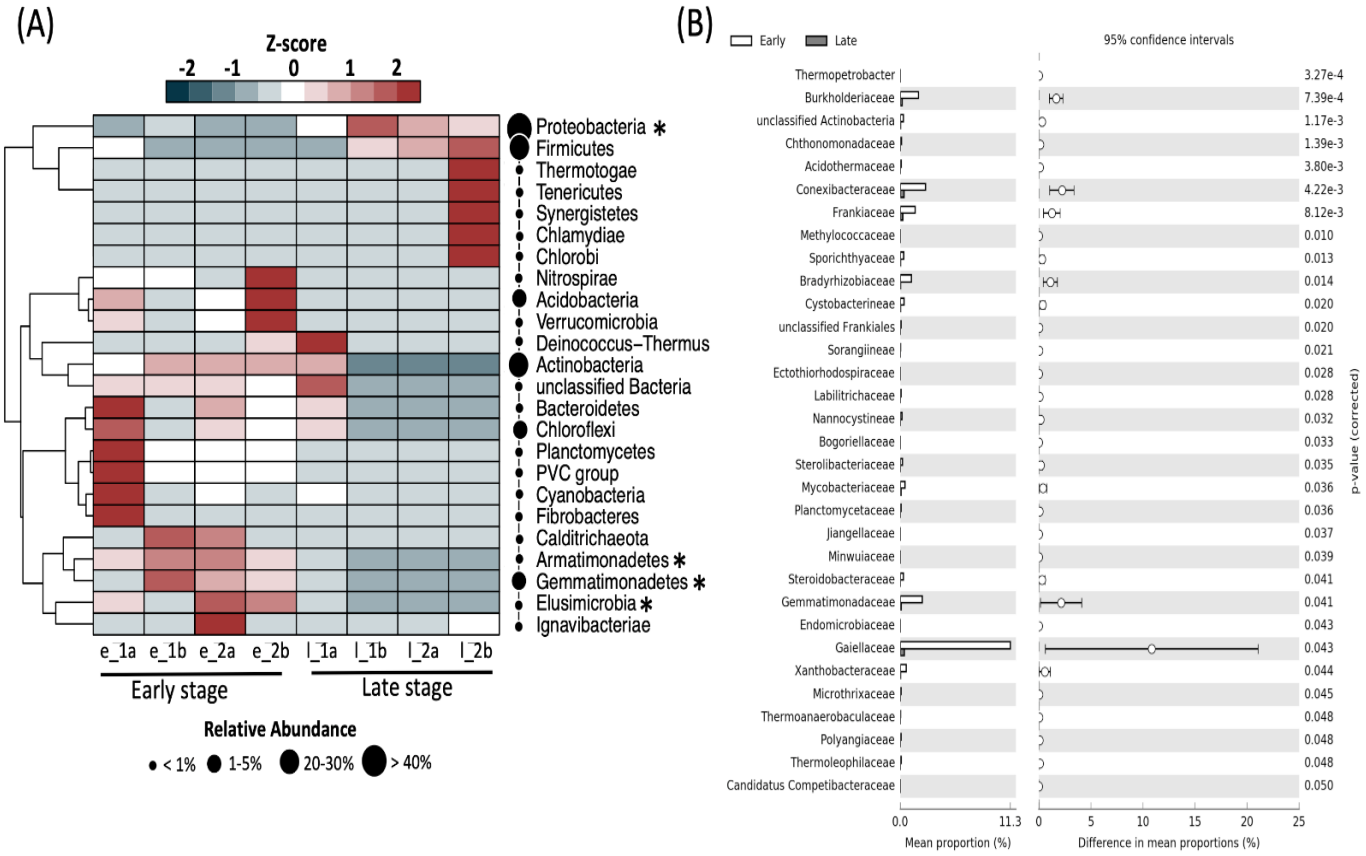


Figure 6.4A: Heatmap of the abundance of bacterial distribution and composition by phylum; B. Family comparism at the two different growth stages using STAMP

* means significant diffence

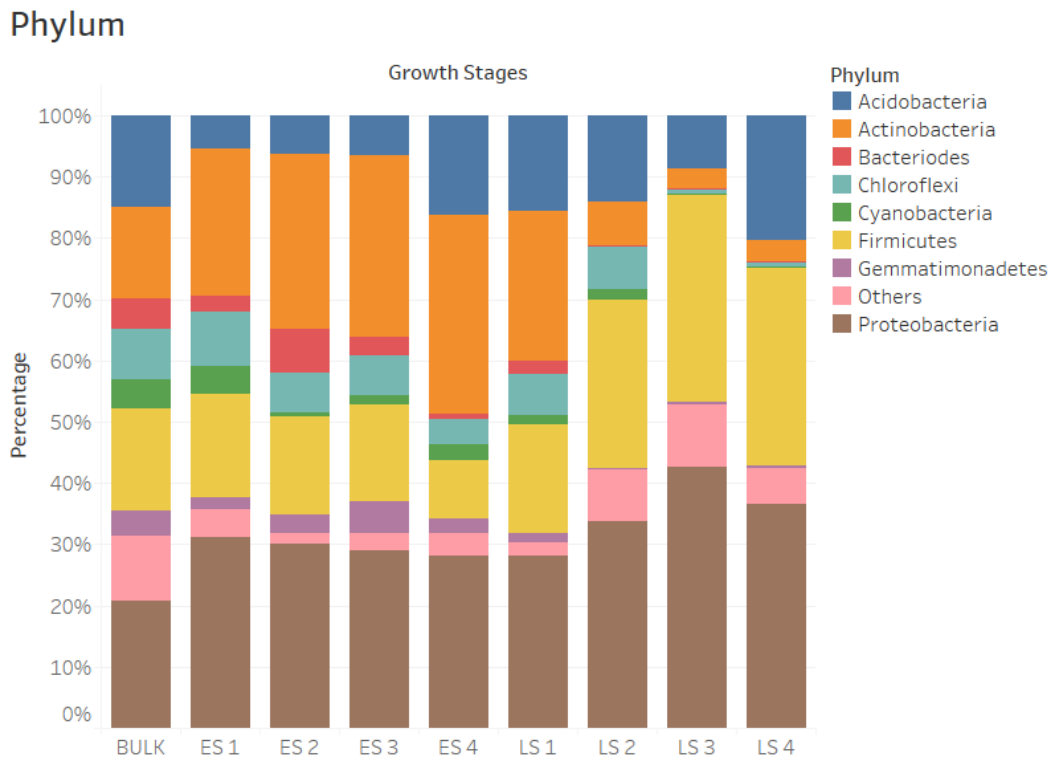
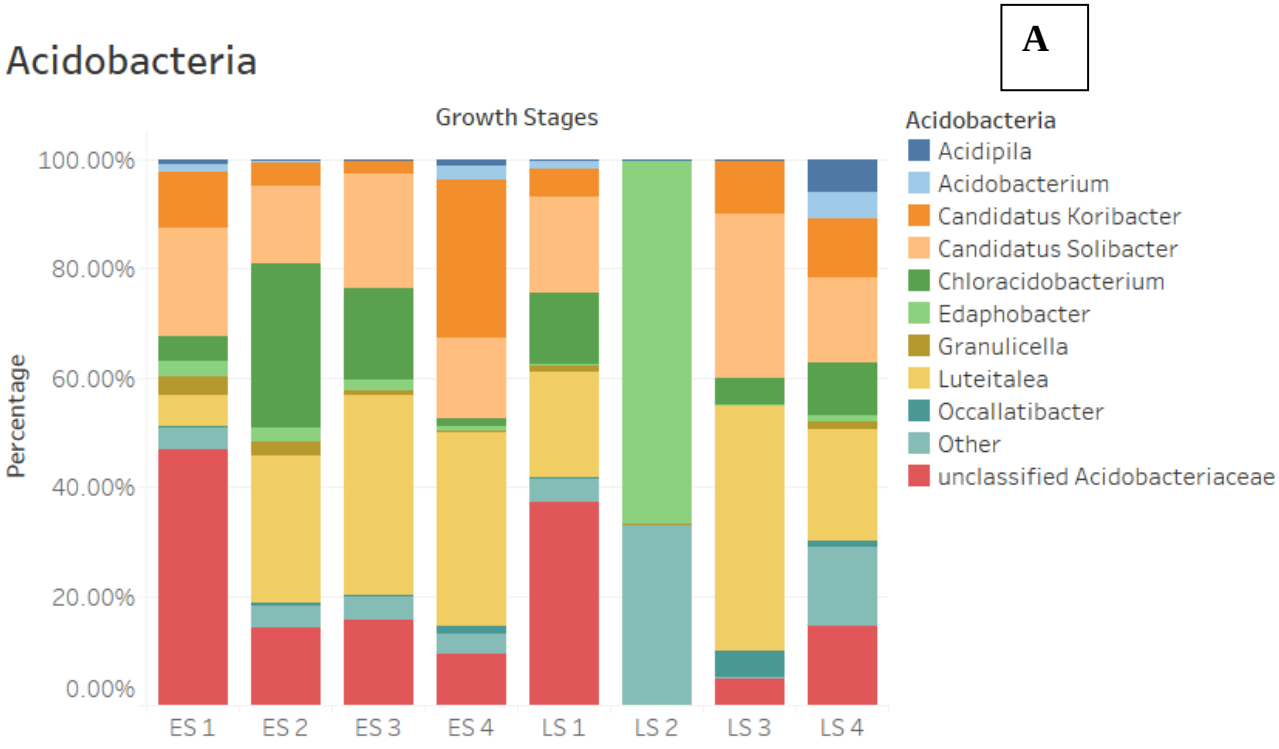
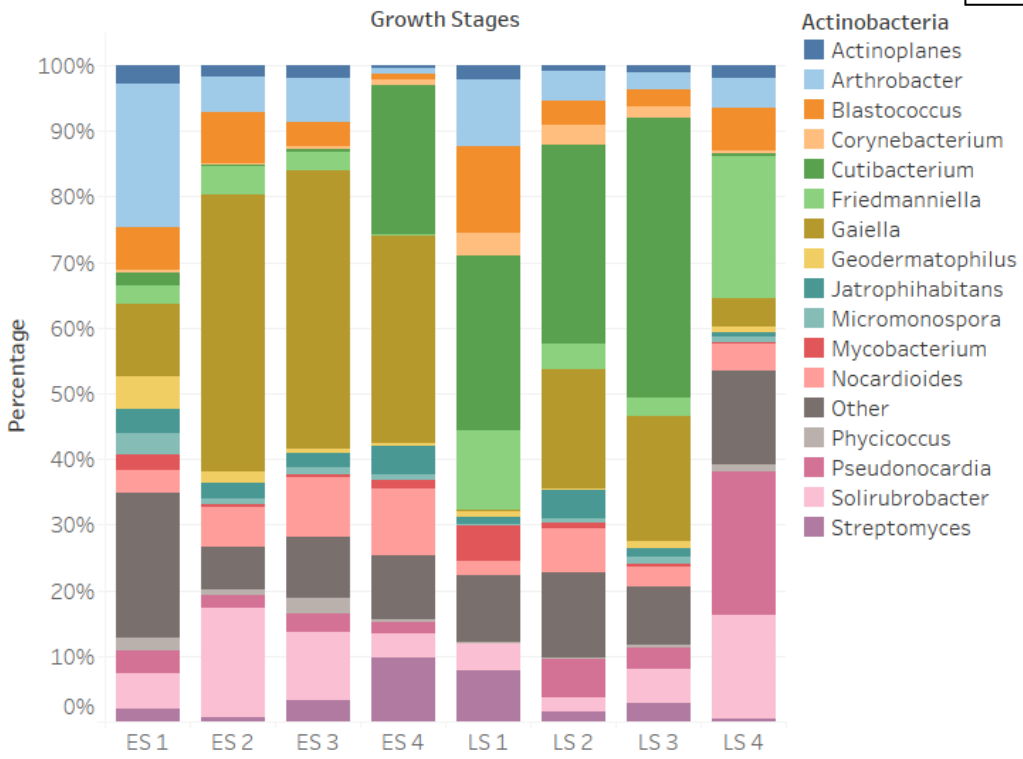


Figure 6.5: Relative abundance of bacterial distribution and composition by phylum



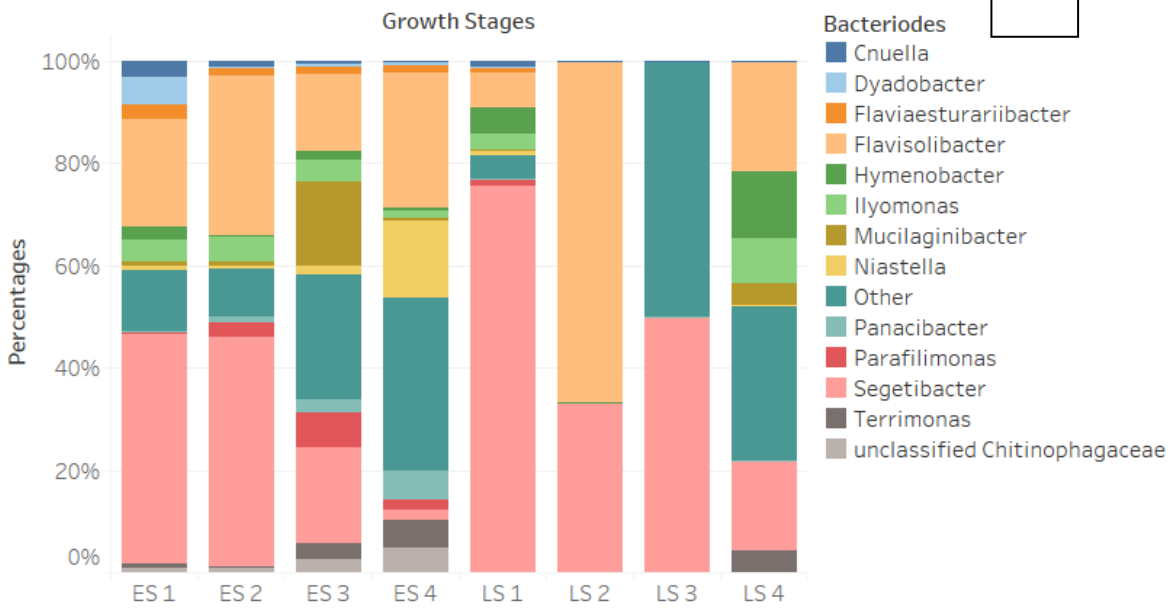
Actinobacteria

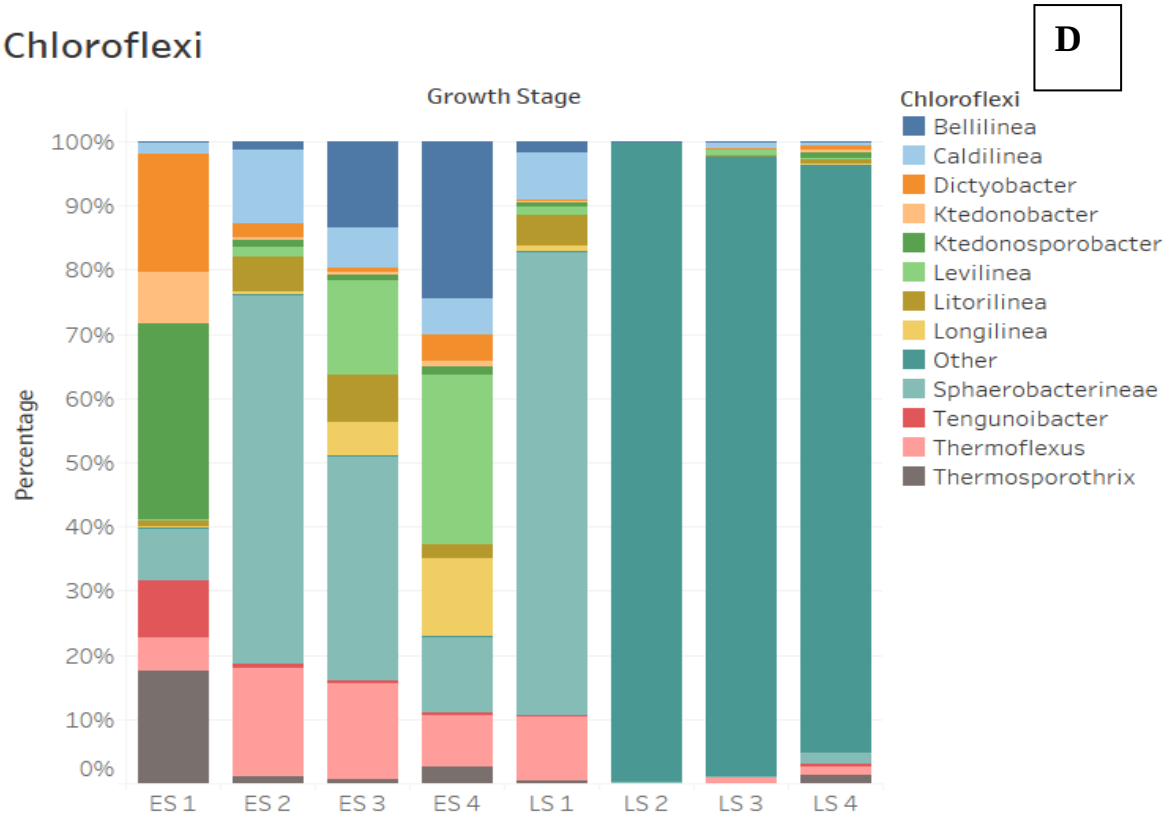
B

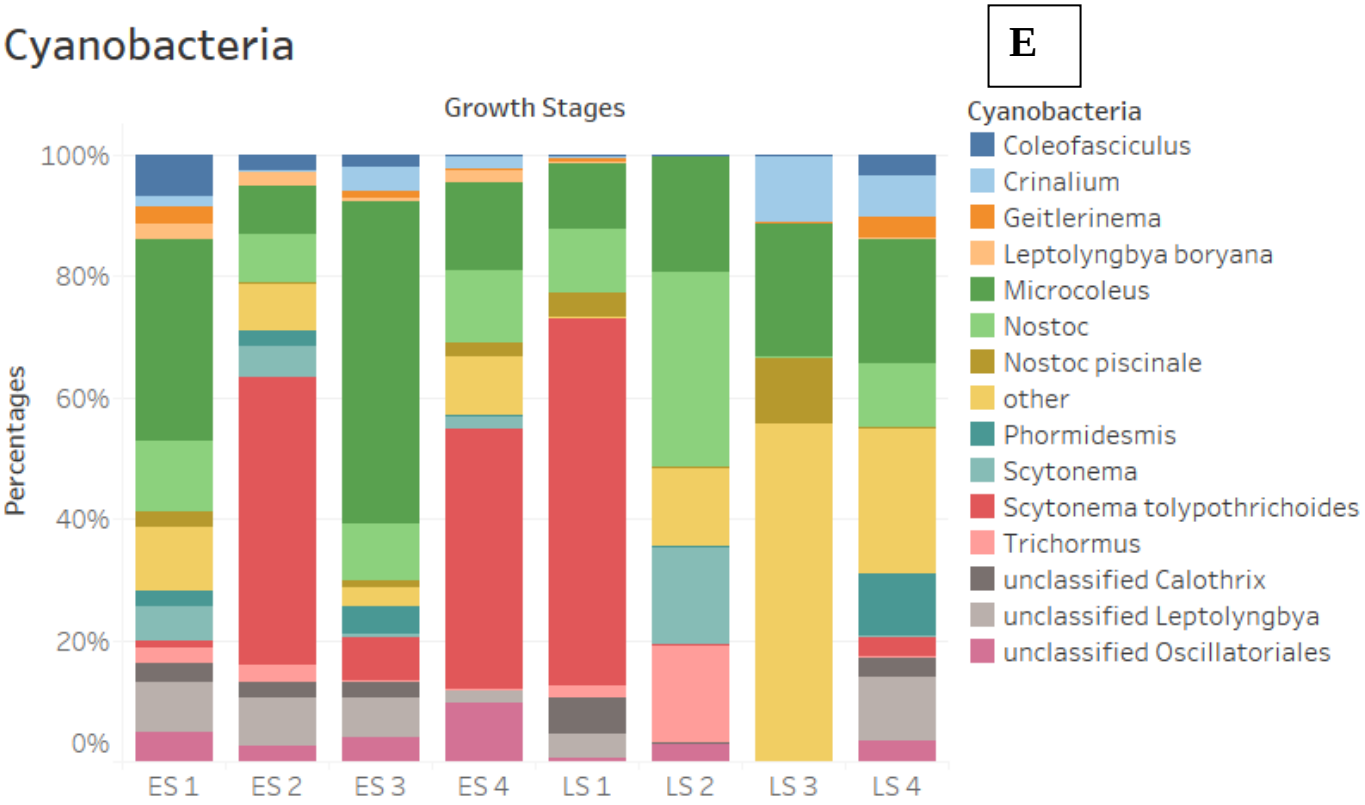


Bacteriodes

C




Chloroflexi
D
Growth Stage
Percentage
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
ES 1
ES 2
ES 3
ES 4
LS 1
LS 2
LS 3
LS 4
Chloroflexi
Bellilinea
Caldilinea
Dictyobacter
Ktedonobacter
Ktedonosporobacter
Levilinea
Litorilinea
Longilinea
Other
Sphaerobacterineae
Tengunoibacter
Thermoflexus
Thermosporothrix


Cyanobacteria
E
Growth Stages
Percentages
100%
80%
60%
40%
20%
0%
ES 1
ES 2
ES 3
ES 4
LS 1
LS 2
LS 3
LS 4
Cyanobacteria
Coleofasciculus
Crinalium
Geitlerinema
Leptolyngbya boryana
Microcoleus
Nostoc
Nostoc piscinale
other
Phormidesmis
Scytonema
Scytonema tolypothrichoides
Trichormus
unclassified Calothrix
unclassified Leptolyngbya
unclassified Oscillatoriales

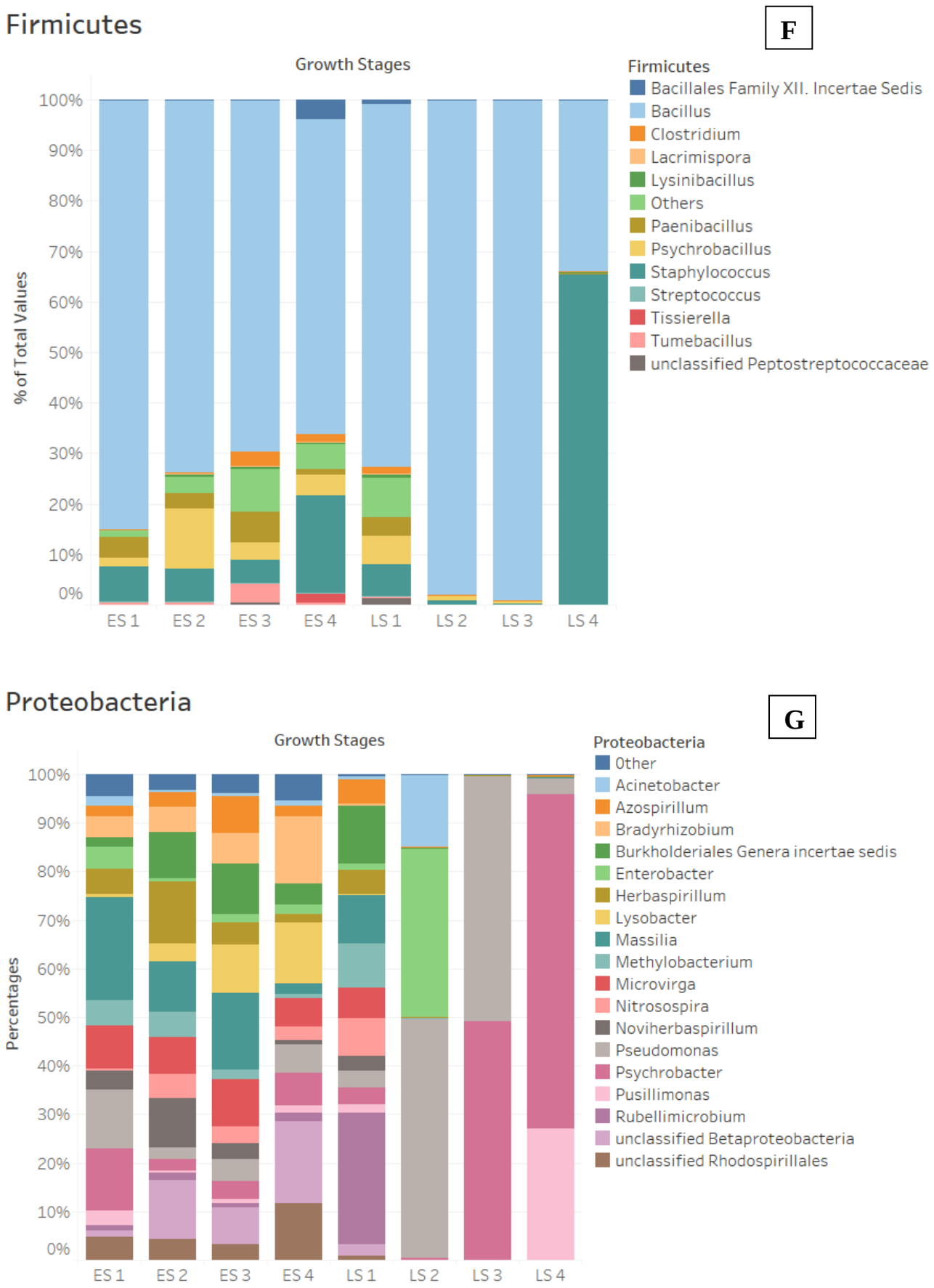


Figure 6.6 A-G: Percentage relative abundance of predominant genera

6.3.5 Correlation between the bacterial community and soil properties

To further explore the correlation between bacterial phyla and soil chemical parameters, we calculated all possible Spearman's rank correlations (Fig 6.7). Most of the correlations were positive. NO_3 presented significant correlation with the most number of phyla and most of these correlations were negative. On the other hand, the phylum Acaryochloris presented correlation with the highest number of chemical parameters. The parameters S, K, and organic C presented only positive correlations with bacterial phyla, while P and total C correlated only negatively.

