Metagenomic analysis of agricultural soils under organic and inorganic fertilization

MC Enebe

orcid.org/0000-0003-0865-8336

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: 31480470

DECLARATION

I, Matthew Chekwube Enebe, declare that this thesis submitted to the North-West University for the degree of Doctor of Philosophy in Biology in the Faculty of Natural and Agricultural Sciences, School of Biological Sciences, is my original work with the exception of 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: **Matthew Chekwube Enebe**

Signature 

Date…21 – 04 – 2020

Supervisor: **Prof. Olubukola Oluranti BABALOLA**

Signature……

Date……

DEDICATION

This work is dedicated to Almighty God for His abundant blessings, provisions and protection upon my family.

ACKNOWLEDGEMENT

I remain ever grateful to my supervisor Prof Olubukola O Babalola for her wonderful support, guidance and mentorship in making this work a success. I say thank you.

I also want to appreciate the financial support I recieved from NRF (National Research Foundation of South Africa) and TWAS (The World Academy of Science) for NRF-TWAS African Renaissance Doctoral Scholarship (Grant UID: 110914). The financial support of North-West University bursary is also greatly appreciated. I thank all the staff members of the Department of Microbiology.

I equally wish to acknowledge my appreciation of outstanding work of my instructional father and mentor Dr. Vincent N Chigor, for being humble and kind enough to support my dream of obtaining a PhD degree abroad and encourage me with words of advice.

My special thanks to my family who made great sacrifices in making a man out of me. My mother, Mrs. Justina N. Enebe and my Sisters; Ogochukwu, Ngozi and Chioma. I love you forever. To my wife Dr (Mrs) Nwabunwanne L. Enebe who contributed greatly in standing by me through thick and thin and for her encouragements, love, prayers, and support

I also acknowledge those who helped me in many ways: Dr Oyeyemi Dada, Prof Damian Onwudiwe, Chief Joseph I. Aguokpo, Dr. Benard Ojuederie, Dr. Monica Uzoh, Mrs Mamsi Khansti, Dr Olayinka S Titilawo and all my friends at the Microbial Biotechnology group.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

GENERAL ABSTRACT

Soil fertilization is an age-old agricultural practice aimed at increasing soil nutrition and soil health. Recently, agriculturists have come to the realization that inorganic fertilizer application over time could result in no corresponding increase in crop yield. Organic manure has been shown to be beneficial to soil health and in promotion of soil ecosystems' biodiversity. A healthy soil is one in which the microbes present are viable and performing their activities in nutrient cycling and sustaining plant health. However, increased fertilization of agricultural soils has resulted in a series of environmental degradation effects. This necessitates the adoption of a suitable strategy for increasing soil fertility without distorting the stability of the soil micro and macro ecosystems. In this study, we employed shotgun metagenomics sequencing to understand the effects of fertilization with organic and inorganic fertilizers at different concentrations on the soil microbiome structure, diversity and function at the maize rhizosphere. The soil samples were sourced from maize plants rhizosphere treated with compost manure at a concentration of 8 tons and 4 tons per hectare as well as from the ones treated with 120 kg and 60 kg per hectare inorganic fertilizers (NPK – 20:7:3) and control. The pH of the soil was 4.97, thus slightly acidic. Other physicochemical properties of the soil were determined. The effects of these fertilizers were investigated on carbon, nitrogen, and phosphorus cycling genes. It was equally evaluated for the genes involved in bacterial chemotaxis and disease suppression in the soil. The different fertilizer doses and types have effects on the microbial communities and their basic functions in the soil. *Proteobacteria* and *Bacteroidetes* were distributed in all the treated samples, with *Proteobacteria and Actinobacteria* being very abundant in high dose of compost manure (Cp8), low inorganic fertilizer (N1) and the control (Cn0) treatments. *Firmicutes* were most abundant in high inorganic fertilizer (N2) treated soils and low compost manure (Cp4) treatments. Besides, the results showed that fungi were selected and enriched by high and low compost manure, while archaea were mostly supported by high dose of inorganic fertilizer and high compost manure treatments. Functional genes involved in carbon, nitrogen and phosphorus cycling were highly enhanced in the maize rhizosphere under high compost, lower inorganic fertilizer and

the control. Disease suppressive genes such as genes for antibiotics, antifungal, nematicides and siderophores production were abundant in the maize rhizosphere. Bacterial chemotaxis were observed to be high in these treatments. On the other hand, low compost manure and high inorganic fertilizers tended to suppress these microbial beneficial functions. This implies that there is higher chance of disease development and poor crop yield in a soil treated with these nutrients at the specified concentrations. They do not promote biodiversity due to inorganic fertilizers' associated soil acidification and salinization and low compost manure induced nutrients starvation to soil microbes and hence their observed negative effects. Therefore, to achieve a sustainable agriculture in maintaining biodiversity within the soil ecosystem and increasing crop yield under a semi-arid condition, higher doses of compost manure and lower inorganic fertilizer should be the desired fertilization choice. Maize plants could be harnessed through intercropping practice to enable other plants to benefit from their enrichment of soil microbial community structure and functions through rhizosphere effects.

Keywords: Soil metagenomes, chemotaxis genes, disease suppression, soil fertilization, biogeochemical cycling, rhizosphere, metagenomics

LIST OF PUBLICATIONS

Chapter Two: The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: a survival strategy.

This chapter is published in Applied Microbiology and Biotechnology. (2018) 102:7821-7835.

Authors: Matthew Chekwube Enebe and Olubukola Oluranti Babalola

Candidate's Contributions: searched the literature, designed the work and wrote the manuscript.

Chapter Three: The impact of microbes in the orchestration of plants' resistance to biotic stress: a disease management approach.

This chapter is published in Applied Microbiology and Biotechnology. (2019) 103:9–25

https://doi.org/10.1007/s00253-018-9433-3

Authors: Matthew Chekwube Enebe and Olubukola Oluranti Babalola

Candidate's Contributions: searched the literature, organized the work and wrote the manuscript.

Chapter Four: The influence of soil fertilization on soil microbial communities and soil health: a guide to nutrient management.

This chapter is undergoing a review process in Archives of Microbiology.

Authors: Matthew Chekwube Enebe and Olubukola Oluranti Babalola

Candidate's Contributions: searched the literature, and wrote the first draft of the manuscript.

Chapter Five: Effects of Inorganic and Organic treatments on the Microbial Community of Maize Rhizosphere by a shotgun metagenomics approach.

This chapter is published in Annals of Microbiology, (2020) 70:49. https://doi.org/10.1186/s13213-020-01591-8

Authors: Matthew Chekwube Enebe and Olubukola Oluranti Babalola

Candidate‘s Contributions: searched the literature, performed the research, analysed data and wrote the manuscript.

Chapter Six: Metagenomics evaluation of the functional diversity of bacteria community in maize rhizosphere grown in a semi-arid region of South Africa.

This chapter is under review by Journal of Soil Science and Plant Nutrition.

Authors: Matthew Chekwube Enebe and Olubukola Oluranti Babalola

Candidate‘s Contributions: searched the literature, performed the research, analysed data and wrote the manuscript.

Chapter Seven: Soil fertilization affects the abundance and distribution of carbon and nitrogen cycling genes in the maize rhizosphere

This chapter is under review in Applied Microbiology and Biotechnology Express (AMBExpress).

Authors: Matthew Chekwube Enebe and Olubukola Oluranti Babalola

Candidate‘s Contributions: performed the research, analysed data and wrote the manuscript.

Chapter Eight: Metagenomics assessment on the effects of soil fertilization on antimicrobial synthesizing, siderophores and chemotaxis genes for induction of disease suppressive soil in the maize rhizosphere

This chapter is under review in Applied Biochemistry and Microbiology

Authors: Matthew Chekwube Enebe and Olubukola Oluranti Babalola

Candidate‘s Contributions: performed the research, analysed data and wrote the manuscript.

Chapter Nine: The influence of soil fertilization on the distribution and diversity of phosphorus cycling genes from maize rhizosphere using shotgun metagenomics

This chapter is under consideration for publication in Current Microbiology

Authors: Matthew Chekwube Enebe and Olubukola Oluranti Babalola

Candidate‘s Contributions: performed the research, analysed data and wrote the manuscript.

CHAPTER ONE

GENERAL INTRODUCTION

1.1 Introduction

Agriculture is the bedrock of the health sustainability of nations. Humans and livestock need food to survive and remain healthy. This need has prompted intensification of agricultural practices to increase crop yield. Plants' performance depends on a wide range of factors but the availability of nutrients is the most influential. These nutrients are in limited supply in the soil and so need to be resupplied back to the soil by either natural or artificial means. Natural means of soil fertilization is usually achieved through the fallow system. However, this takes a lot of time and requires that the land be abandoned for years. The limited supply of farmlands makes this method practically unacceptable and unrealistic. The only suitable alternative is through artificial means of applying inorganic fertilizer, compost manure, treated sewage sludge or animal droppings. Chemical fertilizers have associated environmental negative effects like eutrophication and soil acidification (Zhu et al., 2016). This change in the pH condition of the soil tends to interfere with the attainment of a viable and balanced microecosystem in the soil.

Application of organic fertilizers have beneficial effects over inorganic fertilizers in promoting the diversity of soil microbial communities and enriching the soil with organic carbon, nitrogen and other nutrients (Chávez-Romero et al., 2016). Microbial biodiversity in the soil is increased by fertilization, and knowledge of the functional diversity is necessary for the comprehension of ecological and microbial functional roles involved in nutrient cycling and plant growth promotion (Zhu et al., 2017, Lahiri et al., 2018).

The availability of nutrients in the soil contributes to microbial viability and improves their ecological functions to the plants such as disease suppression, nutrient cycling, priming of plant immunity,

production of phytohormones and enhancement of plants' tolerance to abiotic soil stress (Van Der Putten et al., 2016, Enebe and Babalola, 2018, Enebe and Babalola, 2019).

Plants, on the other hand, regulate the biodiversity and activities of rhizosphere microbes through root exudation, temperature and moisture control (Denef et al., 2009). The rhizosphere is an ecological niche where the activities of microbes are dependent on the level of plant – microbe interactions. Plants excrete sugars, minerals, carbon compounds and a range of secondary metabolites which support or suppress the proliferation of these microbes. This makes plants to have a selective role in the number and type as well as the microbial activities that will prevail in the soil (Yu and Hochholdinger, 2018, Mendes et al., 2013). Maize (*Zea mays averta*), an important staple crop, has been found to support soil dwelling microbes. They do this by exudation of amino acids, sugars, and organic acids during their vegetative growth (Nunes Da Rocha et al., 2009). Both maize plants, inorganic and organic fertilizers are key drivers of soil microbial communities biological and physiological functions like chemotaxis, stress tolerance and nutrient cycling. The metabolic activities of microbes are often higher in a fertilized soil with an increase in the abundance of chemotaxis, nitrogen cycling, carbon and phosphorus cycling genes (Parkinson et al., 2015, Zeng et al., 2016a).

Although a number of studies have studied rhizosphere microbial communities of plants, little has been done on the area of understanding the effects of soil fertilization on the structural, molecular and functions of the maize rhizosphere dwelling microbes. Accurate knowledge of the relationship between fertilization and ecological functions present in the rhizosphere is needed for the manipulation of the soil microecosystem. To achieve a sustainable agriculture in increasing crop yield and maintaining soil health, proper fertilization management and determination of the influence of maize plants on the soil microbial community is the focus of this study and needed detailed evaluation.

The objectives of this study were:

1. To investigate the taxonomic abundance and diversity of the rhizosphere microbial community.
2. To evaluate the maize rhizosphere bacterial functional diversity.
3. To evaluate the influence of soil fertilization on the abundance and distribution of carbon, phosphorus and nitrogen microbial cycling genes within the maize rhizosphere.
4. To determine the effects of soil fertilization on the abundance of disease suppressive genes: antimicrobial, chemotaxis and siderophores genes at the maize rhizosphere.

CHAPTER TWO

The Influence of Plant Growth Promoting Rhizobacteria in Plant Tolerance to Abiotic Stress: A Survival Strategy

Abstract

Action is needed to face the global threat arising from inconsistent rainfall, increase in temperature, and salinization of farm lands which may be the product of climate change. As crops are adversely affected, man and animals may face famine. Plants are severely affected by abiotic stress (drought, salinity, alkalinity and temperature), which impairs yield and results in loss to farmers and to the nation at large. However, microbes have been shown to be of great help in the fight against abiotic stress, via their biological activities at the rhizosphere of plants. The external application of chemical substances such as glycine betaine, proline and nutrients has helped in sustaining plant growth and productive ability. In this review, we tried to understand the part played by bioinoculants in aiding plants to resist the negative consequences arising from abiotic stress and to suggest better practices that will be of help in today's farming systems. The fact that absolute protection and sustainability of plant yield under stress challenges has not been achieved by microbes, nutrients, nor the addition of chemicals (osmo-protectants) alone suggests that studies should focus on the integration of these units (microbes, nutrients, chemical stimulants and osmo-protectants) into a strategy for achieving a complete tolerance to abiotic stress. Also, other species of microbes capable of shielding plants from stress, boosting yield and growth, providing nutrient and protecting the plants from harmful invading pathogens should be sought.

2.1 Introduction

The unpredictability of the hydrological cycle has posed a serious challenge to farmers, horticulturists and to the global community, concerning its effect in meeting food needs of mankind and animals.

The number of people to be fed are constantly increasing and food supplies are not meeting the demand.

In order to increase the quantity and quality of crops grown, agriculturists have intensified the use of open and ground water sources for irrigation purposes, which has a corresponding salinization implication.

However, the use of bioinoculants (Plant growth promoting rhizobacteria) has been of great help in combating this abiotic – climate - induced change that limits the overall performance of plants under stress (Alori et al., 2017a, Staudinger et al., 2016). The use of biofertilizers to enhance successful adaptation and survival of plants will gear towards ensuring a sustainable crop yield and improvement of soil fertility and structure. This is a good approach to stress management (Alori et al., 2017a). These microbes through deaminase enzyme production (Saleem et al., 2015), nodulation (Masciarelli et al., 2014) and other physiological activities at the rhizosphere help the plants tolerate stress.

External (exogenous) introduction of beneficial chemical substances as supplements has been used to improve resilience, yield and tolerance of plants to the toxicity of these stress imposed conditions, such as the application of caffeic acid (Klein et al., 2015), sodium polyacrylate (Hong et al., 2016b), jasmonic acid (Khan et al., 2017) genistein (Hasanah and Rahmawat, 2012), chitosan (Bistgani et al., 2017), humic acid (Kasmani et al., 2013), gibberellic acid and other hormones (Shaddad et al., 2013), and molybdenum (Bouzid and Rahmoune, 2012). These chemicals aid the physiological performance and adaptation that manifest in a well-balanced morphological state of the plant growing in a stressful environment. The stresses ranges from drought, salinity, alkalinity, pathogenic microbial attack to chemicals.

Abiotic stresses are stress conditions to plants arising from the environment. They are the non-living part of the ecosystem whose effect is felt by the living component of the system. Nature is balance sensitive, and at short supply or deviation from the normal occurrence of these conditions will create a stress in the ecosystem and jeopardize the well-being of living things. Water, nutrients, salts,

temperature and pH are among the basic abiotic components of the agricultural ecosystem that have an influence on plants.

These abiotic stresses not only affect plants but also affect microbes. In an effort to survive and prevail in spite of the stiff competition among microbes particularly at the rhizosphere, some microbes possessing the cellulase enzyme capable of dissolving the cellulose cell wall of plant roots gain entrance into the apoplast of plants, the cell wall interior as well as the vascular bundle – xylem, where they live and undergo normal metabolic activities.

Although researchers have looked at various means of handling the stress inducers to enable plants to increase their survival and performance, little has been done on the integrative aspect of this enhancement strategy of protecting and empowering plants to resist and grow better under drought, salinity or alkaline conditions.

This review aims to understand the influence of plant growth promoting rhizobacteria in plant tolerance to abiotic stress, and to suggest a new trend involving the application of microbes, nutrients, nodule inducers, growth hormones and osmo-protecting substances on growing plants (Fig. 1) exposed to abiotic stress, in order to achieve better performance and adaptation of crops and to increase the overall return on investment/expenditure of farmers.

2.2 Sustainable Agriculture – The Influence of Climate Change and Human Population Growth on Crop Yield

The two major challenges affecting crop production in this modern time are climate change and escalation of human population number. The two are interrelated, one influences the other. As the human population increases, the need for fuel energy increases. Also the need to generate heat for cooking food leads to deforestation. These human activities elevate the concentration of carbon dioxide in the atmosphere.

Climate change could result in an unregulated supply of rain water which could either occur in excess or in prolonged short supply. Whichever the case, crop production is affected negatively. Extreme

rain fall gives rise to erosion and flooding, while drought results in loss and poor performance of crops. In a bid to boost crop productivity and yield and to combat drought induced stress, farmers resort to the use of irrigation which adds salt to the soil. Drought is a great cause of poor yield of crops leading to food shortages.

Carbon dioxide increase on the other hand, may aid the growth and nitrogen assimilation of plants. For example, although water limitation affects the clover plant's fixation of nitrogen, in the presence of a moderate supply of water, the plant could still perform excellently in the presence of carbon (IV) oxide and increased atmospheric heat (Lazzarotto et al., 2010).

However, adequate nutrient management with respect to improving the fertility of the soil could be an aid in solving the problem of climate change challenges on crops (Clair and Lynch, 2010) together with the use of rhizobacteria to boost crop production (Babalola, 2010).

Population explosion also creates an urgent need for the establishment of shelter, recreation centers, industrialization, urbanization etc, to accommodate human activities, and this affects land availability for mechanized farming.

To sustain the nutritional need of the growing human population, agricultural practices must be intensified, irrespective of short supply of rain water, environmental effects of the use of inorganic chemicals (fertilizers), and salinity issues. The biotechnological application of microbes may address the rising problem and ensure sustainability in the provision of food for all. In addition, the use of biofuels in automobiles as well as the practice of afforestation should be encouraged to help cut down on greenhouse gas accumulation for effective control of climate change. The use of electric automobiles should also be incentivized.

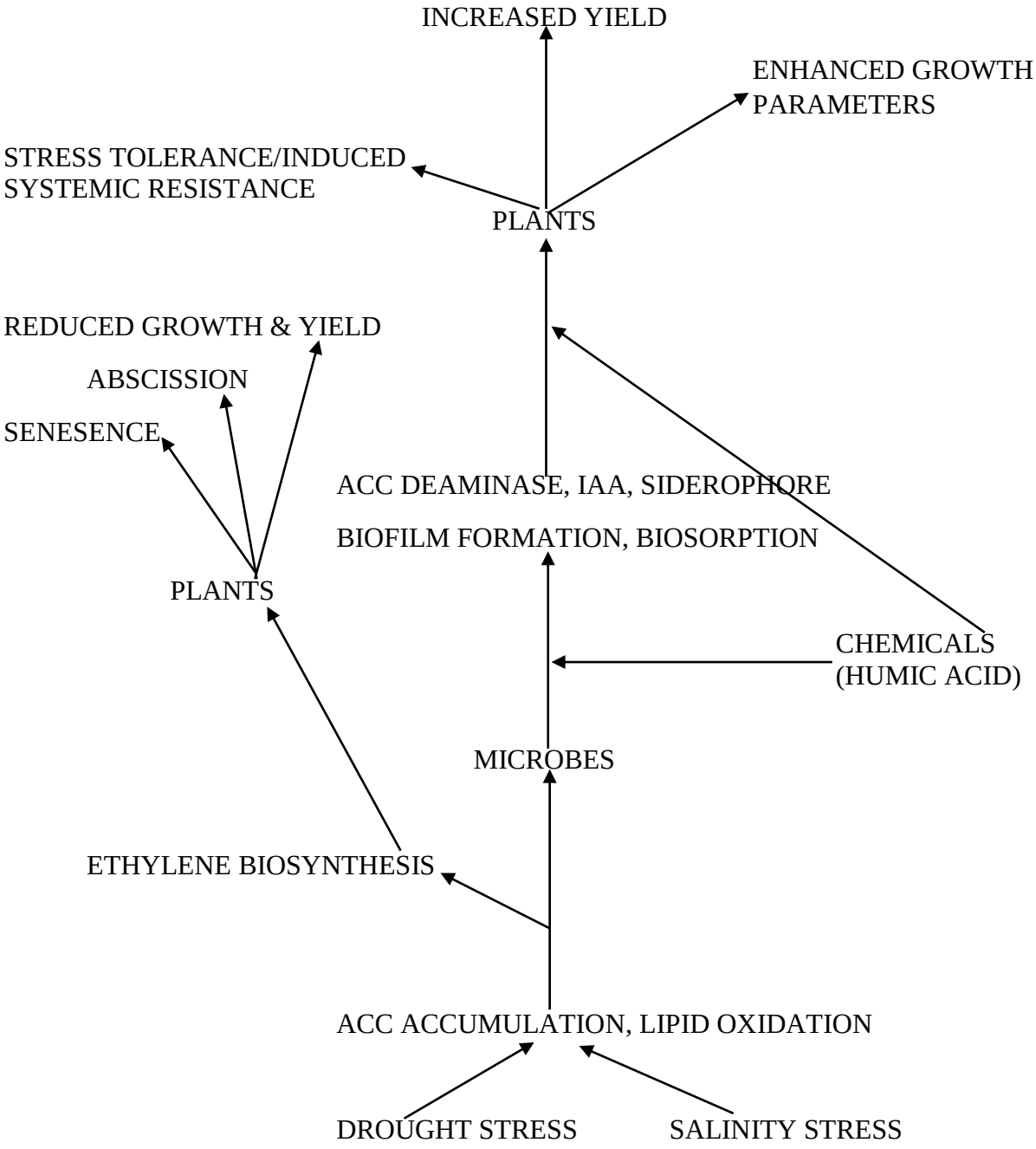


Figure 2. 1: The interactions of microbes, useful chemicals, and their overall influence in plant stress tolerance, growth, and productivity

2.3 Plant Growth Promoting Rhizobacteria – The Redeemer of Plants

Plant growth promoting rhizobacteria are those indispensable microbes possessing the unique abilities of supporting directly and indirectly the wellbeing of plants. In order to survive in the

rhizosphere, these microbes expanded their biological activities that influence the survival and growth of plants (Babalola, 2010).