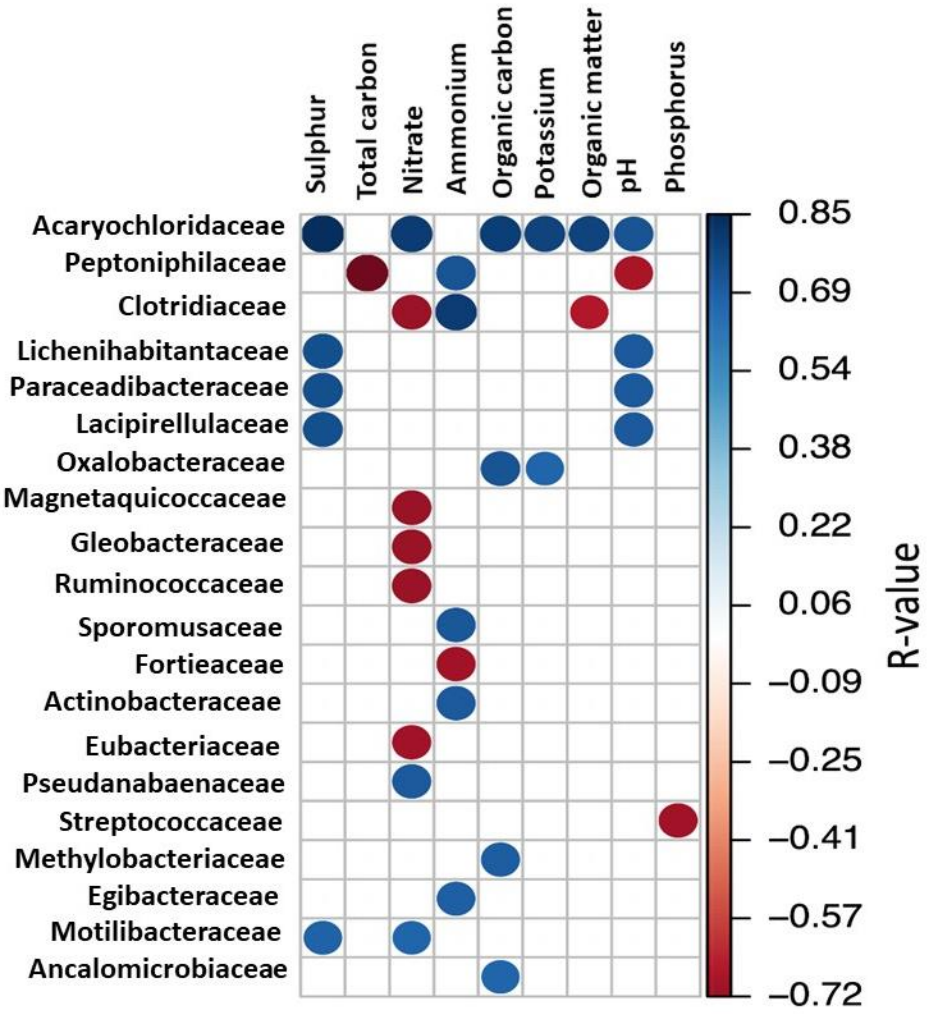


Figure 6.7: Bubble plot showing the Spearman's rank correlation between phyla abundance relative to soil factors. Blue and red colors indicate significant positive and negative correlations, respectively ($P < 0.05$), while empty boxes indicate no significant correlation ($P > 0.05$).

6.4 Discussion

The rhizosphere is often seen as a complex ecosystem and hotspot for microbial activity. This region possesses distinct metabolic abilities that play critical roles in plant health and soil fertility. Notwithstanding the importance of this zone to the growth and development of plants, our knowledge overtime have been limited, as several microbial species could not be easily cultured in the laboratory. It is noteworthy that some of our preceding investigations on maize rhizosphere have used different culture-dependent and independent methods (Babalola et al., 2002). Here, we used Oxford nanopore sequencing platform (MinION), a third-generation DNA sequencing technology to assess the diversity and composition of soil bacterial community in the near root zone of maize plants at two different growth stages, i.e. flowering and senescence, which we often referred as early and late stages of growth in this study. Our results provided an initial view of the effect of growth stages of maize plants on rhizobacteria.

Owing to this peculiar attribute, there is a need to explore the influence of the selected physicochemical properties of the soil of the maize plants. Our study revealed noticeable influence of the physicochemical properties on the bacterial community distribution at the two stages of growth. Although, bacteria distribution were driven by pH, NH_4, P, K during the early stages (Fig 6.3A). Spearman's rank correlations also revealed that some of the families showed positive correlations (Fig 6.7). Our result was very similar to the work Mhete et al. (2020) who concluded that soil bacteria community could be impacted by land-use types, disturbances and physicochemical properties. Commonly, bacteria communities have got preferential living conditions; thus, the variance in the bacterial living condition causes different vigor of the bacterial groups and then influences the composition of the bacterial communities (de Vries et al., 2013). Our inference for such impact in the rhizosphere was that as soil properties are altered due to plants' physiological responses to growth, bacterial communities are fostered to better acclimatize to the new changing conditions, these changes as a consequence alter the environmental conditions, making the near root unfavorable for some particular bacteria community. Furthermore, commonly,

bacteria distributions are viewed to be a consequence of integrated edaphic and environmental factors (Xue et al., 2018) and soil environments are characterized by both edaphic and climatic conditions which microbes must adapt to with synchrony (Schimel and Schaeffer, 2012). In our study, prevailing climatic conditions, such as temperature fluctuations, and the intensity and distribution of rainfall could be of consequence on the variability and relative abundance of the bacteria community. The climate of Mmabatho, where the soil samples were collected, is usually a uniformly semi-arid environment; as a consequence, the nature and attributes of the soils could be a strong link to the bacterial community in the soil environment. In our investigation, a significant correlation was seen between the soil properties and bacteria communities with slightly stronger responses to pH, NH_4, N, P, and K (Fig 6.6). These elements tends to be the driver of the bacteria community at the early stages of growth.

It is noteworthy that the impact of pH can be influenced by other soil properties and could be the reason for its positive correlations with bacterial distribution. Our findings did not come to us as a surprise, as verbal information gathered from the farmers indicated that fertilizers added to this maize plants were NPK based and that these fertilizers were added at the early stages. Our results were consistent with previous findings (Leff et al., 2015; Ling et al., 2017; Lopez-Fernandez et al., 2018). Suggestively, this could indicate that the chronic input of fertilizers could serve as the driver for the diversification and alterations of the bacterial community.

Previously, several studies on rhizobacteria have been described at the genus through phylum levels using the V1-V6 variable regions of the 16S rRNA genes. But in contrast to the V1-V6 method that was commonly used, MinION profiling of rRNA operons affords 10-100x longer reads. This with an OTU calling at ~80% accuracy, species-level resolution and no detectable chimeras. Also, these longer MinION raw reads are used to form a strong consensus sequence from the rRNA operons which gives a higher taxonomic resolution than the shorter reads 16S rRNA gene fragment (Kerkhof et al., 2017).

From our study, the analysis of the overall bacterial community in the rhizosphere under field conditions revealed that changes in the maize rhizobacteria community structure were strongly influenced by the growth stages of the plant (Fig. 6.4A), which suggestively point to the soil physicochemical properties, soil structure, root exudates, nutrients, and other factors. There was significant difference in the communities concerning richness, diversity, and evenness (Table 6.3C). Early growth-stage related communities had a distinct separation from the later ones (Fig. 6.5), and we generally noticed a significant decrease in the bacteria communities at the late stages of growth across the investigated soil samples. Our finding was in agreement with a similar study by Lundberg et al. (2012), which also showed a decreased diversity at the late stages of plant development. A clear look at the bacterial community through the two examined growth stages revealed few taxa to be consistently enriched in the near root with a high abundance of Proteobacteria, Firmicutes, Acidobacteria, and Gemmatiminadetes, which represented > 30% of the entire sequence. This did not come as a surprise as orders Burkholderiales, Oceanospirillales, and Sphingobacteriales of the Proteobacteria have been shown previously to be enriched in the maize rhizosphere (Aira et al., 2010; Chauhan et al., 2011). Also, our findings were quite similar to several other studies that reported the dominance of Proteobacteria, Firmicutes, Acidobacteria in the rhizosphere of maize and cowpea plants (de Araujo et al., 2017; Johnston-Monje et al., 2016). In contrast to the near root region (rhizosphere), the bulk soil is commonly viewed to be enriched with slower growing microbiome with relatively stable population sizes. In our study the bulk soil were enriched uniformly through out the growth stages with phyla such Proteobacteria, Acidobacteria, Chloroflexi, Actinobacteria (Figure 6.4C), and similar result have been previously described by Fierer et al. (2007). Suggesively our findings could be attributed to suitable living conditions of the farm such as the pH (6.4-7.4) and nutrient contents, a condition that contributes to their existence (Yang et al., 2017). Also, our investigation showed that Proteobacteria and Firmicutes, were dominantly present in both stages (Figure 6.4C), however, Acidobacteria, Bacteroidetes, and Actinobacteria changed with plant development, which was suggestive that plants can select a

subcategory of microorganisms at different stages of growth. In terrestrial ecological systems, Acidobacteria is one of the most abundant phyla in the soil (Barns et al., 1999), and they play crucial roles in carbon cycles owing to their capacity to break down plant-derived complex polymers such as lignin and cellulose (Chaves et al., 2019). However, aside from this, there is a paucity of information regarding their other roles in the rhizosphere. A published article by Mendes et al. (2011) linked the activities of Actinobacteria to diseases suppressive soils. *Streptomyces*, a genera under Actinobacteria was commonly found to be enriched in the our study. *Streptomyces* is viewed to produce antibiotics which act as an antagonist to several other different phytopathogens as serves as a promising biocontrol agency.

Generally, the roles of Bacteroidetes in the near root region are not known precisely yet, however, they have been linked to being a vital contributor to the turnover of nutrients in the soil (Yousuf et al., 2012). Also, genes associated with denitrification have been identified in some bacteria belonging to Bacteroidetes and they play a possible role in nitrogen cycling. Unfortunately, some enteric bacteria were identified, which possible route could be traced to organic animal manures added to amend the soil. Consequently, some of the maize plants might pose a public health concern.

Several key microbial groups (at both family and genus levels) exhibited different varied diversity in the rhizosphere as compared to the bulk soil. Across the bulk soil and the two developmental stages, family Gaiellaceae and Gemmatimonadeceae showed the most significant difference. The genera *Pseudomonas, Acinetobacter*, *Psychrobacter,* and *Bacillus* dominated the relative abundance across the growth stages and were in the highest proportion. These are common PGPR that stimulates several growth promoting activities such as nitrogen fixation, phosphorus solubulization, siderophore prouction, etc. in plants (Adedeji et al., 2020). Moreover, our findings were buttressed by changes in the bacteria community in the near root as seen by CCA (Fig 6.3A), which revealed significant differences in the bacteria community of bulk and rhizosphere soils at different

developmental stages. This outcome was projected since bacterial communities are impacted by environmental changes and these bacteria species can multiply by harnessing organic substances produced in the root region (McNear Jr, 2013). They could also show positive chemotactic movements towards plant exudates using numerous chemosensors (García-Salamanca et al., 2013).

We also discovered significant growth-linked changes among the bacteria community. Plant's ability to cause a shift in bacterial diversity and structure in the rhizosphere could be attributed to its ability to initiate a microenvironment that is rich in amino acids, carbohydrates, and carboxylic acids, and it is suggestive that exudates and nutrients in this region could be the driving factor and that the predominant bacteria inhabiting the rhizosphere of young plants preferentially make use of such substances the more. Consequently, Proteobacteria, Actinobacteria, and Firmicutes' increased abundance in the rhizospheric soil compared to the bulk soil. Members of this community were enriched mostly at the later stages, possibly due to their versatility to utilize various plant substrates (mostly carbohydrates during the late stages), degrade aromatic compounds, and also produce antibiotic substances (Li et al., 2014). Members of Burkholderiales were enriched in the rhizosphere, possibly due to their versatile abilities to utilize root metabolites, degrade aromatic compounds (Lopez et al., 2019) and produce anti-microbial substances (Cheng et al., 2020).Acidobacteria was observed to be reduced during the early stages as they are unsually not well adapted to nutirent rich environment due to their oligotrophic nature. Nevertheless, noticeable increase was seen at the latter stages of growth. Our findings were also in agreement with previous study by Mendes et al. (2014).

Generally, we could be deduced that changes in the root region are usually due to the physiological and biochemical alterations (Xue et al., 2018). Sometimes, the extent of these differences could be traced to the quantity and quality of root exudates that change over time as plants develop. However, in our investigations, we did not measure or characterize the released chemical substances around the roots (i.e exudates), but published articles have well documented the roles of

these root exudates (Sasse et al., 2018; Steinauer et al., 2016). Plants release varied chemical profiles of exudates around the roots, and the bacterial population reacts to these substances causing a shift in their community (Strickland et al., 2015). Uroz et al. (2013) reported that these chemical substances in the form of carbohydrates, carboxylic acids, and amino acids alter the chemical attributes of the rhizosphere and cause increased competition or eradication of unsuited bacteria from these regions. There is a distinct pattern of carbon sources around the root at different growth stages, such that at the early stages of growth, there is more carbon available for the bacterial population that resultantly could be the reason for the increased diversity during the early stages of growth. Previous studies have also shown that, at the latter stages of growth of plants, activities are reduced and lower amounts of carbon can be found during this phase in the root regions (de Araujo et al., 2019). Acidobacteria, as earlier highlighted, were impacted due to this reason, as they are better suited for the oligotrophic environment and their increased abundance during the senescence stage can be associated with depletion in the rhizospheric effects.

Rhizodeposition of nutrients is commonly high in plants during flowering stages but decreases as they grow older, which may be the reason for the differential communities and abundance between the early and late growth stages. Furthermore, during the early stages of growth, near root bacteria communities have a preference for simple amino acids, whereas at the latter stages they tend to prefer complex carbohydrates. This is an indication of how the quality of exudates could influence changes in the bacterial community. Firmicutes plays a very significant role in the initiation of degradation of complex substances such as mucin, polysaccharides, and cell walls (Flint et al., 2012), and the increased abundance of this phylum during the late stages of growth could be linked to its capacity to metabolize complex carbohydrates under depleting nutrient conditions. Our data are in alignment with previous studies that also indicated that rhizospheric communities were different at different growth stages (PERMANOVA $P < 0.05$)(de Araujo et al., 2019). Thus, while

the inputted nutrients around the root are reduced, the abundance of Proteobacteria and Firmicutes at the late stages of growth could be attributed to the shift in exudate production.

During the early stages of growth, maize plants produce diverse metabolites that dictate the bacteria environment, a reason for the contrast in diversity when compared with bulk soils. Also, this facilitates the vast growth of Proteobacteria and also contributes to the increased abundance of other predominant phyla during the early stages of growth as these bacteria grow heterotrophically in a sugar-rich environment. Proteobacteria are highly versatile metabolically in the soil environment and they consist of large groups of bacterial communities that occupy the near root regions (Sun et al., 2018). Previous studies by Yang et al. (2017) and Chauhan et al. (2011) also reported similar abundance in their examined maize rhizosphere. Furthermore, changes during the stages of growth could have been contributed by changes in the pH of the rhizosphere. Similarly, Xue et al. (2018) also reported chemical properties as a key factor that drives soil microbiome, and pH being a key chemical driver of the soil microbiome.

The richness and diversity varied between bulk soil and the different growth stages of the maize plants. This was expected as commonly the rhizosphere harbors decreased diversity and richness as compared to bulk soils since plants can select their community (Morella et al., 2020), as earlier highlighted. Some bacteria in the near roots commonly known as plant growth-promoting rhizobacteria (PGPR) are more closely linked with plant growth than bulk soils. Among them, some can produce substances that could restrict or inhibit the proliferation of other organisms including pathogens by producing antimicrobial chemical substances (Giorgio et al., 2015). The production of antibiotics by Streptomyces, a member of the phylum Actinobacteria - the second most dominant phyla, as earlier mentioned, opens the possibility of utilizing them against hazardous plant disease-causing organisms. Also, these microbes function in the solubilization of nutrients and nitrogen fixation, which could consequently alter the pH. Our study revealed the genera *Bacillus, Psychrobacter, Enterobacter, Pseudomonas,* which were present more abundantly throughout the

early stages of growth, are popular plant growth-promoting bacteria (PGPR) in the rhizosphere (Adedeji et al., 2020).

PGPR benefit plant growth and health to a large extent. The bacteria can increase the level of the available nutrients in the soil near the roots. Prior studies have reported the significant positive impact these microbes exhibited, even when compared to conventional fertilizers (Igiehon and Babalola, 2017; Lawal and Babalola, 2014). Most of these bacteria species are responsible for nitrogen fixation, phosphate solubilization, and plant disease suppression. In lieu of this, we further examined the relative distributions of the genera and families between the identified bacterial phyla in the rhizosphere across the growth stages of the maize plants.

6.5 Conclusion

In this study we showed that molecular techniques based on ribosomal genes using the Minon allow a detailed description of rhizobacteria communities independent of the uncertainties of older methods of cultivation. In addition to varying environmental factors and plant types, the physiochenical properties of the soil and growth phase of the plant impact the richness and diversity of bacteria in the rhizosphere of maize plants as there were significant changes in the soil bacteria community across the growth stages and in comparism with the bulk soils. Remarkably, the abundance of PGPRs suggests plant's ability to select a beneficial bacteria community as they grow which could have resultant effects on the growth and health of the plant. Nevertheless, some limitations have to be considered; the PCR maybe susceptible to amplification bias and although our statistical analysis showed good coverage of the rhizobacteria community, our sample size may not be sufficient to indicate absolute bacterial community representation of the farmland.

CHAPTER SEVEN

PROFILING FUNCTIONAL DIVERSITY OF MAIZE PLANT RHIZOSPHERE AS REVEALED BY SHOTGUN METAGENOMICS

Abstract

Reports on bacterial functionality in agricultural soils are still limited, and in this study, we employed a shotgun metagenomic method to compare bulk and maize rhizosphere soil collected from North-West University agricultural farmland. The data obtained in the near root community showed significant differences in some functional subsystems such as nitrogen fixation, carbohydrate metabolism, and metabolism of aromatic compounds which could be linked to pesticide usage and break down and also the role they play in growth promotion and nutrition. On the other hand, dormancy and sporulation, and motility had more sequences when compared with bulk soil. Nevertheless, it was striking that the variations in the diversity of taxa beforehand reported in the previous chapter were far greater than the functional diversity reported now, highlighting a high level of bacteria functional redundancy.

7.1 Introduction

In the soil, microorganisms play significant roles in dictating soil fertility, health, and nutrient cycling (Johns, 2017). In a gram of soil, there are thousands of bacteria, fungi and archaea taxa (Tkacz et al., 2020) which are characterized and mirrored by the variability of their protein functions with identifiable roles in the soil. Some of these functions have been well studied over the years, however, novel developments in high throughput sequencing technology empower scientists to further elucidate taxonomic, phylogenetic and functional diversities among soil microbes. In contrast to culture-based techniques which are known to limit viable but non-culturable bacteria, metagenomics has unlocked the prospect of studying not only unculturable microbes but also previously unknown bacterial phyla and new protein families that are yet to be detailed.

Conceivably, the soil habitat is one of the most studied ecosystems and is known to support almost all forms of life (Lladó et al., 2017; Ponge, 2015). Within the soil system and around the plant roots

are an enriched region commonly identified as a microbial hotspot termed “rhizosphere”. This near-root region is often considered to be a dynamic and complex microbiome (Philippot et al., 2013) owing to the complex enzymatic activities, microbe-plant and microbe-microbe interactions. In this region, microorganisms derive nutrients via the utilization of organic substances secreted by plants, such that it forms a key determinant of their assemblage and also activities around this plant terrain (Mendes et al., 2014). Additionally, the bulk soils influence the rhizosphere microbial community, and alterations in land use types, growth stages and other agricultural activities could impact the community structure and final composition of the soil microbiome (Pérez-Jaramillo et al., 2016).

It is well documented that maize plants secrete varied organic substances from amino acids, sugars and other organic substances (Lakshmanan et al., 2014), such that a continuous exudation of these substances could allow the establishment of a dynamic bacteria community. Activities of various enzymes are greater in the near root than in bulk soil (Asmar et al., 1995), as a consequence of increased microbial activity and enzymes produced in roots and by microbes. It has been well documented that biochemical measurements of soil enzyme activities could provide important information about microbial activities in the soil (Siczek et al., 2020), however, quantification and qualitative estimation could be challenging. Nevertheless, with high throughput sequencing, bacteria composition, assemblage, and metabolic profile can be rapidly analyzed (Feng et al., 2018; Zafra et al., 2016).

Considering microbiome studies, several hypotheses have been proposed, from the neutral theory to niche theory (Dumbrell et al., 2010). The neutral theory suggests that community structures and the compositions of species could be linked to the geographical distances in-between points of sample collections due to dispersal limitations, and microbes are usually well adapted functionally to an environment where they exploit a niche. Consequently, their abundance will tend towards a zero-sum multinomial distribution (McGill et al., 2006). The niche-based theory proposes that alterations in species compositions could be linked to differences in environmental variabilities (Mendes et al.,

2014) and could have consequent effects on soil functionality, as microorganisms have unique abilities to adapt and explore distinct ecological niches (McGill et al., 2007). Both theories highlight the link between soil microbial distribution and functions to environmental factors. As a consequence, we consider this information to be crucial for a more specific prediction and hence for the development of improved soil management strategies and sustainable agricultural practices. Prior studies on rhizobacteria communities have pinpointed the crucial roles plant species play in influencing community distribution. Investigations among others include cowpea (de Araujo et al., 2019), Arabidopsis (Bulgarelli et al., 2015), Oak (Uroz et al., 2010), Potato (Puri et al., 2019), Rice (Hong et al., 2016). Nevertheless, there is a paucity of information as to how the plant selects a particular rhizobacteria community from a highly contrasting pool of bulk soil communities, particularly in a semi-arid region.

Although knowledge on the taxonomy and phylogenetic distributions of bacteria communities is increasing, little has been suggested as to how the functional abilities of these communities change across biomes. From prior reports, distinct microbes have been implicated in individual processes such as N_2 fixation, specific extracellular enzymes and variations across space. Nevertheless, we lack an integrated understanding of how a pool of functional genes acts to structure communities across agricultural farmland. It is quite suggestive that there could be a correlation between functional characteristics and taxonomic compositions of the rhizobacteria community, however, this might differ as different taxa may have different responses to changes in the physiologies and environmental conditions and tolerance.

For several years, investigations have tried to determine the consequence of the plant community on resident soil microbes' diversity and function, but with limited success and using sometimes expensive methods that are restricted to few microorganisms and can only detect the activities of microbial biomass (Kaschuk et al., 2011). Now, employing the nanopore sequencing technology (MinION), the direct sequencing of raw DNA samples provides researchers notable perspicacity

into possible functions of rhizobacteria communities (Fadiji and Babalola, 2020) at a cheaper cost. A more comprehensive understanding of rhizobacteria attributes can be easily elucidated at far less cost, difficult traits that could not be identified with cultural techniques can also be viewed. To our knowledge, there are reports addressing the composition and community structures of the rhizobacteria community across several plant species, however, this is the first study that used the nanopore sequencing technology (MinION) and presents functional data of maize plant rhizosphere. With recent advancements in microbial ecology, it is vital to establish the functionality of bacteria in agricultural farmland, as our knowledge about this, especially in semi-arid soils where the samples for this study were collected, is still far from being clarified.

7.2 Materials and methodology

7.2.1 Sample collection

Soil samples were collected from North-West Teaching Farm, Mmabatho, Mafikeng, South Africa (see map in previous chapter). Sampling was carried out in November 2018 during the maize planting period. Six soil samples (3 bulk + 3 Rhizosphere) (~50 g each consisting of a pool of five subsamples collected at different points) were retrieved at 10 cm depth using a soil corer. Bulk soil were soil samples collected without vegetation. After extracting the plant, the soil that remained adhered to the root hairs after mild pulsation was considered as rhizosphere soils according to an operational definition (Chen et al., 2008). On dry ice, the soil samples were taken to the laboratory for further analysis.

7.2.2 DNA extraction and sequencing

Total DNA was extracted from 0.5 g (total humid weight) of soil using the Power Lyzer Power Soil DNA Isolation Kit (MoBIO Laboratories, Carlsbad, CA, USA), according to the manufacturer's instructions. The DNA extraction was performed in duplicate for each soil sample and pooled.

7.2.3 Amplification of rRNA operons

Touchdown PCR was used for the amplification of ribosomal operon. 2 μL of extracted soil DNA at the different stages of growth, high-quality Taq polymerase (Bimake LLC, Houston, TX, USA), Modified forward 16S rRNA primers, 27 Forward primer (5′ TTT CTG TTG GTG CTG ATA TTG C-[barcode overhang for PCR labeling]-AGA GTT TGA TCC TGG CTC AG 3′) (Kerkhof et al., 2017) and modified 23S rRNA-2241Reverse primer (5′ ACT TGC CTG TCG CTC TAT CTT C-[barcode overhang for PCR labeling]-ACC GCC CCA GTH AAA CT 3′) were utilized as described by (Ibironke et al., 2020). Briefly, initial denaturing temperature was set at 95^{o}C for 5 min, Double cycles for 20 sec at 95^{o}C for denaturation, primer annealing temperature was at 68^{o}C for 15 sec, extension was 72^{o}C for 75 sec. These were followed by 2 cycles of 66^{o}C, 64^{o}C, 62^{o}C for primer annealing, 22 cycles of denaturation, then 60^{o}C for primer annealing, extension at 72^{o}C for 90 sec and an ultimate extension at 72^{o}C for 5 min. After the 16th cycles consisting of 8 touch down and 8 standard cycles, 12 μL of amplification mix was withdrawn and kept at -80^{o}C. Next, amplification continued for up to 30 cycles, and PCR products were viewed using agarose gel electrophoresis. Purification of the 16-cycles PCR products was carried out and bead cleaned as stipulated by the above. Further, barcode amplification was carried out. Initial temperature of 95^{o}C for 5 min, then 30 cycles at 95^{o}C for 20 sec, 60^{o}C for 15 sec and 72^{o}C for 1.5 sec, these were followed by extension at 72^{o}C for 5 min. rRNA amplicons of sample were viewed an measured by gel electrophoresis7.2.4 Library preparation and sequencing by MinION

7.2.4 Library preparation

For the MinION library preparation, 2D sequencing kit (SQKLSK108-Oxford Nanopore; Oxford, UK). Barcoded amplicons were pooled together in low binding tubes, end-repaired, dA-tailed as described by Kerkhof et al. (2017) with little modifications. Blunt/ TA ligase master mix (NEB) was used for the ligation of oxford nanopore technology adaptor by adding 1 μL newly prepared

ATP solution (~3 mg/mL) to expedite the process. Libraries were analyzed on R9.4 flow cells. Next, poretools were used to open the 2D reads and respective fasta format files were exported.

7.2.5 Metagenome analysis pipeline in MG-RAST

Functional characterization for all samples (bacteria community) was executed utilizing the MG-RAST server, an open-source service for pipelines built for high-performance computing of taxonomic classification and functional analysis of metagenomes and a free receptacle for generated metagenomic data. Clean sequences generated by MinION were uploaded to the MG-RAST server for analysis. MG-RAST matches DNA sequences against a comprehensive collection of protein and nucleotide databases for the computerized assignment of metagenomic sequences to their corresponding groups. We utilized the pipeline options using the default settings in MG-RAST. Additional data quality control was done in MG-RAST, along with a normalization step.

For bacteria taxonomic designation, MG-RAST server distinguishes candidate RNA genes by matching the sequence data against rRNA databases Greengenes (DeSantis et al., 2006), RDP-II (Cole et al., 2007), the European 16S RNA database (Wuyts et al., 2002), and boutique databases (Leplae et al., 2004) concurrently. For functional characterization, we analyzed the gene subsystems—assortment of genes with functional designations that are linked in a biological process, for instance, in a metabolic pathway. Sequences obtained from the MinION were submitted to the MG-RAST (the Metagenomics RAST – http://metagenomics.anl.gov) and matched against SEED database for the functional annotation in subsystems (Overbeek et al., 2005). Considering the SEED website (http://theseed.org/wiki/Home_of_the_SEED), a subsystem describes a set of functional attributes that make up a metabolic pathway, a complex, or a class of proteins. The levels of subsystems studied in SEED are: (1) highest level; (2) second highest level; (3) similar to a KEGG pathway; (4) actual functional assignment to the feature in question. Data were matched with KEGG (Kyoto encyclopedia of genes and genomes) database (Kanehisa and Goto, 2000); For the BLAST (basic local alignment search tool, National Center for Biotechnology Information,

http:// www.ncbi.nlm.nih.gov/) search, a cut-off of minimum identity of 60% and E-value of $1x10^{-5}$ were considered.

Considering the solicitudes of agriculture in C and N metabolism in crop production, the sequences were also examined for the metabolic profile towards processes related to C and N metabolism, based on MG-RAST with the KEGG map.

7.2.6 Statistical analysis

Data generated from MG-RAST were subjected to statistical analysis using the STAMP (statistical analysis of metabolic profile) software (Parks and Beiko, 2010), to determine the statistical differences in the metabolic profiles of the metagenomes looking at all combinations in pairs. Statistical significance was evaluated with the Fisher's test for $p \leq 0.05$, adopting the method of Newcombe-Wilson with the correction of Benjamini–Hochberg FDR. Furthermore, Statistical analysis software (SAS) and STAMP were utilized, also, as STAMP exclusively allows the comparison of pairs of treatments, replicated data were also analyzed taking into account the number of reads in each functional category (Tukey, $p \leq 0.05$, ANOVA).

7.3 Results and Discussion

In our prior study, we detailed the variations in the community structures and compositions of bacterial diversity with respect to both maize rhizosphere and bulk soils. Our principal objective now is to view to what extent do the differences identified in the bacterial diversity would influence or reflect in potential functionalities in the soil. The significance of our investigation is based on the hypothesis that since there were variations in the bacterial population and the reported selectivity of plants, there will be changes in functionality (Kaschuk et al., 2010).

7.3.1 General sequencing analysis and functional profile classification

In this study, the six collected soil samples, comprising a pool of bulk and rhizospheric soils, resulted in over 2 million reads. One could suppose that metagenome describes the first step of study, then metatranscriptome and followed by metaproteome. However, in our investigation, all

assumptions will be referred to as potential functionality. In analyzing the sequences with the MG-RAST, we observed that 0.2% was classified as unknown sequences, 56.4% as nucleic sequences coding for known protein, 42.7% as nucleic sequences related to unknown proteins and 0.7% ribosome (Figure 7.1).

The MG-RAST analysis shows metabolic functions at four distinct levels, with subsystem denoting the highest level and specific genes being least of the levels. Our analysis revealed subsystem classification showed 28 functional categories (Figure 7.2). Notwithstanding the similarity within each gene category, there were some observable significant differences which could be of huge consequence or impact. For example, considering 1g of soil, resident are billions of bacterial species (Torsvik and Øvreås, 2002), a variation of as little as 0.4% could mean a difference of over 20 million metabolic functions. Generally, the six soil metagenome is made up of sequences classified in pathways of constitutive genes, enveloping proteins related to essential metabolism needed for bacteria survival in the ecosystem.

Of the 28 metabolic classifications identified, carbohydrate represents the highest, then amino acids and derivatives, while the subsystems of dormancy and sporulation showed the lowest number of sequences. Our result corroborates the finding of Frisli et al. (2013). In the clustering-based subsystems, there are indications of coupling of functional genes, displaying a group of hypothetical proteins described by joint localization of conserved patterns in different genomes (Gerdes et al., 2011) suggesting that these genes might possess some unknown functions. This is common in subsystems classification of functional metagenomic investigations (Delmont et al., 2012), which is an indication that only little is still known regarding the dynamics and structure of bacteria in our environment. These subsystems contain varied sequences coding for assumed genes linked to the metabolism of fatty acids, biosynthesis of lipopolysaccharides and galactoglucans and other functions.

Comparing the metabolic profile analysis using the level classification with STAMP showed significant differences in the two soil samples. Considering bulk soils, there are more sequences related to fatty acids, DNA metabolism, clustering-based subsystems, lipids and isoprenoids (Fig 7.2) (Table 1S). On the other hand, maize rhizosphere soils showed more sequences related to carbohydrates, amino acids and derivatives. It is suggestive that the inclusion of plant species influenced the higher carbohydrate and amino acid derivative sequences.

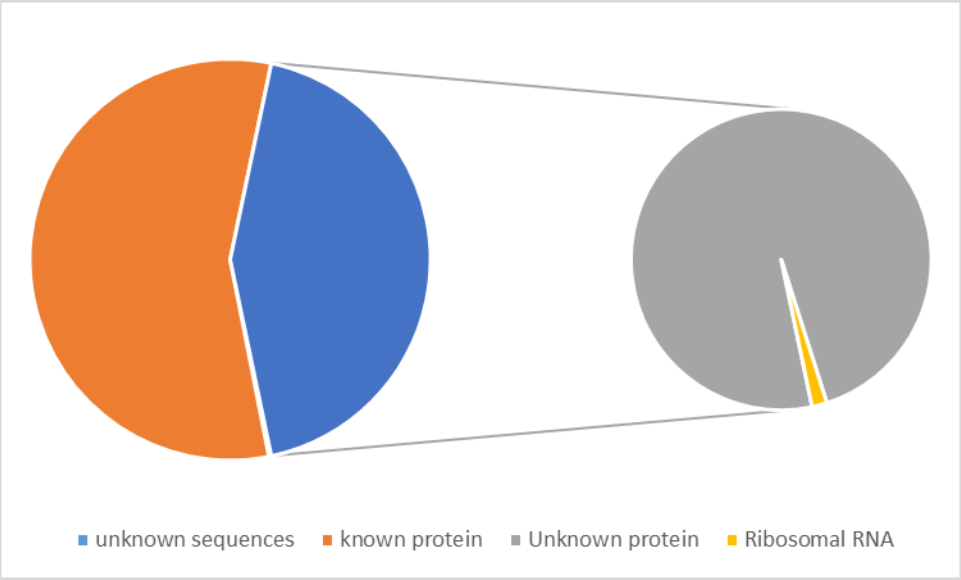
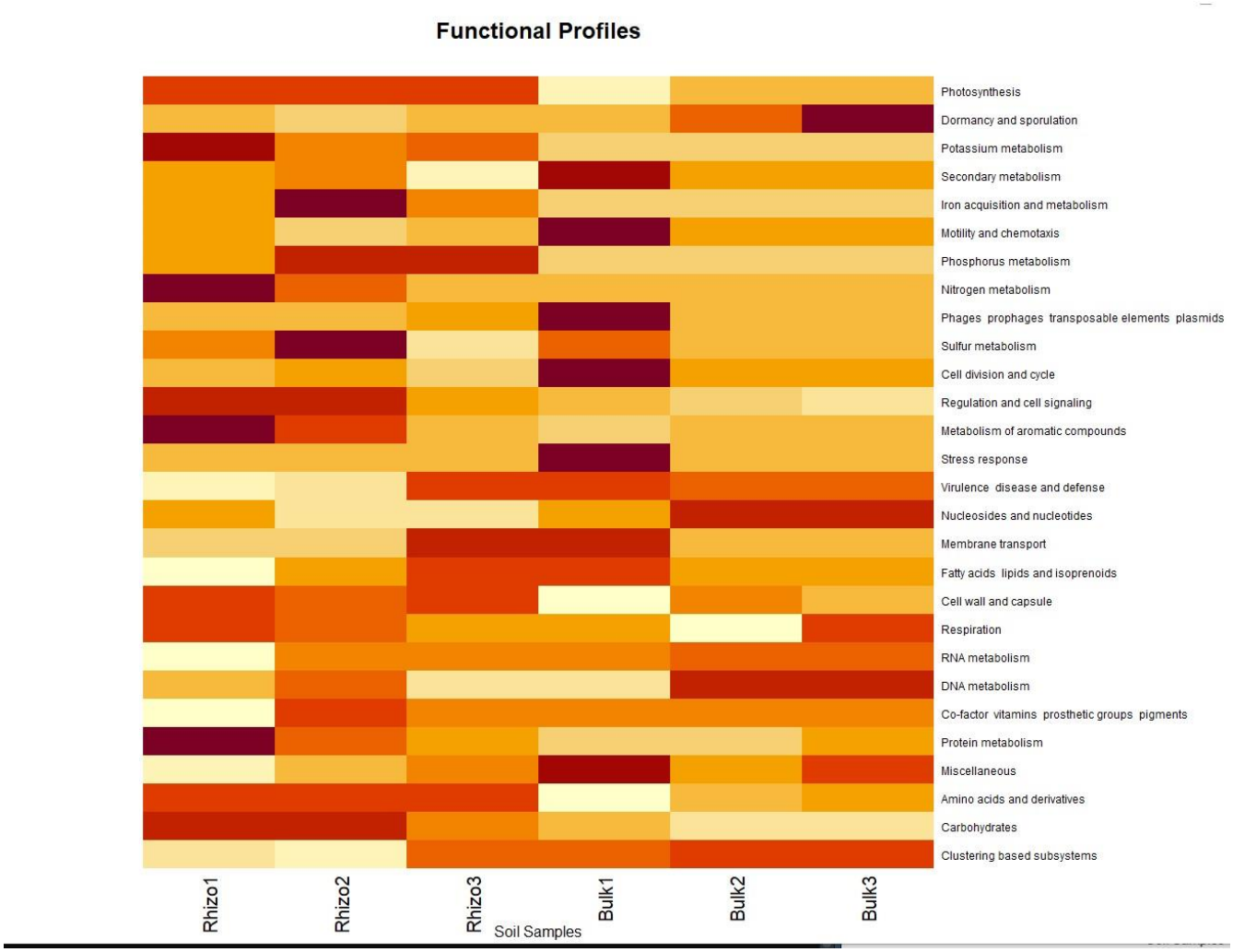


Figure 7.1: Classification of the sequences generated by MG-RAST annotation platform



Fugure 7.2: Heatmap of functional diversity and novelties related to and maize rhizosphere and bulk soils

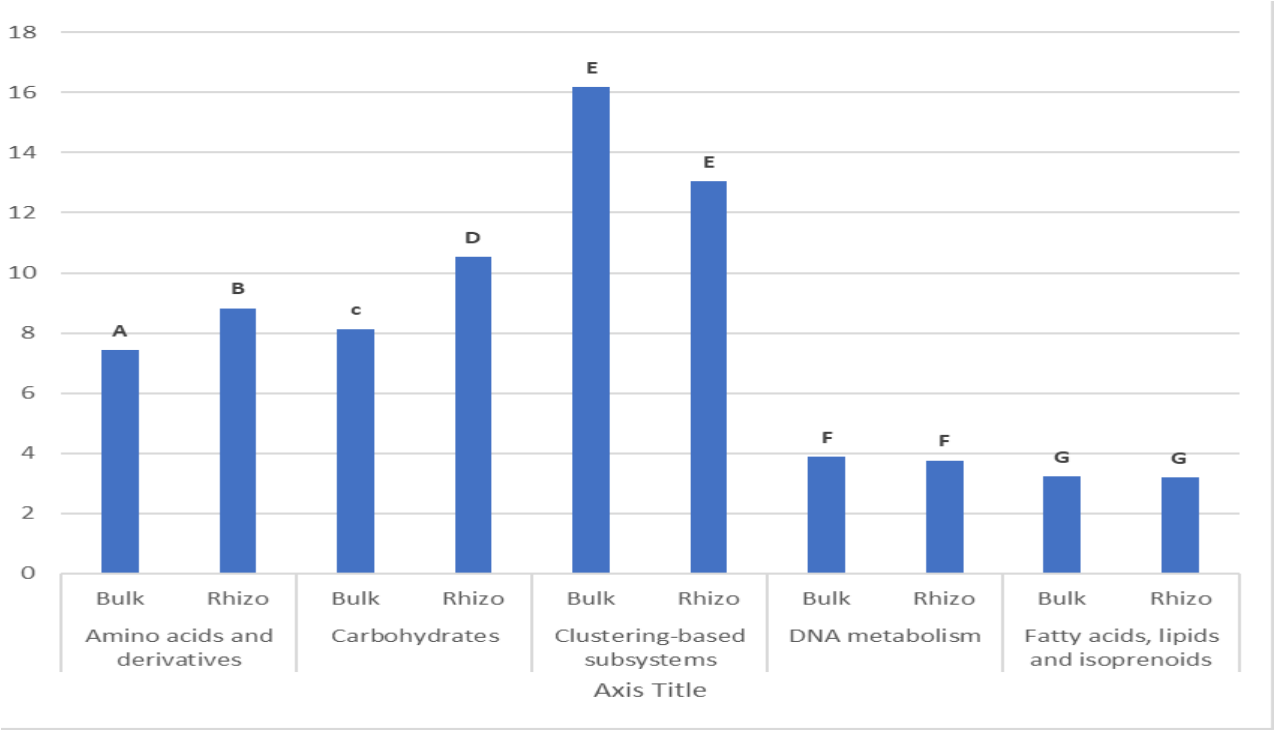


Figure 7.3: Statistically representations of the functional subsystem in level 1 of classification using the MG-RAST annotation platform.Bar chart comparing the average amount of selected functions present in the two samples. For amino acids and derivatives profile, the Bulk bar (7.42) is significantly different from that of the Rhizo (8.81). Means values statistically differ, hence the different letters. In the Carbohydrate profile, the Bulk bar (8.14) is significantly different from that of the Rhizo (10.52). These two means are statistically different, hence the different letters. In the Clustering-based subsystems profile, the Bulk bar (16.16) is not significantly different from that of the Rhizo (13.04), hence the same letters. In the DNA metabolism profile, the Bulk bar (3.87) is not significantly different from that of the Rhizo (3.75), hence the same letters. In the Fatty acids, Lipids and isoprenoids profile, the Bulk bar (3.22) is not significantly different from that of the Rhizo (3.19), hence the same letters.

7.3.2 Functional diversity and novelties related to bulk and maize rhizosphere soils

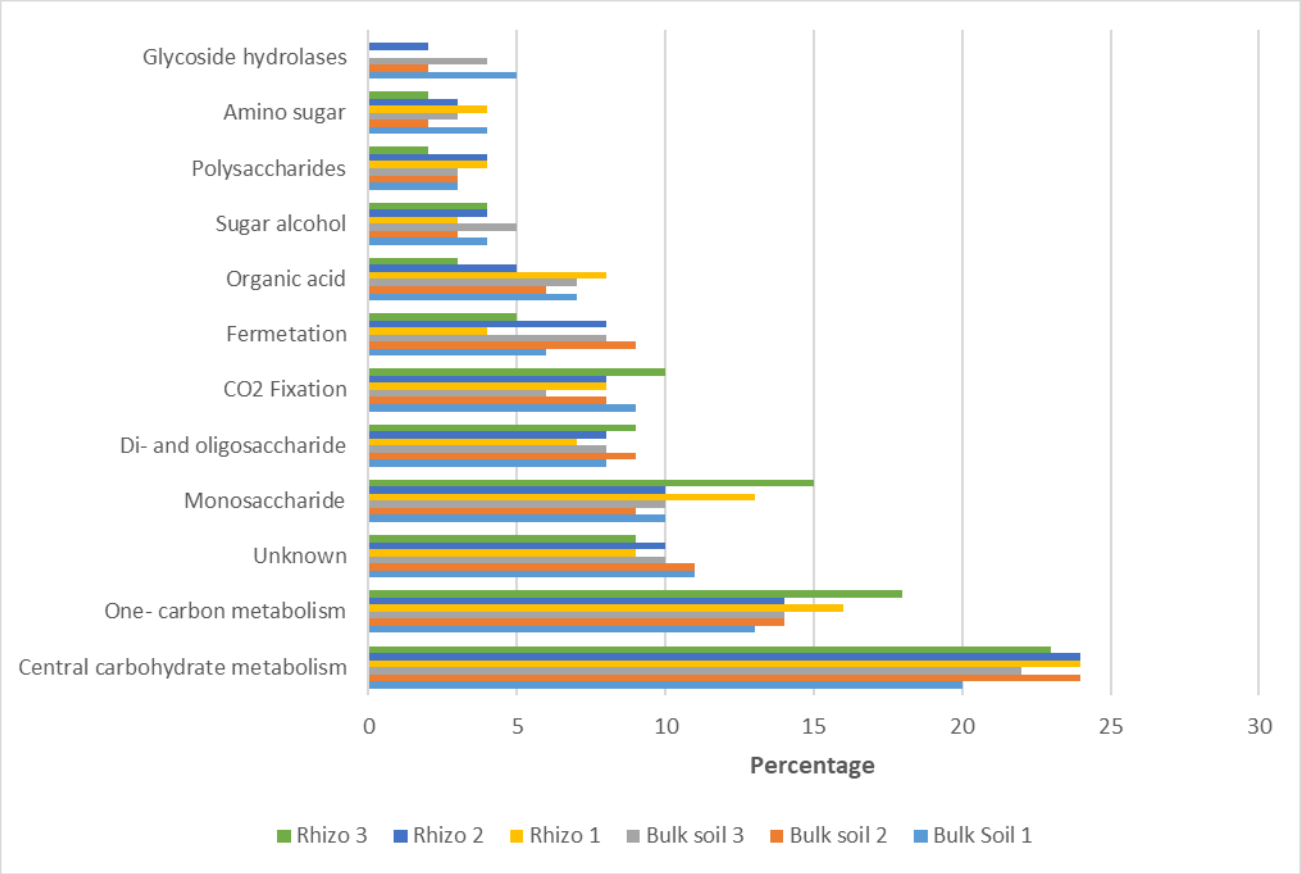


Figure 7.4. Distribution of level 2 of the carbohydrate subsystem generated by the MG-RAST in six metagenomes of the collected soil samples.

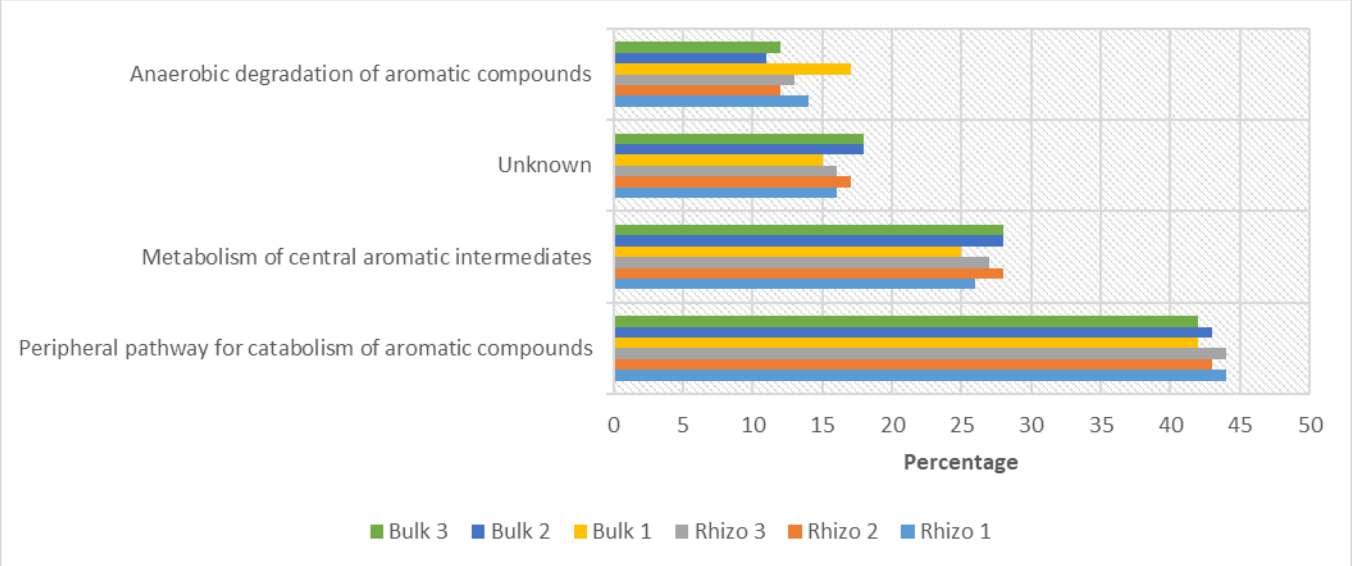


Figure 7.5: Distribution of level 2 of the metabolism of aromatic compounds generated by the MG-RAST in six metagenomes of both bulk and maize rhizosphere soil sample

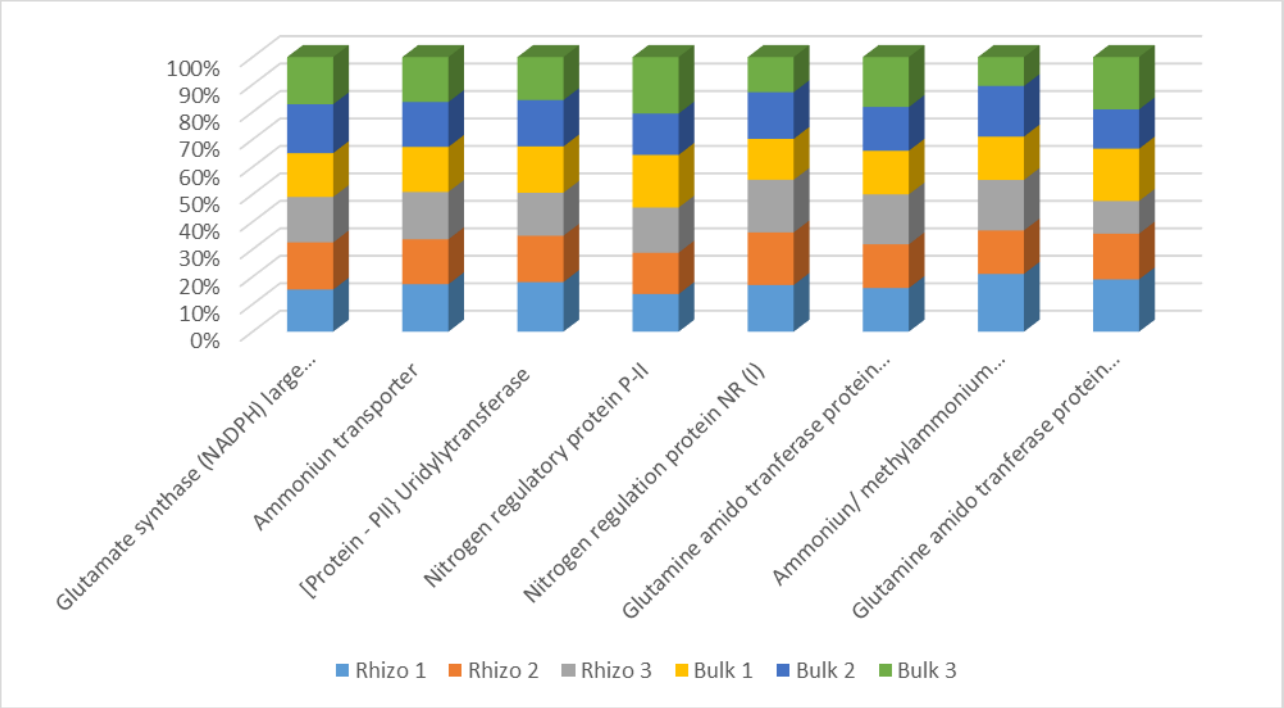


Figure 7.6: Functional distribution of the assimilation of ammonia in the subsystem of metabolism of N generated by MG-RAST in the six metagenomes

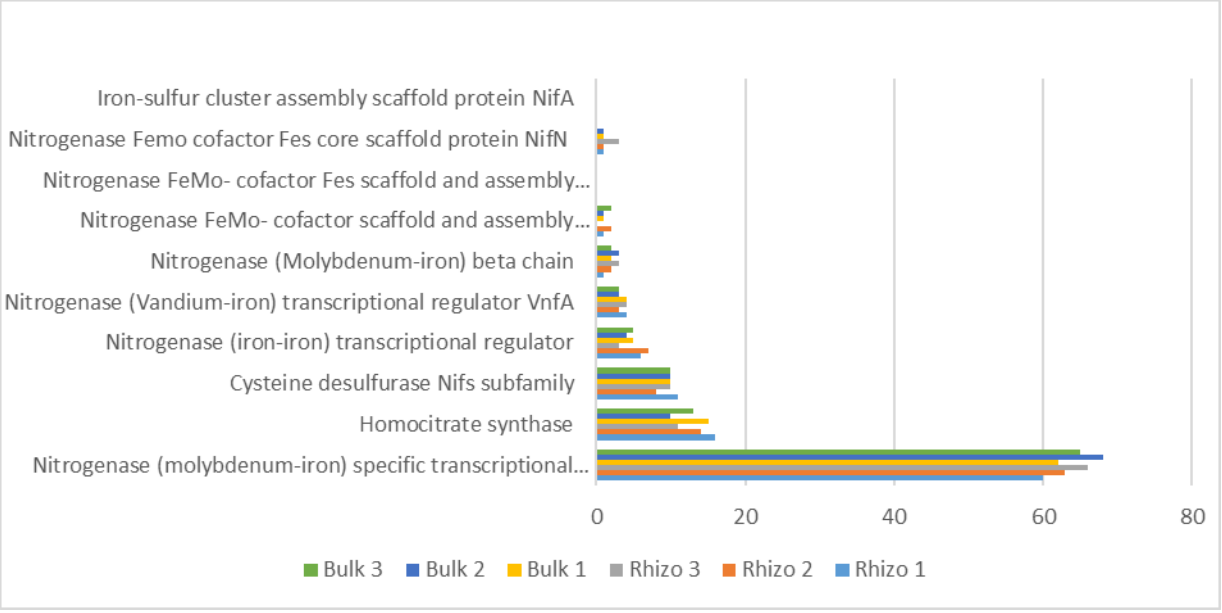


Figure 7.7: Functional distribution of the nitrogen fixation-related genes in the subsystem of metabolism of N generated by MG-RAST in six metagenomes of bulk and maize rhizosphere soil.