A number of these microbes with the enzyme machinery necessary for the breakdown of the exudates of plants are able to protect the plant from stress arising from water scarcity and salt pollution. They produce a variety of substances such as deaminase enzyme (Saleem et al., 2015), plant hormone – indole acetic acid (Gujral et al., 2013), siderophore (Stajković-Srbinović et al., 2014), $PO4^{2-}$ solubilizing enzyme (Kumari and Khanna, 2016), salicylic acid (Ekinci et al., 2014), and microbiocidal/biostatic enzyme (Moustaine et al., 2017).

Some of these microbes contribute to plants nutritionally by trapping and integrating nitrogen into the plant via nitrogen fixation (Attitalla 2012). However, as a means of surviving the stiff competition for existence and dominance at the root environment, some microbes devise a means of burrowing/penetrating the tissues of plant and establish themselves as endophyte within the plant (Latif Khan et al., 2016), contributing not only to the nutrition of the plant but also to enhancing plants' survival rate and adaptation to their environment.

PGPR contribute to sustaining the intrinsic resistance of plants to pathogenic and environmental challenges. Some of these organisms are excellent in biofilm formation and secretion of polysaccharide substances which confer stability to plants during stress (Kasim et al., 2016). As microbes contribute significantly in biohydrometallurgy by the generation of metal rich solution through the biological oxidation of sulphur containing ore, they also play a role in the immobilization of metals or cations and render them non-bioavailable using polymeric substances and other chemicals they produced (siderophores etc) (Fashola et al., 2015). Their presence can contribute to the reduction in metal stress on plants when applied to them as bioinoculants. The growth and survival of plants will not be possible without the help of these "farmers' friends" living both within and around the plant surfaces.

A healthy plant is a product of a healthy relationship between the plants and the growth promoting microbes; while an unhealthy relationship could be observed in the degradation of the physical and physiological wellbeing of the plant. PGPR are the key players in the fight for sustainable plant development amidst stress conditions arising from climatic as well as manmade activities. The classes of microbes belonging to these groups/genera are *Micrococcaceae* HW-2 (Hong et al., 2016b), *Bradyrhizobium* (Masciarelli et al., 2014), *Bacillus* (Kasim et al., 2016), *Microbacterium*, *Pseudomonas*, *Curtobacterium* (Cardinale et al., 2015), *Variovorax*, *Paenibacillus* (Yolcu et al., 2011), *Pantoea* (Damam et al., 2014) and many others.

2.4 Drought Stress in the Reduction of Plant Performance

Drought has been implicated in the reduction of plant metabolic and physiological activities. It reveals its effect in the degree of biomass yield, growth, nodulation and chlorophyll greenness of plants. It is a major agricultural crop impairment inducer and a global concern as it affects the supply and sustainability of food availability to people and livestock. Rainfall is the major source of water for growing crops/plants by farmers (in a subsistent agriculture) in many parts of the world. The gradual delay or low level of rainfall due to the influence of climate adjustment to manmade activities, is affecting the biotic aspect of the ecosystem particularly green plants which are the primary and major producers of food upon which all life forms depend for meeting their daily nutritional needs.

The effect of drought is obvious, yet the microbes domiciled at the root zone of plants known as plant growth promoting rhizobacteria, have proven to contribute to the tolerance and quick adaptation and adjustment of plants to drought stress (Table 2.1) via supplementary production of phytohormones that induce root growth, thus allowing a better water absorption, production of deaminase enzyme responsible for the disintegration of ACC molecules, nitrogen fixation and solubilization of P (Vurukonda et al., 2016a, Olanrewaju et al., 2017).

The survival and enhanced influence of endophytic microbes towards poplar adaptation to water stress, for example, revealed that the presence of these organisms aided the physiological wellbeing

of the plant and enabled it to tolerate water scarcity in the soil. The result showed 28 percent increase in biomass (shoot and roots) as against the control (Khan et al., 2016b).

The increase in root number and tissue mass promoted the wider reach of plant roots for water and nutrient absorption, which enabled them to thrive in water scarce environments. This survival approach of plants is akin to the aid of microbial symbionts capable of plant hormone production (indole acetic acid, gibberellin), which stimulate growth and the resultant stress annulment for better performance of the plant.

In line with the survival induced strategy, the endophytes will produce a poly-sugar substance known as trehalose, capable of protecting biologically produced compounds and molecules from breakdown during water induced osmotic tension (Khan et al., 2016b).

It was observed that *Ocimum basilicum L.,* the plant popularly known as basil, had improved chlorophyll pigment content and antioxidant activity under water induced stress in the presence of mixed rhizobacteria consortium - *Pseudomonas sp., Bacillus lentus* and *Azospirillum brasilens* (Heidari and Golpayegani, 2012), which indicated the aid of microbes in the synthesis of useful substances in the plant in spite of the prevailing stress challenges. Among the nitrogen fixers of atmospheric N to plants for its nutritional needs, *Azospirillum* is a good farmers' friend that contribute in enriching the soil and enabling plants to thrive under abiotic stress. This microbe, which possesses a list of unique attributes such as auxin production, deaminase enzyme production, siderophore – iron trapping substance, nitrogen fixation, exopolysaccharide etc, enables its associates (plants) to tolerate drought as well as salt disturbances (Cruz et al., 2017, Vacheron et al., 2015).

Table 2. 1: Contributions of microbes in the induction of drought tolerance by plants

Plant Species	Microbes	Microbial enhanced plant productivity under stress	References
Tomato	*Rhizophagus irregularis* *Variovorax paradoxus 5C-2*	Enhanced photosynthetic rate, reduced lipid oxidation and increase root water conductivity and oxidative phosphorylation in the plant	(Calvo-Polanco et al., 2016)
Chickpea	*Pseudomonas putida* MTCC5279 (RA)	Reduced/control the expression of stress response gene, maintain water content, osmolyte, membrane structure and germination rate of the plant	(Tiwari et al., 2016)
Maize	*Azospirillum* spp (Az19)	Improve the growth and productivity of the plant under water stress compared to the control	(García et al., 2017)
Wheat	*Piriformospora indica*	Enhanced adaptation of plant by promoting nutrient and water absorption, improved root growth, biomass, water, chlorophyll and modulate the activities of antioxidant molecules	(Hosseini et al., 2017)
Trifolium repens	*Rhizophagus intraradices* *Bacillus megaterium*	A consortium of these microbes increased plant nutrient and water contents, reduced stomatal conductance and stress enzyme activities for better adaptation to drought	(Ortiz et al., 2015)

	Pseudomonas putida		
Wheat	*Azospirillum brasilense* Sp245	Increased growth and expansion of xylem in the coleoptile of inoculated plant for easy conduction of water	(Pereyra et al., 2012)
Lavandula dentata	Arbuscular mycorrhizal fungi *Bacillus thuringiensis*	Co-inoculation enhanced plant growth, nutrient content, biomass and reduction in lipid oxidation of the plant	(Armada et al., 2015a)
Maize	Arbuscular mycorrhizal fungi *Bacillus thuringiensis*	Improved nutrient content and water transport protein as well as reduce lipid oxidation in the stressed plant	(Armada et al., 2015a)
Brassica oxyrrhina	*Pseudomonas libanensis* TR1 *Pseudomonas reactans* Ph3R3	Enhanced growth, pigment and water content as well as phytoaccumulation of heavy metals in the plant	(Ma et al., 2016)
Maize	*Pseudomonas putida* (FBKV2)	Encouraged root and shoot growth, dried biomass weight and reduced stomatal conductance in the plant	(Vurukonda et al., 2016b)
Common Bean	*Rhizobium*	Promoted plant weight, nutrient content and increased *Phaseolus vulgaris* yield	(Yanni et al., 2016)

Azospirillum generates biocidal substances (bacteriocins, hydrogen cyanide, proteolytic enzymes, siderophores) capable of destroying pathogen/invaders of plants and encourages plants to tolerate abiotic stresses as well as living organisms inducing stress on plants. It could be regarded as a plant growth promoting bacteria and is widely studied by researchers (Vacheron et al., 2015, Creus et al., 2010). The contribution of this organism in nutrient uptake by plant as well as water facilitated uptake is well documented. Therefore, *Azospirillum* and other microbes capable of enriching and unbinding trapped nutrients and making them available for easy taking up by plants are called biofertilizer and they in turn depend on plant excretory products (root exudates) for their nutrition (Babalola, 2010).

A closer look at the biosynthesis of siderophore by *Gordonia rubripertincta* CWB2, suggest that *gorA* gene under expression in *Escherichia coli* produce GorA hydroxylase enzyme (N – hydroxylating monooxygenase) which was able to hydroxylate the substrate putrescine (1, 4 diaminobutane) in the presence of coenzyme (FAD and NADPH). The hydroxamate is a crucial part in siderophore synthesis. It is responsible for binding to irons and other metallic elements in the rhizosphere (Esuola et al., 2016).

A screening study on rhizobacteria obtained from cacti rhizosphere showed that the predominant isolate belonging to the *Bacillus* sp exhibited amazing plant growth promoting traits like the production of indole acetic acid, PO_4^{2-} – solubilization, hydrogen cyanide and NH_3 production. *Bacillus* has the attribute of producing exopolysaccharide substances that enable them to form biofilms and survive harsh environments, and has contributed to the survival, growth and performance of *Zea mays L.* seedlings leaf area, stem and biomass shoot dry weight in a simulated water stress experiment (Kavamura et al., 2013). This attribute of poly-sugar production by microbes strengthens the resistance of inoculated plants to water stress inhibitory effects and thus ensures their growth and survival in a water deficient environment (Nocker et al., 2012).

To understand the consequences of water deficiency on plant physiological condition and activities, groundnut plants were subjected to water stress using a hydrocarbon compound - polyethylene glycol, which neither penetrated nor posed any toxicity effect to the plant, rather it caused a decrease in the plant RNA, chlorophyll composition and water content shortfall in the plant. The observed effect is concentration based. The higher the concentration of the polyethylene glycol, the higher the water stress effect it exerts on the plant and the higher the corresponding reduction effect of RNA in the leaves and roots. This effect was observed in the quantity of water content of the plant leaf (relative water content). The drought interfered with chlorophyll a more than b (Shivakrishna et al., 2018). The production of oxygen radicals and hydrogen peroxide molecules are responsible for destruction of lipids and the breakdown of chlorophyll which leads to reduction and yellowing of the leaves (Shivakrishna et al., 2018).

In another experiment, a legume plant (*Aspalathus linearis*) was subjected to drought condition via withholding of water supply to the plant. This led to an observable effect on the photosynthetic rate of the plant by 40 percent reduction as well as 61 percent reduction in stomatal conductance, as a consequence of continuous closure of stomata to maintain intracellular water content and cut down on water loss from the leaves. This is one of the strategies plants adopt to increase their efficiency in the use of water during drought stress periods. Drought induced closure of stomata has a direct connection with the reduction in plant assimilation and fixation of carbon dioxide from the atmosphere. Inadequate supply of water directly impedes the process of photosynthesis in plants. Preferential development and growth of underground plant parts (roots) to shoot parts of the plant undergoing stress was also observed, which enabled the plant to absorb more water from the inner layer of the earth (Lotter et al., 2014).

Although *Aspalathus linearis* is capable of forming a symbiotic relationship with rhizobium microbe, drought on the other hand, affected the nitrogen nutritional content of the plant as a result of its influence on the activities of the nodules and the N_2 fixing enzyme (nitrogenase). This consequently makes the plant more dependent on the available nitrogen in the soil (Lotter et al., 2014).

Periodic or interval water supply through rain water to rice farming has proven to contribute to water stress in rice plants. Rice, being a moderately water loving plant, finds it difficult to cope with dryness arising from drought, and requires a suitable measure for increasing the adaptability and productivity of the plant in a low water vicinity. The application of potassium salts as nutrient (potassium chloride and potassium sulphate) could be a good measure in reducing water stress on rice plants. Potassium nutrient applied at a concentration of 120 kg per hectare was able to increase rice yield and the index of its harvest within 15 days of water scarcity (Zain and Ismail, 2016). Potassium has a link with the efficiency of water use by the plant and improves it via the promotion of transpiration-pull-boost with the assistance of potassium ions which are components of cell membranes mediating active transport of nutrients as well as water into the rice plant.

The ease of water entry from the soil into the plant contributes in cancelling the potential damage of tissues by oxygen radicals during water deficient condition. A 338 percent increase in proline concentration within the plant treated with K^+ salt was observed (Zain and Ismail, 2016).

The use of sugar beet fermented by *Aspergillus niger* as soil amendment in the presence of bioinoculant *Bacillus megaterium* and a consortium of arbuscular mycorrhizal fungi promoted plant growth, biomass, nutritional content, water level and reduced the conductance of stomata and the activities of enzymatic antioxidants in plants faced with drought stress. The amendment boosted the effectiveness of the bacteria towards supporting the growth and productivity of the stressed plant (Armada et al., 2014).

The importance of water as a medium for transport and biochemical reaction in a living system cannot be overemphasized. A limited water environment interferes with the smooth biochemical processes and interaction of molecules in the plant as well as its associated microbes in the rhizosphere. The performance of *Glycine max* under drought stress was increased in the presence of *Bradyrhizobium japonicum* and a nodulation stimulant – genistein. Genistein as well as daidzein iso-flavone substances are capable of inducing response reaction of nodule gene expression in *Bradyrhizobium* sp. These compounds are components of exudates from legume roots. Genistein treatment of *Glycine max* seed together with a bioinoculant (*Bradyrhizobium japonicum)* greatly encouraged the resistance of soybean to drought stress and decreased its detrimental effect on the formation of nodules by the microbe (Hasanah and Rahmawat, 2012).

The tolerance of plants to drought stress is dependent on their own genetic makeup. The physical manifestation observed in a plant is in direct correlation with the quality and quantity of the physiological activities taking place at the gene level. All biomolecules produced are the product of gene expression. The easier the expression of genes encoding the biomolecules that protect the plant cellular component from drought induced cellular destruction/damage, the more the viability and productivity of the plant at a given stress condition. Different species of soybean undergoing a 15 days vegetative growth under drought stress revealed that the electrolyte loss was more in plant tissue

stressed for 10 days and above. The shoot dry matter or biomass concentration decreased (by 79.18 percent) at the 10^{th} day – 15^{th} day of stress, while the root biomass or dry weight/ root growth increased (by 100.96 percent). This means that drought promotes the accumulation of nutrients and photosynthetic products at the root and stimulates its growth and development to maintain balance, adjustment and sustainability of plant physiological adaptation to the stress problem. Soybean specie (SJ-4) possessing unique genetic/physiological characteristics performed better than others under drought stress and should be the crop of choice for drought predominant locations (Aung et al., 2011).

Also, two soybean species showed slight yield reduction of 8 percent and 12 percent respectively as compared to when grown under consistent water supply via irrigation. This implies that they are good in tolerating drought as a result of their inherent delay in wilting and a corresponding elongation of fixation of carbon dioxide in their leaves (Pathan et al., 2014).

In the same vain, the accumulation of amino acid (proline) in the leaves of tomato plant and increased value of the relative water content of the leaves of the plant, confers resistance to drought by tomato plant. Proline is responsible for sustaining the movement of water molecules from a region of higher concentration to that of a lower concentration in response to concentration gradient between the plant and its environment. It helps the plant maintain its turgor intracellular pressure by facilitating movement of available water from the soil into the root of the plant (Jureková et al., 2011).

A non-bioinoculant phyto-drought-stress mediated strategy involving the application of chitosan to a plant (thyme) undergoing drought stress helped to offset the effect of drought on the plant by 20 percent compared to the untreated control which showed a decrease in dry matter of the plant by 54 and 56 percent on the photosynthesis retardation of the plant under drought stress (Bistgani et al., 2017). In the same manner, it encouraged the synthesis of essential oil in the thyme plant, reduced the degree of peroxidation, sustained the cell membrane conformation and function and stimulated the accumulation of proline amino acid in the leaves of the plant by 20 percent (Bistgani et al., 2017). Being a polysaccharide, chitosan is generated from chitin alkalinization of N-deacetylation. These

molecules are abundant in the outer-skeletons of insects, fungi and algae cell wall composition and are implicated in secondary metabolite production in plants when applied to them (Lei et al., 2011).

A sainfoin plant inoculated with arbuscular mycorrhizal fungi exhibited 7.27, 4.21, and 2.40 percent increase for relative water content, N_2 and P leaves total content respectively at 40^{th} day of the experiment. This fungus enabled the plant to adapt and survive during drought limitations by enhancing plant growth and protecting it from damage induced by stress (Kong et al., 2014). Generally, an external-internal symbiotic relationship by soil fungi (arbuscular mycorrhizal fungi) performs the same protective role as endophyte does to plants. There is a biomass increase as well as a decrease of *Fusarium oxysporum* infectivity of tomato plant inoculated with *Glomus intraradices* and *Piriformospora indica* irrespective of the nutritional content of the soil used (Cruz et al., 2017). Also the involvement of mycorrhizal fungi in nutrient dissolution/solubilization particularly as it concerns phosphorus gives credence to its participation in the direct promotion of plant health and productivity.

These microbes form a channel (protein transport channel) for dissolution and absorption of solubilized PO_4^{2-} from the rhizosphere into the host plant in a mutualistic dependent relationship. They are generally used as biofertilizers for immobilized nutrient dissolution via the organic acids produced which are capable of reacting with the compounds of trapped minerals and converting them to soluble forms for easy assimilation by plants (Igiehon and Babalola, 2017).

The presence of mycorrhizal fungi adds further surface area for water and nutrient acquisition, thereby making the plant more resilient and tolerant to the climate change induced stress. This notwithstanding, they also partake in structure building, arrangement and improvement of soil for proper aeration and migration of water together with nutrients in the soil, making the soil healthy and fertile (Igiehon and Babalola, 2017, Alori et al., 2017a). It is a known fact that fungi can survive and thrive well in a dry or semi-dry soil and still perform their normal activities. This property is exploited by arbuscular mycorrhizal fungi in making an inoculated plant perform its activities under drought

condition as the organism reaches out for more water within the soil where the roots of the plant can possibly not reach (Alori et al., 2017a).