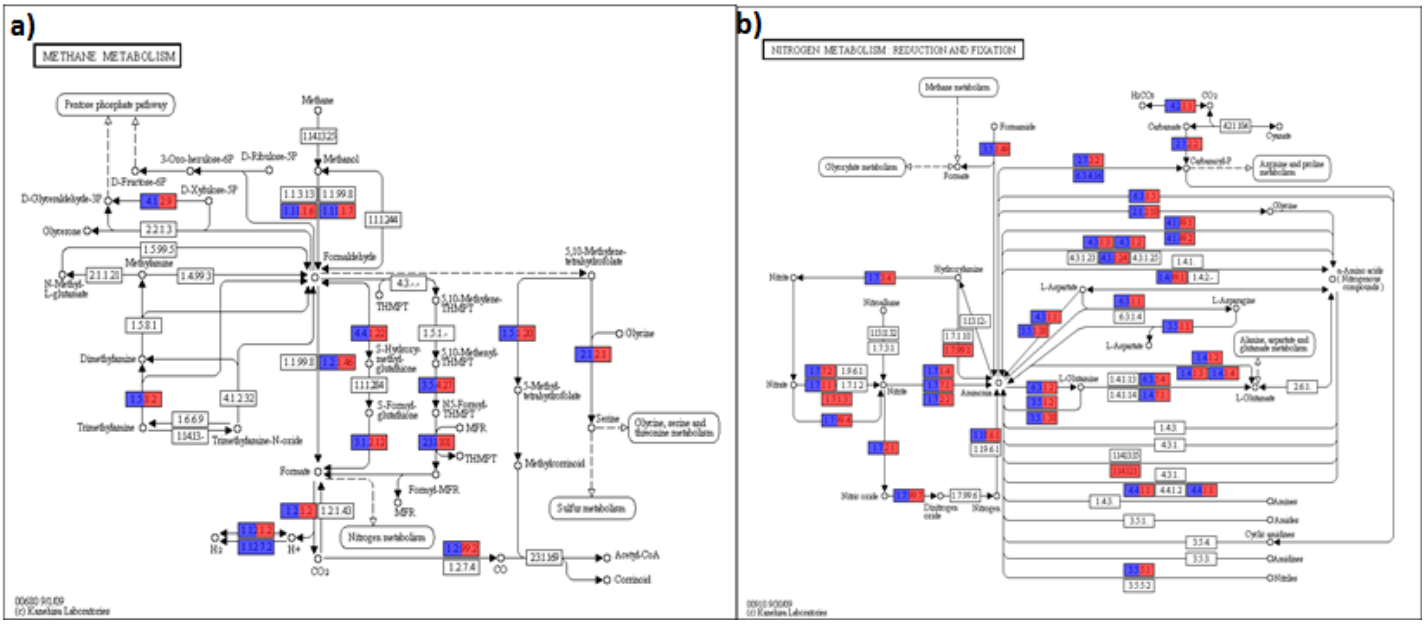


Figure 7.8: Metabolic route of KEGG, for the (a) methane and (b) nitrogen; in blue is the bulk soil and in red the rhizosphere soil.

Functional profiles of maize rhizosphere sample and bulk soil predicted carbohydrate as core functions of the samples as analyzed according to SEED databases while iron acquisition and metabolism and membrane transport were also identified according to the SEED database (Table 1S).

Considering metabolism of carbohydrates at subsystem level 2 classification, the central carbohydrate metabolism showed highest percentage of sequences (figure 7.3), comprising of genes related to pentose-phosphate, tricarboxylic acid (TCA), glycolytic and Entner–Doudoroff pathways. These genes are very critical for the energy-demanding microbial processes.

The measure of carbohydrate present in the soil depends on the plant and animal input, and also their decomposition rate. Carbon sources are required as energy for microbial breakdown processes to be achieved (da Silva et al., 2014). Our study showed the content of C and N were more in the maize rhizosphere soil than bulk soil. It is suggestive that root exudates might favor this and also carbon metabolic pathway which is commonly utilized when carbon sources (exudates) are broken down. We may hypothesize that the increasing diversity of crop residues cause increase in diversity of pathways to use varied sources of carbohydrates.

Furthermore, considering the location and activities around the site of soil collection, we also gave considerations to subsystems of aromatic compounds as farmlands commonly experience an influx of heavy application of pesticides, consequently, microbes develop ways to obtain energy from these substances. Although with fewer sequences shown, we obtained central and peripheral pathways of the catabolism of aromatic compounds (Fig 7.4). For example, from prior studies on degradation of n-phenyl alkanoic acid by *Pseudomonas putida* (Olivera et al., 2001), degradation of N-heterocyclic aromatics sequences attributed have been identified (Arora, 2015b). Bacteria belonging to the genera *Pseudomonas, Rhodococcus,* and *Acinetobacter* all can degrade anaerobically, which may be associated with the breakdown of xenobiotics (Zhang et al., 2020). From our previous study, we observed that *Pseudomonas* spp and other bacteria with degradative abilities were prevalent in the maize rhizosphere soils, suggesting their role as a key contributor in the breakdown processes in the near root. Considering the breakdown of aromatic compounds, maize rhizosphere soil showed significantly more sequences than bulk soils**.** We viewed that this may be attributed to the addition, breakdown of pesticides and other organic compounds. As also earlier highlighted in prior studies that soils with large quantities of organic matter content have a prominent impact on degradation (Uroz et al., 2013), as higher organic matter content causes absorbsion of pesticides and also increased activities of bacteria that can use carbon sources (Souza et al., 2013).

Considering our matches with the KEGG database, it is important to pinpoint that we identified in both bulk soil and rhizosphere genes related to the metabolism of methane (Fig. 7.7). Several hits were linked to the transformation of methanol to formaldehyde, and one ferredoxin hydrogenase (EC 1.12.7.2) was found exclusively in the bulk soil, indicating that methane pathways may occur not only in anaerobic conditions (Angel et al., 2012) but also in aerobic soils.

KEGG maps provide a definite view of the metabolic pathways. For instance, in the metabolism of mannose and fructose, some essential enzymes were found particularly in bulk soil (EC 2.4.1.217; EC 1.1.1.17) and others solely in rhizosphere soil (EC 2.7.1.51; EC 3.1.3.70). Fundamental

enzymes for the breakdown of aminobenzoate, fluorobenzoate and dioxin were seen exclusively in the bulk soil (EC 5.5.1.7 and EC 1.14.12.18), and in the rhizospheric soil, there were enzymes for the breakdown of benzoate (EC 1.14.12.1; EC 5.3.3.4) and naftalene (EC 1.13.11.38). These suggest notable facilities break down some xenobiotics in bulk and rhizosphere soils; notwithstanding, there are possibilities that despite the deficiency of distinct enzymes in some of the soil samples, breakdown of these compounds may still be carried out.

Concerning subsystems of hydrogen metabolism, the majority were linked to ammonia assimilation, large proportions of key enzymes glutamine synthase and glutamate synthase (Figure 7.5). (Bernard and Habash, 2009). Our findings support the report of Fierer et al. (2012) on soil microbial communities across nitrogen gradients, which inferred shifts in the prevalence of microbial population, favoring the breakdown of proteins, related to a more active copiotrophic. Biological nitrogen fixation is an important process in crop productivity, we identified significant difference in the soil samples. We recovered more genes associated with nitrogen fixation, ammonification, denitrification in the rhizosphere. The prevalence of a considerable contingent of nitrogen fixation gene could be linked to increased amino acids and derivatives (Signorelli et al., 2020). We noticed more sequences correlated with nitrogenase genes transcriptional regulation nifA (Fig 7.6). Strikingly, the amount of genes linked with various components and types of nitrogenase varies. Nevertheless, little is known regarding the influence on the soil. With respect to membrane transport, we also identified higher sequences in maize rhizosphere soil affiliated with secretion system IV, which is commonly linked with mutual interactions among bacteria species and other microorganisms (Burke et al., 2011). Annotation of sequences associated with phosphorus metabolism in the collected soil samples revealed the predominance of genes linked with the uptake of P and utilization of alkylphosphonate, which shows the augmentation of plant P availability by P solubilization and mineralization bacteria (Marschner et al., 2011). Potassium is one of the vital macronutrients required for adequate crop growth (Adedeji et al., 2020). Microbes have a pivotal role in the K cycle, with specific groups of bacteria that can mobilize in an accessible in soil (Kour

et al., 2020). Generally, iron is deficient in most soils, in the root region, Fe is mobilized by plant-microbe interactions and there is always a strong competition for iron uptake (Concha and Doerner, 2020).

MG-RAST also revealed another remarkable subsystem in regulation and signaling with more sequences in the rhizosphere soil (Fig 7.2). Considering this subsystem, importance was placed on genes of the enzyme adenylate cyclase (cAMP) signaling system in bacteria. cAMP is viewed as an essential secondary messenger employed in intracellular transduction in bacteria. It is involved in the regulations of vital physiological processes which could include virulence factors in disease-causing bacteria (Venturi and Keel, 2016). It is also seen as an internal cell warning for abiotic and biotic stresses. Commonly, bacteria are constantly undergoing varied changes at substrate level, and thus cAMP controls energy and cell metabolism, as well as communications between cells. The prevalence of this subsystem in the rhizosphere suggests the reasons for the stability of certain communities and the selectivity of bacteria in the near root. Other subsystems were also considered in comparison e.g of virulence disease and defense, regulation and cell signaling, sulfur metabolism, phages and correlates, fatty acids, lipids and isoprenoids and secondary metabolism (Fig. 7.2).

7.4 Conclusion

Whole-genome sequencing has been reaching fitness proving and standardizing experimental and bioinformatics tools and methods to elucidate typical targeted ecological queries. Results obtained helps not only microbial community analysis, alike the quantification of gene families, prevailing metabolism and functional modules. Our study suggests that maize plants select bacterial communities based on their functional attributes, which may be linked to the roles they play in the near root. From our previous study, we identified more abundance of bacteria which might explain some versatility in functionality. Nevertheless, differences in the taxa identified do not correspond with functional traits identified, which suggests some level of bacterial redundancy.

CHAPTER EIGHT

CONCLUSION, SUMMARY AND RECOMMENDATION

Plants and microorganisms are in a partnership. Rhizosphere microbiome presents a significant role in the development and functioning of plant growth and health. In this region, bacteria, fungi, viruses, and archaea are part of a complex trophic web that uses an extensive spectrum of nutrients secreted by the plant root. On the other hand, plants are viewed to be associated with particular groups of microorganisms interacting with one another and forming a structure of individuals commonly called holobionts. As it is well-established that beneficial relationship exists between most plants and bacterial communities, plants can also impact the chemical and microbial composition of the rhizosphere, which is the soil area under the influence of the root.

To select or shape the plant-associated bacteria community and also their functional attributes, these requires a highly selective pressure that acts upon distinctive components of the holobiont which put an immense influence on the fitness of plant species. One of such is the changes in physiological conditions of the plant (e.g growth stages of the plant) and edaphic properties. However, little has been known about how these cause changes through the plant's development, as most investigations focus either on later developmental stages of the plants alone when the root system is already firmly established or on planting had been done in the green house.

Our study successfully investigated the influence of physicochemical attributes of maize rhizosphere soils at two different growth stages (where the root was just developing and when fully developed) and the influences of these parameters on bacterial diversity. We also considered the functional profiles of both the rhizospheric and bulk soils. Some identified physicochemical properties of the soils were noticed to be strong drivers of bacterial diversity shift during the early stages. These key drivers were chemical elements commonly found in the conventional fertilzers applied in this farmland. Also, the soil pH was immensly implicated. We hypothesised that soil pH also causes this changes by modifying enzyme activity through controlling the accessibility of nutrients and moisture by changing the ionisation balance in soil. Soil pH influenced the ionisation

equilibrium of ammonia and nitrare in soils and as consequence drove community composition and the biogeography of ammonia oxidisers. Considering bacteria diversity, several plant growth promoting bacteria were recovered during the stages. This emphasised the important roles they play in land developmental processes. Some bacteria species increased while some decreased as we presumed nutrients were limited at the latter stages. Strikingly we also recovered some pathogenic enteric organisms, an indication of the manure input in the soil. This calls for public health concern as innoculation of such might be dangerous.

In the near root, rhizo- deposits or exudates fuel substrate driven community shifts that cause the biggest influence on rhizobacteria.The different organic substances secreted in the root commonly serve as chemo-attaractants for specific groups of bacteria. Also, the chemical conditions of the root are altered and as a consquence favor the existence of certain bacteria species. In our study we did not determine the quantity or quality of the depositions by these maize plants. Nevertheless, functional profiles as revealed by MG-Rast predicted likely activities that go on in the root. For further study, we recommend that the quality and quantity of these organic compounds be determined.

Our study revealed some specific functional attributes linked with both bulk and rhizospheric soils in high proportion, for example, carbohydrate metabolism. Nevertheless, it was viewed that considering the diversity reported in the two samples, one should expect far greater functional differences than what we recorded. We consequently infered some level of functional redundancy, indicating that two or more taxa could be performing alike roles within the bacteria community.

Conclusively, we recommend that bacteria succession, diversity and functionality be determined beyound just two stages. In our case we were limited by funds at time of sampling and also accessibility to the farms was also restricted due to security reasons. Furthermore, although considerable levels of normalization have been achieved concerning metagenomics, metatranscriptomics and metaproteomics, more still need to be done pertaining experimental,

computational pipelines which can be significant to the efficient investigation of transcriptional regulation and metabolite dynamics of microbial population. Also, Systemic variations in dissimilar project platforms, technical inconsistencies between data sets need to be addressed. Nevertheless, we believe integrations of metabolomics and metatranscriptomics will present a more comprehensive detailed overview of dynamic microbial systems. More advanced computer operating systems amalgamated with an improved meta-omics will further enhance our knowledge of functional aspects of microorganisms.

SUPPLEMENATRY DATA

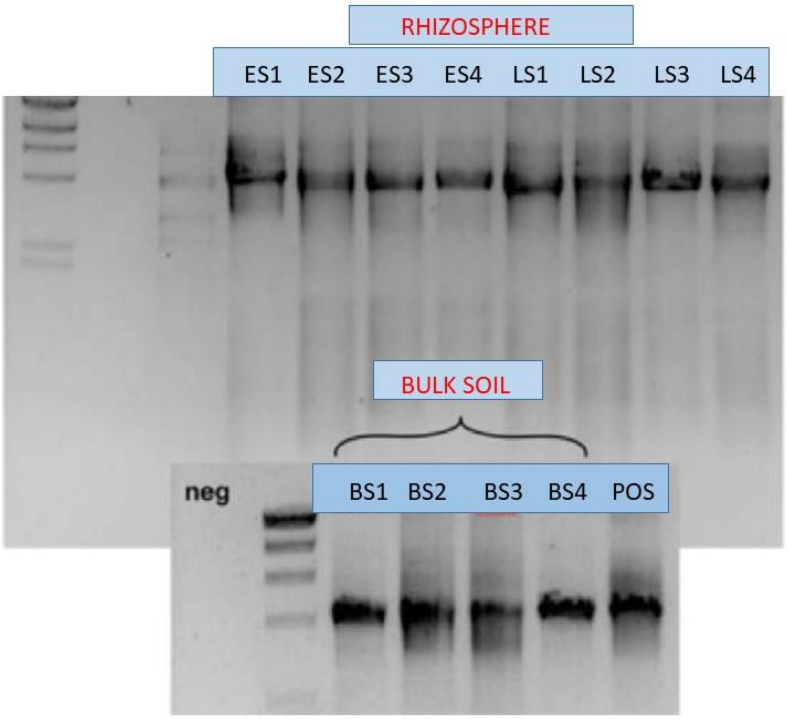


Figure 1S: PCR amplification of RNA operon from the soil samples

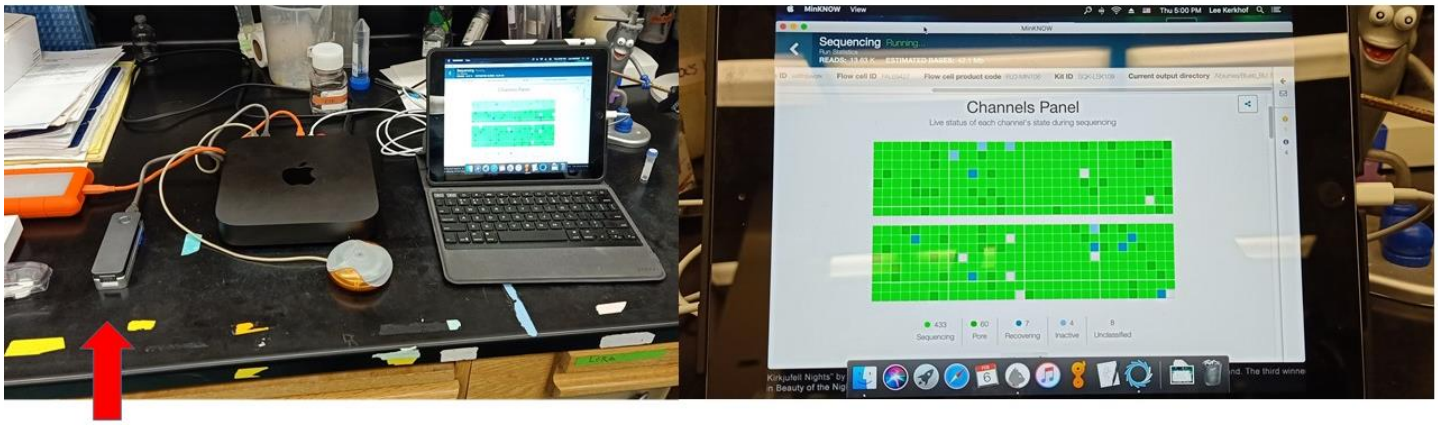


Figure 2S: A running Oxford nanopore sequencing technology (MinION) sequencer

Table 1S: Functional classification of the genes detected in six metagenomes of both bulk and rhizospheric soils.

	Rhizo 1	Rhizo 2	Rhizo 1	Bulk 1	Bulk 2	Bulk3
Clustering-based subsystems	12.31	11.3	15.5	15.5	16.5	16.5
Carbohydrates	10.94	10.81	9.82	8.82	7.8	7.8
Amino acids and derivatives	8.86	8.78	8.8	6.6	7.78	7.9
Miscellaneous	6.43	7.59	8.58	10.58	8.39	9.59
Protein metabolism	7.57	7.32	7.2	7.11	7.1	7.2
Co-factor, vitamins, prosthetic groups, pigments	3.72	5.9	5.32	5.32	5.39	5.39
DNA metabolism	3.75	3.87	3.63	3.63	3.99	3.99
RNA metabolism	3.1	3.6	3.61	3.61	3.71	3.71
Respiration	3.53	3.51	3.48	3.48	3.42	3.52
Cell wall and capsule	3.77	3.62	3.63	1.67	3.1	2.6
Fatty acids, lipids and isoprenoids	3.11	3.2	3.25	3.25	3.2	3.2
Membrane transport	3.82	3.82	3.99	3.99	3.83	3.83
Nucleosides and nucleotides	3.01	1.02	1.02	3.02	5.03	5.03
Virulence, disease and defense	1.03	1.15	3.16	3.16	3.02	3.02
Stress response	2.64	2.61	2.62	3.62	2.66	2.63
Metabolism of aromatic compounds	4.11	3.28	2.29	2	2.19	2.4
Regulation and cell signaling	2.64	2.65	1.9	1.78	1.59	1.4
Cell division and cycle	1.36	1.38	1.22	1.85	1.37	1.39
Sulfur metabolism	2.33	3.35	1.48	2.48	1.84	1.9
Phages, prophages, transposable elements, plasmids	1.33	1.32	1.48	2.48	1.34	1.34
Nitrogen metabolism	3.08	2.12	1.13	1.13	1.09	1.09
Phosphorus metabolism	1.28	1.92	1.89	0.89	0.91	0.91

Motility and chemotaxis	1.07	0.92	0.93	1.93	1.08	1.08
Iron acquisition and metabolism	0.88	1.61	1	0.6	0.65	0.65
Secondary metabolism	0.51	0.53	0.46	0.58	0.52	0.52
Potassium metabolism	2.42	1.43	1.59	0.59	0.44	0.44
Dormancy and sporulation	0.23	0.2	0.22	0.21	0.3	0.43
Photosynthesis	1.18	1.19	1.21	0.21	0.71	0.61

Table 2S: Stastistical tables of SAS analysis

Analysis Variable : Values Values			
Functions	**Cat2**	**N Obs**	**Mean**
Amino acids and derivatives	Bulk	3	7.4266667
	Rhizo	3	8.8133333
Carbohydrates	Bulk	3	8.1400000
	Rhizo	3	10.5233333
Clustering-based subsystems	Bulk	3	16.1666667
	Rhizo	3	13.0366667
DNA metabolism	Bulk	3	3.8700000
	Rhizo	3	3.7500000
Fatty acids, lipids and isoprenoids	Bulk	3	3.2166667
	Rhizo	3	3.1866667

Dependent Variable: Values Values

Nutrient=Amino acids and derivatives

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	2.88426667	2.88426667	11.14	0.0289
Error	4	1.03573333	0.25893333		
Corrected Total	5	3.92000000			

Nutrient=Amino acids and derivatives

Levene's Test for Homogeneity of Values Variance
ANOVA of Squared Deviations from Group Means

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Cat2	1	0.1764	0.1764	3.97	0.1170
Error	4	0.1776	0.0444		

Carbohydrate

Nutrient=Carbohydrates

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	8.52041667	8.52041667	23.60	0.0083
Error	4	1.44406667	0.36101667		
Corrected Total	5	9.96448333			

R-Square	Coeff Var	Root MSE	Values Mean
0.855079	6.438792	0.600847	9.331667

Clustering based system

Nutrient=Clustering-based subsystems

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	14.69535000	14.69535000	5.72	0.0751
Error	4	10.27873333	2.56968333		
Corrected Total	5	24.97408333			

Nutrient=DNA metabolism

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.02160000	0.02160000	0.75	0.4353
Error	4	0.11520000	0.02880000		
Corrected Total	5	0.13680000			

Nutrient=Fatty acids, lipids and isoprenoids

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	1	0.00135000	0.00135000	0.46	0.5347
Error	4	0.01173333	0.00293333		
Corrected Total	5	0.01308333			

A one factor analysis of variance (ANOVA) test was conducted to evaluate the null hypothesis that there is no difference in the selected functional compositon of the soil used. SAS software was used for the analysis and a statistical significance level of 0.05 was used to determine the difference in the **LandCat** for the Rhizo and Bulk**.** Also, the data did not violate any assumption of the test used (i.e data is normally distributed, does not violate homoscedasticity and the variables are independent). Between the Rhizo and the Bulk soil, at 0.05 significance level, there exist significant variations for the selected Nutrients (Amino Acids and Derivatives [F (1, 4) = 11.4, p-value = 0.0289], carbohydrates [F (1, 4) = 23.60, p-value = 0.0083]). However, there exist no significant variation on average in the amount of functional profiles (Clustering based system, DNA metabolism and fatty acids, lipids and isoprenoids) with p-values 0.0751, 0.4353 and 0.5347 respectively between the Rhizo and Bulk soil.

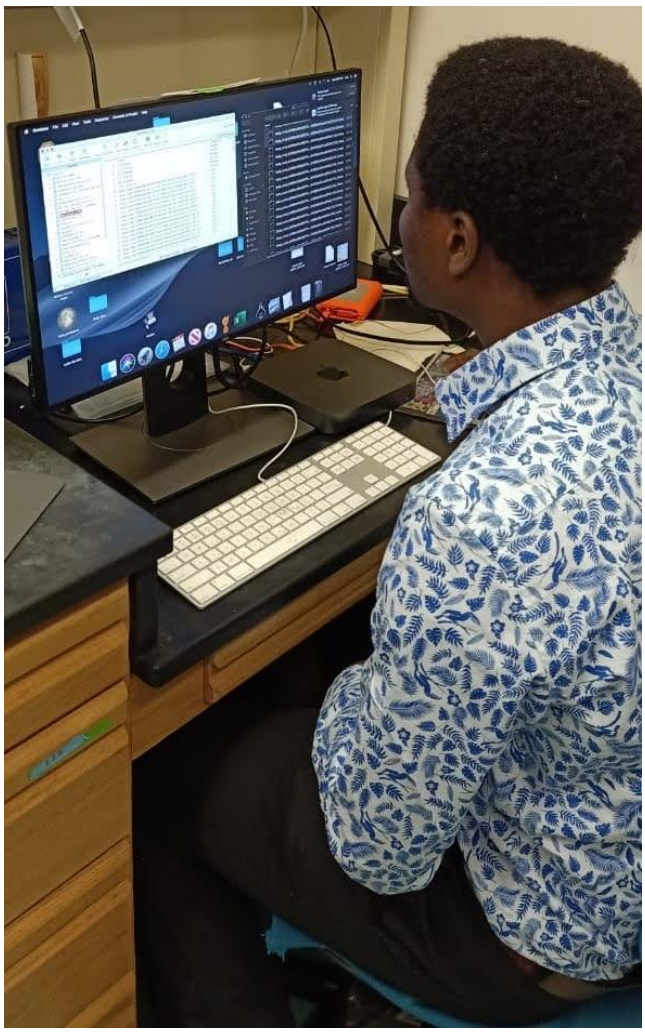


Figure 3S: Deciphering microbial community on the macintosh

REFERENCES

Abdallah, R. A. B., Mejdoub-Trabelsi, B., Nefzi, A., Jabnoun-Khiareddine, H., and Remadi, M. (2016). Isolation of endophytic bacteria from Withania somnifera and assessment of their ability to suppress Fusarium wilt disease in tomato and to promote plant growth. *J Plant Pathol Microbiol* **7**.

Abdel-Lateif, K., Bogusz, D., and Hocher, V. (2012). The role of flavonoids in the establishment of plant roots endosymbioses with arbuscular mycorrhiza fungi, rhizobia and Frankia bacteria. *Plant signaling & behavior* **7**, 636-641.

Abhilash, P., Powell, J. R., Singh, H. B., and Singh, B. K. (2012). Plant–microbe interactions: novel applications for exploitation in multipurpose remediation technologies. *Trends in biotechnology* **30**, 416-420.

Aboody, M. S. A., and Mickymaray, S. (2020). Anti-Fungal Efficacy and Mechanisms of Flavonoids. *Antibiotics* **9**, 45.

Adedeji, A. A., Häggblom, M. M., and Babalola, O. O. (2020). Sustainable agriculture in Africa: Plant growth-promoting rhizobacteria (PGPR) to the rescue. *Scientific African*, e00492.

Afifi, A.-A. M., El-Laithy, A. Y., Shehata, S. A., and El-Saiedy, E.-S. M. (2010). Resistance of strawberry plants against the two-spotted spider mite, Tetranychus urticae (Acari: Tetranychidae). *In* "Trends in Acarology", pp. 505-507. Springer.

Agbodjato, N. A., Amogou, O., ewadjou, Noumavo, P. A., Dagb, enonbakin, G., Salami, H. A., Karimou, R., Allad, e, A.-M., Adedayo, O., Baba-Moussa, F., and Adjanohoun, A. (2018). Biofertilising, plant-stimulating and biocontrol potentials of maize plant growth promoting rhizobacteria isolated in central and northern Benin. *African Journal of Microbiology Research* **12**, 664-672.

Ahemad, M., and Khan, M. S. (2011). Effect of tebuconazole-tolerant and plant growth promoting Rhizobium isolate MRP1 on pea–Rhizobium symbiosis. *Scientia horticulturae* **129**, 266-272.

Ahemad, M., and Khan, M. S. (2012). Effect of fungicides on plant growth promoting activities of phosphate solubilizing Pseudomonas putida isolated from mustard (Brassica compestris) rhizosphere. *Chemosphere* **86**, 945-950.

Ahemad, M., and Kibret, M. (2014). Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. *Journal of King saud University-science* **26**, 1-20.

Ahmad, N., Bibi, Y., Raza, I., Zahara, K., Khalid, N., Bashir, T., and Tabassum, S. (2015). Traditional uses and pharmacological properties of Alhagi maurorum: A review. *Asian Pacific Journal of Tropical Disease* **5**, 856-861.

Ahmed, E., and Holmström, S. J. (2014). Siderophores in environmental research: roles and applications. *Microbial biotechnology* **7**, 196-208.

Aira, M., Gómez-Brandón, M., Lazcano, C., Bååth, E., and Domínguez, J. (2010). Plant genotype strongly modifies the structure and growth of maize rhizosphere microbial communities. *Soil Biology and Biochemistry* **42**, 2276-2281.

Aislabie, J., Deslippe, J. R., and Dymond, J. (2013). Soil microbes and their contribution to soil services. *Ecosystem services in New Zealand–conditions and trends. Manaaki Whenua Press, Lincoln, New Zealand*, 143-161.

Ajilogba, C. F., and Babalola, O. O. (2016). RAPD Profiling of Bacillus spp with PGPR Potential and Their Effects on Mineral Composition of Tomatoes. *Journal of Human Ecology* **56**, 42-54.

Akiyama, K., Matsuzaki, K.-i., and Hayashi, H. (2005). Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* **435**, 824-827.

Alawiye, T. T., and Babalola, O. O. (2019). Bacterial diversity and community structure in typical plant rhizosphere. *Diversity* **11**, 179.

Aleklett, K., Leff, J. W., Fierer, N., and Hart, M. (2015). Wild plant species growing closely connected in a subalpine meadow host distinct root-associated bacterial communities. *PeerJ* **3**, e804.

Amborabé, B.-E., Fleurat-Lessard, P., Chollet, J.-F., and Roblin, G. (2002). Antifungal effects of salicylic acid and other benzoic acid derivatives towards Eutypa lata: structure–activity relationship. *Plant Physiology and Biochemistry* **40**, 1051-1060.

Amoo, A. E., and Babalola, O. O. (2019). Impact of land use on bacterial diversity and community structure in temperate pine and indigenous forest soils. *Diversity* **11**, 217.

Angel, R., Claus, P., and Conrad, R. (2012). Methanogenic archaea are globally ubiquitous in aerated soils and become active under wet anoxic conditions. *The ISME journal* **6**, 847-862.

Armanhi, J. S. L., de Souza, R. S. C., Damasceno, N. d. B., de Araújo, L. M., Imperial, J., and Arruda, P. (2018). A community-based culture collection for targeting novel plant growth-promoting bacteria from the sugarcane microbiome. *Frontiers in plant science* **8**, 2191.

Arora, N. K. (2015a). "Plant microbes symbiosis: Applied facets," Springer.

Arora, P. K. (2015b). Bacterial degradation of monocyclic aromatic amines. *Frontiers in microbiology* **6**, 820.

Asad, S. A., Farooq, M., Afzal, A., and West, H. (2019). Integrated phytobial heavy metal remediation strategies for a sustainable clean environment-a review. *Chemosphere* **217**, 925-941.

Asmar, F., Singh, T., and Nielsen, N. E. (1995). Barley genotypes differ in activity of soluble extracellular phosphatase and depletion of organic phosphorus in the rhizosphere soil. *Plant and Soil* **172**, 117-122.

Asogwa, E., and Dongo, L. (2009). Problems associated with pesticide usage and application in Nigerian cocoa production: A review. *African Journal of Agricultural Research* **4**, 675-683.

Atanasova-Penichon, V., Barreau, C., and Richard-Forget, F. (2016). Antioxidant secondary metabolites in cereals: potential involvement in resistance to Fusarium and mycotoxin accumulation. *Frontiers in microbiology* **7**, 566.

Babalola, O. O. (2010). Beneficial bacteria of agricultural importance. *Biotechnology letters* **32**, 1559-1570.

Babalola, O. O., and Glick, B. R. (2012). Indigenous African agriculture and plant associated microbes: current practice and future transgenic prospects. *Scientific Research and Essays* **7**, 2431-2439.

Babalola, O. O., Osir, E. O., and Sanni, A. I. (2002). Characterization of potential ethylene-producing rhizosphere bacteria of Striga-infested maize and sorghum. *African journal of Biotechnology* **1**, 67-69.

Babu, S., Prasanna, R., Bidyarani, N., Nain, L., and Shivay, Y. S. (2015). Synergistic action of PGP agents and Rhizobium spp. for improved plant growth, nutrient mobilization and yields in different leguminous crops. *Biocatalysis and Agricultural Biotechnology* **4**, 456-464.

Backer, R., Rokem, J. S., Ilangumaran, G., Lamont, J., Praslickova, D., Ricci, E., Subramanian, S., and 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**, 1473.

Badri, D. V., Chaparro, J. M., Zhang, R., Shen, Q., and Vivanco, J. M. (2013). Application of natural blends of phytochemicals derived from the root exudates of Arabidopsis to the soil reveal that phenolic-related compounds predominantly modulate the soil microbiome. *Journal of Biological Chemistry* **288**, 4502-4512.

Báidez, A., Gómez, P., Del Río, J., and Ortuño, A. (2006). Antifungal capacity of major phenolic compounds of Olea europaea L. against Phytophthora megasperma Drechsler and Cylindrocarpon destructans (Zinssm.) Scholten. *Physiological and Molecular Plant Pathology* **69**, 224-229.

Bainard, L., Koch, A., Gordon, A., and Klironomos, J. (2013). Growth response of crops to soil microbial communities from conventional monocropping and tree-based intercropping systems. *Plant and Soil* **363**, 345-356.

Baker, N. R., Khalili, B., Martiny, J. B., and Allison, S. D. (2018). Microbial decomposers not constrained by climate history along a Mediterranean climate gradient in southern California. *Ecology* **99**, 1441-1452.

Bakker, M. G., Bradeen, J. M., and Kinkel, L. L. (2013). Effects of plant host species and plant community richness on streptomycete community structure. *FEMS microbiology ecology* **83**, 596-606.

Baldan, E., Nigris, S., Romualdi, C., D'Alessandro, S., Clocchiatti, A., Zottini, M., Stevanato, P., Squartini, A., and Baldan, B. (2015). Beneficial bacteria isolated from grapevine inner tissues shape Arabidopsis thaliana roots. *PLoS One* **10**, e0140252.

Barnawal, D., Bharti, N., Maji, D., Chanotiya, C. S., and Kalra, A. (2014). ACC deaminase-containing Arthrobacter protophormiae induces NaCl stress tolerance through reduced ACC oxidase activity and ethylene production resulting in improved nodulation and mycorrhization in Pisum sativum. *Journal of plant physiology* **171**, 884-894.

Barnes, J., and Putnam, A. (1983). Rye residues contribute weed suppression in no-tillage cropping systems. *Journal of Chemical Ecology* **9**, 1045-1057.

Barns, S. M., Takala, S. L., and Kuske, C. R. (1999). Wide distribution and diversity of members of the bacterial kingdom Acidobacterium in the environment. *Applied and environmental microbiology* **65**, 1731-1737.

Basten, S., Lutz, W., and Scherbov, S. (2013). Very long range global population scenarios to 2300 and the implications of sustained low fertility. *Demographic Research* **28**, 1145-1166.

Baudoin, E., Benizri, E., and Guckert, A. (2003). Impact of artificial root exudates on the bacterial community structure in bulk soil and maize rhizosphere. *Soil Biology and Biochemistry* **35**, 1183-1192.

Becker, J., Eisenhauer, N., Scheu, S., and Jousset, A. (2012). Increasing antagonistic interactions cause bacterial communities to collapse at high diversity. *Ecology Letters* **15**, 468-474.

Bender, S. F., Wagg, C., and van der Heijden, M. G. (2016). An underground revolution: biodiversity and soil ecological engineering for agricultural sustainability. *Trends in ecology & evolution* **31**, 440-452.

Beneduzi, A., Ambrosini, A., and Passaglia, L. M. (2012). Plant growth-promoting rhizobacteria (PGPR): their potential as antagonists and biocontrol agents. *Genetics and molecular biology* **35**, 1044-1051.

Bennett, R. N., and Wallsgrove, R. M. (1994). Secondary metabolites in plant defence mechanisms. *New phytologist* **127**, 617-633.

Berendsen, R. L., Vismans, G., Yu, K., Song, Y., de Jonge, R., Burgman, W. P., Burmølle, M., Herschend, J., Bakker, P. A., and Pieterse, C. M. (2018). Disease-induced assemblage of a plant-beneficial bacterial consortium. *The ISME journal* **12**, 1496-1507.

Berg, G., and Smalla, K. (2009). Plant species and soil type cooperatively shape the structure and function of microbial communities in the rhizosphere. *FEMS microbiology ecology* **68**, 1-13.

Berg, M., and Koskella, B. (2018). Nutrient-and dose-dependent microbiome-mediated protection against a plant pathogen. *Current Biology* **28**, 2487-2492. e3.

Bergaglio, M. (2017). The contemporary illusion: population growth and sustainability. *Environment, Development and Sustainability* **19**, 2023-2038.

Berger, B., Patz, S., Ruppel, S., Dietel, K., Faetke, S., Junge, H., and Becker, M. (2018). Successful formulation and application of plant growth-promoting Kosakonia radicincitans in maize cultivation. *BioMed research international* **2018**.

Bernard, S. M., and Habash, D. Z. (2009). The importance of cytosolic glutamine synthetase in nitrogen assimilation and recycling. *New Phytologist* **182**, 608-620.

Berninger, T., González López, Ó., Bejarano, A., Preininger, C., and Sessitsch, A. (2018). Maintenance and assessment of cell viability in formulation of non-sporulating bacterial inoculants. *Microbial biotechnology* **11**, 277-301.

Bhattacharjee, R., and Dey, U. (2014). Biofertilizer, a way towards organic agriculture: A review. *African Journal of Microbiology Research* **8**, 2332-2343.

Block, A. K., Vaughan, M. M., Schmelz, E. A., and Christensen, S. A. (2019). Biosynthesis and function of terpenoid defense compounds in maize (Zea mays). *Planta* **249**, 21-30.

Boyd, E., and Peters, J. W. (2013). New insights into the evolutionary history of biological nitrogen fixation. *Frontiers in microbiology* **4**, 201.

Boyer, E. W., Howarth, R. W., Galloway, J. N., Dentener, F. J., Cleveland, C., Asner, G. P., Green, P., and Vörösmarty, C. (2004). Current nitrogen inputs to world regions. *In* "Agriculture and the nitrogen cycle: assessing the impacts of fertilizer use on food production and the environment", Vol. 65, pp. 221-230. Island Press Washington, DC.

Brader, G., Compant, S., Vescio, K., Mitter, B., Trognitz, F., Ma, L.-J., and Sessitsch, A. (2017). Ecology and genomic insights into plant-pathogenic and plant-nonpathogenic endophytes. *Annual Review of Phytopathology* **55**, 61-83.

Bragazza, L., Bardgett, R. D., Mitchell, E. A., and Buttler, A. (2015). Linking soil microbial communities to vascular plant abundance along a climate gradient. *New Phytologist* **205**, 1175-1182.

Bragina, A., Berg, C., Cardinale, M., Shcherbakov, A., Chebotar, V., and Berg, G. (2012). Sphagnum mosses harbour highly specific bacterial diversity during their whole lifecycle. *The ISME journal* **6**, 802-813.

Braud, A., Jézéquel, K., Léger, M. A., and Lebeau, T. (2006). Siderophore production by using free and immobilized cells of two pseudomonads cultivated in a medium enriched with Fe and/or toxic metals (Cr, Hg, Pb). *Biotechnology and bioengineering* **94**, 1080-1088.

Brígido, C., Singh, S., Menéndez, E., Tavares, M. J., Glick, B. R., Félix, M. d. R., Oliveira, S., and Carvalho, M. (2019). Diversity and functionality of culturable endophytic bacterial communities in chickpea plants. *Plants* **8**, 42.

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

Bronick, C. J., and Lal, R. (2005). Soil structure and management: a review. *Geoderma* **124**, 3-22.

Bulgarelli, D., Garrido-Oter, R., Münch, P. C., Weiman, A., Dröge, J., Pan, Y., McHardy, A. C., and Schulze-Lefert, P. (2015). Structure and function of the bacterial root microbiota in wild and domesticated barley. *Cell host & microbe* **17**, 392-403.

Burke, C., Steinberg, P., Rusch, D., Kjelleberg, S., and Thomas, T. (2011). Bacterial community assembly based on functional genes rather than species. *Proceedings of the National Academy of Sciences* **108**, 14288-14293.

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., and Holmes, S. P. (2016). DADA2: high-resolution sample inference from Illumina amplicon data. *Nature methods* **13**, 581-583.

Campisano, A., Antonielli, L., Pancher, M., Yousaf, S., Pindo, M., and Pertot, I. (2014). Bacterial endophytic communities in the grapevine depend on pest management. *PloS one* **9**, e112763.

Canarini, A., Wanek, W., Merchant, A., Richter, A., and Kaiser, C. (2019). Root exudation of primary metabolites: mechanisms and their roles in plant responses to environmental stimuli. *Frontiers in Plant Science* **10**, 157.

Cardinale, M., Ratering, S., Suarez, C., Montoya, A. M. Z., Geissler-Plaum, R., and Schnell, S. (2015). Paradox of plant growth promotion potential of rhizobacteria and their actual promotion effect on growth of barley (Hordeum vulgare L.) under salt stress. *Microbiological research* **181**, 22-32.

Carrión, V. J., Cordovez, V., Tyc, O., Etalo, D. W., de Bruijn, I., de Jager, V. C., Medema, M. H., Eberl, L., and Raaijmakers, J. M. (2018). Involvement of Burkholderiaceae and sulfurous volatiles in disease-suppressive soils. *The ISME journal* **12**, 2307-2321.

Caruso, F., Mendoza, L., Castro, P., Cotoras, M., Aguirre, M., Matsuhiro, B., Isaacs, M., Rossi, M., Viglianti, A., and Antonioletti, R. (2011). Antifungal activity of resveratrol against Botrytis cinerea is improved using 2-furyl derivatives. *PLoS One* **6**.

Cesco, S., Neumann, G., Tomasi, N., Pinton, R., and Weisskopf, L. (2010). Release of plant-borne flavonoids into the rhizosphere and their role in plant nutrition. *Plant and Soil* **329**, 1-25.

Chakraborty, U., Chakraborty, B., and Basnet, M. (2006). Plant growth promotion and induction of resistance in Camellia sinensis by Bacillus megaterium. *Journal of basic microbiology* **46**, 186-195.

Chamam, A., Sanguin, H., Bellvert, F., Meiffren, G., Comte, G., Wisniewski-Dyé, F., Bertrand, C., and Prigent-Combaret, C. (2013). Plant secondary metabolite profiling evidences strain-dependent effect in the Azospirillum–Oryza sativa association. *Phytochemistry* **87**, 65-77.

Chaparro, J. M., Badri, D. V., Bakker, M. G., Sugiyama, A., Manter, D. K., and Vivanco, J. M. (2013). Root exudation of phytochemicals in Arabidopsis follows specific patterns that are developmentally programmed and correlate with soil microbial functions. *PloS one* **8**, e55731.

Chauhan, P. S., Chaudhry, V., Mishra, S., and Nautiyal, C. S. (2011). Uncultured bacterial diversity in tropical maize (Zea mays L.) rhizosphere. *Journal of basic microbiology* **51**, 15-32.

Chaves, M. G. d., Silva, G. G. Z., Rossetto, R., Edwards, R. A., Tsai, S. M., and Navarrete, A. A. (2019). Acidobacteria subgroups and their metabolic potential for carbon degradation in sugarcane soil amended with vinasse and nitrogen fertilizers. *Frontiers in microbiology* **10**, 1680.

Chen, L., Liu, Y., Wu, G., Veronican Njeri, K., Shen, Q., Zhang, N., and Zhang, R. (2016). Induced maize salt tolerance by rhizosphere inoculation of Bacillus amyloliquefaciens SQR9. *Physiologia plantarum* **158**, 34-44.

Chen, X., Cromer, B. A., and Lynch, J. W. (2009). Molecular determinants of β-carboline inhibition of the glycine receptor. *Journal of neurochemistry* **110**, 1685-1694.

Chen, X. P., Kong, W. D., He, J. Z., Liu, W. J., Smith, S. E., Smith, F. A., and Zhu, Y. G. (2008). Do water regimes affect iron-plaque formation and microbial communities in the rhizosphere of paddy rice? *Journal of Plant Nutrition and Soil Science* **171**, 193-199.

Chen, Z., Zheng, Y., Ding, C., Ren, X., Yuan, J., Sun, F., and Li, Y. (2017). Integrated metagenomics and molecular ecological network analysis of bacterial community composition during the phytoremediation of cadmium-contaminated soils by bioenergy crops. *Ecotoxicology and environmental safety* **145**, 111-118.

Cheng, Z., Lei, S., Li, Y., Huang, W., Ma, R., Xiong, J., Zhang, T., Jin, L., Xu, X., and Tian, B. (2020). Revealing the Variation and Stability of Bacterial Communities in Tomato Rhizosphere Microbiota. *Microorganisms* **8**, 170.

Chodak, M., Gołębiewski, M., Morawska-Płoskonka, J., Kuduk, K., and Niklińska, M. (2015). Soil chemical properties affect the reaction of forest soil bacteria to drought and rewetting stress. *Annals of microbiology* **65**, 1627-1637.

Choudhary, S., Kumar, R., Dalal, U., Tomar, S., and Reddy, S. N. (2020). Green synthesis of nanometal impregnated biomass–antiviral potential. *Materials Science and Engineering: C*, 110934.

Claire Horner-Devine, M., Leibold, M. A., Smith, V. H., and Bohannan, B. J. (2003). Bacterial diversity patterns along a gradient of primary productivity. *Ecology letters* **6**, 613-622.

Clairmont, L. K., Stevens, K. J., and Slawson, R. M. (2019). Site-specific differences in microbial community structure and function within the rhizosphere and rhizoplane of wetland plants is plant species dependent. *Rhizosphere* **9**, 56-68.

Cline, L. C., and Zak, D. R. (2015). Soil microbial communities are shaped by plant-driven changes in resource availability during secondary succession. *Ecology* **96**, 3374-3385.

Cole, J. R., Chai, B., Farris, R. J., Wang, Q., Kulam-Syed-Mohideen, A., McGarrell, D. M., Bandela, A., Cardenas, E., Garrity, G. M., and Tiedje, J. M. (2007). The ribosomal database project (RDP-II): introducing myRDP space and quality controlled public data. *Nucleic acids research* **35**, D169-D172.

Compant, S., Clément, C., and Sessitsch, A. (2010). Plant growth-promoting bacteria in the rhizo-and endosphere of plants: their role, colonization, mechanisms involved and prospects for utilization. *Soil Biology and Biochemistry* **42**, 669-678.

Compant, S., Mitter, B., Colli-Mull, J. G., Gangl, H., and Sessitsch, A. (2011). Endophytes of grapevine flowers, berries, and seeds: identification of cultivable bacteria, comparison with other plant parts, and visualization of niches of colonization. *Microbial ecology* **62**, 188-197.

Compant, S., Samad, A., Faist, H., and Sessitsch, A. (2019). A review on the plant microbiome: Ecology, functions, and emerging trends in microbial application. *Journal of advanced research* **19**, 29-37.

Concha, C., and Doerner, P. (2020). The impact of the rhizobia–legume symbiosis on host root system architecture. *Journal of Experimental Botany*.

Cotton, T. A., Pétriacq, P., Cameron, D. D., Al Meselmani, M., Schwarzenbacher, R., Rolfe, S. A., and Ton, J. (2019). Metabolic regulation of the maize rhizobiome by benzoxazinoids. *The ISME journal* **13**, 1647-1658.

Craig, J. W., Chang, F.-Y., Kim, J. H., Obiajulu, S. C., and Brady, S. F. (2010). Expanding small-molecule functional metagenomics through parallel screening of broad-host-range cosmid environmental DNA libraries in diverse proteobacteria. *Applied and environmental microbiology* **76**, 1633-1641.

D'Amelia, L., Dell'Aversana, E., Woodrow, P., Ciarmiello, L. F., and Carillo, P. (2018a). Metabolomics for crop improvement against salinity stress. *In* "Salinity Responses and Tolerance in Plants, Volume 2", pp. 267-287. Springer.

D'Amelia, V., Aversano, R., Chiaiese, P., and Carputo, D. (2018b). The antioxidant properties of plant flavonoids: their exploitation by molecular plant breeding. *Phytochemistry Reviews* **17**, 611-625.

da Costa, P. B., Granada, C. E., Ambrosini, A., Moreira, F., de Souza, R., dos Passos, J. F. M., Arruda, L., and Passaglia, L. M. (2014). A model to explain plant growth promotion traits: a multivariate analysis of 2,211 bacterial isolates. *PLoS One* **9**, e116020.

da Silva, A. P., Babujia, L. C., Franchini, J. C., Ralisch, R., Hungria, M., and de Fátima Guimarães, M. (2014). Soil structure and its influence on microbial biomass in different soil and crop management systems. *Soil and Tillage Research* **142**, 42-53.

Dafoe, N. J., Thomas, J. D., Shirk, P. D., Legaspi, M. E., Vaughan, M. M., Huffaker, A., Teal, P. E., and Schmelz, E. A. (2013). European corn borer (Ostrinia nubilalis) induced responses enhance susceptibility in maize. *PLoS One* **8**.

Dangi, S. R., Tirado-Corbalá, R., Gerik, J., and Hanson, B. D. (2017). Effect of long-term continuous fumigation on soil microbial communities. *Agronomy* **7**, 37.

de Araujo, A. S. F., Bezerra, W. M., dos Santos, V. M., Rocha, S. M. B., da Silva Carvalho, N., Figueiredo, M. d. V. B., de Almeida Lopes, Â. C., and Melo, V. M. M. (2017). Distinct bacterial communities across a gradient of vegetation from a preserved Brazilian Cerrado. *Antonie van Leeuwenhoek* **110**, 457-469.

de Araujo, A. S. F., Miranda, A. R. L., Sousa, R. S., Mendes, L. W., Antunes, J. E. L., de Souza Oliveira, L. M., de Araujo, F. F., Melo, V. M. M., and Figueiredo, M. d. V. B. (2019). Bacterial community associated with rhizosphere of maize and cowpea in a subsequent cultivation. *Applied soil ecology* **143**, 26-34.

de Boer, W., Li, X., Meisner, A., and Garbeva, P. (2019). Pathogen suppression by microbial volatile organic compounds in soils. *FEMS microbiology ecology* **95**, fiz105.

De Coninck, B., Timmermans, P., Vos, C., Cammue, B. P., and Kazan, K. (2015). What lies beneath: belowground defense strategies in plants. *Trends in plant science* **20**, 91-101.

de Jesus Jatoba, L., Varela, R. M., Molinillo, J. M. G., Din, Z. U., Gualtieri, S. C. J., Rodrigues-Filho, E., and Macías, F. A. (2016). Allelopathy of bracken fern (Pteridium arachnoideum): new evidence from green fronds, litter, and soil. *PloS one* **11**.

de Lamo, F. J., and Takken, F. L. (2020). Biocontrol by Fusarium oxysporum using endophyte-mediated resistance. *Frontiers in Plant Science* **11**.

de Vries, F. T., Thébault, E., Liiri, M., Birkhofer, K., Tsiafouli, M. A., Bjørnlund, L., Jørgensen, H. B., Brady, M. V., Christensen, S., and de Ruiter, P. C. (2013). Soil food web properties explain ecosystem services across European land use systems. *Proceedings of the National Academy of Sciences* **110**, 14296-14301.

de Vries, S., von Dahlen, J. K., Schnake, A., Ginschel, S., Schulz, B., and Rose, L. E. (2018). Broad-spectrum inhibition of Phytophthora infestans by fungal endophytes. *FEMS Microbiology Ecology* **94**, fiy037.

De Vrieze, M., Germanier, F., Vuille, N., and Weisskopf, L. (2018). Combining different potato-associated Pseudomonas strains for improved biocontrol of Phytophthora infestans. *Frontiers in microbiology* **9**, 2573.

Deacon, J. W., and Berry, L. A. (1993). Biocontrol of soil-borne plant pathogens: Concepts and their application. *Pesticide Science* **37**, 417-426.

Deaker, R., Roughley, R. J., and Kennedy, I. R. (2004). Legume seed inoculation technology—a review. *Soil Biology and Biochemistry* **36**, 1275-1288.

Delmont, T. O., Prestat, E., Keegan, K. P., Faubladier, M., Robe, P., Clark, I. M., Pelletier, E., Hirsch, P. R., Meyer, F., and Gilbert, J. A. (2012). Structure, fluctuation and magnitude of a natural grassland soil metagenome. *The ISME journal* **6**, 1677-1687.

DeSA, U. (2013). World population prospects: the 2012 revision. *Population division of the department of economic and social affairs of the United Nations Secretariat, New York* **18**.

DeSantis, T. Z., Hugenholtz, P., Larsen, N., Rojas, M., Brodie, E. L., Keller, K., Huber, T., Dalevi, D., Hu, P., and Andersen, G. L. (2006). Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. *Applied and environmental microbiology* **72**, 5069-5072.

Devi, K. K., Seth, N., Kothamasi, S., and Kothamasi, D. (2007). Hydrogen cyanide-producing rhizobacteria kill subterranean termite Odontotermes obesus (Rambur) by cyanide poisoning under in vitro conditions. *Current microbiology* **54**, 74-78.

Dolman, A. M. (2014). Macrophyte effects on algal turbidity in subtropical versus temperate lakes: a comment on Wang et al.(2014). *Freshwater biology* **59**, 2656-2658.

Donn, S., Kirkegaard, J. A., Perera, G., Richardson, A. E., and Watt, M. (2015). Evolution of bacterial communities in the wheat crop rhizosphere. *Environmental microbiology* **17**, 610-621.

Du, Y., Han, H., Wang, Y., Zhong, M., Hui, D., Niu, S., and Wan, S. (2018). Plant functional groups regulate soil respiration responses to nitrogen addition and mowing over a decade. *Functional Ecology* **32**, 1117-1127.

Dumbrell, A. J., Nelson, M., Helgason, T., Dytham, C., and Fitter, A. H. (2010). Relative roles of niche and neutral processes in structuring a soil microbial community. *The ISME journal* **4**, 337-345.

Dupuy, L. X., Mimault, M., Patko, D., Ladmiral, V., Ameduri, B., MacDonald, M. P., and Ptashnyk, M. (2018). Micromechanics of root development in soil. *Current opinion in genetics & development* **51**, 18-25.

Durán, P., Tortella, G., Viscardi, S., Barra, P. J., Carrión, V. J., Mora, M. d. l. L., and Pozo, M. J. (2018). Microbial community composition in take-all suppressive soils. *Frontiers in microbiology* **9**, 2198.

Edwards, J., Johnson, C., Santos-Medellín, C., Lurie, E., Podishetty, N. K., Bhatnagar, S., Eisen, J. A., and Sundaresan, V. (2015). Structure, variation, and assembly of the root-associated microbiomes of rice. *Proceedings of the National Academy of Sciences* **112**, E911-E920.

Eisenhauer, N., Lanoue, A., Strecker, T., Scheu, S., Steinauer, K., Thakur, M. P., and Mommer, L. (2017). Root biomass and exudates link plant diversity with soil bacterial and fungal biomass. *Scientific reports* **7**, 1-8.