In addition to fungi solubilizing PO_4^{2-} bacteria, the enzymes (phytase, phosphatase) can produce organic acid (acetic acid, citric acid) and will be able to mineralize or solubilize P via ionic interaction of the charged group in these molecules. These solubilizers of P play a part in the enrichment of soil fertility and supporting plant nutritional requirements irrespective of the prevailing bioavailability or non-bioavailability of the nutrient in the soil. Though the level of available P can determine the function of the microbes as it relates to solubilization of P, the higher the available nutrients, the less the quest to solubilize already immobilized phosphorus and vice versa. Microbes are the key players in the replenishment of P in the soil (Alori et al., 2017b, Zhu et al., 2011).

Also, bacterial inoculation of rice plant using *Pseudomonas fluorescens* promoted the intrinsic tolerance of rice plant to drought stress and encouraged the expression of abscisic acid synthetic genes, particularly at the stage of reproduction by the plant. This confers an induced systemic plant resistance to drought stress. It implies that microbes, especially the PGPR colonizing the rhizosphere of plants, have an indirect as well as a direct role in enhancing the expression of genes by water deficient plants through a process known as induced systemic resistance. Microbes in their various capacities have been shown to aid plants in their tolerance to drought (Saakre et al., 2017, Bresson et al., 2013).

The symbiotic N_2 fixers (*Sinorhizobium meliloti* and *Sinorhizobium medicae*) on the other hand, possess the ability of protecting a legume (*Medicago truncatula*) from senescence of the leaves under drought stress (Staudinger et al., 2016). The rhizobial inoculants aid in the delay of the process of senescence by promoting the accumulation of potassium ions, reduction in the protein mediating ethylene production, induction of the production of cytokinins which inhibit senescence and accumulation of sugars and amino acids that aided in the plant survival during stress.

Nodulated medicago plants recovered from drought stress faster than the non-nodulated legumes as a result of the shifts in the carbon partitioning from starch to sugars and thus the enhanced allocation of reserves to osmolytes during drought, enabling a stay green with the ability of fast recovery after rewatering (Staudinger et al., 2016).

Drought stress reduced relative water content and chlorophyll and increased proline concentration of the plant (wheat) (Keyvan, 2010). Obviously, drought affects root, shoot, yield and overall performance of the affected plant. Yet at moderate water scarcity or even severe conditions, the intrinsic qualities of the plant determine to a large extent its sensitivity or tolerance to the drought condition. The osmotic adjustment and performance of three barley plant species (yousof, fajr 30 and morocco) was measured when they were subjected to different drought treatments (moderate and severe). The results showed that yousof specie possesses a drought tolerance trait and was able to have an increased root length to shoot length ratio under severe stress conditions.

The test plants survived the water stress by employing an adjustment in the osmotic behaviour of their root systems, leading to the accumulation of solutes such as proline in their cells to maintain the cell structure and function during water scarcity. This adjustment in osmosis to sustain the turgidity of the plant cells directly influences the escalation level of photosynthesis and tissue growth of plant during drought stress (Afshari-Behbahanizadeh et al., 2014).

The symbiont (*Bradyrhizobium* sp) aided the tolerance of cowpea plant to water scarcity stress and boosted the quality of NO_3^- (nitrate) and amino acid (proline) in the inoculated plant (Barbosa et al., 2013).

Groundnut inoculated by *Bradyrhizobium* under drought conditions had a large quantity of amino acids which was derived from the nitrogenase catalyzed conversion of atmospheric N to NH_4^+ ions necessary for amino acid and protein formation in the plant (Delfini et al., 2010). The observed increase in protein level of legume inoculated plants, implied that these microbes aid in the supply of nitrogen via fixation in the presence of the nitrogenase enzyme operating at the root nodules of the

plant. Bioinoculation of legume plant with rhizobium has helped to encourage the development of many leaves in the plant due to the greater number of root nodules in the plant roots that aid in nitrogen fixation (Ferreira et al., 2011).

The treatment of annual medic plant growing in sufficient water (irrigated) and water deficient (dry farming) systems with a mixture of biofertilizer and chemical fertilizer in an integrated fertilization practice encouraged the plant to efficiently adapt to the water deficient condition and accumulate both macro and micro-nutrients in the tissues of the plant compared to the use of chemical fertilizer alone. The application of bioinoculants and mineral fertilizer to a plant growing in a water limiting environment is more effective (Table 2.2) in boosting plant productivity than in water sufficient soil (Shabani et al., 2015).

The attenuation capacity of microbes to the negative effects of drought on plant development was facilitated by the use of a combination of *Azotobacter chroococcum and Pseudomonas fluorescence* together with phosphorus fertilizer, and was able to boost the inorganic P content of the plant (soybean) growing under insufficient and abundant water content of the soil. The N content of the leaves and root of the soybean plant were increased by six (6) percent and eight (8) percent under water stress condition. The absorption of phosphorus in the soybean plant under the influence of bioinoculant increased by sixteen (16) percent (Vladimir, 2012).

Although drought negatively affected plant development such as water content, chlorophyll of the leaves and the dry weight of the root with a corresponding increase in the amino acid proline and abscisic acid – osmoprotecting molecules, yet the application of humic acid to the drought stressed plant has reversed the observed effect by decreasing the quantity of proline and absicsic acid content of the treated plant, with resultant increases in chlorophyll content, dry weight of the root and water content of the pistachio plant during drought stress circumstances (Kasmani et al., 2013).

However, from the above reviewed information, it is clear that bioinoculants and the application of useful chemicals and amino acid could help plants avert the challenges of drought conditions and aid in the promotion of a sustainable production of crops especially in those regions prone to water scarcity (insufficient rain fall).

2.5 Salt Induced Challenges on Plant

The deleterious nature of sodium chloride and other salt compounds on the growth and development of plants particularly in inducing water limitation stress and uncontrollable negative stomata closure cannot be overemphasized. This necessitates the application of an attenuation strategy to cancel the effect of salt on crops.

The co-inoculation of *Pseudomonas* and *endomycorrhizae* on cowpea plant undergoing salt induced stress showed a decrease in the mycorrhizal infection of the plant. Treatment of 6000 ppm sodium chloride in the presence of the fungi and bacteria increased the carotenoids concentration (0.449 mg/g). Also, osmolytes (proline and sugars) increased in the presence of endomycorrhizae inoculation in the plant. Irrigation with tap water in the midst of *endomycorrhizae* and *Pseudomonas fluorescens* gave higher fresh and dry weight, pod length, seed number and protein content of the cowpea plant (Manaf and Zayed, 2015) compared to the one irrigated with salt water.

As part of microbial facilitated plant tolerance to salinity, biofilm formation is a suitable strategy which microbes employ in enhancing barley plant tolerance to soil salinity. This was observed in the aversion/reversal of the harmful effect of salt on a number of growth parameters such as seedling length, relative water content of the plant's leaf as well as fresh and dry weight of barley plant. This biofilm formation strategy by microbes is actually a means of protecting themselves from the harsh conditions they found themselves in (short supply of nutrient, ionic toxicity as well as water limitation induced osmotic stress), enhancing survival within the presence of these limited resources by aggregating in masses (sessile compartmentalization) of cells at both living and non-living surfaces

present at the root environment and contributing indirectly to tolerance ability of the plant to stress (Kasim et al., 2016, Qurashi and Sabri, 2012).

Table 2. 2: Plant drought stress tolerance mediated by synergy between microbes and soil amendment

Plant species	**Microbes**	**Amendment**	**Plant productivity and tolerance outcome**	**References**
Arundo donax	*Micrococcaceae* HW-2	Sodium polyacrylate	Microbial attributes of plant hormone, deaminase and siderophores production and enhanced water retention capacity of sodium polyacrylate promoted shoot growth, biomass and root of the plant	(Hong et al., 2016b)
Thymus vulgaris ***Santolina chamaecyparissus*** ***Lavandula dentate*** ***Salvia officinalis***	*Enterobacter sp.* *Bacillus thuringiensis* *Bacillus megaterium* *Bacillus sp.*	Fermented agrowaste	The amendment enhanced nutrient uptake via bacterial stimulated activities for proper nutrient absorption by plants and stomatal conductance during drought stress	(Armada et al., 2015b)
Pinus halepensis	*Azospirillum brasilense* *Pantoea dispersa*	Olive-mill waste	The treatment and the inoculants promoted the carbohydrate and microbial biomass carbon as well as soil nutrient and consequently increased growth, water content and nutrient uptake by the plant	(Mengual et al., 2014a)
Lavandula dentata L.	*Bacillus megaterium* *Enterobacter sp* *Bacillus thuringiensis* *Bacillus sp.*	Composted sugar beet	It increased biomass shoot dry weight, root and nutrient content of the plant. The amendment increased the concentration of bioavailable phosphorus and nitrogen in the plant rhizosphere	(Mengual et al., 2014b)

Cistus albidus L.	*Azospirillum brasilense* *Pantoea dispersa*	Olive residue	Increased dry root, shoot weight, organic carbon, soil enzymes and microbial biomass carbon	(Schoebitz et al., 2014)

Bacillus amyloliquefaciens (SQR9) contributed to maize salt tolerance by enhancing its chlorophyll production level via colonizing and interacting with the plant roots. It also aids in the exclusion of sodium from the plant root, and stimulates the production of sugars and antioxidant within the tissues of the maize plant (Chen et al., 2016). This implies that a sensitive plant could become tolerant to salt stress in the presence of a bioinoculant.

This unique contribution to the wellbeing of the plant (Table 2.3) could be ascribed to its ability to produce indole acetic acid, solubilize phosphorus, colonize the plant root and produce enzyme deaminase (ACC-deaminase) capable of maintaining optimum biological function of the plant in the midst of stress inducers.

With a shift in perspective, it was understood that opportunistic pathogens of humans can aid in plant tolerance to salt stress. The organism *Methylobacterium mesophilicum* could efficiently colonize and dominate the root rhizosphere region of three plants (cucumber, tomato and paprika) under the study at salt concentration as high as 6 percent, and possess the capability of phytohormone (IAA) production together with fungicidal enzyme production that contributes to the growth, protection and tolerance of the plant to biotic and abiotic stress (Egamberdieva et al., 2015b).

Sensitive crops have remarkable reduction in viability and performance when challenged with salt stress as compared to tolerant species, following the observed accumulation of proline and reduced leaf area in a sensitive wheat plant compared to a tolerant plant. However, the application of synthesized phyto-hormone (gibberellic acid) on salt stressed wheat plants has resulted in adequate adaptation of two wheat plant species to salt influenced stress and improved its yield (Shaddad et al., 2013).

Moreover, the efficiency of microbial exopolysaccharide formation and the formation of biofilm during environmental stress at the plant rhizosphere is a contributory factor towards plant adjustment to salinity or salt induced osmotic shock. The polysaccharide molecules produced by microbes in a bid to protect themselves from desiccation and ionic toxicity have a charged part which reacts with dissociated sodium ions and chelates them. This reduces the toxicity and abundance of these ions in the rhizosphere, making the soil suitable for root proliferation (Arora et al., 2010).

The quest for a better way to reduce or alleviate stress inducers, has led researchers to investigate the use of non-biological methods such as the external application of micronutrient Mo (molybdenum) as an additive to plants facing salinity stress. When molybdenum was applied to a bean plant (*Phaseolus vulgaris*) subjected to salt stress, the plant was able to maintain its chlorophyll composition and tissue components. Mo being a unique player (cofactor) in cellular enzyme biosynthesis and activities enhanced the formation of chlorophyll and maintenance of adequate cellular function (Bouzid and Rahmoune, 2012).

The critical evaluation of the microbial community of *Aster tripolium* L showed the predominance of gram positive bacteria residing in the root of the halophyte, while gram negative bacteria exist more in the rhizosphere and bulk part of the soil. The isolated organism exhibited the ability of forming 1-aminocyclopropane-1-carboxylate deaminase, indole acetic acid etc, that contribute to the survival and tolerance of the plant to salt induced stress and toxicity (Szymańska et al., 2016).

It was noted that rhizosphere bacteria of a desert adapted plant (*Suaeda fruticosa*) from Kutch Desert has a unique strain of bacteria (*Bacillus licheniformis* strain A2), which solubilized phosphate, produced indole acetic acid, siderophore etc, contributed to 31 percent groundnut height and 43 percent biomass increment of the plant and 24 percent and 28 percent rise when grown in 50mM sodium chloride supplemented soil (Goswami et al., 2014).

Another non-biological stress alleviation technique showed that caffeic acid was able to aid the plant (soybean) tolerate salt stress. The applied caffeic acid stimulated the NO (nitric oxide) composition

of the nodules, which has a direct relationship with the induction of cyclic guanine monophosphate responsible for controlling reactive oxygen radicals produced in the stressed plant. Caffeic acid help to shield nitrogenase enzyme and legheamoglobin of the soybean root nodules from destructive effect of sodium and chloride ions, thereby adding to the growth of the plant by enabling the symbionts to fix nitrogen adequately to meet plant nitrogen requirements. Caffeic acid has also been implicated in chlorosis suppression of treated plants undergoing salinity induced stress (Klein et al., 2015, Wang et al., 2011).

On the other hand, it was demonstrated that sodium chloride impedes the uptake of potassium ions by *Broussonetia papyrifera* plant, resulting in high sodium to potassium ratio. Sodium ion equally decreased the calcium, magnesium and phosphorus content of the plant root system and caused an induced ion imbalance in the plant rhizosphere. Bio-active enzymes responsible for controlling oxygen radicals (catalase, peroxidase, superoxide dismutase) were also decreased by the inhibitory effect of the high salt concentration in the plant environment. Protein content of the plant was also affected as observed in the appearance and disappearance of protein bands in the plant samples analysed (Zhang et al., 2013a). Plant growth promoting rhizobacteria inoculated on wheat plant promoted its root and shoot growth and the weight of the fresh tissue component of the plant. The observed rates of plant growth were from 62.2 to 78.1 percent in the presence of the bioinoculant (Orhan, 2016). This could be attributed to the production of phyto-hormone by the microbes. Stress adapted bacteria (*Pseudomonas putida* R4 and *Pseudomonas chlororaphis* R5) capable of producing plant hormone (IAA) at the rhizosphere of inoculated cotton plant subjected to salt stress completely averted salinity stress on cotton plant (Egamberdieva et al., 2015a). This was also found in cotton plant inoculated with *Klebsiella oxytoca* (Rs-5) that resulted in the increase in seed germination (15.40 percent), growth and overall tolerance of cotton to salinity stress (Wu et al., 2014).

Table 2. 3: The unique contributions of microbes to salinity tolerance by plants

Plants	Microbes	Microbial influenced plant productivity and salinity tolerance	Reference
Chilli	*Bacillus spp* *Alcaligenes spp* *Proteus spp* *Aneurinibacillus aneurinilyticus*	Significantly increased root and shoot length more than the control	(Patel et al., 2017)
Rice	*Enterobacter* sp	Promoted the growth of rice seedling and reduced ethylene production and antioxidant enzyme activities in the plant	(Sarkar et al., 2018)
Rice	*Bacillus* sp	Aided the alleviation of salt stress by increasing the biomass and growth of rice seedling via production of indole acetic acid and deaminase enzyme	(Misra et al., 2017)
Festuca arundinacea	*Enterobacter ludwigii*	Membrane transport protein in the microbe that control sodium and hydrogen ion movement across bacteria cell and the production of plant hormone, phosphate solubilization, nitrogen fixation contribute towards the growth, tolerance and plant productivity	(Kapoor et al., 2017)
Alfalfa	*Halomonas maura* *Ensifer meliloti*	Increased the weight of shoot dry weight, yield and water content of the plant	(Martínez et al., 2015)
Rice	*Thalassobacillus denorans* (NCCP-58) *Oceanobacillus kapialis* (NCCP-76)	Inoculated plant was observed to have increased germination ability, root and shoot growth, protein and chlorophyll contents as well as nutrient contents with reduced sodium ion accumulation in the plant	(Shah et al., 2017)
Rice	*Bacillus pumilus*	Enhanced plant growth and decreased the accumulation of sodium ions without having an effect on boron accumulation in the leaf tissues	(Khan et al., 2016a)
Oat	*Klebsiella* sp	It boosted plant growth, water content, dry shoot and root weight of inoculated plant	(Sapre et al., 2018)

Wheat	*Bacillus subtilis*	Enhanced salicyclic acid content of the plant, leaf water content and reduced proline and malondialdehyde content of the plant for better induction of systemic resistance	(Lastochkina et al., 2017)
Barley	*Hartmannibacter diazotrophicus*	The production of deaminase enzyme enhanced percentage root and shoot dry weight and growth of the plant	(Suarez et al., 2015)
Canola	*Enterobacter cloacae* HSNJ4	Enhanced canola tolerance via promotion of plant hormone content of the plant and reduced ethylene and malondialdehyde content. Root, shoot and chlorophyll contents were improved	(Li et al., 2017)
Sunflower	*Rhizophagus irregularis* *Chryseobacterium humi* ECP37 *Ochrobacterium haematophilum* ZR3-5	Improved biomass and nutritional content of the plant as well as antioxidant response in the plant and lowered sodium ion content in the plant	(Pereira et al., 2016)
Lettuce Radish Chinese cabbage –	*Arthrobacter scleromae* SYE-3	Increased all the plant shoot length and leaf number in lettuce by 45.1 percent	(Hong and Lee, 2017)

A newly identified rhizobacteria (*Ochrobactrum intermedium*) possessing the survival ability of changing its membrane phospholipid composition when subjected to stress condition and also producing indole acetic acid, siderophores, deaminase enzyme and utilization of nitrate happens to improve the growth (root and shoot) of groundnut plant subjected to salt stress better than a well-known classified *Bradyrhizobium* sp (C145) accepted as a suitable groundnut inoculant by Argentine INTA organization. The organism also performed better in the production of biofilm than *Bradyrhizobium* and recorded higher tolerance to 300 millimole sodium chloride and higher temperature (Paulucci et al., 2015).

Soil additives containing a variety of organic matter (poultry droppings, olive plant waste, rice straw, molasses) and a consortium of microbes known as "*effective microbe*" (bacteria, yeast, photosynthetic bacteria, actinomycetes etc.) collectively regarded as bokashi, have proven to be effective in shielding Mandarin tree (citrus plant) from salt stress and improving its productivity, and the nutritional content of the fruit, and also in enhancing the rhizosphere microbial community count and population (El-Hamied, 2014). The bokashi treatment has aided greatly in the salted soil fertility improvement and protection of the plant from osmotic-salt induced stress (El-Hamied, 2014).

In a salt toxicity alleviation study, microbial species known as *Kocuria erythromyxa* EY43 and *Staphylococcus kloosii* EY37 were found to be very potent in increasing strawberry plant growth, chlorophyll, mineral content and fruit yield. The organism was able to inhibit the absorption of toxic ions (sodium and chloride ions) from the plant rhizophere making a salinity sensitive strawberry plant insensitive and tolerant to the condition of salt stress (Karlidag et al., 2013). The integration of endophytic bacteria (*Sphingomonas* sp LK11) with applied jasmonic acid synergistically improved the overall tolerance of tomato plants (wild type and mutant specie) to salinity. The combined applied microbe-jasmonic acid enhanced root and shoot growth of the plant, brought about proper regulation of glutathione content of the plant and enabled it to overcome the detrimental effects of salinity. It was noted that the microbial-jasmonate treatment lowered the intracellular abscisic acid level of the plants (Khan et al., 2017).

Although application of nutrients or minerals is an alternative measure to curbing salinity stress, introduction of excess of these nutrients will result in phytotoxicity instead of phytoenhancement and will necessitate remediation.

Three hundred (300) percent growth in barley plant growing in salt containing soil was recorded when inoculated with *Curtobacterium flaccumfaciens* which did not fully express the attributes of plant growth promotion in pure culture growth medium. This organism, being relatively new in this branch of study, was originally associated with the infection and destruction of crops but was now found to protect plants (barley) from salt stress better than the well-known microbes exhibiting excellent

characteristics of plant growth promotion such as *Microbacterium natoriense* and *Pseudomonas brassicacearum* (Cardinale et al., 2015).

Rhizobium naturally adapted to desert environments has been shown to perform well in establishing nodules on the roots of legumes at a high level of salt-soil-content and to contribute to growth and better performance of the legume (Sobti et al., 2015). Also, an endophytic bacteria (*Bacillus subtilis* LK14) isolated from the plant (*Moringa peregrina*) bark possesses high indole acetic acid production capacity and ACC-deaminase was able to improve the growth and development of tomato plant seedlings (Khan et al., 2016b).