El-Sayed, W. S., Akhkha, A., El-Naggar, M. Y., and Elbadry, M. (2014). In vitro antagonistic activity, plant growth promoting traits and phylogenetic affiliation of rhizobacteria associated with wild plants grown in arid soil. *Frontiers in microbiology* **5**, 651.

el Zahar Haichar, F., Marol, C., Berge, O., Rangel-Castro, J. I., Prosser, J. I., Balesdent, J. m., Heulin, T., and Achouak, W. (2008). Plant host habitat and root exudates shape soil bacterial community structure. *The ISME journal* **2**, 1221-1230.

el Zahar Haichar, F., Santaella, C., Heulin, T., and Achouak, W. (2014). Root exudates mediated interactions belowground. *Soil Biology and Biochemistry* **77**, 69-80.

Enebe, M. C., and 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.

Erb, M., and Lu, J. (2013). Soil abiotic factors influence interactions between belowground herbivores and plant roots. *Journal of experimental botany* **64**, 1295-1303.

Etesami, H., and 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.

Eviner, V. T., Chapin, I., F Stuart, and Vaughn, C. E. (2006). Seasonal variations in plant species effects on soil N and P dynamics. *Ecology* **87**, 974-986.

Fadiji, A. E., and Babalola, O. O. (2020). Metagenomics methods for the study of plant-associated microbial communities: A review. *Journal of Microbiological Methods* **170**, 105860.

Falcone Ferreyra, M. L., Rius, S., and Casati, P. (2012). Flavonoids: biosynthesis, biological functions, and biotechnological applications. *Frontiers in plant science* **3**, 222.

Fang, W., and Leger, R. J. S. (2010). Mrt, a gene unique to fungi, encodes an oligosaccharide transporter and facilitates rhizosphere competency in Metarhizium robertsii. *Plant physiology* **154**, 1549-1557.

FAO, W. (2015). IFAD, 2012: The State of Food Insecurity in the World: Economic Growth is Necessary but not Sufficient to Accelerate Reduction of Hunger and Malnutrition. *Food and Agriculture Organization of the United Nations, Rome, Italy*.

Feng, G., Xie, T., Wang, X., Bai, J., Tang, L., Zhao, H., Wei, W., Wang, M., and Zhao, Y. (2018). Metagenomic analysis of microbial community and function involved in cd-contaminated soil. *BMC microbiology* **18**, 11.

Ferrari, B. C., Binnerup, S. J., and Gillings, M. (2005). Microcolony cultivation on a soil substrate membrane system selects for previously uncultured soil bacteria. *Applied and environmental microbiology* **71**, 8714-8720.

Fierer, N. (2017). Embracing the unknown: disentangling the complexities of the soil microbiome. *Nature Reviews Microbiology* **15**, 579-590.

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

Fierer, N., Lauber, C. L., Ramirez, K. S., Zaneveld, J., Bradford, M. A., and Knight, R. (2012). Comparative metagenomic, phylogenetic and physiological analyses of soil microbial communities across nitrogen gradients. *The ISME journal* **6**, 1007-1017.

Fitzpatrick, C. R., Copeland, J., Wang, P. W., Guttman, D. S., Kotanen, P. M., and Johnson, M. T. (2018). Assembly and ecological function of the root microbiome across angiosperm plant species. *Proceedings of the National Academy of Sciences* **115**, E1157-E1165.

Flint, H. J., Scott, K. P., Duncan, S. H., Louis, P., and Forano, E. (2012). Microbial degradation of complex carbohydrates in the gut. *Gut microbes* **3**, 289-306.

Friebe, A., Klever, W., and Sikora, R. (2012). ALLELOCHEMICALS IN ROOT EXUDATES. *Phytochemical Signals and Plant-Microbe Interactions* **32**, 71.

Frisli, T., Haverkamp, T., Jakobsen, K., Stenseth, N. C., and Rudi, K. (2013). Estimation of metagenome size and structure in an experimental soil microbiota from low coverage next-generation sequence data. *Journal of applied microbiology* **114**, 141-151.

Fu, L., Penton, C. R., Ruan, Y., Shen, Z., Xue, C., Li, R., and Shen, Q. (2017). Inducing the rhizosphere microbiome by biofertilizer application to suppress banana Fusarium wilt disease. *Soil Biology and Biochemistry* **104**, 39-48.

Fukami, J., Nogueira, M. A., Araujo, R. S., and Hungria, M. (2016). Accessing inoculation methods of maize and wheat with Azospirillum brasilense. *Amb Express* **6**, 3.

Fürstenberg-Hägg, J., Zagrobelny, M., and Bak, S. (2013). Plant defense against insect herbivores. *International journal of molecular sciences* **14**, 10242-10297.

Gaonkar, T., and Bhosle, S. (2013). Effect of metals on a siderophore producing bacterial isolate and its implications on microbial assisted bioremediation of metal contaminated soils. *Chemosphere* **93**, 1835-1843.

Garbeva, P., Van Elsas, J., and Van Veen, J. (2008). Rhizosphere microbial community and its response to plant species and soil history. *Plant and soil* **302**, 19-32.

García-Orenes, F., Morugán-Coronado, A., Zornoza, R., and Scow, K. (2013). Changes in soil microbial community structure influenced by agricultural management practices in a Mediterranean agro-ecosystem. *PloS one* **8**, e80522.

García-Salamanca, A., Molina-Henares, M. A., van Dillewijn, P., Solano, J., Pizarro-Tobías, P., Roca, A., Duque, E., and Ramos, J. L. (2013). Bacterial diversity in the rhizosphere of maize and the surrounding carbonate-rich bulk soil. *Microbial biotechnology* **6**, 36-44.

Gerdes, S., El Yacoubi, B., Bailly, M., Blaby, I. K., Blaby-Haas, C. E., Jeanguenin, L., Lara-Núñez, A., Pribat, A., Waller, J. C., and Wilke, A. (2011). Synergistic use of plant-prokaryote comparative genomics for functional annotations. *BMC genomics* **12**, S2.

Germida, J. J., Siciliano, S. D., Renato de Freitas, J., and Seib, A. M. (1998). Diversity of root-associated bacteria associated with field-grown canola (Brassica napus L.) and wheat (Triticum aestivum L.). *FEMS Microbiology Ecology* **26**, 43-50.

Ghosh, P., Tripathi, A., Bandyopadhyay, K., and Manna, M. (2009). Assessment of nutrient competition and nutrient requirement in soybean/sorghum intercropping system. *European Journal of Agronomy* **31**, 43-50.

Gindling, T. H., and Newhouse, D. (2012). "Self-employment in the developing world," The World Bank.

Giorgio, A., De Stradis, A., Lo Cantore, P., and Iacobellis, N. S. (2015). Biocide effects of volatile organic compounds produced by potential biocontrol rhizobacteria on Sclerotinia sclerotiorum. *Frontiers in microbiology* **6**, 1056.

Glick, B. R. (2018). Soil microbes and sustainable agriculture. *Pedosphere* **28**, 167-169.

Gocke, M. I., Huguet, A., Derenne, S., Kolb, S., Dippold, M. A., and Wiesenberg, G. L. (2017). Disentangling interactions between microbial communities and roots in deep subsoil. *Science of the Total Environment* **575**, 135-145.

Gomes, E. A., Lana, U. G., Quensen, J. F., de Sousa, S. M., Oliveira, C. A., Guo, J., Guimarães, L. J., and 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., and Raaijmakers, J. M. (2017). Current insights into the role of rhizosphere bacteria in disease suppressive soils. *Frontiers in Microbiology* **8**, 2529.

González-Chang, M., Wratten, S. D., Lefort, M.-C., and Boyer, S. (2016). Food webs and biological control: A review of molecular tools used to reveal trophic interactions in agricultural systems. *Food Webs* **9**, 4-11.

Gopal, M., and Gupta, A. (2016). Microbiome selection could spur next-generation plant breeding strategies. *Frontiers in microbiology* **7**, 1971.

Gore, M. A., Chia, J.-M., Elshire, R. J., Sun, Q., Ersoz, E. S., Hurwitz, B. L., Peiffer, J. A., McMullen, M. D., Grills, G. S., and Ross-Ibarra, J. (2009). A first-generation haplotype map of maize. *Science* **326**, 1115-1117.

Goudjal, Y., Toumatia, O., Yekkour, A., Sabaou, N., Mathieu, F., and Zitouni, A. (2014). Biocontrol of Rhizoctonia solani damping-off and promotion of tomato plant growth by endophytic actinomycetes isolated from native plants of Algerian Sahara. *Microbiological Research* **169**, 59-65.

Gracheva, M. E., Xiong, A., Aksimentiev, A., Schulten, K., Timp, G., and Leburton, J.-P. (2006). Simulation of the electric response of DNA translocation through a semiconductor nanopore–capacitor. *Nanotechnology* **17**, 622.

Gundel, P. E., Omacini, M., Sadras, V. O., and Ghersa, C. M. (2010). The interplay between the effectiveness of the grass-endophyte mutualism and the genetic variability of the host plant. *Evolutionary applications* **3**, 538-546.

Gupta, D., Huang, H., and Corpas, F. (2013). Lead tolerance in plants: strategies for phytoremediation. *Environmental Science and Pollution Research* **20**, 2150-2161.

Habib, E., Linher-Melville, K., Lin, H.-X., and Singh, G. (2015). Expression of xCT and activity of system xc− are regulated by NRF2 in human breast cancer cells in response to oxidative stress. *Redox biology* **5**, 33-42.

Hacquard, S. (2016). Disentangling the factors shaping microbiota composition across the plant holobiont. *New Phytologist* **209**, 454-457.

Haldar, S., and Sengupta, S. (2015). Plant-microbe cross-talk in the rhizosphere: insight and biotechnological potential. *The open microbiology journal* **9**, 1.

Hamner, S., Brown, B. L., Hasan, N. A., Franklin, M. J., Doyle, J., Eggers, M. J., Colwell, R. R., and Ford, T. E. (2019). Metagenomic profiling of microbial pathogens in the little Bighorn river, Montana. *International Journal of Environmental Research and Public Health* **16**, 1097.

Han, H.-S., and Lee, K. (2006). Effect of co-inoculation with phosphate and potassium solubilizing bacteria on mineral uptake and growth of pepper and cucumber. *Plant soil and Environment* **52**, 130.

Hannula, S. E., Kielak, A. M., Steinauer, K., Huberty, M., Jongen, R., Jonathan, R., Heinen, R., and Bezemer, T. M. (2019). Time after time: temporal variation in the effects of grass and forb species on soil bacterial and fungal communities. *MBio* **10**.

Hardoim, P. R., Hardoim, C. C., Van Overbeek, L. S., and Van Elsas, J. D. (2012). Dynamics of seed-borne rice endophytes on early plant growth stages. *PloS one* **7**, e30438.

Hardoim, P. R., Van Overbeek, L. S., Berg, G., Pirttilä, A. M., Compant, S., Campisano, A., Döring, M., and Sessitsch, A. (2015). The hidden world within plants: ecological and evolutionary considerations for defining functioning of microbial endophytes. *Microbiology and Molecular Biology Reviews* **79**, 293-320.

Hartman, K., van der Heijden, M. G., Wittwer, R. A., Banerjee, S., Walser, J.-C., and Schlaeppi, K. (2018). Cropping practices manipulate abundance patterns of root and soil microbiome members paving the way to smart farming. *Microbiome* **6**, 1-14.

Hartmann, A., Rothballer, M., and Schmid, M. (2008). Lorenz Hiltner, a pioneer in rhizosphere microbial ecology and soil bacteriology research. *Plant and Soil* **312**, 7-14.

Hartmann, M., Frey, B., Mayer, J., Mäder, P., and Widmer, F. (2015). Distinct soil microbial diversity under long-term organic and conventional farming. *The ISME journal* **9**, 1177-1194.

Hassan, M. Z., Osman, H., Ali, M. A., and Ahsan, M. J. (2016). Therapeutic potential of coumarins as antiviral agents. *European journal of medicinal chemistry* **123**, 236-255.

Havlíčková, H., Cvikrová, M., and Eder, J. (1996). Phenolic acids in wheat cultivars in relation to plant suitability for and response to cereal aphids/Phenolsäuren in Weizensorten im Zusammenhang mit ihrer Eignung als Wirt und ihre Reaktion auf Blattläuse. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz/Journal of Plant Diseases and Protection*, 535-542.

Heanes, D. (1984). Determination of total organic-C in soils by an improved chromic acid digestion and spectrophotometric procedure. *Communications in soil science and plant analysis* **15**, 1191-1213.

Hein, J. W., Wolfe, G. V., and Blee, K. A. (2008). Comparison of rhizosphere bacterial communities in Arabidopsis thaliana mutants for systemic acquired resistance. *Microbial ecology* **55**, 333-343.

Hendgen, M., Hoppe, B., Döring, J., Friedel, M., Kauer, R., Frisch, M., Dahl, A., and Kellner, H. (2018). Effects of different management regimes on microbial biodiversity in vineyard soils. *Scientific reports* **8**, 1-13.

Hernández-Montiel, L. G., Rueda-Puente, E. O., Cordoba-Matson, M. V., Holguín-Peña, J. R., and Zulueta-Rodríguez, R. (2013). Mutualistic interaction of rhizobacteria with arbuscular mycorrhizal fungi and its antagonistic effect on Fusarium oxysporum in Carica papaya seedlings. *Crop Protection* **47**, 61-66.

Herrera Paredes, S., Gao, T., Law, T. F., Finkel, O. M., Mucyn, T., Teixeira, P. J. P. L., Salas González, I., Feltcher, M. E., Powers, M. J., and Shank, E. A. (2018). Design of synthetic bacterial communities for predictable plant phenotypes. *PLoS biology* **16**, e2003962.

Hider, R. C., and Kong, X. (2010). Chemistry and biology of siderophores. *Natural product reports* **27**, 637-657.

Hiltner, L. (1904). Uber nevere Erfahrungen und Probleme auf dem Gebiet der Boden Bakteriologie und unter besonderer Beurchsichtigung der Grundungung und Broche. *Arbeit. Deut. Landw. Ges. Berlin* **98**, 59-78.

Hol, W. G., Garbeva, P., Hordijk, C., Hundscheid, M. P., Gunnewiek, P. J. K., Van Agtmaal, M., Kuramae, E. E., and De Boer, W. (2015). Non-random species loss in bacterial communities reduces antifungal volatile production. *Ecology* **96**, 2042-2048.

Hong, X., Chen, J., Liu, L., Wu, H., Tan, H., Xie, G., Xu, Q., Zou, H., Yu, W., and Wang, L. (2016). Metagenomic sequencing reveals the relationship between microbiota composition and quality of Chinese Rice Wine. *Scientific Reports* **6**, 1-11.

Hu, J., Wei, Z., Friman, V.-P., Gu, S.-h., Wang, X.-f., Eisenhauer, N., Yang, T.-j., Ma, J., Shen, Q.-r., and Xu, Y.-c. (2016). Probiotic diversity enhances rhizosphere microbiome function and plant disease suppression. *MBio* **7**.

Hu, J., Wei, Z., Weidner, S., Friman, V.-P., Xu, Y.-C., Shen, Q.-R., and Jousset, A. (2017). Probiotic Pseudomonas communities enhance plant growth and nutrient assimilation via diversity-mediated ecosystem functioning. *Soil Biology and Biochemistry* **113**, 122-129.

Hu, L., Robert, C. A., Cadot, S., Zhang, X., Ye, M., Li, B., Manzo, D., Chervet, N., Steinger, T., and 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.

Huang, X.-F., Chaparro, J. M., Reardon, K. F., Zhang, R., Shen, Q., and Vivanco, J. M. (2014). Rhizosphere interactions: root exudates, microbes, and microbial communities. *Botany* **92**, 267-275.

Hungria, M., Campo, R. J., Souza, E. M., and Pedrosa, F. O. (2010). Inoculation with selected strains of Azospirillum brasilense and A. lipoferum improves yields of maize and wheat in Brazil. *Plant and soil* **331**, 413-425.

Hunt, D. E., Klepac-Ceraj, V., Acinas, S. G., Gautier, C., Bertilsson, S., and Polz, M. F. (2006). Evaluation of 23S rRNA PCR primers for use in phylogenetic studies of bacterial diversity. *Applied and environmental microbiology* **72**, 2221-2225.

Ibiene, A. A., Agogbua, J. U., Okonko, I., and Nwachi, G. (2012). Plant growth promoting rhizobacteria (PGPR) as biofertilizer: Effect on growth of Lycopersicum esculentus. *Journal of American Science* **8**, 318-324.

Ibironke, O., McGuinness, L. R., Lu, S.-E., Wang, Y., Hussain, S., Weisel, C. P., and Kerkhof, L. J. (2020). Species-level evaluation of the human respiratory microbiome. *GigaScience* **9**, giaa038.

Igiehon, N. O., and Babalola, O. O. (2017). Biofertilizers and sustainable agriculture: exploring arbuscular mycorrhizal fungi. *Applied Microbiology and Biotechnology* **101**, 4871-4881.

Igiehon, N. O., and Babalola, O. O. (2018). Rhizosphere microbiome modulators: contributions of nitrogen fixing bacteria towards sustainable agriculture. *International journal of environmental research and public health* **15**, 574.

Igiehon, N. O., Babalola, O. O., and Aremu, B. R. (2019). Genomic insights into plant growth promoting rhizobia capable of enhancing soybean germination under drought stress. *BMC microbiology* **19**, 159.

Illeghems, K., Weckx, S., and De Vuyst, L. (2015). Applying meta-pathway analyses through metagenomics to identify the functional properties of the major bacterial communities of a single spontaneous cocoa bean fermentation process sample. *Food microbiology* **50**, 54-63.

Imchen, M., Kumavath, R., Barh, D., Azevedo, V., Ghosh, P., Viana, M., and Wattam, A. R. (2017). Searching for signatures across microbial communities: Metagenomic analysis of soil samples from mangrove and other ecosystems. *Scientific reports* **7**, 1-13.

İnceoğlu, Ö., Salles, J. F., and van Elsas, J. D. (2012). Soil and cultivar type shape the bacterial community in the potato rhizosphere. *Microbial ecology* **63**, 460-470.

Islam, W., Adnan, M., Tayyab, M., Hussain, M., and Islam, S. U. (2018). Phyto-metabolites; an impregnable shield against plant viruses. *Natural Product Communications* **13**, 1934578X1801300131.

Jacobsen, C. S., and Hjelmsø, M. H. (2014). Agricultural soils, pesticides and microbial diversity. *Current Opinion in Biotechnology* **27**, 15-20.

Jacoby, R., Peukert, M., Succurro, A., Koprivova, A., and Kopriva, S. (2017). The role of soil microorganisms in plant mineral nutrition—current knowledge and future directions. *Frontiers in plant science* **8**, 1617.

Jahanian, A., Chaichi, M., Rezaei, K., Rezayazdi, K., and Khavazi, K. (2012). The effect of plant growth promoting rhizobacteria (PGPR) on germination and primary growth of artichoke (Cynara scolymus). *International Journal of Agriculture and Crop Sciences (IJACS)* **4**, 923-929.

Jenkins, J. N., Hedin, P., Parrott, W., McCarty Jr, J., and White, W. (1983). Cotton Allelochemics and Growth of Tobacco Budworm Larvae 1. *Crop science* **23**, 1195-1198.

Jiang, H., Huang, L., Yang, J., and Wu, G. (2018). A microbial analysis primer for biogeochemists. *In* "Environmental geochemistry", pp. 599-609. Elsevier.

Jiménez, D. J., Chaves-Moreno, D., and Van Elsas, J. D. (2015). Unveiling the metabolic potential of two soil-derived microbial consortia selected on wheat straw. *Scientific reports* **5**, 13845.

Johns, C. (2017). Living soils: the role of microorganisms in soil health. *Fut Direct Intl*, 1-7.

Johnston-Monje, D., Lundberg, D. S., Lazarovits, G., Reis, V. M., and Raizada, M. N. (2016). Bacterial populations in juvenile maize rhizospheres originate from both seed and soil. *Plant and Soil* **405**, 337-355.

Jones, P., Garcia, B., Furches, A., Tuskan, G., and Jacobson, D. (2019a). Plant host-associated mechanisms for microbial selection. *Frontiers in Plant Science* **10**, 862.

Jones, S. E., Pham, C. A., Zambri, M. P., McKillip, J., Carlson, E. E., and Elliot, M. A. (2019b). Streptomyces volatile compounds influence exploration and microbial community dynamics by altering iron availability. *MBio* **10**.

Joseph, S. J., Hugenholtz, P., Sangwan, P., Osborne, C. A., and Janssen, P. H. (2003). Laboratory cultivation of widespread and previously uncultured soil bacteria. *Applied and environmental microbiology* **69**, 7210-7215.

Jousset, A., Bienhold, C., Chatzinotas, A., Gallien, L., Gobet, A., Kurm, V., Küsel, K., Rillig, M. C., Rivett, D. W., and Salles, J. F. (2017). Where less may be more: how the rare biosphere pulls ecosystems strings. *The ISME journal* **11**, 853-862.

Kabaluk, J. T., Svircev, A. M., Goettel, M. S., and Woo, S. G. (2010). "The use and regulation of microbial pesticides in representative jurisdictions worldwide," International Organization for Biological Control of Noxious Animals and ….

Kabanda, T., and Palamuleni, L. (2011). Seasonal weather events and their impacts on buildings around Mafikeng, North West province, South Africa. *Life Science Journal* **8**, 64-69.

Kanehisa, M., and Goto, S. (2000). KEGG: kyoto encyclopedia of genes and genomes. *Nucleic acids research* **28**, 27-30.

Karuppiah, P., and Rajaram, S. (2011). Exploring the potential of chromium reducing Bacillus sp. and there plant growth promoting activities. *Journal of Microbiology Research* **1**, 17-23.

Kaschuk, G., Alberton, O., and Hungria, M. (2010). Three decades of soil microbial biomass studies in Brazilian ecosystems: lessons learned about soil quality and indications for improving sustainability. *Soil Biology and Biochemistry* **42**, 1-13.

Kaschuk, G., Alberton, O., and Hungria, M. (2011). Quantifying effects of different agricultural land uses on soil microbial biomass and activity in Brazilian biomes: inferences to improve soil quality. *Plant and soil* **338**, 467-481.

Kaur, T., Rani, R., and Manhas, R. K. (2019). Biocontrol and plant growth promoting potential of phylogenetically new Streptomyces sp. MR14 of rhizospheric origin. *AMB Express* **9**, 125.

Kawasaki, A., Donn, S., Ryan, P. R., Mathesius, U., Devilla, R., Jones, A., and Watt, M. (2016). Microbiome and exudates of the root and rhizosphere of Brachypodium distachyon, a model for wheat. *PloS one* **11**, e0164533.

Ke, S., Fang, W., Huang, W., Zhang, Z., Shi, L., Wan, Z., Wang, K., Cao, C., and Huang, D. (2020). Sulfur-Containing Natural Hinduchelins Derivatives as Potential Antifungal Agents against Rhizoctonia solani. *Bioorganic & Medicinal Chemistry Letters*, 127245.

Kecskés, M., Choudhury, A., Casteriano, A., Deaker, R., Roughley, R., Lewin, L., Ford, R., and Kennedy, I. (2016). Effects of bacterial inoculant biofertilizers on growth, yield and nutrition of rice in Australia. *Journal of Plant Nutrition* **39**, 377-388.

Kerkhof, L. J., Dillon, K. P., Häggblom, M. M., and McGuinness, L. R. (2017). Profiling bacterial communities by MinION sequencing of ribosomal operons. *Microbiome* **5**, 116.

Khan, N., Anwer, A. H., Ahmad, A., Sabir, S., and Khan, M. Z. (2020). Investigating microbial fuel cell aided bio-remediation of mixed phenolic contaminants under oxic and anoxic environments. *Biochemical Engineering Journal* **155**, 107485.

Kiers, E. T., Duhamel, M., Beesetty, Y., Mensah, J. A., Franken, O., Verbruggen, E., Fellbaum, C. R., Kowalchuk, G. A., Hart, M. M., and Bago, A. (2011). Reciprocal rewards stabilize cooperation in the mycorrhizal symbiosis. *science* **333**, 880-882.

Kleinheinz, G., Bagley, S., John, W. S., Rughani, J., and McGinnis, G. (1999). Characterization of alpha-pinene-degrading microorganisms and application to a bench-scale biofiltration system for VOC degradation. *Archives of Environmental Contamination and Toxicology* **37**, 151-157.

Köhl, J., Postma, J., Nicot, P., Ruocco, M., and Blum, B. (2011). Stepwise screening of microorganisms for commercial use in biological control of plant-pathogenic fungi and bacteria. *Biological control* **57**, 1-12.

Kour, D., Rana, K. L., Kaur, T., Yadav, N., Halder, S. K., Yadav, A. N., Sachan, S. G., and Saxena, A. K. (2020). Potassium solubilizing and mobilizing microbes: Biodiversity, mechanisms of solubilization, and biotechnological implication for alleviations of abiotic stress. *Trends of Microbial Biotechnology for Sustainable Agriculture and Biomedicine Systems: Diversity and Functional Perspective. Elsevier, Amsterdam*, 177-202.

Kramshøj, M., Albers, C. N., Svendsen, S. H., Björkman, M. P., Lindwall, F., Björk, R. G., and Rinnan, R. (2019). Volatile emissions from thawing permafrost soils are influenced by meltwater drainage conditions. *Global change biology* **25**, 1704-1716.

Kristin, A., and Miranda, H. (2013). The root microbiota—a fingerprint in the soil? *Plant and soil* **370**, 671-686.

Kujur, A., Kumar, A., Yadav, A., and Prakash, B. (2020). Antifungal and aflatoxin B1 inhibitory efficacy of nanoencapsulated Pelargonium graveolens L. essential oil and its mode of action. *LWT*, 109619.

Kumar, A., Singh, M., Singh, P. P., Singh, S. K., Singh, P. K., and Pandey, K. D. (2016). Isolation of plant growth promoting rhizobacteria and their impact on growth and curcumin content in Curcuma longa L. *Biocatalysis and Agricultural Biotechnology* **8**, 1-7.

Kumar, A., Singh, V. K., Tripathi, V., Singh, P. P., and Singh, A. K. (2018). Plant growth-promoting rhizobacteria (PGPR): perspective in agriculture under biotic and abiotic stress. *In* "Crop improvement through microbial biotechnology", pp. 333-342. Elsevier.

Kumar, K. V., Srivastava, S., Singh, N., and Behl, H. (2009). Role of metal resistant plant growth promoting bacteria in ameliorating fly ash to the growth of Brassica juncea. *Journal of Hazardous Materials* **170**, 51-57.

Kumar, M., and Ashraf, S. (2017). Role of Trichoderma spp. as a biocontrol agent of fungal plant pathogens. *In* "Probiotics and Plant Health", pp. 497-506. Springer.

Kumari, A., and Kumar, R. (2018). Exploring Phyllosphere Bacteria for Growth Promotion and Yield of Potato (Solanum tuberosum L.). *Int. J. Curr. Microbiol. App. Sci* **7**, 1065-1071.

Ladygina, N., and Hedlund, K. (2010). Plant species influence microbial diversity and carbon allocation in the rhizosphere. *Soil Biology and Biochemistry* **42**, 162-168.

Lakshmanan, V., Selvaraj, G., and Bais, H. P. (2014). Functional soil microbiome: belowground solutions to an aboveground problem. *Plant physiology* **166**, 689-700.

Lal, R. (2013). Food security in a changing climate. *Ecohydrology & Hydrobiology* **13**, 8-21.

Larsen, J., Jaramillo-López, P., Nájera-Rincon, M., and González-Esquivel, C. (2015). Biotic interactions in the rhizosphere in relation to plant and soil nutrient dynamics. *Journal of soil science and plant nutrition* **15**, 449-463.

Latif Khan, A., Ahmed Halo, B., Elyassi, A., Ali, S., Al-Hosni, K., Hussain, J., Al-Harrasi, A., and Lee, I.-J. (2016). Indole acetic acid and ACC deaminase from endophytic bacteria improves the growth of Solarium lycopersicum. *Electronic Journal of Biotechnology* **19**, 58-64.

Lawal, T. E., and Babalola, O. O. (2014). Relevance of biofertilizers to agriculture. *Journal of Human Ecology* **47**, 35-43.

Leake, J. R. (2004). Myco-heterotroph/epiparasitic plant interactions with ectomycorrhizal and arbuscular mycorrhizal fungi. *Current opinion in plant biology* **7**, 422-428.

Leff, J. W., Jones, S. E., Prober, S. M., Barberán, A., Borer, E. T., Firn, J. L., Harpole, W. S., Hobbie, S. E., Hofmockel, K. S., and Knops, J. M. (2015). Consistent responses of soil microbial communities to elevated nutrient inputs in grasslands across the globe. *Proceedings of the National Academy of Sciences* **112**, 10967-10972.

Lei, S., Xu, X., Cheng, Z., Xiong, J., Ma, R., Zhang, L., Yang, X., Zhu, Y., Zhang, B., and Tian, B. (2019). Analysis of the community composition and bacterial diversity of the rhizosphere microbiome across different plant taxa. *MicrobiologyOpen* **8**, e00762.

Lemanceau, P., Blouin, M., Muller, D., and Moënne-Loccoz, Y. (2017). Let the core microbiota be functional. *Trends in Plant Science* **22**, 583-595.

Leplae, R., Hebrant, A., Wodak, S. J., and Toussaint, A. (2004). ACLAME: a CLAssification of Mobile genetic Elements. *Nucleic acids research* **32**, D45-D49.

Li, H., Su, J.-Q., Yang, X.-R., and Zhu, Y.-G. (2019). Distinct rhizosphere effect on active and total bacterial communities in paddy soils. *Science of The Total Environment* **649**, 422-430.

Li, X., Rui, J., Mao, Y., Yannarell, A., and Mackie, R. (2014). Dynamics of the bacterial community structure in the rhizosphere of a maize cultivar. *Soil Biology and Biochemistry* **68**, 392-401.

Ling, N., Chen, D., Guo, H., Wei, J., Bai, Y., Shen, Q., and Hu, S. (2017). Differential responses of soil bacterial communities to long-term N and P inputs in a semi-arid steppe. *Geoderma* **292**, 25-33.

Ling, N., Zhang, W., Wang, D., Mao, J., Huang, Q., Guo, S., and Shen, Q. (2013). Root exudates from grafted-root watermelon showed a certain contribution in inhibiting Fusarium oxysporum f. sp. niveum. *PLoS One* **8**.

Liu, F., Qu, Y.-K., Wang, A.-M., Yu, Y.-B., Yang, W.-P., Lv, F., and Nie, Q. (2019). Effects of carotenoids on the growth performance, biochemical parameters, immune responses and disease resistance of yellow catfish (Pelteobagrus fulvidraco) under high-temperature stress. *Aquaculture* **503**, 293-303.

Liu, W., Kohlen, W., Lillo, A., den Camp, R. O., Ivanov, S., Hartog, M., Limpens, E., Jamil, M., Smaczniak, C., and Kaufmann, K. (2011). Strigolactone biosynthesis in Medicago truncatula and rice requires the symbiotic GRAS-type transcription factors NSP1 and NSP2. *The Plant Cell* **23**, 3853-3865.

Liu, W., Zhang, Y., Jiang, S., Deng, Y., Christie, P., Murray, P. J., Li, X., and Zhang, J. (2016). Arbuscular mycorrhizal fungi in soil and roots respond differently to phosphorus inputs in an intensively managed calcareous agricultural soil. *Scientific reports* **6**, 1-11.

Lladó, S., López-Mondéjar, R., and Baldrian, P. (2017). Forest soil bacteria: diversity, involvement in ecosystem processes, and response to global change. *Microbiology and Molecular Biology Reviews* **81**.

Longa, C. M., Nicola, L., Antonielli, L., Mescalchin, E., Zanzotti, R., Turco, E., and Pertot, I. (2017). Soil microbiota respond to green manure in organic vineyards. *Journal of applied microbiology* **123**, 1547-1560.

Loper, J. E., and Gross, H. (2007). Genomic analysis of antifungal metabolite production by Pseudomonas fluorescens Pf-5. *In* "New Perspectives and Approaches in Plant Growth-Promoting Rhizobacteria Research", pp. 265-278. Springer.

Lopez-Fernandez, M., Åström, M., Bertilsson, S., and Dopson, M. (2018). Depth and dissolved organic carbon shape microbial communities in surface influenced but not ancient saline terrestrial aquifers. *Frontiers in microbiology* **9**, 2880.

López-Gámez, G., Elez-Martínez, P., Martín-Belloso, O., and Soliva-Fortuny, R. (2020). Enhancing phenolic content in carrots by pulsed electric fields during post-treatment time: Effects on cell viability and quality attributes. *Innovative Food Science & Emerging Technologies* **59**, 102252.

López, A. C., Alvarenga, A. E., Zapata, P. D., Luna, M. F., and Villalba, L. L. (2019). Trichoderma spp. from Misiones, Argentina: effective fungi to promote plant growth of the regional crop Ilex paraguariensis St. Hil. *Mycology* **10**, 210-221.

Lopez, S., Goux, X., Van Der Ent, A., Erskine, P. D., Echevarria, G., Calusinska, M., Morel, J. L., and Benizri, E. (2019). Bacterial community diversity in the rhizosphere of nickel hyperaccumulator species of Halmahera Island (Indonesia). *Applied Soil Ecology* **133**, 70-80.

Lucas, J. A., García-Cristobal, J., Bonilla, A., Ramos, B., and Gutierrez-Manero, J. (2014). Beneficial rhizobacteria from rice rhizosphere confers high protection against biotic and abiotic stress inducing systemic resistance in rice seedlings. *Plant Physiology and Biochemistry* **82**, 44-53.

Lugtenberg, B. (2015). Life of microbes in the rhizosphere. *In* "Principles of plant-microbe interactions", pp. 7-15. Springer.

Lundberg, D. S., Lebeis, S. L., Paredes, S. H., Yourstone, S., Gehring, J., Malfatti, S., Tremblay, J., Engelbrektson, A., Kunin, V., and Del Rio, T. G. (2012). Defining the core Arabidopsis thaliana root microbiome. *Nature* **488**, 86-90.

Ma, Y., Rajkumar, M., Luo, Y., and Freitas, H. (2011). Inoculation of endophytic bacteria on host and non-host plants—effects on plant growth and Ni uptake. *Journal of Hazardous Materials* **195**, 230-237.

Macías, F. A., Galindo, J. L., García-Díaz, M. D., and Galindo, J. C. (2008). Allelopathic agents from aquatic ecosystems: potential biopesticides models. *Phytochemistry Reviews* **7**, 155-178.

Mafu, S., Ding, Y., Murphy, K. M., Yaacoobi, O., Addison, J. B., Wang, Q., Shen, Z., Briggs, S. P., Bohlmann, J., and Castro-Falcon, G. (2018). Discovery, biosynthesis and stress-related accumulation of dolabradiene-derived defenses in maize. *Plant physiology* **176**, 2677-2690.

Magurran, A. E., and Henderson, P. A. (2003). Explaining the excess of rare species in natural species abundance distributions. *Nature* **422**, 714-716.

Mahoney, A. K., Yin, C., and Hulbert, S. H. (2017). Community structure, species variation, and potential functions of rhizosphere-associated bacteria of different winter wheat (Triticum aestivum) cultivars. *Frontiers in plant science* **8**, 132.

Makowska-Grzyska, M., Kim, Y., Gorla, S. K., Wei, Y., Mandapati, K., Zhang, M., Maltseva, N., Modi, G., Boshoff, H. I., and Gu, M. (2015). Mycobacterium tuberculosis IMPDH in complexes with substrates, products and antitubercular compounds. *PloS one* **10**.

Mallon, C. A., Poly, F., Le Roux, X., Marring, I., van Elsas, J. D., and Salles, J. F. (2015). Resource pulses can alleviate the biodiversity–invasion relationship in soil microbial communities. *Ecology* **96**, 915-926.

Mandal, L., and Kotasthane, A. (2014). Isolation and assessment of plant growth promoting activity of siderophore producing Pseudomonas fluorescens in crops. *International Journal of Agriculture, Environment and Biotechnology* **7**, 63-67.

Manoj, S. R., Karthik, C., Kadirvelu, K., Arulselvi, P. I., Shanmugasundaram, T., Bruno, B., and Rajkumar, M. (2020). Understanding the molecular mechanisms for the enhanced phytoremediation of heavy metals through plant growth promoting rhizobacteria: A review. *Journal of environmental management* **254**, 109779.

Mansfield, J., Genin, S., Magori, S., Citovsky, V., Sriariyanum, M., Ronald, P., Dow, M., Verdier, V., Beer, S. V., and Machado, M. A. (2012). Top 10 plant pathogenic bacteria in molecular plant pathology. *Molecular plant pathology* **13**, 614-629.

Marks, B. B., Megías, M., Nogueira, M. A., and Hungria, M. (2013). Biotechnological potential of rhizobial metabolites to enhance the performance of Bradyrhizobium spp. and Azospirillum brasilense inoculants with soybean and maize. *AMB Express* **3**, 21.

Marks, B. B., Megias, M., Ollero, F. J., Nogueira, M. A., Araujo, R. S., and Hungria, M. (2015). Maize growth promotion by inoculation with Azospirillum brasilense and metabolites of Rhizobium tropici enriched on lipo-chitooligosaccharides (LCOs). *Amb Express* **5**, 1-11.

Marschner, P., Crowley, D., and Rengel, Z. (2011). Rhizosphere interactions between microorganisms and plants govern iron and phosphorus acquisition along the root axis–model and research methods. *Soil Biology and Biochemistry* **43**, 883-894.

Marschner, P., Yang, C.-H., Lieberei, R., and Crowley, D. E. (2001). Soil and plant specific effects on bacterial community composition in the rhizosphere. *Soil biology and biochemistry* **33**, 1437-1445.

Mašínová, T., Bahnmann, B. D., Větrovský, T., Tomšovský, M., Merunková, K., and Baldrian, P. (2017). Drivers of yeast community composition in the litter and soil of a temperate forest. *FEMS microbiology ecology* **93**.

McGill, B. J., Etienne, R. S., Gray, J. S., Alonso, D., Anderson, M. J., Benecha, H. K., Dornelas, M., Enquist, B. J., Green, J. L., and He, F. (2007). Species abundance distributions: moving beyond single prediction theories to integration within an ecological framework. *Ecology letters* **10**, 995-1015.

McGill, B. J., Maurer, B. A., and Weiser, M. D. (2006). Empirical evaluation of neutral theory. *Ecology* **87**, 1411-1423.

McNear Jr, D. H. (2013). The rhizosphere-roots, soil and everything in between. *Nature Education Knowledge* **4**, 1.

McPherson, M. R., Wang, P., Marsh, E. L., Mitchell, R. B., and Schachtman, D. P. (2018). Isolation and analysis of microbial communities in soil, rhizosphere, and roots in perennial grass experiments. *JoVE (Journal of Visualized Experiments)*, e57932.

Meena, V. S., Meena, S. K., Verma, J. P., Kumar, A., Aeron, A., Mishra, P. K., Bisht, J. K., Pattanayak, A., Naveed, M., and Dotaniya, M. (2017). Plant beneficial rhizospheric microorganism (PBRM) strategies to improve nutrients use efficiency: A review. *Ecological Engineering* **107**, 8-32.

Mendes, L. W., Kuramae, E. E., Navarrete, A. A., Van Veen, J. A., and Tsai, S. M. (2014). Taxonomical and functional microbial community selection in soybean rhizosphere. *The ISME journal* **8**, 1577-1587.

Mendes, L. W., Raaijmakers, J. M., de Hollander, M., Mendes, R., and Tsai, S. M. (2018). Influence of resistance breeding in common bean on rhizosphere microbiome composition and function. *The ISME journal* **12**, 212-224.

Mendes, R., Kruijt, M., De Bruijn, I., Dekkers, E., van der Voort, M., Schneider, J. H., Piceno, Y. M., DeSantis, T. Z., Andersen, G. L., and Bakker, P. A. (2011). Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science* **332**, 1097-1100.

Mesterházy, Á., Oláh, J., and Popp, J. (2020). Losses in the grain supply chain: Causes and solutions. *Sustainability* **12**, 2342.

Mhete, M., Eze, P. N., Rahube, T. O., and Akinyemi, F. O. (2020). Soil properties influence bacterial abundance and diversity under different land-use regimes in semi-arid environments. *Scientific African* **7**, e00246.

Mia, M. A. B., Shamsuddin, Z. H., Wahab, Z., and Marziah, M. (2005). High-yielding and quality banana production through plant growth-promoting rhizobacterial (PGPR) inoculation. *Fruits* **60**, 179-185.

Micallef, S. A., Channer, S., Shiaris, M. P., and Colón-Carmona, A. (2009). Plant age and genotype impact the progression of bacterial community succession in the Arabidopsis rhizosphere. *Plant signaling & behavior* **4**, 777-780.

Mierziak, J., Kostyn, K., and Kulma, A. (2014). Flavonoids as important molecules of plant interactions with the environment. *Molecules* **19**, 16240-16265.

Mikić, S., and Ahmad, S. (2018). Benzoxazinoids-protective secondary metabolites in cereals: biochemistry and genetic control. *Ratarstvo i povrtarstvo* **55**, 39-48.

Mishra, J., Prakash, J., and Arora, N. K. (2016). Role of beneficial soil microbes in sustainable agriculture and environmental management. *Climate Change and Environmental Sustainability* **4**, 137-149.

Mitter, B., Pfaffenbichler, N., Flavell, R., Compant, S., Antonielli, L., Petric, A., Berninger, T., Naveed, M., Sheibani-Tezerji, R., and von Maltzahn, G. (2017). A new approach to modify plant microbiomes and traits by introducing beneficial bacteria at flowering into progeny seeds. *Frontiers in Microbiology* **8**, 11.

Mitter, B., Pfaffenbichler, N., and Sessitsch, A. (2016). Plant–microbe partnerships in 2020. *Microbial biotechnology* **9**, 635-640.

Mmbaga, G. W., Mtei, K. M., and Ndakidemi, P. A. (2014). Extrapolations on the use of rhizobium inoculants supplemented with phosphorus (P) and potassium (K) on growth and nutrition of legumes. *Agricultural Sciences* **5**, 1207.

Mohamed, I., Eid, K. E., Abbas, M. H., Salem, A. A., Ahmed, N., Ali, M., Shah, G. M., and Fang, C. (2019). Use of plant growth promoting Rhizobacteria (PGPR) and mycorrhizae to improve the growth and nutrient utilization of common bean in a soil infected with white rot fungi. *Ecotoxicology and Environmental Safety* **171**, 539-548.

Molina-Montenegro, M. A., Ballesteros, G. I., Castro-Nallar, E., Meneses, C., Torres-Díaz, C., and Gallardo-Cerda, J. (2018). Metagenomic exploration of soils microbial communities associated to Antarctic vascular plants. *PeerJ Preprints* **6**, e26508v1.

Molina-Romero, D., Baez, A., Quintero-Hernández, V., Castañeda-Lucio, M., Fuentes-Ramírez, L. E., Bustillos-Cristales, M. d. R., Rodríguez-Andrade, O., Morales-García, Y. E., Munive, A., and Muñoz-Rojas, J. (2017). Compatible bacterial mixture, tolerant to desiccation, improves maize plant growth. *PloS one* **12**, e0187913.

Molinski, T. F. (1993). Marine pyridoacridine alkaloids: structure, synthesis, and biological chemistry. *Chemical Reviews* **93**, 1825-1838.

Mommer, L., Kirkegaard, J., and van Ruijven, J. (2016). Root–root interactions: towards a rhizosphere framework. *Trends in Plant Science* **21**, 209-217.

Morella, N. M., Weng, F. C.-H., Joubert, P. M., Metcalf, C. J. E., Lindow, S., and Koskella, B. (2020). Successive passaging of a plant-associated microbiome reveals robust habitat and host genotype-dependent selection. *Proceedings of the National Academy of Sciences* **117**, 1148-1159.

Mueller, U. G., and Sachs, J. L. (2015). Engineering microbiomes to improve plant and animal health. *Trends in microbiology* **23**, 606-617.

Nacke, H., Goldmann, K., Schöning, I., Pfeiffer, B., Kaiser, K., Castillo-Villamizar, G. A., Schrumpf, M., Buscot, F., Daniel, R., and Wubet, T. (2016). Fine spatial scale variation of soil microbial communities under European beech and Norway spruce. *Frontiers in microbiology* **7**, 2067.

Nacke, H., Will, C., Herzog, S., Nowka, B., Engelhaupt, M., and Daniel, R. (2011). Identification of novel lipolytic genes and gene families by screening of metagenomic libraries derived from soil samples of the German Biodiversity Exploratories. *FEMS microbiology ecology* **78**, 188-201.

Nannipieri, P., Kandeler, E., and Ruggiero, P. (2002). Enzyme activities and microbiological and biochemical processes in soil. *Enzymes in the environment. Marcel Dekker, New York*, 1-33.

Navarro-Noya, Y. E., Hernández-Mendoza, E., Morales-Jiménez, J., Jan-Roblero, J., Martínez-Romero, E., and Hernández-Rodríguez, C. (2012). Isolation and characterization of nitrogen fixing heterotrophic

bacteria from the rhizosphere of pioneer plants growing on mine tailings. *Applied Soil Ecology* **62**, 52-60.
Neal, A. L., Ahmad, S., Gordon-Weeks, R., and Ton, J. (2012). Benzoxazinoids in root exudates of maize attract Pseudomonas putida to the rhizosphere. *PloS one* **7**.
New, P., and Kerr, A. (1972). Biological control of crown gall: field measurements and glasshouse experiments. *Journal of Applied Bacteriology* **35**, 279-287.
Nisar, N., Li, L., Lu, S., Khin, N. C., and Pogson, B. J. (2015). Carotenoid metabolism in plants. *Molecular plant* **8**, 68-82.
Nnedinma, U. (2016). Approaches, drivers and motivators of health and safety self-regulation in the Nigerian construction industry: a scoping study. *Architectural Engineering and Design Management* **12**, 460-475.
O'Callaghan, M. (2016). Microbial inoculation of seed for improved crop performance: issues and opportunities. *Applied microbiology and biotechnology* **100**, 5729-5746.
Odelade, K. A., and 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.
Olanrewaju, O. S., Ayangbenro, A. S., Glick, B. R., and Babalola, O. O. (2019). Plant health: feedback effect of root exudates-rhizobiome interactions. *Applied microbiology and biotechnology* **103**, 1155-1166.
Olanrewaju, O. S., Glick, B. R., and Babalola, O. O. (2017). Mechanisms of action of plant growth promoting bacteria. *World Journal of Microbiology and Biotechnology* **33**, 197.
Oldroyd, G. E. (2013). Speak, friend, and enter: signalling systems that promote beneficial symbiotic associations in plants. *Nature Reviews Microbiology* **11**, 252-263.
Oliveira, A. L., Santos, O. J., Marcelino, P. R., Milani, K. M., Zuluaga, M. Y., Zucareli, C., and Gonçalves, L. S. (2017). Maize inoculation with Azospirillum brasilense Ab-V5 cells enriched with exopolysaccharides and polyhydroxybutyrate results in high productivity under low N fertilizer input. *Frontiers in microbiology* **8**, 1873.
Olivera, E. R., Carnicero, D., García, B., Miñambres, B., Moreno, M. A., Cañedo, L., DiRusso, C. C., Naharro, G., and Luengo, J. M. (2001). Two different pathways are involved in the β-oxidation of n-alkanoic and n-phenylalkanoic acids in Pseudomonas putida U: genetic studies and biotechnological applications. *Molecular microbiology* **39**, 863-874.
Omomowo, I., Ola, I., Akintokun, A., Bankole, M., and Babalola, O. (2009). Direct and residual influence of inoculation with Glomus mosseae and Bradyrhizobium japonicum on proximate and nutrient element content of cowpea seeds. *Am.-Eurasian J. Sustain. Agr* **3**, 435-441.
Omomowo, O. I., and Babalola, O. O. (2019). Bacterial and fungal endophytes: Tiny giants with immense beneficial potential for plant growth and sustainable agricultural productivity. *Microorganisms* **7**, 481.
Ortuño, A., Díaz, L., Alvarez, N., Porras, I., García-Lidón, A., and Del Rio, J. (2011). Comparative study of flavonoid and scoparone accumulation in different Citrus species and their susceptibility to Penicillium digitatum. *Food chemistry* **125**, 232-239.
Otani, T., and Ae, N. (1999). Extraction of organic phosphorus in andosols by various methods. *Soil Science and Plant Nutrition* **45**, 151-161.
Ouma, S. (2016). From financialization to operations of capital: Historicizing and disentangling the finance–farmland-nexus. *Geoforum* **72**, 82-93.
Overbeek, R., Begley, T., Butler, R. M., Choudhuri, J. V., Chuang, H.-Y., Cohoon, M., de Crécy-Lagard, V., Diaz, N., Disz, T., and Edwards, R. (2005). The subsystems approach to genome annotation and its use in the project to annotate 1000 genomes. *Nucleic acids research* **33**, 5691-5702.
Pagare, S., Bhatia, M., Tripathi, N., Pagare, S., and Bansal, Y. (2015). Secondary metabolites of plants and their role: Overview. *Current Trends in Biotechnology and Pharmacy* **9**, 293-304.
Palla, M. S., Guntuku, G. S., Muthyala, M. K. K., Pingali, S., and Sahu, P. K. (2018). Isolation and molecular characterization of antifungal metabolite producing actinomycete from mangrove soil. *Beni-Suef University Journal of Basic and Applied Sciences* **7**, 250-256.
Pandey, V., Awasthi, M., Singh, S., Tiwari, S., and Dwivedi, U. (2017). A comprehensive review on function and application of plant peroxidases. *Biochemistry and Analytical Biochemistry* **6**, 1-16.
Panke-Buisse, K., Poole, A. C., Goodrich, J. K., Ley, R. E., and Kao-Kniffin, J. (2015). Selection on soil microbiomes reveals reproducible impacts on plant function. *The ISME journal* **9**, 980-989.

Parks, D. H., and Beiko, R. G. (2010). Identifying biologically relevant differences between metagenomic communities. *Bioinformatics* **26**, 715-721.
Parnell, J. J., Berka, R., Young, H. A., Sturino, J. M., Kang, Y., Barnhart, D., and DiLeo, M. V. (2016). From the lab to the farm: an industrial perspective of plant beneficial microorganisms. *Frontiers in plant science* **7**, 1110.
Pascale, A., Proietti, S., Pantelides, I. S., and Stringlis, I. A. (2020). Modulation of the root microbiome by plant molecules: the basis for targeted disease suppression and plant growth promotion. *Frontiers in Plant Science* **10**, 1741.
Pavela, R. (2014). Acute, synergistic and antagonistic effects of some aromatic compounds on the Spodoptera littoralis Boisd.(Lep., Noctuidae) larvae. *Industrial Crops and Products* **60**, 247-258.
Peiffer, J. A., Spor, A., Koren, O., Jin, Z., Tringe, S. G., Dangl, J. L., Buckler, E. S., and Ley, R. E. (2013). Diversity and heritability of the maize rhizosphere microbiome under field conditions. *Proceedings of the National Academy of Sciences* **110**, 6548-6553.
Pérez-Jaramillo, J. E., Mendes, R., and Raaijmakers, J. M. (2016). Impact of plant domestication on rhizosphere microbiome assembly and functions. *Plant molecular biology* **90**, 635-644.
Perez, J., Francois, N., Maroniche, G. A., Borrajo, M. P., Pereyra, M., and Creus, C. M. (2018). A novel, green, low-cost chitosan-starch hydrogel as potential delivery system for plant growth-promoting bacteria. *Carbohydrate polymers* **202**, 409-417.
Philippot, L., Raaijmakers, J. M., Lemanceau, P., and Van Der Putten, W. H. (2013). Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology* **11**, 789-799.
Phillips, D., and Tsai, S. (1992). Flavonoids as plant signals to rhizosphere microbes. *Mycorrhiza* **1**, 55-58.
Pii, Y., Mimmo, T., Tomasi, N., Terzano, R., Cesco, S., and 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.
Piñar, G., Poyntner, C., Lopandic, K., Tafer, H., and Sterflinger, K. (2020). Rapid diagnosis of biological colonization in cultural artefacts using the MinION nanopore sequencing technology. *International Biodeterioration & Biodegradation* **148**, 104908.
Pinton, R., Varanini, Z., and Nannipieri, P. (2007). "The rhizosphere: biochemistry and organic substances at the soil-plant interface," CRC press.
Ponge, J.-F. (2015). The soil as an ecosystem. *Biology and Fertility of Soils* **51**, 645-648.
Postma-Blaauw, M. B., de Goede, R. G. M., Bloem, J., Faber, J. H., and Brussaard, L. (2010). Soil biota community structure and abundance under agricultural intensification and extensification. *Ecology* **91**, 460-473.
Preininger, C., Sauer, U., Bejarano, A., and Berninger, T. (2018). Concepts and applications of foliar spray for microbial inoculants. *Applied microbiology and biotechnology* **102**, 7265-7282.
Prudent, M., Salon, C., Souleimanov, A., Emery, R. N., and Smith, D. L. (2015). Soybean is less impacted by water stress using Bradyrhizobium japonicum and thuricin-17 from Bacillus thuringiensis. *Agronomy for sustainable development* **35**, 749-757.
Puri, R. R., Adachi, F., Omichi, M., Saeki, Y., Yamamoto, A., Hayashi, S., Ali, M. A., and Itoh, K. (2019). Metagenomic study of endophytic bacterial community of sweet potato (Ipomoea batatas) cultivated in different soil and climatic conditions. *World Journal of Microbiology and Biotechnology* **35**, 176.
Qi, J., ul Malook, S., Shen, G., Gao, L., Zhang, C., Li, J., Zhang, J., Wang, L., and Wu, J. (2018). Current understanding of maize and rice defense against insect herbivores. *Plant diversity* **40**, 189-195.
Qiu, Z., Egidi, E., Liu, H., Kaur, S., and Singh, B. K. (2019). New frontiers in agriculture productivity: Optimised microbial inoculants and in situ microbiome engineering. *Biotechnology advances* **37**, 107371.
Qurashi, A. W., and Sabri, A. N. (2012). Bacterial exopolysaccharide and biofilm formation stimulate chickpea growth and soil aggregation under salt stress. *Brazilian Journal of Microbiology* **43**, 1183-1191.
Ramirez, K. S., Lauber, C. L., Knight, R., Bradford, M. A., and Fierer, N. (2010). Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology* **91**, 3463-3470.
Rasmann, S., Bennett, A., Biere, A., Karley, A., and Guerrieri, E. (2017). Root symbionts: Powerful drivers of plant above-and belowground indirect defenses. *Insect science* **24**, 947-960.
Rasouli-Sadaghiani, M., Malakouti, M. J., Khavazi, K., and Miransari, M. (2014). Siderophore Efficacy of Fluorescent Pseudomonades Affecting Labeled Iron (59 Fe) Uptake by Wheat (Triticumaestivum L.)