Microbial facilitated survival of plants under saline tension is dependent on microbial production of deaminase enzyme (1-aminocyclopropane-1-carboxylate deaminase) responsible for degrading plant product known as aminocyclopropane-1-carboxylate, the precursor of the plant hormone (ethylene) (Adams and Yang, 1979, Dong et al., 1992, Honma and Shimomura, 1978, Glick et al., 2007). This hormone is responsible for the interference of root development when challenged with stress substances that induce elevated levels of ethylene production and is well documented by Tak et al., (2013).

Chickpea plant affected by an increase in salinity was rescued by co-inoculation with two deaminase enzyme producing microbes (*Mesorhizobium* MBD26 and *Rhizobacteria* RHD 18) with an observed nodulation capacity of 49 nodules per plant, 201 mg weight of nodules, 12.28 mg per plant nitrogen and a rise in 31.2 percent of the above the soil plant part dry weight (shoot dry weight). The result was further increased to 53 nodules and 116.9 percent grain produced, with N_2 level spanning between 9.59 – 27.36 mg per plant investigated (Chaudhary and Sindhu, 2017).

The production of deaminase by rhizobacteria, on the other hand, encouraged the root growth of velvet beans undergoing drought stress by catalyzing the disintegration of 1-aminocyclopropane-1-carboxylate to generate nutrients for the microbes and in turn create a shortage of the ingredient (ACC) necessary for the production of ethylene. This helped to limit the action of this hormone

(Saleem et al., 2015). The inoculation of *Bradyrhizobium japonicum* together with *Bacillus amyloliquefaciens* enabled better nodule formation on the soybean roots by the *B. japonicum* and resulted in better fixation of nitrogen and plant viability (Masciarelli et al., 2014).

2.6 Alkalinity Stress in Phyto-Retardation

Apart from stress induced by sodium chloride, sodium carbonate (Na_2CO_3) and sodium hydrogen carbonate ($NaHCO_3$) also constitute a problem to crops. They are implicated in the formation of alkaline soils that result in elevated soil pH and interfere with the bioavailability of phosphorus, iron, copper, manganese and zinc resulting in induced nutrient deficiency and osmotic stress capable of interfering with the proper biological function of the plant (Chen et al., 2011). The high pH has its own inhibitory challenges on non-alkaliphiles inhabiting the rhizosphere. It also interferes with their biological activities as well as physiological functions of the plant.

However, fertility issues of alkaline soil could be handled by the application of bioinoculants. These microbes ameliorate the alkalinity effect on plant by supporting increase in the number of nodules formed. They boost nitrogenase enzyme activities for efficient nitrogen fixation and improve mycorrhizal dominance in the root of faba bean inoculated with *Rhizobium leguminosarum* and Mycorrhizal fungi. The team work between the two organisms promoted faba bean productivity and resistance to alkalinity stress (Abd-Alla et al., 2014).

2.7 Conclusion

The reality of abiotic stress in reducing the availability of food for the growing human population is obvious. The temperature of the atmosphere is rising and deviation or instability in rainfall is frequently observed in our environment today. This creates a tension in the sustainability of farming practice for food production. And as human beings devise an alternative to combat these challenges by adopting irrigation methods, salinity becomes the end product of this alternative practice. This also affects plants negatively.

Significant effects of plant tolerance to abiotic stress are that it will result in promoting yield and production of crops to feed humans and livestock. This can be achieved via the search, selection and engineering of plant species capable of resisting salinity and drought stress.

The use of plant growth promoting rhizobacteria will go a long way in supporting the plant to develop both intrinsic and extrinsic ability to tolerate stressful conditions and sustain yield. An integrated abiotic stress management strategy of co-integration of external application of proline, caffeic acid, nutrients, synthetic plant hormones and microbes (PGPR) could aid greatly in ensuring continuous and efficient agricultural practices that will manage the stress and boost the yield of crops.

CHAPTER THREE

The Impact of Microbes in the Orchestration of Plants Resistance to Biotic Stress: A Disease Management Approach

Abstract

Struggle for survival is a natural and a continuous process. Microbes are struggling to survive by depending on plants for their nutrition while plants on the other hand, are resisting the attack of microbes in order to survive. This interaction is a tug of war and the knowledge of microbe-plant relationships will enable farmers/agriculturists to improve crop health and yield, sustain regular food supply and minimize the use of agrochemicals such as fungicides and pesticides in the fight against plant-pathogens. Although these chemicals are capable of inhibiting pathogens, they also constitute an environmental hazard. However, certain microbes known as plant growth promoting microbes (PGPM) aid in the sensitization and priming of the plant immune defence arsenal for it to conquer invading pathogens. PGPM perform this function by the production of elicitors such as volatile organic compounds, antimicrobials and/or through competition. These elicitors are capable of inducing the expression of pathogenesis related genes in plants through induced systemic resistance or acquired systemic resistance channels. This review discussed current findings on the influence and participation of microbes in plants' resistance to biotic stress and suggests an integrative approach as a better practice in disease management and control for the achievement of sustainable environment, agriculture and increasing food production.

3.1 Introduction

Defense is a strategy for survival and for any organism to survive in this interdependent environmental ecosystem, it must defend itself or experience extinction. Every creature possesses one or more defence tools and plants are no exception. To sustain their health, vitality and existence, plants must ward off and counteract the actions of their enemies (pathogens) through many different

modes including the production of secondary metabolites known as phytoalexins or phytoanticipins (Khare et al., 2017).

The survival of plants depends on their ability to defend themselves through local and systemic responses with respect to an invasion or sensing of the presence of pathogens. These defence signals are triggered by microbes (Figure. 1) at the site of infection that leads to multiple protective responses against the invader and other unrelated pathogenic species (Pieterse et al., 2014). Biotic stress induces the production of oxygen derived radicals such as H_2O_2 (hydrogen peroxide), superoxide molecules, hydroxyl and/or oxygen radicals) that are the first lines of defence for a stressed plant (Nanda et al., 2010). However, certain plant hormones (salicylic acid, jasmonic acid, ethylene) and substances like hydrogen peroxide and oxygen radicals are often implicated in the initiation and control of these phytodefence activities that trigger the production of phytoalexins, callose depositions, cell wall thickening/strengthening, metabolite production and pathogenesis related protein synthesis. Together these responses intercept and inhibit the action of the invading pathogens (Vinale et al., 2008, Singh et al., 2016, Nie et al., 2017). These defence proteins (enzymes) are remarkable in the protection of the plant via the reaction processes they catalyse. The biosynthesis of phytoalexins and/or phenolic compounds as well as salicylic acid production is catalysed by phenylalanine ammonia lyase. Polyphenol oxidase facilitates the redox reaction that converts polyphenol to quinone antimicrobial compounds (Gong et al., 2017). However, in the absence of biocontrol microbes, pest or pathogen challenged plants could produce in excess of PAL - phenylalanine ammonia lyase (Fukasawa-Akada et al., 1996, Vanitha et al., 2009), beta 1,3-glucanase, chitinase (Mauch et al., 1988), peroxidase (Van Lelyveld and Brodrick, 1975), superoxide dismutase (Lu et al., 2017) and peroxidase biomolecules (Hammerschmidt et al., 1982). Pathogen infected plants produce many compounds including alkaloids, phenolics, glucosinolates, betanins, terpenoids, cyanogenic glucosides etc. These compounds are produced by infected cells and surrounding tissues during and after the infection. These substances can prevent pathogens from further infection of the plant (Sirikantaramas et al.,

2008). These defence mechanisms could best be described as an intrinsic resistant strategy by plants to biotic stress.

In the presence of beneficial microbes, pathogen stressed plants undergo a partial or complete reprogramming of their metabolic pathways involved in the defence signalling processes to activate appropriate pathogenesis related pathways (Singh et al., 2016). These defence mechanisms are metabolically costly to the plant. Semi intrinsic part of plant defence process involves reprogramming of the defensive response in plants as engineered by the rhizosphere soil microbiome. These microbiome populations are attracted by plants. Plants play a central role in the selection, initiation and recruitment of potential microbes that will form their rhizosphere microbiome through the type and nature of exudates they release into the rhizosphere (Spence et al., 2014, Berendsen et al., 2012). These recruited microbes include some that are beneficial and others that are antagonistic, and will interact with the plant receptors and prime their immunity. The defence immune priming in plants is initiated once the microbial extracellular structures and molecules such as exopolysaccharide, proteins, flagellins etc, come in contact with the cell receptors on the surfaces of plant. Also, local infection of plants by pathogens as well as herbivore attack will result in structural and functional damage of the affected part. This disruption in the structural and functional network perhaps will initiate a signal transduction from the local site of attack to other parts of the plant for proper immune sensitization. This process is mediated by the amino acid glutamate. The glutamate receptor-like family bearing charged groups and ions will pick up these signals associated with tissue damage and hence induce the accumulation of calcium ions within the plant cells. The accumulated ions will relay the impulse to distant organs responsible for the activation of the defence response genes. Therefore, Glutamate triggers long distance, calcium-based plant defence signalling (Toyota et al., 2018). In other words, this initiates the activation of a cascade of defence genes to produce reactive oxygen molecules, superoxide dismutase, peroxidase and a host of other biomolecules (Luiz et al., 2015). These chemical substances work both within and outside plants to bring forth desired inhibitory

effects on the pathogen. Priming of defence genes in plants as a result of inducers (microbes) or elicitors is termed induced systemic resistance (Stangarlin et al., 2011).

Beneficial non-pathogenic microbes interact directly with the pathogens by secreting chemicals metabolites that will suppress their growth and/or render them avirulent, thereby protecting the host plant (Dey et al., 2014). This mechanism is a direct plant pathogen assisted control by rhizomicrobes. A gram-positive microbe *Micromonospora* obtained from the root nodules of legumes has exhibited a direct biocontrol by inhibiting the growth of many fungal pathogens. This microbe also induces jasmonic acid signalling defence in tomato plants exposed to the fungus *Botrytis cinerea* (Martínez-Hidalgo et al., 2015).

Over the years, the use of agrochemicals (fungicides and pesticides) to control pathogens of crops has been found to constitute an environmental hazard and causes bioaccumulation of toxic substances in the food chain. This necessitates the adoption of an ecofriendly alternative in solving the problem and in sustaining the environment. The use of plant growth promoting microbes has been shown to be a good option in the fight against pathogen invasion of crops (Ashwin et al., 2017, Böhm et al., 2014). With the need to boost the yield and health of crops and to minimize the involvement of agrochemicals in crop disease management, identification of viable biocontrol agents as well as uncovering mechanisms and mediators of plants resistance to biotic stress (as seen in glutamate induced long-distance defense signaling above) is of paramount importance.

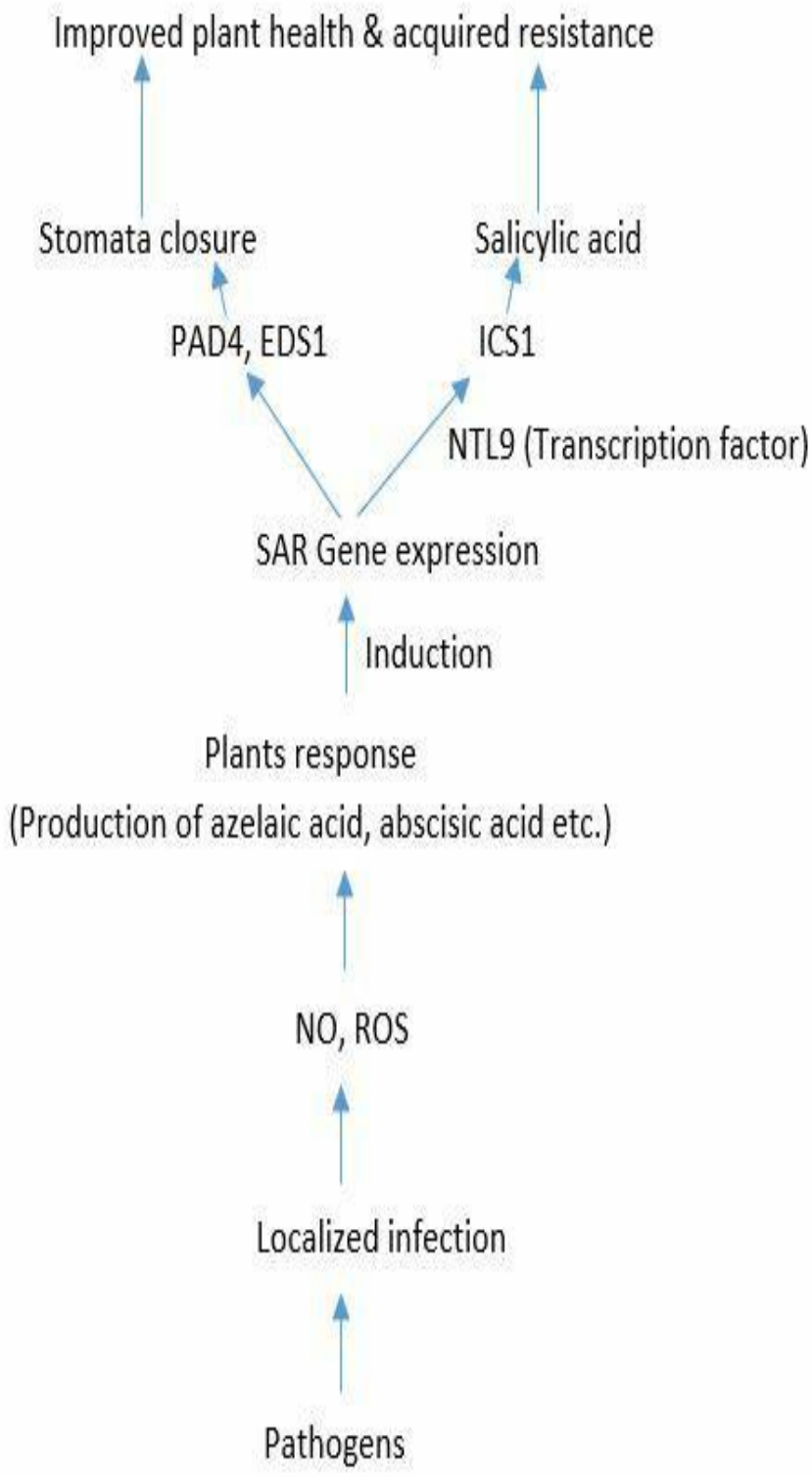


Figure 3. 1: The impact of pathogen infection in the acquisition of systemic resistance in plant. PAD4 phytoalexin deficient 4, EDS1 enhanced disease susceptibility 1, ICS1 isochorismate synthase 1, NTL9 NTM1-LIKE9 transcriptional factor, NO nitric oxide, ROS reactive oxygen specie

3.2 Microbial Induction of Systemic Resistance in Plant to Biotic Stress

The production of chemical substances and their transportation through protein channels to the site of infection and to other parts of the plant (Khare et al., 2017) undergoing stressful condition is a direct approach to the sustenance of plant's health and vitality. The plant *Arabidopsis thaliana* possesses high numbers of transport proteins (ABCG34) and has the ability to resist invasion of the fungus (*Alternaria brassicicola*) by producing and transporting the fungicidal substance camelexin to the surface of the plant leaves. The presence of this fungus stimulates the production of metabolites as well as the expression of genes (*AtABCG34*) responsible for the production of the transport protein (Khare et al., 2017). *Nicotiana tabacum* producing sclareol (diterpene alcohol) enhances the plant defences against invading pathogens (Crouzet et al., 2013).

The biosynthesis/production of jasmonic acid within plants as a result of physiological defense impact-response of plants to pathogen invasion/attack contributes to the growth of the plant and also inhibits pathogenic infection reoccurrence. Jasmonate induced oxygenase inactivates the jasmonic acid activity by hydroxylation reaction to bring its activities to normal as seen in *Arabidopsis*. An Arabidopsis mutant possessing a dysfunctional gene responsible for production of the oxygenase enzyme produced an over production of jasmonic acid that resulted in the inhibition of plant growth but increased the plant resistance to invasive pathogens (Caarls et al., 2017). In the presence of pathogens, salicylic acid is produced through the transcription and induction of the major synthetic gene known as isochorismate synthase 1 which is activated by the transcriptional factors (NTM1 – LIKE 9 and CCA1 HIKING EXPEDITION). As seen in the induction of acquired immunity in plants, salicylic acid not only serves as a hormone but is also responsible for local and systemic resistance of plants to pathogens. It facilitates the production of plant proteins that are microbiocidal in nature. It is involved in the stomatal regulation/behaviour in the presence of pathogenic microbes on the phylloplane and enhances efficient closure of this pathway against the entrance of the pathogen into the plant tissue. To perform this regulatory role, plants engage their surface receptors (flagellin sensing 2) that sense the presence of microbe associated protein such as flagellin which triggers the

closure of the stomata and prevent the entrance of pathogen into the plant (Zheng et al., 2015, Zeng and He, 2010).

The rhizobacteria flora native to the soil have significantly reduced the incidence of disease and death of tobacco (*Nicotiana attenuata*) inflicted by *Fusarium* or *Alternaria* compared to plants growing in a fungi infested agricultural soil (Santhanam et al., 2015).

The application of exopolysaccharides produced by *Lactobacillus plantarium* on tomato plant, elicited/induced the expression of defensive genes as observed with increased expression of the intracellular defense enzyme: CAT (catalase), PPO (polyphenoloxidase), SOD (superoxide dismutase), and H_2O_2 (hydrogen peroxide). These substances (Figure. 2) enhanced plant resistance to the destructive pathogen *Xanthomonas gardneri*, the agent that causes bacterial spot disease on the tomato leaves. Exopolysaccharide treatment influenced the lowering of water movement and escape from the leaves stomata pores by 36 percent (Blainski et al., 2018). Also, a protein molecule (*Colletotrichum falcatum* plant defense inducing protein 1) produced by the fungus *Colletotrichum falcatum* (a pathogen of sugar cane) was able to prime the expression of the defense machinery of sugar cane. This induction of the defense genes led to the inhibition of the fungus associated cellular lesion on the treated sugar cane plant that was challenged with the pathogen *Colletotrichum falcatum*. Noticed also was stimulation of the plant's production of hydrogen peroxide and deposition of callose on the affected plant parts (Ashwin et al., 2018).

A tomato rhizosphere associated bacteria – *pseudomonas* sp. capable of producing antimicrobial substance phenazine enhanced the intrinsic resistance of the tomato root and shoot to the attack of pathogens. It stimulated the intracellular accumulation of organic compounds (phenolics, lipoxygenase and jasmonic acid) in the treated plant and provided protection against a wide range of pathogenic microbes (fungi, bacteria and/or viruses) (Hariprasad et al., 2014). The gene products responsible for the control of pathogenesis-related genes has shown that *Lox3-4*, *ZmLox3* (lipoxygenase gene), negatively control the expression of genes capable of promoting systemic resistance in plant. But the disruption of this gene will generate a Lox3-4 mutant in maize plant. This

significantly increased the leaves' systemic resistance to the pathogen *Colletotrichum graminicola*. In the absence of this negative control gene, the pathogenesis genes were constitutively expressed to sustain the resistance of the plant to pathogens (Constantino et al., 2013).

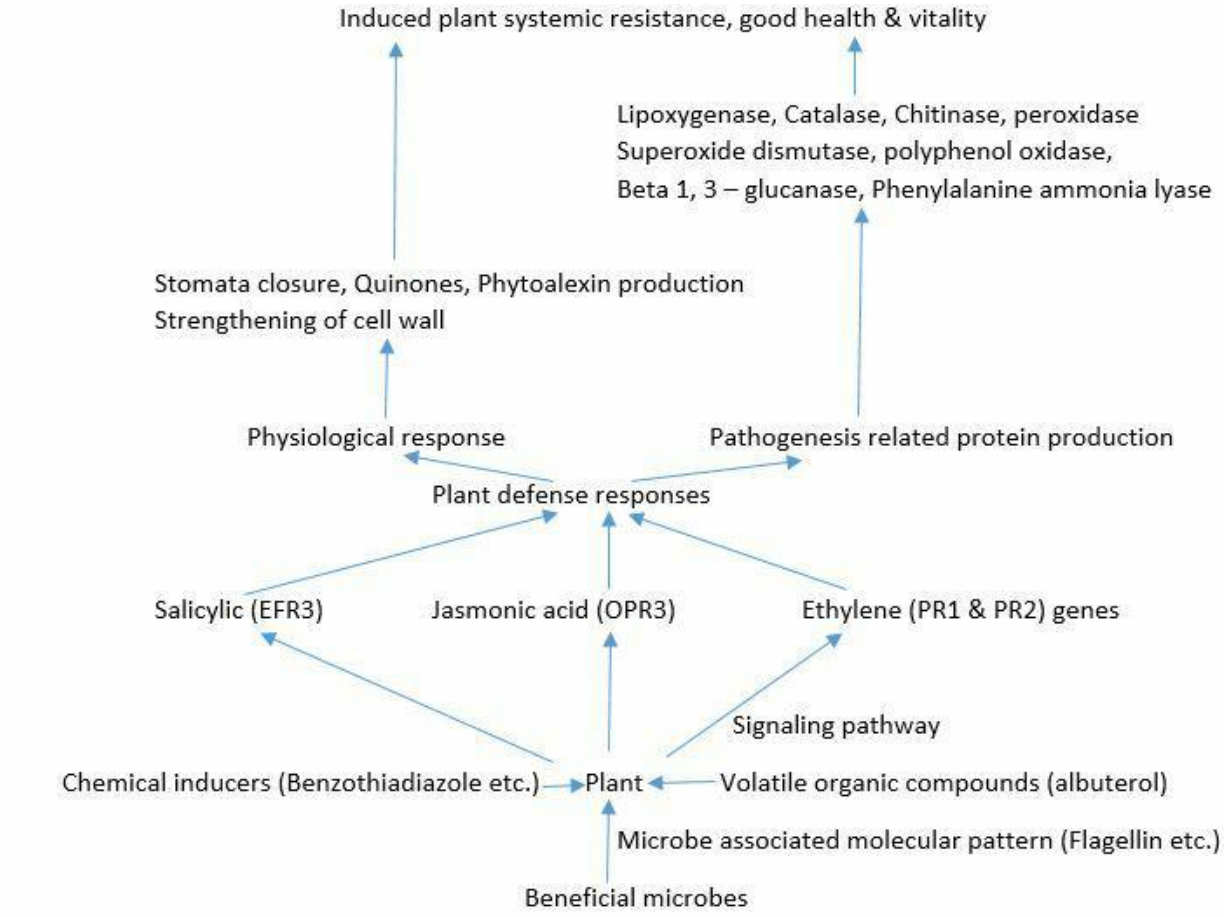


Figure 3. 2: The interrelatedness of beneficial microbes, chemical inducers, and elicitors in the induction of systemic resistance in plants. EFR3 ethylene response factor, OPR3 jasmonic acid signaling gene, PR1 pathogenesis-related protein, PR2 beta 1,3 – glucanase

In the same vein, soybean treated with the bacterium *Bacillus* sp. CHEP5 remarkably stimulated the intrinsic resistance of the plant to the fungus *Cercospora sojina* Hara, an agent that causes FLS (frogeye leaf spot) disease in soybean plants. It equally empowered the plant to express defense genes responsible for jasmonic acid synthesis (Tonelli and Fabra, 2014). Although bacteria involvement in

induction of systemic resistance has been known, the issue is whether this is scalable and feasible for a field crop disease control. With respect to the dynamic climate condition and varying agricultural practices and soil management, mycorrhizal fungi are equally implicated in the priming process of plant protective genes. They do this by root colonization and through signaling pathway, prime jasmonic and salicylic acid synthetic genes (OPR3 and PR1). These primed genes will then boost tomato resistance (for instance) to *Alternaria alternata* infection. Also primed were the genes involved in the biosynthesis of enzymes such as LOX (Lipoxygenase) and PAL (Phenyl ammonia lyase) (Nair et al., 2015).

From a different perspective, bacterial involvement in systemic resistance of barley plants could proceed through an indirect priming of the non-expressor pathogenesis related genes by activating the transcriptional factors (WRKY and Ethylene responsive factor) which then resulted in transcriptional reprogramming of the plant for effective induction of the expression of pathogenesis related genes. Also, exogenous application of salicylic acid (Table 3.1) could play a major role in plant immunity, as well as jasmonic acid methyl ester and/or abscisic acid which induce systemic resistance of plant to *Xanthomonas translucens* pathovar *cerecilis* infection (Dey et al., 2014). Among the myriad of microbes found at the rhizosphere of maize plant, *Pseudomonas putida* KT2440 is an excellent root associated bacterium and could tolerate the toxicity of maize root exudates that are inhibitory to other microbes. The mutual affinity between maize and pseudomonas enabled the bacterial presence to trigger the expression of jasmonic and abscisic acid production in the early phase of the plant relationship with the bacteria. This relationship manifested in the suppression of the gene expression of salicylic acid. However, the plant response to the bacteria presence gradually faded away as the plant began to adjust and accommodate the presence of the associative partner. This microbe equally induced systemic resistance of maize to the infection of the fungus *Colletotrichum graminicola* which causes leaf necrosis (Planchamp et al., 2015). To achieve this immune enhancement of plants by microbial influence, effective communication is prerequisite.

This communication is mediated by exchange of signaling molecules or proteins at the rhizosphere (Babalola, 2014). *Trichoderma virens* have been found to excrete small, secreted cysteine proteins (SSCPs) which enhance the symbiosis between the microbe and the plant. These molecules perform a positive effector role in the sustenance of the plant's defense to parasites as well as pathogens and in promoting the establishment of a symbiotic relationship between the plant and fungi. They are also involved in the control of induced systemic resistance of plant by *Trichoderma virens*. These beneficial rhizosphere interactions empower the plant to tolerate and/or resist pathogen infection such as resistance of maize to *Cochliobolus heterostrophus* (Lamdan et al., 2015).

Co-inoculation of plant growth promoting rhizobacteria (*Pseudomonas* sp. R41805) with mycorrhizal fungi (*Rhizophagus irregularis* MUCL 41833) stimulated the activation of systemic defense genes of potato plant against *Rhizoctonia solani* infection through the priming of ethylene resistance network. This is an indirect approach to biocontrol of plant pathogens (Velivelli et al., 2015). In a direct biocontrol, *Pseudomonas fluorescens* LBUM223 possessing the intrinsic ability to produce Phenazine – 1 – carboxylic acid was found to control *Streptomyces* sp. involvement in infection of potato by negatively regulating the gene (*txtA*) expression of the *Streptomyces*. This virulence and pathogenicity gene in *Streptomyces* responsible for thaxtomic production is involved in scab disease development in potato (Arseneault et al., 2015). The influence of root associated *pseudomonas fluorescens* PTA-CT2 was felt in grapevine both local and systemic, as the bacteria induced systemic resistance of the plant to the pathogen *Botrytis cinerea*. This point source as well as systemic (in roots and leaves) influence could be attributed to the transfer of metabolites from the root to the upper chamber of the plant. It also induced the expression of phytoalexin and glutathion – 3 – transferase with a marked decrease in the expression of hypersensitive related genes. Cell death was also observed in the plant roots (Gruau et al., 2015). Indeed, *Phytophthora cactorum* pathogen of Korean ginseng plant that caused the dreaded root disease was efficiently controlled by a plant growth promoting rhizobacteria (*Bacillus amyloliquefaciens* strain HK34) which induced systemic resistance

in ginseng plant via inducing the expression of pathogenesis related genes in the treated plant (Lee et al., 2015).

Table 3. 1: Microbial elicitors that induce systemic resistance in plants

Microbes	Organic substance produced	Phytopathogens	Plants	References
Bacillus subtilis 985, *Bacillus amyloliquefaciens* 5499	Surfactin	*Botrytis cinerea*	Tobacco	(Cawoy et al., 2014)
Escherichia coli (Recombinant)	PevD1 protein	*Verticillium dahlia*	Cotton	(Bu et al., 2014)
Cladosporium sp., *Ampelomyces* sp.	m-cresol methyl benzoate	*Pseudomonas syringae* (pv. tomato DC3000)	*Arabidopsis thaliana*	(Naznin et al., 2014)
Bacillus subtilis	Culture supernatant	*Meloidogyne incognita*	Tomato	(Adam et al., 2014)
Phytophthora parasitica OPEL protein from Recombinant *E. coli*	OPEL protein	*Tobacco mosaic virus, Ralstonia solanacearum, Phytophthora parasitica*	*Nictiana tabacum* (cv. Samsun NN)	(Chang et al., 2015)

Bacillus subtilis	Surfactin, Mycosubtilin	*Botrytis cinerea*	Grapevine	(Farace et al., 2015)
Pseudomonas fluorescens RRLJ134, *Pseudomonas aeruginosa* RRLJ04	Phenazine analogues	*Fomes lamoensis, Ustulina zonata*	Tea	(Mishra et al., 2014)
Trichoderma virens, Trichoderma atroviride	SM1 (small protein1) and EPl1 proteins (eliciting plant response-like protein)	*Alternaria solani, Botrytis cinerea, Pseudomonas syringae* pv. tomato (Pst DC3000)	Tomato	(Salas-Marina et al., 2015)
Bacillus sp. SJ	Volatile compounds	*Rhizoctonia solani, Phytophthora nicotianae*	Tobacco	(Kim et al., 2015)
Bacillus fortis IAGS 162	Phenylacetic acid	*Fusarium oxysporum f.sp. lycopersici*	Tomato	(Akram et al., 2016)
Bacillus subtilis DZSY21	Lipopeptides	*Bipolaris maydis*	Maize	(Ding et al., 2017)

Pseudomonas aeruginosa PM12	3-hydroxy-5-methoxy benzene methanol (HMB)	*Fusarium oxysporum*	Tomato	(Fatima and Anjum, 2017)
Bacillus subtilis SYST2	Albuterol, 1,3-propanediol	*Ralstonia solanacearum* TBBS1	Tobacco	(Tahir et al., 2017)
Pseudomonas protegens CHAO	Orfamide A	*Cochlibolus miyabeanus*	Rice	(Ma et al., 2017)
Bacillus amyloliquefaciens (UCMB5113)	Fengycins	*Alternaria brassicicola*	*Arabidopsis thaliana*	(Asari et al., 2017)
Saccharothrix yanglingensis (Hhs.015)	BAR11 protein	*Pseudomonas syringae* pv. tomato DC3000	*Arabidopsis thaliana*	(Zhang et al., 2018)
Bacillus amyloliquefaciens, Bacillus subtilis	Iturin A, Fengycin, Bacillomycin	*Fusarium moniliforme*	Maize	(Gond et al., 2015)

The immune response of plants to microbes could follow one of two main routes: SAR - systemic acquired resistance and/or ISR - induced systemic resistance. These two routes will arrive at the same point of boosting the plant immunity. Induced systemic resistance involves the activation of the plant immunity by plant interaction with beneficial nonpathogenic microbes such as the plant growth promoting rhizomicrobes. These microbes stimulate the activation of plant immunity via its contact with the plant receptor responsible for sensing the microbe associated molecular pattern of the microbe (Pieterse et al., 2012, Zamioudis and Pieterse, 2012). Moreover, the mechanisms and

signaling processes involved in microbial induction of systemic resistance have been well reviewed by Shine et al., (2019).

The two main signaling pathways (salicylic acid and jasmonic acid/ethylene) were primed simultaneously by *Bacillus cereus* AR156 in *Arabidopsis thaliana* plant. The activation of these pathways/defense genes gave rise to improved plant immunity and resistance to pathogenic microbial infection. These genes are found to be controlled by two transcriptional factors (WRKY11 and WRKY70). The presence of *Bacillus cereus* has a positive stimulatory effect on the activity of WRKY70 but negatively suppresses WRKY11 in the plant. Nevertheless, transcriptional factors enable proper transcription of DNA and contribute greatly in the process of *Bacillus cereus* induction of systemic resistance in plant. The microorganisms also have the tendency to activate both salicylic acid and jasmonic acid signaling pathways simultaneously in the green vegetative plant (Jiang et al., 2016).

In line with the forgoing, mycorrhizal fungi, a beneficial symbiotic microbe, facilitated the induction of systemic resistance in tomato plant. This fungus on its own does not induce the priming of the pathogenesis-related-genes but only does so in the presence of a pathogen. The arbuscular mycorrhizal fungus (*Funneliformis mosseae*) facilitated tomato resistance to *Alternaria solani sorauer* infection. The organism led to an increase in beta 1,3 – glucanase, chitinase, PAL (phenylalanine ammonia lyase) and Lox (Lipoxygenase) in the tomato leaves when inoculated with the microbial pathogen. Therefore, in the presence of a pathogen, arbuscular mycorrhizal fungi pre-inoculated tomato plants had the highest defensive gene response involved in pathogenesis (namely PR1 – pathogenesis related protein, PR2 – Beta 1,3 – glucanase, and PR3 - chitinase) and defense related genes (Lox – lipoxygenase, AOC (allene oxide cyclase), PAL (phenylalanine ammonia lyase) in the leaves of tomato plant (Song et al., 2015).

A nonpathogenic bacteria *Rhizobium radiobacter,* a close cousin of *Agrobacterium tumifaciens* (now called *Rhizobium radiobacter* biovar 1 strain C58), has the ability to activate/induce the expression of plant defense-genes and boost plant immunity through jasmonic acid signaling pathway induction.

This was observed in *Arabidopsis* challenged with the microbe *Pseudomonas syringae* (pv. tomato DC3000). Similar effects were observed in wheat plants challenged with *Xanthomonas translucens* (pv. Translucens*)* (xtt) (Glaeser et al., 2016). The contributions of beneficial microbes in food production through the induction of systemic resistance of plants to pathogens cannot be overemphasized. This is clearly observed in the influence of *Bacillus cereus* C1L to increase the vegetative growth of maize plant and improve its resistance/tolerance to pathogenic fungal induced disease condition (southern leaf blight that is caused by *Cochliobolus heterostrophus*). This alternative and ecofriendly approach to biocontrol and promotion of plant immunity through the application of microorganisms that prime plant defensive genes has a significant substituting effect to farmers' dependence on fungicides (Dithiocarbamate and mancozeb) in the control of fungi infection of plants. Unfortunately, these fungicides are capable of causing neurological disease/ disorder in human beings (parkinson) and necessitate the use of ecofriendly microbes as a substitute (Lai et al., 2016, Ferraz et al., 1988, Meco et al., 1994).

A look at induced systemic resistance from another dimension suggested that yeast (*Pseudozyma churashimaensis* strain RGJ1) isolated from a pepper leaf surface exerted a protective role on the plant against the viral infections caused by Cucumber mosaic virus (CMV), Pepper mottle virus (PMV), Pepper mild mottle virus (PMMV), and Broad bean wilt virus (BBWV) and against bacterial pathogen *Xanthomonas axonopodis*. The yeast was able to boost plant immunity through the induction of plant pathogenesis related genes involved in salicylic/jasmonic acid signaling pathway (CaPR4) and ethylene (CaPR5) signaling pathway (Lee et al., 2017). Whenever microbes succeed in infecting a plant, the cellular level of hydrogen peroxide will increase, as well as the deposition of callose in the affected plant part as a first line of defense response by the plant (Nie et al., 2017). However, treatment of *Arabidopsis thaliana* with *Bacillus cereus* (AR156) promoted the plant immunity against *Botrytis cinerea*. The protection involved many phases of induced systemic responses that encompass the expression of protein (PR1), H_2O_2 and deposition of callose. These physiological activities were observed more in *Arabidopsis* pretreated with *Bacillus cereus* and later

challenged with *Botrytis cinerea* pathogen. Induced resistance is as a result of the activation of jasmonic acid/ethylene dependent signaling pathway and NPR1 (NON EXPRESSOR OF PR1) signaling pathway (Nie et al., 2017).

Synergy is often the best approach to achieving excellent results in any biological system. The co-inoculation of *Bacillus* sp. (CHEP5) and *Bradyrhizobium japonicum* (E109) enhanced the induction of soybean resistance to *Cercospora sojina* infection. This agent causes frog leaf spot disease in soybean plant. The inductive capacity of these microbes could be ascribed to their ability to form biofilm when grown together as well as priming of the plant defense immune system (Tonelli et al., 2017).

The microbial approach to pathogen infestation control through induction of systemic resistance in plants can be perpetuated by antagonistic microbes (*Pseudomonas* sp S2 and S4) which not only enhanced plant growth but also reduced and controlled the epiphyte microbe – *Salmonella enterica*, the agent of food crop associated with salmonellosis in tomato, spinach and lettuce. Inoculation of this microbe on the root of vegetables had an indirect biocontrol effect on the phylloplane microbial pathogen through the induction of systemic resistance in the inoculated plant (Hsu and Micallef, 2017). The effect of *Burkholderia phytofirmans* (PsJN) a plant useful endophyte was observed in the suppression of the pathogen *Pseudomonas syringae* (pv. tomato DC3000) against its infection on *Arabidopsis thaliana*. The presence of this microbe (*Burkholderia phytofirmans*) on the roots of *Arabidopsis* caused the expression of salicylic acid defense gene (PR1) and the expression of PDF1.2 (a jasmonic acid and ethylene regulated gene) which fortified the immune strength of the plant against infection (Su et al., 2017a). A similar event was noticed in a cucumber plant pretreated with the fungus *Trichoderma atroviride* (TRS25) which induced resistance in the plant against *Rhizoctonia solani* infection. The pretreatment exercise that resulted in *Rhizoctonia* inhibition was a result of increased treatment activation of plant defense enzymes – GPX (guaiacol peroxidase), SPX (syringaldazine peroxidase), PAL - phenylalanine ammonia lyase and PPO - polyphenol oxidase. Also increased was the concentration of intracellular phenolic compound, hydrogen peroxide and a

corresponding decrease in thiobarbituric acid concentration. The fungus also promoted the accumulation of derivatives of salicylic acid – MeSA (methyl salicylate), EHS (ethylhexyl salicylate), SAGC (salicylic acid glucosylated conjugates), beta cyclocitral and VOC (volatile organic compound – 2,3 hexanal, 2,3-hexenol and E-2-hexenal). These compounds, particularly the volatile organic compound, contributed greatly in the fungal induction of salicylic acid defense genes (PR1 and PR5) involved in the plant's systemic acquired resistance (Nawrocka et al., 2018).

The influence of BjNPR1 protein was found to contribute significantly to the *Brassica juncea* resistance to *Alternaria brassicae* and *Erysiphe cruciferarum* infection in a transgenic Brassica plant overexpressing *BjNPR1* gene (Ali et al., 2017). The bacterium *Bacillus* sp. capable of inducing the production of antioxidant defense enzymes (superoxide dismutase, peroxidase, polyphenol oxidase, phenylalanine ammonia lyase) in rice plant strengthened its resistance to *Pyricularia oryzae* infection (Rais et al., 2017).

Although beneficial microbes induce systemic resistance in plants against microbial infection, yet the struggle for dominance and survival allows some pathogens to constantly devise a means to avert the inhibitory effect of plant immune components. These microbes end up producing HC-Toxin (a histone deacetylase inhibitor) which reprogrammes the transcriptional response of plants to microbial infection and succeeds in making the plants' immune defenses ineffective. This HC-Toxin is produced by the pathogen (*Cochliobolus carbonum* race 1). The toxic substance increased the virulence and infection capacity of the microbe and also changed the acetylation of proteins in maize plant (Walley et al., 2018). The big problem is whether this HC-Toxin gene will be transferred to other microbes that are beneficial to the plant. If this toxin producing gene were to be transferred either horizontally or vertically, this will render the farmers' effort in crop production futile. For this reason, more studies should be channeled toward the identification of potential biocontrol agents that can antagonize *C. carbonum* without picking up the toxin gene from it. At present, there is very little work done in this area to help solve the problem. A good strategy will always involve cooperation and/or division of labor. Co-inoculation of peanut (groundnut) plants with *Bacillus* sp (CHEP5

specie) and *Bradyrhizobium* sp. (SEMIA6144 specie) remarkably protected the plants from the attack of the *Sclerotium rolfsii* (the agent that causes plant stem wilt disease) and increased the plant immunity together with the yield of the treated plant (Figueredo et al., 2017). Also, *Bacillus pumilus* and *Paenibacillus* sp were able to secrete volatile compounds (2,5 dimethyl pyrazine and 1 – octen – 3 - ol) which inhibited the proliferation of the fungus (*Phaeomoniella chlamydospora*, the agent that causes grapevine trunk disease). These microbes induced the expression/activation of the pathogenesis-related genes and callose synthase-genes in the plant. Therefore, production of antimicrobial/antagonistic substances take priority in the control of fungus which is followed by induction and/or priming of systemic resistance in the plant (Haidar et al., 2016).