Genotypes Differing in Fe Efficiency. *In* "Use of microbes for the alleviation of soil stresses", pp. 121-132. Springer.

Rastogi, G., Coaker, G. L., and Leveau, J. H. (2013). New insights into the structure and function of phyllosphere microbiota through high-throughput molecular approaches. *FEMS microbiology letters* **348**, 1-10.

Raynaud, X., and Nunan, N. (2014). Spatial ecology of bacteria at the microscale in soil. *PLoS One* **9**, e87217.

Raza, W., Yuan, J., Ling, N., Huang, Q., and Shen, Q. (2015). Production of volatile organic compounds by an antagonistic strain Paenibacillus polymyxa WR-2 in the presence of root exudates and organic fertilizer and their antifungal activity against Fusarium oxysporum f. sp. niveum. *Biological Control* **80**, 89-95.

Reinhold-Hurek, B., Bünger, W., Burbano, C. S., Sabale, M., and Hurek, T. (2015). Roots shaping their microbiome: global hotspots for microbial activity. *Annual review of phytopathology* **53**, 403-424.

Richardson, A. E., and Simpson, R. J. (2011). Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant physiology* **156**, 989-996.

Ritz, K. (2005). Underview: origins and consequences of belowground biodiversity. *Biological diversity and function in soils. Cambridge University Press, Cambridge*, 381-401.

Rizvi, A., Khan, M. S., and Ahmad, E. (2014). Inoculation impact of phosphate-solubilizing microorganisms on growth and development of vegetable crops. *In* "Phosphate Solubilizing Microorganisms", pp. 287-297. Springer.

Robin, A., Vansuyt, G., Hinsinger, P., Meyer, J. M., Briat, J.-F., and Lemanceau, P. (2008). Iron dynamics in the rhizosphere: consequences for plant health and nutrition. *Advances in agronomy* **99**, 183-225.

Rodríguez, C. E., Mitter, B., Barret, M., Sessitsch, A., and Compant, S. (2018). Commentary: seed bacterial inhabitants and their routes of colonization. *Plant and soil* **422**, 129-134.

Roesch, L. F., Fulthorpe, R. R., Riva, A., Casella, G., Hadwin, A. K., Kent, A. D., Daroub, S. H., Camargo, F. A., Farmerie, W. G., and Triplett, E. W. (2007). Pyrosequencing enumerates and contrasts soil microbial diversity. *The ISME journal* **1**, 283-290.

Roger-Estrade, J., Anger, C., Bertrand, M., and Richard, G. (2010). Tillage and soil ecology: partners for sustainable agriculture. *Soil and Tillage Research* **111**, 33-40.

Rokhbakhsh-Zamin, F., Sachdev, D., Kazemi-Pour, N., Engineer, A., Pardesi, K. R., Zinjarde, S., Dhakephalkar, P. K., and Chopade, B. A. (2011). Characterization of plant-growth-promoting traits of Acinetobacter species isolated from rhizosphere of Pennisetum glaucum. *J Microbiol Biotechnol* **21**, 556-566.

Rolli, E., Marasco, R., Vigani, G., Ettoumi, B., Mapelli, F., Deangelis, M. L., Gandolfi, C., Casati, E., Previtali, F., and Gerbino, R. (2015). Improved plant resistance to drought is promoted by the root-associated microbiome as a water stress-dependent trait. *Environmental microbiology* **17**, 316-331.

Romão, D., Staley, C., Ferreira, F., Rodrigues, R., Sabino, R., Veríssimo, C., Wang, P., Sadowsky, M., and Brandão, J. (2017). Next-generation sequencing and culture-based techniques offer complementary insights into fungi and prokaryotes in beach sands. *Marine Pollution Bulletin* **119**, 351-358.

Rúa, M. A., Antoninka, A., Antunes, P. M., Chaudhary, V. B., Gehring, C., Lamit, L. J., Piculell, B. J., Bever, J. D., Zabinski, C., and Meadow, J. F. (2016). Home-field advantage? Evidence of local adaptation among plants, soil, and arbuscular mycorrhizal fungi through meta-analysis. *BMC Evolutionary Biology* **16**, 122.

Ruíz-Sánchez, M., Armada, E., Muñoz, Y., de Salamone, I. E. G., Aroca, R., Ruíz-Lozano, J. M., and Azcón, R. (2011). Azospirillum and arbuscular mycorrhizal colonization enhance rice growth and physiological traits under well-watered and drought conditions. *Journal of plant physiology* **168**, 1031-1037.

Ruiz-Sola, M. Á., Arbona, V., Gómez-Cadenas, A., Rodríguez-Concepción, M., and Rodríguez-Villalón, A. (2014). A root specific induction of carotenoid biosynthesis contributes to ABA production upon salt stress in Arabidopsis. *PLoS One* **9**.

Sablon, L., Dickens, J. C., Haubruge, É., and Verheggen, F. J. (2013). Chemical ecology of the Colorado potato beetle, Leptinotarsa decemlineata (Say)(Coleoptera: Chrysomelidae), and potential for alternative control methods. *Insects* **4**, 31-54.

Samad, A., Antonielli, L., Sessitsch, A., Compant, S., and Trognitz, F. (2017a). Comparative genome analysis of the vineyard weed endophyte Pseudomonas viridiflava CDRTc14 showing selective herbicidal activity. *Scientific reports* **7**, 1-15.

Samad, A., Trognitz, F., Compant, S., Antonielli, L., and Sessitsch, A. (2017b). Shared and host-specific microbiome diversity and functioning of grapevine and accompanying weed plants. *Environmental Microbiology* **19**, 1407-1424.

Sánchez-Pérez, R., Jørgensen, K., Olsen, C. E., Dicenta, F., and Møller, B. L. (2008). Bitterness in almonds. *Plant physiology* **146**, 1040-1052.

Santhanam, R., Weinhold, A., Goldberg, J., Oh, Y., and Baldwin, I. T. (2015). Native root-associated bacteria rescue a plant from a sudden-wilt disease that emerged during continuous cropping. *Proceedings of the National Academy of Sciences* **112**, E5013-E5020.

Santi, C., Certini, G., and 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**, 155-162.

Sasse, J., Martinoia, E., and Northen, T. (2018). Feed your friends: do plant exudates shape the root microbiome? *Trends in plant science* **23**, 25-41.

Sattar, A., Naveed, M., Ali, M., Zahir, Z. A., Nadeem, S. M., Yaseen, M., Meena, V. S., Farooq, M., Singh, R., and Rahman, M. (2019). Perspectives of potassium solubilizing microbes in sustainable food production system: A review. *Applied soil ecology* **133**, 146-159.

Schadt, E. E., Turner, S., and Kasarskis, A. (2010). A window into third-generation sequencing. *Human molecular genetics* **19**, R227-R240.

Schimel, J., and Schaeffer, S. M. (2012). Microbial control over carbon cycling in soil. *Frontiers in microbiology* **3**, 348.

Schlatter, D., Kinkel, L., Thomashow, L., Weller, D., and Paulitz, T. (2017). Disease suppressive soils: new insights from the soil microbiome. *Phytopathology* **107**, 1284-1297.

Shade, A., and Handelsman, J. (2012). Beyond the Venn diagram: the hunt for a core microbiome. *Environmental microbiology* **14**, 4-12.

Shahid, M., Ahmad, A., Khalid, S., Siddique, H. F., Saeed, M. F., Ashraf, M. R., Sabir, M., Niazi, N. K., Bilal, M., and Naqvi, S. T. A. (2016). Pesticides pollution in agricultural soils of Pakistan. *In* "Soil science: Agricultural and environmental prospectives", pp. 199-229. Springer.

Shakeel, M., Rais, A., Hassan, M. N., and Hafeez, F. Y. (2015). Root associated Bacillus sp. improves growth, yield and zinc translocation for basmati rice (Oryza sativa) varieties. *Frontiers in Microbiology* **6**, 1286.

Sharma, A., Johri, B., Sharma, A., and Glick, B. (2003). Plant growth-promoting bacterium Pseudomonas sp. strain GRP3 influences iron acquisition in mung bean (Vigna radiata L. Wilzeck). *Soil Biology and Biochemistry* **35**, 887-894.

Sharma, S. K., Johri, B. N., Ramesh, A., Joshi, O. P., and Prasad, S. S. (2011). Selection of plant growth-promoting Pseudomonas spp. that enhanced productivity of soybean-wheat cropping system in central India. *J Microbiol Biotechnol* **21**, 1127-1142.

Siczek, A., Frąc, M., Gryta, A., Kalembasa, S., and Kalembasa, D. (2020). Variation in soil microbial population and enzyme activities under faba bean as affected by pentachlorophenol. *Applied Soil Ecology* **150**, 103466.

Siddique, S., Hamid, M., Tariq, A., and Kazi, A. G. (2014). Organic farming: the return to nature. *In* "Improvement of crops in the era of climatic changes", pp. 249-281. Springer.

Signorelli, S., Sainz, M., Tabares-da Rosa, S., and Monza, J. (2020). The role of nitric oxide in nitrogen fixation by legumes. *Frontiers in Plant Science* **11**, 521.

Singh, A., Moody, G., Wu, S., Wu, Y., Ghimire, N. J., Yan, J., Mandrus, D. G., Xu, X., and Li, X. (2014). Coherent electronic coupling in atomically thin MoSe 2. *Physical review letters* **112**, 216804.

Singh, J. S., and Gupta, V. K. (2018). Soil microbial biomass: a key soil driver in management of ecosystem functioning. *Science of the Total Environment* **634**, 497-500.

Singh, N., Singh, R., Meena, V., and Meena, R. (2015). Can we use maize (Zea mays) rhizobacteria as plant growth promoter. *Vegetos* **28**, 86-99.

Smalla, K., Wieland, G., Buchner, A., Zock, A., Parzy, J., Kaiser, S., Roskot, N., Heuer, H., and Berg, G. (2001). Bulk and rhizosphere soil bacterial communities studied by denaturing gradient gel electrophoresis: plant-dependent enrichment and seasonal shifts revealed. *Applied and environmental microbiology* **67**, 4742-4751.

Smith, O. P. (2013). Allelopathic Potential of the Invasive Alien Himalayan Balsam (Impatiens glandulifera Royle).
Smith, S. E., and Smith, F. A. (2011). Roles of arbuscular mycorrhizas in plant nutrition and growth: new paradigms from cellular to ecosystem scales. *Annual review of plant biology* **62**, 227-250.
Spence, C., and Bais, H. (2015). Role of plant growth regulators as chemical signals in plant–microbe interactions: a double edged sword. *Current opinion in plant biology* **27**, 52-58.
Steinauer, K., Chatzinotas, A., and Eisenhauer, N. (2016). Root exudate cocktails: the link between plant diversity and soil microorganisms? *Ecology and Evolution* **6**, 7387-7396.
Steven, B., Huntley, R. B., and Zeng, Q. (2018). The influence of flower anatomy and apple cultivar on the apple flower phytobiome. *Phytobiomes* **2**, 171-179.
Stone, B. W., Weingarten, E. A., and Jackson, C. R. (2018). The role of the phyllosphere microbiome in plant health and function. *Annual Plant Reviews online*, 533-556.
Strickland, M. S., McCulley, R. L., Nelson, J. A., and Bradford, M. A. (2015). Compositional differences in simulated root exudates elicit a limited functional and compositional response in soil microbial communities. *Frontiers in Microbiology* **6**, 817.
Sturz, A., and Christie, B. (2003). Beneficial microbial allelopathies in the root zone: the management of soil quality and plant disease with rhizobacteria. *Soil and Tillage Research* **72**, 107-123.
Sugiyama, A., Bakker, M. G., Badri, D. V., Manter, D. K., and Vivanco, J. M. (2013). Relationships between Arabidopsis genotype-specific biomass accumulation and associated soil microbial communities. *Botany* **91**, 123-126.
Sun, X., Zhou, Y., Tan, Y., Wu, Z., Lu, P., Zhang, G., and Yu, F. (2018). Restoration with pioneer plants changes soil properties and remodels the diversity and structure of bacterial communities in rhizosphere and bulk soil of copper mine tailings in Jiangxi Province, China. *Environmental Science and Pollution Research* **25**, 22106-22119.
Sundram, S., Meon, S., Seman, I. A., and Othman, R. (2015). Application of arbuscular mycorrhizal fungi with Pseudomonas aeruginosa UPMP3 reduces the development of Ganoderma basal stem rot disease in oil palm seedlings. *Mycorrhiza* **25**, 387-397.
Syranidou, E., Christofilopoulos, S., Gkavrou, G., Thijs, S., Weyens, N., Vangronsveld, J., and Kalogerakis, N. (2016). Exploitation of endophytic bacteria to enhance the phytoremediation potential of the wetland helophyte Juncus acutus. *Frontiers in microbiology* **7**, 1016.
Szoboszlay, M., Lőrincz, A., Lanore, F., Vervaeke, K., Silver, R. A., and Nusser, Z. (2016). Functional properties of dendritic gap junctions in cerebellar Golgi cells. *Neuron* **90**, 1043-1056.
Tatangelo, V., Franzetti, A., Gandolfi, I., Bestetti, G., and Ambrosini, R. (2014). Effect of preservation method on the assessment of bacterial community structure in soil and water samples. *FEMS microbiology letters* **356**, 32-38.
Tatsumi, K., Yano, M., Kaminade, K., Sugiyama, A., Sato, M., Toyooka, K., Aoyama, T., Sato, F., and Yazaki, K. (2016). Characterization of shikonin derivative secretion in Lithospermum erythrorhizon hairy roots as a model of lipid-soluble metabolite secretion from plants. *Frontiers in plant science* **7**, 1066.
Tenaillon, M. I., Hufford, M. B., Gaut, B. S., and Ross-Ibarra, J. (2011). Genome size and transposable element content as determined by high-throughput sequencing in maize and Zea luxurians. *Genome biology and evolution* **3**, 219-229.
Thrall, P. H., Laine, A.-L., Broadhurst, L. M., Bagnall, D. J., and Brockwell, J. (2011). Symbiotic effectiveness of rhizobial mutualists varies in interactions with native Australian legume genera. *PLoS One* **6**, e23545.
Timmusk, S., Behers, L., Muthoni, J., Muraya, A., and Aronsson, A.-C. (2017). Perspectives and challenges of microbial application for crop improvement. *Frontiers in plant science* **8**, 49.
Tiwari, P., Indoliya, Y., Singh, P. K., Singh, P. C., Chauhan, P. S., Pande, V., and Chakrabarty, D. (2019). Role of dehydrin-FK506-binding protein complex in enhancing drought tolerance through the ABA-mediated signaling pathway. *Environmental and Experimental Botany* **158**, 136-149.
Tkacz, A., Bestion, E., Bo, Z., Hortala, M., and Poole, P. S. (2020). Influence of Plant Fraction, Soil, and Plant Species on Microbiota: a Multikingdom Comparison. *Mbio* **11**.
Toju, H., Peay, K. G., Yamamichi, M., Narisawa, K., Hiruma, K., Naito, K., Fukuda, S., Ushio, M., Nakaoka, S., and Onoda, Y. (2018). Core microbiomes for sustainable agroecosystems. *Nature Plants* **4**, 247-257.

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

Townsend, C., and Ebizuka, Y. (2010). Natural Products Structural Diversity-I Secondary Metabolites: Organization and biosynthesis. Elsevier, UK.

Traveset, A., and Richardson, D. M. (2014). Mutualistic interactions and biological invasions. *Annual Review of Ecology, Evolution, and Systematics* **45**, 89-113.

Tringe, S. G., Von Mering, C., Kobayashi, A., Salamov, A. A., Chen, K., Chang, H. W., Podar, M., Short, J. M., Mathur, E. J., and Detter, J. C. (2005). Comparative metagenomics of microbial communities. *Science* **308**, 554-557.

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hamonts, K., Anderson, I. C., and Singh, B. K. (2017). Keystone microbial taxa regulate the invasion of a fungal pathogen in agro-ecosystems. *Soil Biology and Biochemistry* **111**, 10-14.

Turra, D., El Ghalid, M., Rossi, F., and Di Pietro, A. (2015). Fungal pathogen uses sex pheromone receptor for chemotropic sensing of host plant signals. *Nature* **527**, 521-524.

Uarrota, V. G., Severino, R. B., and Maraschin, M. (2011). Maize landraces (Zea mays L.): a new prospective source for secondary metabolite production. *International Journal of Agricultural Research* **6**, 218-226.

Uren, N. C. (2007). Types, amounts, and possible functions of compounds released into the rhizosphere by soil-grown plants. *The rhizosphere: biochemistry and organic substances at the soil-plant interface* **2**, 1-21.

Urich, T., Lanzén, A., Qi, J., Huson, D. H., Schleper, C., and Schuster, S. C. (2008). Simultaneous assessment of soil microbial community structure and function through analysis of the meta-transcriptome. *PloS one* **3**, e2527.

Uroz, S., Buée, M., Murat, C., Frey-Klett, P., and Martin, F. (2010). Pyrosequencing reveals a contrasted bacterial diversity between oak rhizosphere and surrounding soil. *Environmental Microbiology Reports* **2**, 281-288.

Uroz, S., Ioannidis, P., Lengelle, J., Cébron, A., Morin, E., Buée, M., and Martin, F. (2013). Functional assays and metagenomic analyses reveals differences between the microbial communities inhabiting the soil horizons of a Norway spruce plantation. *PLoS One* **8**, e55929.

Vahidinia, A., Samiee, F., Faradmal, J., Rahmani, A., Javad, M. T., and Leili, M. (2019). Mercury, lead, cadmium, and barium levels in human breast milk and factors affecting their concentrations in Hamadan, Iran. *Biological trace element research* **187**, 32-40.

van Dam, N. M., and Bouwmeester, H. J. (2016). Metabolomics in the rhizosphere: tapping into belowground chemical communication. *Trends in plant science* **21**, 256-265.

Vaughan, M. M., Tholl, D., and Tokuhisa, J. G. (2011). An aeroponic culture system for the study of root herbivory on Arabidopsis thaliana. *Plant Methods* **7**, 5.

Venturi, V., and Keel, C. (2016). Signaling in the rhizosphere. *Trends in plant science* **21**, 187-198.

Viebahn, M., Veenman, C., Wernars, K., van Loon, L. C., Smit, E., and Bakker, P. A. (2005). Assessment of differences in ascomycete communities in the rhizosphere of field-grown wheat and potato. *FEMS microbiology ecology* **53**, 245-253.

Vitorino, L., and Bessa, L. (2018a). Microbial Diversity: The Gap between the Estimated and the Known. *Diversity* **10**, 46.

Vitorino, L. C., and Bessa, L. A. (2018b). Microbial diversity: the gap between the estimated and the known. *Diversity* **10**, 46.

Vivas, A., Barea, J., Biro, B., and Azcon, R. (2006). Effectiveness of autochthonous bacterium and mycorrhizal fungus on Trifolium growth, symbiotic development and soil enzymatic activities in Zn contaminated soil. *Journal of applied microbiology* **100**, 587-598.

Vos, M., Wolf, A. B., Jennings, S. J., and Kowalchuk, G. A. (2013). Micro-scale determinants of bacterial diversity in soil. *FEMS microbiology reviews* **37**, 936-954.

Wallace, J. G., Kremling, K. A., and Buckler, E. S. (2018). Quantitative Genetic Analysis of the Maize Leaf Microbiome. *BioRxiv*, 268532.

Wang, G., Zhao, Y., Gao, H., Yue, W., Xiong, M., Li, F., Zhang, H., and Ge, W. (2013). Co-metabolic biodegradation of acetamiprid by Pseudoxanthomonas sp. AAP-7 isolated from a long-term acetamiprid-polluted soil. *Bioresource technology* **150**, 259-265.

Wang, R., Wu, T., Dai, W., Liu, H., Zhao, J., Wang, X., Huang, F., Wang, Z., and Shi, C. (2015). Effects of straw return on C2–C5 non-methane hydrocarbon (NMHC) emissions from agricultural soils. *Atmospheric Environment* **100**, 210-217.

Wang, Y.-H., Zhang, Z.-K., Yang, F.-M., Sun, Q.-Y., He, H.-P., Di, Y.-T., Mu, S.-Z., Lu, Y., Chang, Y., and Zheng, Q.-T. (2007). Benzylphenethylamine alkaloids from Hosta plantaginea with inhibitory activity against tobacco mosaic virus and acetylcholinesterase. *The Journal of Natural Products* **70**, 1458-1461.

Wardle, D., Nicholson, K., Bonner, K., and Yeates, G. (1999). Effects of agricultural intensification on soil-associated arthropod population dynamics, community structure, diversity and temporal variability over a seven-year period. *Soil Biology and Biochemistry* **31**, 1691-1706.

Wassermann, B., Cernava, T., Müller, H., Berg, C., and Berg, G. (2019). Seeds of native alpine plants host unique microbial communities embedded in cross-kingdom networks. *Microbiome* **7**, 108.

Weidenhamer, J. D., and Callaway, R. M. (2010). Direct and indirect effects of invasive plants on soil chemistry and ecosystem function. *Journal of chemical ecology* **36**, 59-69.

Weir, T., Perry, L., Gilroy, S., and Vivanco, J. (2010). The role of root exudates in rhizosphere interactions with plants and other organisms. *Annual Review of Plant Biology*.

Wu, T., Cheng, D., He, M., Pan, S., Yao, X., and Xu, X. (2014). Antifungal action and inhibitory mechanism of polymethoxylated flavones from Citrus reticulata Blanco peel against Aspergillus niger. *Food Control* **35**, 354-359.

Wuyts, J., Van de Peer, Y., Winkelmans, T., and De Wachter, R. (2002). The European database on small subunit ribosomal RNA. *Nucleic acids research* **30**, 183-185.

Xue, P.-P., Carrillo, Y., Pino, V., Minasny, B., and McBratney, A. B. (2018). Soil properties drive microbial community structure in a large scale transect in south eastern Australia. *Scientific reports* **8**, 1-11.

Xun, F., Xie, B., Liu, S., and Guo, C. (2015). Effect of plant growth-promoting bacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) inoculation on oats in saline-alkali soil contaminated by petroleum to enhance phytoremediation. *Environmental Science and Pollution Research* **22**, 598-608.

Yan, X.-H., Chen, J., Di, Y.-T., Fang, X., Dong, J.-H., Sang, P., Wang, Y.-H., He, H.-P., Zhang, Z.-K., and Hao, X.-J. (2010). Anti-tobacco mosaic virus (TMV) quassinoids from Brucea javanica (L.) Merr. *Journal of agricultural and food chemistry* **58**, 1572-1577.

Yang, Y., Wang, N., Guo, X., Zhang, Y., and Ye, B. (2017). Comparative analysis of bacterial community structure in the rhizosphere of maize by high-throughput pyrosequencing. *PLoS One* **12**, e0178425.

Yarzábal, L. A., and Chica, E. J. (2019). Role of Rhizobacterial Secondary Metabolites in Crop Protection Against Agricultural Pests and Diseases. *In* "New and Future Developments in Microbial Biotechnology and Bioengineering", pp. 31-53. Elsevier.

Yousuf, B., Keshri, J., Mishra, A., and Jha, B. (2012). Application of targeted metagenomics to explore abundance and diversity of CO2-fixing bacterial community using cbbL gene from the rhizosphere of Arachis hypogaea. *Gene* **506**, 18-24.

Yu, H., Ling, N., Wang, T., Zhu, C., Wang, Y., Wang, S., and Gao, Q. (2019). Responses of soil biological traits and bacterial communities to nitrogen fertilization mediate maize yields across three soil types. *Soil and Tillage Research* **185**, 61-69.

Zafra, G., Taylor, T. D., Absalón, A. E., and Cortés-Espinosa, D. V. (2016). Comparative metagenomic analysis of PAH degradation in soil by a mixed microbial consortium. *Journal of hazardous materials* **318**, 702-710.

Zagrobelny, M., Bak, S., and Møller, B. L. (2008). Cyanogenesis in plants and arthropods. *Phytochemistry* **69**, 1457-1468.

Zarea, M., Hajinia, S., Karimi, N., Goltapeh, E. M., Rejali, F., and Varma, A. (2012). Effect of Piriformospora indica and Azospirillum strains from saline or non-saline soil on mitigation of the effects of NaCl. *Soil Biology and Biochemistry* **45**, 139-146.

Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N. A., Mills, D. A., Martin, G., and Taghavi, S. (2015). The soil microbiome influences grapevine-associated microbiota. *MBio* **6**.

Zaynab, M., Fatima, M., Abbas, S., Sharif, Y., Umair, M., Zafar, M. H., and Bahadar, K. (2018). Role of secondary metabolites in plant defense against pathogens. *Microbial pathogenesis* **124**, 198-202.

Zerbe, P. (2015). Small molecules with big impact: terpenoid phytoalexins as key factors in maize stress tolerance. *Plant, cell & environment* **38**, 2193-2194.

Zhalnina, K., Louie, K. B., Hao, Z., Mansoori, N., da Rocha, U. N., Shi, S., Cho, H., Karaoz, U., Loqué, D., and Bowen, B. P. (2018). Dynamic root exudate chemistry and microbial substrate preferences drive patterns in rhizosphere microbial community assembly. *Nature microbiology* **3**, 470-480.

Zhang, W., Lin, Z., Pang, S., Bhatt, P., and Chen, S. (2020). Insights into the biodegradation of lindane (γ-hexachlorocyclohexane) using a microbial system. *Frontiers in Microbiology* **11**, 522.

Zhao, J., Zhang, D., Yang, Y., Pan, Y., Zhao, D., Zhu, J., Zhang, L., and Yang, Z. (2020). Dissecting the effect of continuous cropping of potato on soil bacterial communities as revealed by high-throughput sequencing. *PloS one* **15**, e0233356.

Zhou, C., Guo, J., Zhu, L., Xiao, X., Xie, Y., Zhu, J., Ma, Z., and Wang, J. (2016). Paenibacillus polymyxa BFKC01 enhances plant iron absorption via improved root systems and activated iron acquisition mechanisms. *Plant Physiology and Biochemistry* **105**, 162-173.

Zhou, Y., Qin, Y., Liu, X., Feng, Z., Zhu, H., and Yao, Q. (2019). Soil bacterial function associated with stylo (legume) and bahiagrass (grass) is affected more strongly by soil chemical property than by bacterial community composition. *Frontiers in microbiology* **10**, 798.

Zhou, Y., Zhu, H., Fu, S., and Yao, Q. (2017). Variation in soil microbial community structure associated with different legume species is greater than that associated with different grass species. *Frontiers in Microbiology* **8**, 1007.

Zilber-Rosenberg, E. R. I. (2013). 15 Role of Microorganisms in Adaptation, Development, and Evolution of Animals and Plants: The Hologenome Concept. *Prokaryotic Biology and Symbiotic Associations*.