3.3 The Dual Role of an Effective-Microbe

An effective microbe has a dual role – disease control and promotion of plant growth. This attribute is found with *Rhodopseudomonas palustris* GJ-22. These photosynthetic bacteria not only promote *Nicotiana benthamiana* (tobacco) growth by producing indole acetic acid and 5-aminolevulinic acid but also improved the plants resistance against tobacco mosaic viral infection through priming of pathogenesis related genes (Su et al., 2017b). The dual role of the bacterium *Azospirillum* sp B510 enable tomato plant grow and be protected against infection by *Pseudomonas syringae* (pv. Tomato) as well as *Botrytis cinerea* which causes (leaf spot and gray mold) in the plant. *Azospirillum* enhances the immunity of the treated plant in a non-acquired systemic resistance manner (Fujita et al., 2017).

The question is, can the use of these microbes be effective in the field? Is it scalable? If it is scalable, what is the probability that these biocontrol microbes will tolerate the stiff-competition at the rhizosphere and adapt to the environmental condition? Would their competitive advantage (if any) enable them carryout their biocontrol activities in the soil? Many a time, an effective microbe at the laboratory or controlled laboratory experiment is usually a failure or ineffective under field condition.

On the other hand, root associated beneficial microbe (*Pseudomonas simiae* WCS417) capable of inducing plant systemic resistance and also boost its growth are able to perform this function by

suppressing quite a number of plant's responses arising from its association with microbes. This is to enable it interact mutually with the plant root without being interfered by the plant immune response at the root region (rhizosphere). The flagellin influenced the transcriptional activity of the plant. Microbial flagellin from live and dead microbes can equally elicit/trigger plant immune response (Stringlis et al., 2018). Microbes have the tendency to trigger plant immunity as well as promote its growth without anyone of these activities interfering with another (Huot et al., 2014). These unique characteristics of beneficial microbes could be exploited in disease control and management. Certain proteins are essential in the induction and expression of pathogenesis genes for a sustainable plant immunity. The protein of importance is NPR1 (known as Non-expressor of pathogen related gene1). They are transcriptional cofactor protein molecules that upon binding to the transcription factor (TGA) can enhance the transcription of salicylic acid pathogenesis genes (Tada et al., 2008, Cao et al., 1994).

3.4 Biocontrol Microbes in Fruits Preservation

Microbes have proven to be good candidate in the control of fruit spoilage organisms and hence useful in fruit preservation. For instance, *Cryptococcus laurentii* a yeast capable of biocontrol of pathogen involved in postharvest fruit and vegetable spoilage has encouraged the activation/priming of defense-related genes (salicylic and jasmonic acid signal pathways) and the expression of pathogenesis-related-proteins genes that together made cherry tomato resistant to *Botrytis cinerea* and *Alternaria alternata* infection of the fruit (Lai et al., 2018, Wei et al., 2016). Fruit preservation could be approached microbiologically by the application of antagonistic microbes that will prime the expression of pathogenesis related genes in the fruit, raise the fruit immunity and enable it resist the infective action of the fruit spoilage organisms. Treatment of tomato with *Clonostachys rosea* excellently inhibited the pathogenic actions of *Botrytis cinerea* on the fruit. *C. rosea* induced systemic resistance condition in the fruit as observed with the elevated level of IAA (indole acetic acid), SA (salicylic acid), nitric oxide, PAL - phenylalanine ammonia lyase, PPO - polyphenol oxidase and decreased the concentration of CAT (catalase) and ABA (abscisic acid) (Gong et al., 2017). However,

utilization of microbes in the control of fruit spoilage organisms for an increase in fruits shelf life has raised quite a number of issues. What is the likelihood that these microbes will not constitute an environmental hazard as well as posse health related challenges when used in fruit preservation? One of the major challenges that could limit the wider adoption of this phytopathogen control method is the possibility of microbial gene exchange in the environment. This requires that caution be applied to avoid pickup and transfer of virulent genes. Although, this fruit preservation method is effective, the fact remains that until these issues are sorted out, using this method of fruit preservation in a commercial scale is full of risk.

3.5 Systemic Acquired Resistance in Plants: A Second Alternative

Systemic acquired immunity usually occurs when a necrophilic/necrotizing pathogen (i.e. pathogens that cause cell death upon infection of a living cell/tissue) attack a plant leading to the priming of pathogenesis related genes responsible for this immune response to be activated. Systemic acquired resistance can as well be described as a wide-spectrum disease resistance of plant following a localized infection that transmit protection/immunity to secondary infection of the same or to a different microbial pathogen in an uninfected part of the plant. It is found that NO (nitric oxide) and O_2 radical species are implicated in the induction/activation of systemic-acquired resistance in plant via cleaving the C9 double bond of C18 unsaturated fatty acid whose product is azelaic acid (the inducer of systemic acquired resistance) (El-Shetehy et al., 2015). Also, the role of abscisic acid in the modulation of salicylic acid biosynthesis during tomato acquired systemic resistance is well documented by Kusajima et al., (2017).

At the gene level, induction of salicylic acid production during systemic acquired resistance by plant involves the transcription of the gene ICS1 (isochorismate synthase 1). Activation of this gene upon pathogen attack is controlled by the transcriptional factors NTL9 (NTM1-LIKE9) and CHE (CCA1 HIKING EXPEDITION) responsible for priming the ICS1 gene for immune responses to specific pathogen. Transcriptional factor NTL9 not only induce the expression of ICS1 but also the expression

of PAD4 - phytoalexin deficient 4 and EDS1 - Enhanced disease susceptibility 1 located within the guard cells of the leaves stomata where sensitization (Figure. 1) and expression of the gene help to boost the immunity and closure behaviour of the stomata in response to pathogen presence (Zheng et al., 2015).

3.6 Plant Influence in Soil Immunity Build-Up

Many soils harbor a consortium of both beneficial and pathogenic soil borne microbes but naturally through root exudate secretions, microbes could be either supported or starved depending on their ability to metabolize the exudates. As the number one major primary producers in the ecosystem, photosynthesis is the only means by which plants synthesize and supply labile carbon as well as poly-sugars to the soil dwelling microbes. This gives them the influence over which organism should prevail and which to suppress through starvation. Aside from photosynthates injection into the soil, plants also introduce a number of antimicrobial substances that could inhibit the growth of certain microbes.

The actual plant protection and disease suppression inherent in the soil could equally be attributed to the rich diversity, structure and function viable microbes attracted and supported by the plants. These microbes will proliferate and out-compete the pathogenic microbes or may secrete antimicrobials into the soil to outwit their competitors, thereby indirectly making the soil healthy for crop production.

However, microbes have been implicated in the induction of plants' immune responses and protection from invasive pathogens. Yet the choice of which microbe to invite, support and sustain is entirely dependent on the plants. Plants carry out these roles through their rhizosphere effects. This type of soil sustains the health of plants in spite of the presence or absence of soil borne pathogens. This "immune fortified" soil often occurs with agricultural practice of continuous cropping system involving planting the same crop such as wheat, sugar beet etc, in the same farmland till the soil enter a disease suppressive mode (Raaijmakers and Mazzola, 2016). *Arabidopsis thaliana* attracted *Xanthomonas* sp. (WCS2014-23), *Stenotrophomonas* sp. (WCS2014-113), and *Microbacterium* sp.

(WCS2014-259) in a defense against the attack of the pathogen *Hyaloperonospora arabidopsidis*, the agent that causes downy mildew in plants. These microbes perform the enhancement of plant protection better as a team that as individual players in the rhizosphere (Berendsen et al., 2018)

3.7 Endophytes in the Activation of Plants Immunity - Systemic Intrinsic Resistance

In an effort to survive and prevail in spite of the stiff competition among microbes particularly at the rhizosphere, some microbes possessing the cellulase enzyme capable of dissolving the cellulose cell wall of plant roots gain entrance into the apoplast of the plant that includes the interior of the cell wall as well as the vascular bundle – xylem, where they live and undergo normal metabolic activities. Any microbe able to gain entrance and dwell within the plant tissue is said to be an endophyte.

These microbes also have plant growth promoting properties such as hormone production (Naveed (Naveed et al., 2015), and deaminase enzyme production etc. for supporting the host in the fight against invading microbes. The endophytes will continue to enjoy the aid the plants render to them until the plant is mechanically uprooted or die, however, during the life of the plant both parties benefit (Miliute et al., 2015). The presence of these organisms do not, in any way, interrupt the proper functioning of the plant and so they are not pathogenic. Other microorganisms reside on the surface of the plant root exorhizosphere microbes and still perform their duty for the interest of the plant.

Biocontrol agents provide pathogen control role by the production of secondary metabolites that inhibit the growth of the pathogen, by out competing them or induction of systemic resistance in the plant. Endophytes as well as non-endophytic microbes can adopt either of the methods mentioned above in the control of pathogens (O'hanlon et al., 2012). Two endophytic microbes (*Diaporthe endophytica* and *Diaporthe terebinthifolii*) exerted a biocontrol effect on *Phyllosticta citricarpa* (a fungus responsible for causing citrus black spot disease in citrus fruit). These biocontrol microbes were able to inhibit the pathogen via competition and colonization of the same citrus plant organ (niche) that the pathogenic fungus will seek to colonize and cause disease in the plant (Dos Santos et al., 2016).

Endophytes found in the seed of plants are likely to enter the seed through the connection of the vascular bundle, where they will ultimately colonize the embryo and/or endosperm. They could enter the plant seed via the reproductive part of the meristems (Malfanova et al., 2013). Endophytic bacteria isolated from wheat seeds promotes vegetative plant growth through phyto-hormone (indole acetic acid) biosynthesis, siderophore and/or phosphate solubilization. Also, observed was its ability to form biofilms. Above all, they were effective in the inhibition of the fungal pathogen *Fusarium graminearum*. These endophytes included *Paenibacillus* sp. and *Pantoea* sp. (Herrera et al., 2016). The plant growth boosting rhizobacterium (*Paenibacillus polymyxa* AC-1) was implicated in the control/inhibition of the pathogen - *Pseudomonas syringae* (pv. Tomato DC 3000) and *Pseudomonas syringae* (pv. *tabaci*). It was able to colonize the interior part of the *Arabidopsis thaliana* plant and induce the expression of pathogenesis related genes (PR1, PDF1.2, WRKY29, FRK1) in the plant responsible for salicylic and jasmonic acid signaling and defense pathway in *Arabidopsis thaliana* (Hong et al., 2016a).

In the same vain, bacteria species identified as *Bacillus amyloliquefaciens* (SB14), *Bacillus pumilus* (SB6), *Bacillus siamensis* (AP2) and *Bacillus siamensis* (AP8) isolated from the rhizosphere of sugar beet, root and shoot of apple and walnut plant controlled the disease of sugar-beet-damping-off that is caused by *Rhizoctonia solani* (AG-4 and AG2-2). Among these isolates, *Bacillus amyloliquefaciens* was the most effective biocontrol agent. However, solutions to every problem lies in the problem. This is supported by the observation that using native microbes associated with plants have higher chances of biocontrol success as a result of their environmental familiarity, adaptation and easy adjustment to the host plant metabolites and the environmental conditions. This enables them to perform well in the fight against pathogens and foreign (allochthonous) microorganisms (Karimi et al., 2016).

From another perspective, the viral pathogen, cucumber mosaic virus of tomato plants has been found to be controlled by *Trichoderma harzianum* through the mechanism of induced systemic-resistance. *T. harzianum* primed the activation and expression of the defense genes for jasmonic acid, ethylene

and salicylic acid production in the tomato plants. It enhanced the growth of the plant, photosynthetic rate/chlorophyll content as well as the gaseous exchange capacity of the inoculated plant (Vitti et al., 2015) providing a suitable alternative to the control of viral pathogen as chemical treatment of plants is ineffective in the control of viral pathogen (Vitti et al., 2015). The saprotrophic beneficial endophytic fungus *Trichoderm harzianum* T-78 via its efficient root colonization of tomato plant not only stop the penetration/invasion and multiplication of *Meloidogyne incognita* but also primed the activation of salicylic and jasmonic acid immune dependent signaling pathway in plant. This induction of pathogenesis related genes was in the presence of the pathogen which elicited the process by sensitizing the plant through pathogen associated molecular pattern induction. At first, *Trichoderma* induced salicylic acid defense against the nematode and when the nematode imped the jasmonic acid signaling/expression in the plant root, the fungus quickly restored the suppressed jasmonic acid pathway and fortified the plant resistance to the nematode reproduction and proliferation (Martínez-Medina et al., 2017).

The molecular approach through which *Trichoderma harzianum* induces systemic resistance in plants involves the expression of hydrolase genes *Thph1* and *Thph2* that is controlled by Thc6 (C6 zinc finger protein). These gene products (Thph1 and Thph2) prime the production of ROS (reactive oxygen species) and increased the cytoplasmic calcium content of maize leaf. They equally increased the expression of jasmonic acid/ethylene defense signaling pathway in the non-genetic modified maize plant for efficient protection against the disease (Saravanakumar et al., 2016). The colonization of maize plant by *Trichoderma harzianum* induced the plant's systemic resistance to the pathogen *Curvularia lunata* by priming the expression of the gene PAF-AH (platelet activating factor acetylhydrolase). This activation factor produced by the fungus primed the expression and production of chitinase and cellulase enzyme including jasmonic acid inducible genes. The intracellular activities breed and enhance the resistance of maize to the pathogen (Yu et al., 2015).

3.8 Elicitors in the Induction of Systemic Resistance to Biotic Stress in Plants

Elicitors are natural or synthesized chemicals either from microbial origin or chemical combination of elements (Table 2) that are capable of initiating systemic resistance action in plants against pathogens when applied. They could cause a physiological condition of programmed cell death/apoptosis (Heath, 1998). Apoptosis could be caused by invasive pathogen attack on plants whose influence increase the intracellular level of reactive oxygen species and initiate calcium buildup within the plant cell that results in apoptotic cell death (Li et al., 2018).

Exogenous and endogenous salicylic acid are important in gene priming for systemic resistance/protection of plants against pathogens. Exogenous applied salicylic acid on tomato plant has induced the activation of genes responsible for pathogenesis and protection of plant. This substance hindered root infection by *Meloidogyne incognita* and boosted the resistance of the plant to the nematode (Molinari et al., 2014).

A variety of metabolites produced by *Azospirillum brasilense* (V5 and V6) which comprises of (IAA) indole acetic acid, (IEA) indole – 3 -ethanol, ILA (indole – 3 – lactic acid) and SA (salicylic acid) promoted the increased expression of oxidative stress genes and pathogenesis related genes in the leaves and roots parts of maize plant. Application of the metabolites and live bacteria on the leaves of maize equally enhanced plant growth as a result of the phytohormone produced and priming of plant defense genes (Fukami et al., 2017, Vacheron et al., 2015).

Also implicated in the protection of plant against pathogen is the volatile organic compounds produced by beneficial rhizomicrobes (Table 2) which performs their role by the initiation of systemic resistance in the plants. Volatile organic compounds are gaseous, low molecular weight organic compounds such as albuterol, 1,3 -propanediol (Tahir et al., 2017), 3 – pentanol, 2 – butanone (Song and Ryu, 2013) which can activate plant immune system and imped pathogenic microbes from a distance. For this type of compound, distance is never a barrier to it action compare with other

chemical substance of higher molecular weight like exopolysaccharide and proteins that are involved in pathogen control which acts only in close proximity/contact with the plant (Raza et al., 2016).

Table 3. 2: The influence of biological and chemical elicitors in plant protection against pathogens

Elicitors/Inducers	Plants	Phytopathogens	Priming actions of elicitor in plants	References
Azelaic acid AZA1	Arabidopsis	*Pseudomonas syringae* pv. maculicola ES4326	Defense genes enabled the movement of AZA by binding to lipid-AZA and induced systemic resistance in the plant	(Cecchini et al., 2015)
Ammonium ion (NH_4^+)	Tomato	*Pseudomonas syringae* pv. tomato DC3000	Improved the accumulation of hydrogen peroxide which triggered abscicis acid signaling pathway and induced the closure of stomata as well as accumulation of putrescine in the plant	(Fernández-Crespo et al., 2015)
PeBA1 protein	Tobacco	*Tobacco mosaic virus, Botrytis cinerea*	Induced defensive genes for the production of salicylic acid, phenylalanine ammonia lyase, jasmonic acid, hydrogen peroxide and phenolic compounds	(Wang et al., 2016)
Benzothiadiazole	Tomato	Tomato spotted wilt virus and Citrus Exocortis viroid	Activated the salicylic acid signaling pathway and improved the plant resistance to the viral infection	(Lopez-Gresa et al., 2016)
Benzothiadiazole	Sunflower	*Sclerotinia sclerotiorum*	Hindered the development of fungal hypae in the plant and increased the establishment of mycorrhizae in the plant root	(Bán et al., 2017)

Methyl jasmonate	Whitebark pine	*Cronartium ribicola* mountain pine beetle (MBP, *Dendroctonus ponderosae*)	It triggered the plant reprogramming of the transcriptome profile, a set of DEG (Differentially expressed genes) associated with plant defense signaling etc.	(Liu et al., 2017)
Salicylic acid or Methyl jasmonate	Cassava	*Xanthomonas axonopodis* pv. manihotis	Elevated the defense action of cassava plant to the bacterial pathogen	(Yoodee et al., 2018)
Benzoylsalicylic acid	Tobacco, Arabidopsis	Tobacco mosaic virus	It enhanced plant resistance to the virus and induce the expression of Non-expressor of pathogenesis related gene 1 (NPR1), hypersensitivity related molecules, mitogen activated protein kinase (MARK) as well as WRKY genes in the plant	(Kamatham et al., 2016)
Ningnanmycin	Tobacco	Tobacco mosaic virus	Inhibited polymerization of tobacco mosaic virus protein coat and induced systemic resistance and accumulation of pathogenesis related proteins in the plant	(Han et al., 2014)
3-Acetonyl-3-hydroxyoxindole (AHO)	*Nicotiana tabacum*	Tomato spotted wilt virus	Induced the activation of differentially expressed genes (PR1 and PR10) that facilitated the priming and expression of metabolic pathways for synthesis of phenyl propanoid, sesquiterpenoid, triterpenoid for	(Chen et al., 2017)

			protecting plant cuticle and wax	
N-decanoyl-homoserine lactone	Tomato	*Botrytis cinerea*	Induced plant jasmonic acid biosynthesis and signal transduction in the treated tomato plant which confer resistance to the fungal infection	(Hu et al., 2018)
PevD1	*Nicotiana benthamiana*	*Verticillium dahliae, Tobacco mosaic virus, Pseudomonas syringae* pv. tabaci	Interacted with asparagine rich protein (Nbnrp1) to regulate PevD1 that is associated with induction of cell death and increased the plant resistance to the virus	(Liang et al., 2018)

Also, an important organic compound produced by the organism *Enterobacter aerogenes* was good in boosting the resistance of maize to the attacking fungus *Setosphaeria turcica* (a leaf blight causing fungus). In as much as this organic compound (2,3 Butanediol) produced by *E. aerogenes* has a remarkable effect in plant resistance to blight causing fungus, it does not necessarily contribute to the resistance of maize plant to the parasitoid (*Cotesia marginiventris*) attack, yet when applied to the soil as an amendment it acta as an attractant of the microbe to the plant root. However, the attraction is inhibited in the presence of the organism (*Enterobacter aerogenes*) (Table 3.3) (D'alessandro et al., 2014). On the other hand, the analogue of salicylic and jasmonic acid facilitate the activation/initiation of systemic resistance in treated tobacco plant. It protects the plant against tobacco mosaic viral infection by priming the activation and expression of the genes responsible for plant systemic protection. Tobacco plants that bear defective salicylic and jasmonic acid genes increased the infectivity and/or susceptibility of tobacco plant to the viral infection (Zhu et al., 2014).

The volatile organic compound 2,3-butanediol, has two enantiomers (2R,3R and 2S,3S) and a meso-type (2R3S) butanediol produced by root associated beneficial microbes are implicated in the systemic resistance induction in pepper against CMV - cucumber mosaic virus and TMV - tobacco

mosaic virus. Amongst the three isomers, 2R, 3R and 2R, 3S – butanediols were the most effective in priming salicylic acid, jasmonic acid and ethylene defense genes in the plant (Kong et al., 2018).

Table 3. 3: Influence of direct microbe-plant association in plant protection

Microbes	Compounds produced in plants	Invading pathogens	Plants	References
Azotobacter sp., *Pseudomonas* sp.	Beta 1,3-glucanase, peroxidase	Cucumber mosaic virus	Cucumber	(El-Borollosy and Oraby, 2012)
Bacillus cereus AR156	Hydrogen peroxide, pathogenesis related protein	*Pseudomonas syringae* pv. tomato	Arabidopsis	(Niu et al., 2016)
Pseudomonas putida CRN-09, *Bacillus subtilis* CRN-16	Peroxidase, polyphenol oxidase, phenylalanine ammonia lyase, Beta 1,3-glucanase, chitinases	*Macrophomina phaseolina*	Mung bean	(Sharma et al., 2018)
Paenibacillus sp. P16	Induced systemic resistance in cabbage plant	*Xanthomonas campestris* pv. campestris	Cabbage	(Ghazalibiglar et al., 2016)
Bacillus amyloliquefaciens	Production of peroxidase, polyphenol oxidase and expression of pathogenesis related genes for (jasmonic and salicylic acid)	*Ralstonia solanacearum*	Tomato	(Li et al., 2017)

Pseudomonas sp. (BaC1-38)	Beta 1,3-glucanase, chitinases	*Xanthomonas campestris*	Rice	(Lucas et al., 2014)

However, the application of Beta aminobutyric acid (BABA) (a non-protein amino acid elicitor) and non-host *phytophthora nicotianae* on chile peppers induced systemic resistance of the plants to the pathogen *phytophthora capsici*. These elicitors influenced the plant by reducing plant cellular sucrose concentration as well as tricarboxylic acid cycle intermediates. It also boosted the concentration of hexose phosphate, hexose disaccharides, amino acids and galactose in the induced plant, thereby building the plant immunity against *Phytophthora capsici* (Stamler et al., 2015).

The dead cells surrounding the area of infection will block the migration of the pathogen from point of infection to other points in the plant. This is to encourage the production of antimicrobial substances that will impede the proliferation of the pathogen (Hammerschmidt, 1999). Exogenous application of synthetic salicylic acid and beta aminobutyric acid (in a concentration of 1.5 and 15mM respectively) was found to induce the activation of the pathogenesis related protein production of chitinase enzyme as well as beta 1,3 – glucanase for effective immunity and resistance of tomato plant to the invasive pathogen *Alternaria solani* (Raut and Borkar, 2014). Also, hydrogen peroxide, abscisic acid and 2,4 dichlorophenoxy acetic acid chemical inducers applied exogenously to potato plant challenged with *Alternaria solani* were able to resist the pathogen infection as a result of increase in plant intracellular concentration of peroxidase, phenylalanine ammonia lyase and polyphenoloxidase enzymes. These synthesized enzymes inhibited the invasion of *Alternaria solani* in tomato plant (Nassar and Adss, 2016).

Calcium treatment of plants is another abiotic approach to enhancing plant resistance to biotic stress. Calcium has been found to boost the activities of peroxidase and strengthen the plant cell wall as well as improve the production of substances that could inhibit fungi development on the plant (Clark, 2013, Xu et al., 2013, Downie, 2014). A combined treatment of tomato plant with calcium salt and

salicylic acid elevated the production of antioxidant proteins, chitinase and pathogenesis related proteins that encouraged tomato resistance to *Botrytis cinerea* infection (Li et al., 2016).

Another elicitor that is ecofriendly and effective in induction of plant resistance to pathogens is plant extract. The plant extract – limonoids (Munronin O) obtained from the plant of *Munronia henryi* Harms is effective in protecting tobacco plants against the Tobacco mosaic virus by enhancing the defense enzyme production and salicylic acid level of the treated tobacco plant and induced systemic acquired resistance in the plant (Yan et al., 2018). However, the interaction of these chemicals with soil humus or particles leaves doubt concerning their biodegradability because they form stable complexes with the soil particles. Therefore, caution must be applied in the use of these chemical analogues to boost plant immunity. However, the use of natural elicitors such as plant extracts and microbial metabolites should be encouraged, although the cost of producing these natural organic elicitors as well as their preservation could be quite expensive.

3.9 Conclusion

The use of pesticides to control plant pathogens and pests causes issues of concern as the majority of the agrochemicals used in biocontrol not only lower the disease severity in the plant but also lower the yield of the crop (Egel et al., 2018). Some of these chemicals can be harmful to human and animals and may constitute environmental pollution. Carbamate and pyrethoid (insecticides) can cause secondary outbreaks of pests such as aphids (Egel et al., 2018). This necessitates the search for a suitable and ecofriendly alternative in disease control and management.

The use of microbes capable of antagonistic behaviour against pathogens for induction of systemic resistance in plants is a good method in crop disease management (Babalola, 2010). Also, the application of elicitors either in a drench form or foliar spray on plants is yet another method of pathogen control. Elicitors are capable of inducing the expression and/or activation of pathogenesis-related-genes and improving the immunity of the treated plant for efficient fight against invaders.

However, to achieve maximum protection of plants against pathogens, an integrated disease management and control approach is needed, which will involve the use of microbes, their metabolites, synthetic chemicals and plant extracts that will be simultaneously applied to plants. This will enable farmers to win the war against plant pathogens, increase crop yield and achieve sustainable agricultural practices in ensuring food security.

CHAPTER FOUR

The Influence of Soil Fertilization on Soil Microbial Communities and Soil Health: A Guide to Nutrient Management

Abstract

The global quest for improving the utility level of agricultural farmland in the production of abundant food crops to feed humans and livestock have necessitated continuous soil fertilization. The two fertilization regimes are the use of organic fertilizer and inorganic fertilizer. These fertilizers have various effects on the community composition of soil microbes and tend to either promote or suppress the function and population of certain groups of microbial taxa. It has been found that the benefits of using organic manure far outweigh its side effects compared to inorganic fertilizer which not only causes soil acidification but also salinization. Inorganic fertilizer stimulates the denitrification processes and the production of nitrous oxide. To create a balanced microbial community composition where all microbes are properly represented, application of high doses of organic or compost manure is recommended, based on the experimental results contained in the preceding chapters. This will help create an enabling environment for microbial proliferation, and plant growth promotion. The taxonomic abundance, diversity and functions of soil microbes is directly proportional to the quantity of organic manure applied and inversely proportional to the quantity of chemical fertilizer used. Therefore, soil fertilization with higher quantity of organic manure carries with it an enrichment potential for promoting soil microbial community and enhances efficient nutrient utilization.

4.1 Introduction

The interactions of plants, microbes and soil constitute the most important ecosystem driver. In these interactions, microbes are responsive to any slight adjustment or modification in the physicochemical properties of the soil initiated by either the plants or the added nutrients (Suleiman et al., 2013, Zhao

et al., 2014a). For proper ecosystem function, the three components must support one another, particularly in enhancing microbial diversity and functions. Increase in diversity, richness, and abundance of the microbial community in the soil agro-ecosystem is paramount for the maintenance of soil quality, enhanced nutrient cycling and in the attainment of ecological balance in the agricultural soil as well as in pasture farmlands (Li et al., 2014a).

Diverse microbes dwell in the soil and play an important role in nutrient cycling, promotion of plant growth, enhancement of plants' nutrient uptake, mitigation of abiotic stress and biotic stress on plants (Enebe and Babalola, 2019, Enebe and Babalola, 2018). The unique roles played by these organisms in ecosystem function is of great importance in the actualization of a sustainable agriculture (Bhat, 2013).

However, sensitivity of microbes, particularly bacteria, to environmental conditional adjustment that might arise from anthropogenic activities or natural events, have led to the use of bacteria as indicative markers in the evaluation of environmental impacts of agricultural practices on soil health and quality. A shift in bacterial community structure in the soil could be proportional to the level of alteration in soil quality. The prolonged use of chemical fertilizer in an agricultural soil could cause a total loss in bacterial diversity (Yin et al., 2010, Sharma et al., 2010, Kennedy and Smith, 1995, Coolon et al., 2013). Thus, there is a need for an effective mitigation approach in the restoration of soil microbial diversity and functions as well as the physical and chemical properties of the soil. This could be achieved through the use of organic manure in soil fertilization and/or a combination of both (Figure 4.1). This organic manure or organic-inorganic fertilizers mix could enhance the pH, phosphorus, nitrate and potassium concentrations in the soil (Ahn et al., 2012, Ding et al., 2016).

The use of animal manures aids in promoting bacterial diversity in the soil, either as a sole fertilizer or in combination with inorganic fertilizer (Chaudhry et al., 2012, Lazcano et al., 2013). Hence, the rationale behind organic manure (animal droppings, compost or plant residues) enrichment of soil microbial diversity and functions is in its unique nutrients composition and activated microbial groups present in the manure that facilitate ease of nutrient mineralization.

Though, prior to the discovery of synthetic chemical fertilizers, organic manure has been the chief source of soil fertilization. In recent years, the need to feed the increasing human population and the development of modern technologies that could help scale up agricultural practices have resulted in an enormous input of mineral fertilizers into arable farmlands. This soil fertilization method of using chemical fertilizers has resulted in the degradation of agricultural soil through soil compaction, nitrate leaching, decrease in organic content of the soil and a resultant decrease in the efficacy of plants utilization of the mineral fertilizer (Savci, 2012, Singh et al., 2014).

Chemical fertilizer has been implicated in soil acidification. Nitrogen fertilizer with its pH reduction effects, plays a selective role on the type and number of microbial species to prevail in the fertilized soil (Schroder et al., 2011, Griffiths et al., 2011). Because of pH adjustment, certain microbes tend to go into dormancy when the condition is not favourable and will emerge when the system adjusts back to optimum conditions. To achieve a balanced micro-ecosystem, proper fertilization regime should be adopted. And a clear understanding of how these fertilizers influence the types of microbes to support or suppress is important for proper management of soil microbial communities and nutrients. (Liu et al., 2010, Bakker et al., 2012, Quiza et al., 2015).

In this review, the different ways in which soil fertilization affects soil microbial communities and how it contributes to plant and soil health are examined.

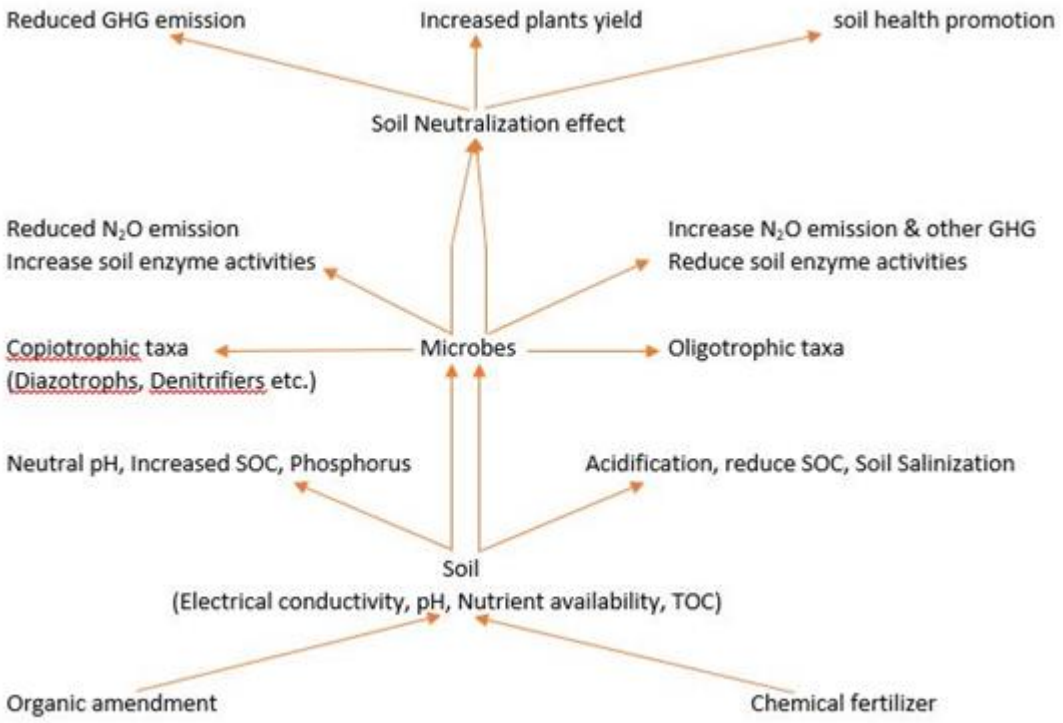


Figure 4. 1: The integrative fertilization approach on soil health and nutrient management under organic and inorganic soil fertilization regime

4.2 Studies showing effects of soil fertilization on Microbial Diversity in Soil

In an agroecosystem, different microbes perform diverse roles to uphold the soil functions such as in nutrient cycling. Fertilization of the soil affects the efficiency of these microbes and influence their diversity, activities and biomass composition. For instance, it was observed that prolonged application of nitrogen fertilizers caused a marked bacterial community change in the composition of ammonia oxidizers, *Nitrobacter*-like-bacteria, cellulose degrader and *Acidobacterial* community (Zhao et al., 2014a, Rousk et al., 2010, Wertz et al., 2012, Fan et al., 2012). Obviously, nitrogen-containing inorganic fertilizer is prone to shifting the balance in the predominant resident microbial community as well as in interfering with the respiration rate of the soil microbial community in the nutrient-enriched soil (Ramirez et al., 2010).

A study by Zhou et al., (2015) showed that in an inorganic fertilized soil, *Acidobacteria* and *Nitrospirae* were decreased while the relative abundance of *Actinobacteria*, TM7, *Verrucomicrobia*,

and *Proteobacteria* were increased across the fertilizer treatments. Bacteria happen to be the most affected among the soil microbiota during the change in nutrient and chemical composition of the soil, unlike fungi which have the ability to adjust metabolically and adapt well to the environmental strain caused by the excessive input of the fertilizers. Also noted were the reduction in the population of ammonia-oxidizing bacteria, nitrogen-fixing bacteria and arbuscular mycorrhizal fungi as affected by the long term use of inorganic nitrogen-containing fertilizer in agricultural soil (Avio et al., 2013, Berthrong et al., 2014, Zhong et al., 2016). Protons released as a result of microbial assimilation of ammonium ions via the microbial porins channels and the electron transport system across the membrane contribute to the reduction of soil pH. Although, microbes are ubiquitous and diverse in nature, their activities are highly influenced by the prevailing environmental conditions. Fertilizer application on agricultural soil is part of human disturbance to microbial physiological activities, richness, and diversity (Zhong et al., 2010).

Also, the decomposition of straw stimulated the proliferation of *Lysobacter*, *Burkholderia* and fungi, *Fusarium*, and *Rhizopus*. Due to the various roles played by the selected microbes, it could be concluded that plant pathogens such as *Fusarium* and /or plant beneficial microbes will dominate in the soil fertilized with plant straw, and depending on which of these microbes are more in population, might result in either infection or growth promotion to the plant (Tardy et al., 2015).

The microbial diversity dynamics that follows soil fertilization gives rise to development of specialization gradient that flows from the dominance of copiotrophic microbes (in the presence of organic matter amendment that contains easily degradable carbon compounds: cellulose, starch, hemicellulose etc) to oligotrophic ones which have well-developed metabolic apparatus for the degradation of recalcitrant carbon polymers – lignin (Pascault et al., 2013, Marschner et al., 2011).

Moderate addition of nitrogen-containing fertilizer in a study by Wang et al., (2015) supported the proliferation of cyanobacteria and Ascomycota fungi, while on the other hand, high doses of the nutrient have a reverse effect on those microbes. High doses of nitrogen fertilizer increased the relative abundance of *Actinobacteria*, *Proteobacteria*, and *Basidiomycota*. And the bacterial

populations far outgrew those of the fungi in a fertilized soil, compared to that of the unfertilized one. The implication is that nitrogen enriched soil has a direct reduction effect on the pH and soil organic carbon contents, which inturn shape the soil microbial community.

Forest, grasslands and agricultural soils have been observed and it has been noticed that *Actinobacteria* are often the dominant groups of bacterial community in a nitrogen-fertilized soil. Nitrogen fertilized soil tends to decrease the biomass and cellular respiration of soil microbes (Ramirez et al., 2010, Entwistle et al., 2013).

A soil rich in microbial diversity as a result of the introduction of a mixture of biofertilizer, inorganic and organic fertilizer, on the other hand, has been shown to increase not only crop yield but also the soil organic matter content. Across unfertilized, chemically fertilized and bioorganic fertilized soil, *Proteobacteria*, *Alphaproteobacteria* and *Gammaproteobacteria*, *Rhizobiales* and *Xanthomonadales* were highly abundant in the soil, while *Acidobacteria*, and *Sphaerobacter* exhibited lower abundance in the soil. But, moving from surface to deeper soil layers within the three fertilization regime, *Verruccomicrobia*, *Candidatus*, *Brocadiales*, and *Skermanella* decreased in their diversity, abundance, and richness. Fertilization has a greater effect in shaping the soil microbial community than the soil depth. Bioorganic fertilized soil supported the proliferation of *Lysobacter* and *Rhodospirillaceae* microbes. These two groups of microbes, for instance, were of greater benefit in the maintenance of plant health and vitality. *Lysobacter* acts as a biological control agent to plant pathogens, while *Rhodospirillaceae* helps to fix atmospheric nitrogen in the soil. Together, they contributed to an increase in apple yield (Wang et al., 2015). Fertilization with manure has an enormous impact on the microbial selection, enzyme activity, and plant performance, and it has been shown that accurate and proper addition of manure to the soil encourages the performance of microbes, microbial enzyme and crop yield (Sun et al., 2014, Zhang et al., 2013b). Therefore, to restore a chemical fertilizer induced degraded agricultural soil, the application of animal manure such as pig or cow dung is preferred as it has shown to perform better in the restoration as well as improvement of soil microbial diversity more than the application of plant residue (wheat straw)

alone. These animal manures help to enrich the soil with nitrate, carbon, and phosphorus. It also create a conducive environment for efficient thriving of a diverse groups of microbes as well as serves the role of microbial carrier for bioaugument. It has an enhancement effect on soil pH (Sun et al., 2015).

Variation in the soil microbial activities and diversity in organic amended soil is time-dependent. The analyses of a digestate (pig slurry) amended soil clearly showed an increase in carbon dioxide evolution rate (a direct reflection of the degree of soil microbial respiration) at the initial time of the soil fertilization due to the high availability of easily degradable carbon compounds in the manure. At the onset of the fertilization, *Proteobacteria*, *Bacteroidetes*, and *Firmicutes* were increased in abundance. And as time elapsed, Gram-negative bacteria and Ascomycota fungi community predominate in the soil and adapted to the limited nutrient availability. Soil microbial biomass also increased with time. The soil microbial community responded to organic matter addition in the soil by moving from copiotrophic dominated microbial taxa to oligotrophic groups. This behaviour of soil microbes is dependent on the soil nutrient gradient (Pezzolla et al., 2015).

Table 4. 1: The effects of soil fertilization on soil microbes

Fertilization type	**Plants**	**Microbiological effects**	**References**
Biochar and compost	Apple orchard	Increased soil organic carbon, reduced the pH and increased the abundance of the soil microbes	(Abujabhah et al., 2016)
Mineral and organo-mineral	grapevine	The organic-inorganic fertilizer mixture increased the soil microbial activities and the organic carbon level of the soil compared to mineral fertilizer alone	(Canfora et al., 2018)
$(NH_4)_2SO_4$	Tulip poplar (*Liriodendron tulipifera*), red maple (*Acer rubrum*), black cherry (*Prunus serotina*), and	Reduced arbuscular mycorrhizal colonization of the plant root, reduced the plant carbon allocation to the roots as well as the soil hydrolytic enzymes, with limited shift effect on the	(Carrara et al., 2018)

	sugar maple (*Acer saccharum*), sweet birch (*Betula lenta*), red oak (*Quercus rubra*), and American beech (*Fagus grandifolia*)	relative abundance of the soil bacterial phyla at high N fertilization level.	
Organic amendments	wheat-maize rotation	It shaped bacterial communities in the soil, increased soil pH by 0.26 as well as soil organic carbon contents	(Dai et al., 2017)
Chicken manure or chicken manure compost	Cucumber (*Cucumis sativus* L.)	The chicken manure compost increased bacterial abundance in the soil, microbial diversity and the yield of cucumber but decreased the fungal abundance and the diversity of microbes in the early spring	(Gao et al., 2015)
Inorganic fertilizers	Pepper (*Capsicum annum* L.)	The N and P contents of inorganic chemical fertilizers exert greater effects on certain specific groups of bacteria than the K component. It alters the bacterial community composition and not their diversity in a fertilized soil	(Eo and Park, 2016)
Organic vs Inorganic fertilization	Rice	The compost greatly supports the fertility of the soil, activates a diverse group of Gram-positive microbes, supports the proliferation of *Rhizobiales* for efficient rice nodulation and *Methylococcales* for combating greenhouse gas	(Daquiado et al., 2016)

		emission compared to inorganic fertilized soil	
Biochar		It increased the fungal richness and diversity, and showed no significant changes/effect on bacterial richness	(Li et al., 2019)
Inorganic and organic fertilizers (sheep manure supplemented with nitrogen)	Potato	The fertilizer treatments did not affect the general microbial community composition in the Greenlandic soils. Bacteria from the phyla *Proteobacteria*, *Actinobacteria* and *Acidobacteria* were the most abundant in all the treatments	(Michelsen et al., 2014)
N fertilizer	Maize (*Zea mays* L.	Across different soil types, N fertilization altered the soil physiochemical properties and shifted the bacterial community structure in the soil with resultant decrease in bacterial abundance, diversity and microbial biomass.	(Yu et al., 2019)
Mineral vs animal slurry fertilisation		Animal slurry applications on the soil increased bacterial community richness and diversity when compared with mineral fertiliser applications. It also increased the relative abundance of several/many copiotrophic bacterial taxa.	(Van Der Bom et al., 2018)
Goat manure (M), sugarcane straw(S), and goat manure plus straw(MS) amendments		Goat manure and goat manure-straw amended soil increased the soil enzyme activity, nutrient content of the soil and plant growth promoting and lignocellulose	(Tayyab et al., 2018)

		degrading bacterial population.	
Organic amendments	spring cereals	No major changes in the community composition due to different fertilizer treatments were found, demonstrating a high robustness of the soil microbiota with *Actinobacteria*, *Acidobacteria* and *Betaproteobacteria*, *Crenarchaeota* (archaea) prevailing in all the treatments	(Poulsen et al., 2013)
Compost and compost-inorganic fertilizer mixture	Citrus	It increased the soil pH to 6 and adjusted the diversity of the soil bacterial community with dominant bacterial group at phylum being *Proteobacteria*, *Acidobacteria*, and *Actinobacteria*.	(Joa et al., 2014)
Mineral vs organic fertilized farming system	timothy grass (*Phleum pratense*) and meadow fescue (*Festuca pratensis*)	Chemical fertilized soil increased the abundance of *Pseudomonas, Oxalobacteriaceae, Koribacteriaceae, Nakamurellaceae and genera Ralstonia, Paenibacillus and Pedobacter* more than the organic fertilized one while organic fertilized soil were enriched with *Comamonadaceae* (genera *Hylemonella*) and *Hyphomicrobiaceae*, actinobacteria from the family *Micrococcaceae*, and bacteria of the genera	(Pershina et al., 2015)

		Geobacter, *Methylotenera*, *Rhizobium* (mainly *Rhizobium leguminosarum*) and *Clostridium*	
Manure and urea fertilizer	*Brassica napus* L, *Triticum aestivum* L, barley	The manure treatment influenced the bacterial diversity with a positive increase while *Actinobacteria*, *Firmicutes*, *Gemmatimonadetes* and *Bacteroidetes* differed between the manure and urea treatments	(Hamm et al., 2016)
Compost and/or inorganic fertilizer-compost mixture	Rice	It enhanced soil microbial biomass, microbial community composition and diversity and increase the abundance of *Cyanobacteria*, *Bacillus*, *Thiobacillus*, *Rhizobium* and *Pseudomonas* sp. with possible N-fixing and/or P solubilizing potentials	(Kuppusamy et al., 2018)

In addition, at taxonomic levels, microbial community composition varies with different fertilizer treatments and a mixture of manure, phosphorus, and potassium gave the best result by encouraging the proliferation of microbial community responsible for disease suppression, and plant growth promotion. It also boosted the soil organic matter content and the soil enzyme activity. These microbes: *Proteobacteria*, *Bacteroidetes*, *Alphaproteobacteria*, *Variovorax*, *Chthoniobacter*, *Massilia*, *Lysobacter*, *Catelliglobosispora*, and *Steroidobacter* were supported by the manure, phosphorus, and potassium nutrients mix. This fertilization regime adjusted the bacterial community in the soil for the attainment of a balanced soil microbial community. These microbes play a unique role in the maintenance of soil health and in disease suppression of soil-borne microbial pathogens (Ma et al., 2018, Makhalanyane et al., 2015, Mendes et al., 2011). Also, a study by Lu et al., (2014)

revealed that manure derived from pigs dung fed with green plants had a better influence in shaping soil microbial community than those from pigs fed with synthetic food. The two components of the swine manure that cause changes in bacterial community composition were the nutrient or organic matter content and toxic metal levels in the manure. The manure from pigs fed with natural feed gotten from plants have no toxic metal compared to those fed with synthetic feed. Therefore, pigs dung from pigs fed with plant material is the ideal source of soil organic amendment. It is also believed that gut microorganisms introduced into the soil alongside with organic amendment during soil fertilization have an effect in changing the resident microbial community structure and activities in the soil. On the other hand, organic manure fertilized soil could prime the proliferation of microbes with various beneficial functions that ranges from antibiotic synthesis to the biogeochemical cycling of nitrogen, carbon, and phosphorus. Long-term organic manure amendment of an agricultural soil in a study by Ling et al., (2016), created an enabling environment that supports active, functional and interactive microbial communities within the soil. Amongst the functional microbial groups supported were the photosynthetic microbes bearing the carbon dioxide fixation gene for the production of a multimeric RuBisCO (ribulose-1,5-bisphosphate carboxylase/oxygenase) enzyme. This implies that organic amendment could support the dominance of diverse microbial groups that participate in a wide range of ecosystem functions. Organic manure fertilized soil has a lasting soil conditioning and enrichment effects. It also boosts the proliferation of both prokaryotic and eukaryotic microbes. *Acidobacteria* and *Gemmatimonadetes* bacteria dominated the amended soil, while *Actinobacteria* and *Proteobacteria* prevail in chemical fertilized soil (Cesarano et al., 2017).

However, nutrient optimization and attainment of balance in the rhizosphere soil nutrient composition following a prolonged fertilization could result in a shift of both fungal and bacterial community composition. Perhaps, long term addition of nutrients in the soil could positively enhance the biodiversity of soil microbes and support proper functioning of the below the ground ecosystem and the free flow of biogeochemical cycling processes in nature (Haas et al., 2018). The availability of carbon compounds has a greater role to play in shaping soil microbial composition and population.

This is because the carbon to nitrogen ratio demand varies across many microbial groups and copiotrophic microbes tend to thrive well at high carbon to nitrogen ratio. The reverse is for oligotrophic taxa which proliferate quite slowly in an environment with less easily degradable carbon sources. The application of maize stubble as an organic amendment in the soil supported an increased bacterial richness and diversity with copiotrophic microbes being dominant at the onset of the experiment and gradually shift to oligotrophic community in response to decrease in stubble carbon. Therefore carbon, water and a host of other factors are responsible for determining microbial community richness and diversity in the fertilized soil (Chen et al., 2015). Using organic manure in soil fertilization will facilitate an increase in microbial diversity and help strengthen the interactions among soil dwelling microbes. It also provides sufficient nutrients to fuel the metabolic activities and needs of these heterogeneous (beta diversity) microbial communities. The more interactions that exist among microbes, the better the health and productive condition of the soil for farming. On the other hand, fertilization of the soil employing chemical fertilizer for a long period of time, as well as the use of agrochemicals like organo-pesticides, will unavoidably create a condition of metabolic stress to the soil microbial community. It often distort the balance and diversity of these soil borne microbes.

In the light of the foregoing, understanding the ways in which soil fertilization practices shape the microbial community could enable agriculturists to adopt better ways of controlling soil-borne pathogens and obtaining the maximum yield of crops from their farmland in arid, semi-arid or savannah regions of the world.

4.3 Diazotrophic Microbes and Soil Fertilization Practices

The agricultural practice of nutrient supplementation and enrichment via fertilizer application to increase crop yield has direct or indirect alteration effects on the abundance, richness, diversity, and composition of microbial communities in the fertilized soils. Diazotrophs are nitrogen-fixing microbes and are among the organisms affected by soil fertilization (Geisseler and Scow, 2014, E Silva et al., 2013). The challenge with external soil nutrient enrichment is its use efficiency by the plants and effect on the soil microbial community. Often, these nutrients are converted into inaccessible forms that cannot be easily absorbed by plants.

A clear understanding on how fertilization (organic or inorganic fertilizer) affects the selection and biological activities of diazotrophs in the soil is important for agriculturists to ensure the sustainability of biodiversity in the soil. An external supply of nitrogen has been found to inhibit or suppress microbial nitrogen fixation (biological nitrogen fixation). It affects the composition of diazotrophs in the soil. The rationale behind this inorganic nitrogen influence on the nitrogen-fixing activities of diazotrophic microbes is that “nature abhors wastage”. If the nitrogen needs of microbes are met through fertilizer application, a repressive feedback mechanism on the *nifH* genes will be exerted by the nutrient and shut off its expression (Wei et al., 2013, Gelfand and Robertson, 2015, Tan et al., 2013, Mirza et al., 2014).

4.4 Nitrifying Bacteria and Nitrification Process in Fertilized Soil

Bacteria and archaea are endowed with ammonia-oxidation genes responsible for catalysing the conversion of soil nitrogen (ammonia) to nitrate. The increase in the availability of nitrate as a result of nitrifying bacterial conversion of ammonium to nitrate will increase the rate of nitrous oxide gas production. The nitrification process is the convertion of ammonium or ammonia to nitrite and nitrite to nitrate. The two main contributors to autotrophic nitrification are the ammonia oxidizing bacteria (*Betaproteobacteria*) and ammonia-oxidizing archaea (*Thaumarchaeota*). These microbes possess the *amoA* gene that produces the enzyme ammonia monooxygenase which catalyses the conversion

of ammonia to nitrite and nitrate respectively. The molecular marker for determining the number, type and diversity of ammonia oxidizers are the presence and quantity of *amoA* genes present in the soil metagenomes (Norton, 2011, Giguere et al., 2015).

In agricultural soils, there is a high rate of conversion of ammonia to nitrate via oxidation process. This oxidation process is mediated by the activities of ammonia-oxidizing bacteria and archaea. Fertilization involving the use of ammonium fertilizer tends to have a slight repressive effect on the ammonia oxidizing bacteria and not on ammonia-oxidizing archaea, whose abundance are usually higher than those of the bacteria. However, Ouyang et al., (2016) noticed that there were more nitrification activities in a moist soil fertilized with ammonium than in a fertilized dry soil. Although, ammonia oxidizing bacteria play an active role in the nitrification process occuring in an ammonium fertilized soil, they are more sensitive to the presence of supplied ammonium than ammonium oxidizing archaea. And the availability of ammonium in the agricultural soil, perhaps, determines the rate of nitrification process in the soil. pH, on the other hand, has an effect on the potential nitrification rate. It was found that nitrification rate in an acidic soil is low even in the presence of more ammonia-oxidizing archaea *amoA* gene copies than in the ammonia-oxidizing bacteria. The addition of biochar to an acidic soil gave rise to increase in nitrification as a result of ammonia-oxidizing bacteria *amoA* genes and increase in the bacterial abundance (He et al., 2018).

On the other hand, chemical fertilizer could cause a shift in the community composition and abundance of ammonia-oxidizing bacteria and archaea in the soil. Soil fertilization with a mixture of chemical fertilizer and organic manure gave rise to an increase in the abundance of ammonia oxidizers and nitrification rate in the soil. This could be attributed to a high available soil nutrients for ammonia oxidizers metabolism and the creation of an enabling environment for the proliferation of these organisms. However, the total available nitrogen and organic carbon in the soil are the main factors driving the shifts in the community composition and structure of nitrifying bacteria and archaea (Xun et al., 2016). As a general rule, soil physicochemical parameters like pH, organic matter and total soil nitrogen largely influence the composition, activities and abundance of soil microbes bearing *AmoA*

genes. These genes are responsible for the production of enzymes that catalyzes ammonia oxidation. Organic amendment increases soil organic matter and nitrogen content as well as pH buffering effect for proper proliferation of nitrifying bacteria (Ling et al., 2014).

Although ammonia-oxidizing bacteria and ammonia-oxidizing archaea are responsible for ammonia oxidation, both microbes respond differently to the presence of urea fertilizer. The population of the former is enhanced in the presence of urea while that of the latter remains unchanged. The variation in their response to urea fertilization implies that ammonia oxidizing bacteria are the initiators of the nitrification process and not the archaea. Archaea unlike the ammonia oxidizing bacteria are less sensitive to the presence of urea amendment and are a good pointer to how nitrogen fertilizers could influence the microbial communities involved in nitrification (Xiang et al., 2017). Studies have shown that soil rich in ammonium will support a higher abundance of ammonia-oxidizing bacteria than soil deficient in ammonium. An ammonium deficient soil will significantly boost the proliferation and abundance of ammonia-oxidizing archaea (Lu et al., 2012, Jia and Conrad, 2009). Therefore, to achieve a balanced microbial community for proper ecosystem function in the soil, adequate supply of ammonium or nitrogen fertilizer and organic manure are required.

4.5 The Fate of Denitrifiers Community in a Fertilized Soil

Denitrifying bacteria facilitate the conversion of soil nitrate to gaseous nitrogen or nitrous oxide. The microbial enzyme that catalyzes the conversion of nitrate through nitrite and finally to nitric oxide, nitrous oxide, and nitrogen molecules are nitrate reductase, nitrite reductase, nitric oxide reductase, and finally nitrous oxide reductase. The genes *nirK*/*nirS* and *nosZ* (for nitrite and nitrous oxide reductase) are the functional markers for the identification of denitrifying microbial community (Azziz et al., 2017, Zumft, 1997, Cui et al., 2016). Fertilization of soil with inorganic fertilizer helps to boost the abundance of denitrifiers in the soil (Chen et al., 2012).

Nitrite reductase gene (*nirK* gene) abundance are high in soil treated with chemical fertilizer, while nitrite reductase gene (*nirS*) and nitrous oxide reductase (*nosZ*) genes are higher in soil treated with a combination of biofertilizer and inorganic fertilizer.

4.6 Fertilization and soil physicochemical properties

Nitrogen chemical fertilization of the soil affects the electrical conductivity, pH, ammonium and nitrate content of the soil. These physicochemical parameters are involved in the restructuring of the soil microbial community and at high nitrogen fertilization, the richness and diversity of bacteria were lowered in the fertilized soil. Therefore, directly or indirectly, nitrogen-containing inorganic fertilizer exerts significant effects on the soil microbial community composition, diversity, and richness (Shen et al., 2016). These effects are canceled by soil nutrient enrichment with organic fertilizer or compost manure (Lloret et al., 2016). In addition, the charge distribution in the soil influence soil electrical conductivity which shape the soil microbial community structure. Organic matter in an amended soil releases carboxylic groups, ammonium ions, hydrogen, hydroxyl ions, which influence the electrical conductivity of the soil. High electrical conductivity favours the proliferation of salt/ionic tolerant microbes and suppresses organisms that are non-tolerant to soil increased ions. This explains how electrical conductivity participates in altering the diversity of soil microbial community. Electrical conductivity and pH work hand in hand. Electrical conductivity affects charge distribution, concentration, and migration. While pH is affected by the degree and concentration of hydrogen ions in the soil environment. Every organism operates at a certain optimal and tolerable level of these chemical conditions of the soil, beyond which the attainment of cellular homeostasis is impossible. At this point, physiological stress sets in and it will cause suppression of growth and metabolic inactivition of the microbes.

However, the remarkable difference between chemical nitrogen-containing fertilizer and organic fertilizer, is that the former slightly increases the total soil nitrogen, microbial biomass nitrogen and

soil nitrate level, while the latter increases significantly the microbial biomass carbon, soil phosphorus, potassium, soil organic carbon, and soil electrical conductivity.

Nitrogen-containing fertilizer has no effect in altering fungal species in the treated soil. Its effect is on the abundance of *Zygomycota* which was decreased and *Fusarium* increased. This effect helps to explain why excessive use of inorganic fertilizer does support the development of plants' disease succeptibility to *Fusarium* fungi infection. Unlike the soil fertilized with organic manure that support the proliferation of disease suppressive microbes which control *Fusarium* pathogens in the soil. The effect of nitrogen fertilizer on the fungal community works by altering the community population and not their diversity. On the other hand, both bacterial diversity, structure, function and number could be affected negatively by higher doses of inorganic fertilization. These observed effects are as a result of fertilizer-induced changes in the soil physicochemical properties. Under moderate nitrogen fertilization rate, soil microbial community and soil fertility of the agricultural soil can be improved considerably (Liu et al., 2018). In addition, soil physicochemical properties under compost amendment were increased, such as soil pH, nitrogen, organic matter, potassium, and phosphorus content. Whereas the soil microbes belonging to *Burkholderia*, *Pseudomonas*, and *Paenibacillus*, were increased together with *Proteobacteria, Bacteroidetes*, and Cyanobacteria in the compost-amended soil, other groups of microbes such as *Nitrospira*, *Gemmatimonas*, and *Phenylobacterium*, *Chloroflexi, Acidobacteria, Nitrospirae, Gemmatimonadetes*, and *Actinobacteria* were not increased in abundance in the compost fertilized soil (Liang et al., 2018). In line with the foregoing, prolonged organic manure fertilization has caused an increase in nitrogen content of the soil and the yield of the crops. It reduced the soil pH by a marginal unit of 0.4. *Pseudomonadaceae* and *Cytophagaceae* were increased and *Acidobacteria* decreased in response to nutrient addition to the soil (Table 4.1). However, the fungi community were less affected by nutrient addition and Bacteria responded more to nutrient amendment than fungi (Ai et al., 2018).

4.7 A guide to nutrient management

Haven considered in detail the influence of soil fertilization to microbial communities and soil physicochemical properties, it is pertinent to suggest suitable approaches for soil nutrient management. The use of organic fertilizer is a sustainable approach to soil fertilization than inorganic fertilizer. However, fertilizers application is aimed at maintaining soil microbial biodiversity and increasing crop yield. To decide which type and quantity of fertilizer or manure to apply, when, where and in what proportion, agriculturist should understand the nutrient requirements of the crops, measure the nutrient composition of the soil and understand the chemical nutrient ratio of the fertilizer to be used.

Various crops have different requirements for macro and micro nutrients needed for proper growth and yield. These nutrient requirements are at its peak during the early vegetative growth phase of the crops, precisely, when the roots and shoot meristems are actively dividing. At this stage, the root exudation is high too and the rhizosphere microbial communities are very abundant and active. These active rhizosphere microbial communities facilitate the nutrient uptake capacity of the plants, thereby resulting in a high nutrient use efficiency of the plants. Any excessive application of nutrients will not result in any significant soil degradation since the assimilation rate is high. However, when these plants reach maturation stage, their nutrient use efficiency might decrease and if there is a continued external application of nutrients, it will result in nutrient wastage. Understanding the plant type, and their growth stages will determine the overall nutrient requirements of the plants for proper fertilization management.

Also, measurement of soil physicochemical properties is another essential factor to be considered for choosing the right fertilization approach. Agricultural soils have different physicochemical properties. These properties are influenced by soil type, soil usage, soil history and location. For instance, the world most fertile soil is Amazonian Dark Earths soil which is rich in organic carbon and other minerals (Glaser et al 2001), this soil will not be suitable for external application of organic or inorganic fertilizer since it is already rich in nutrients. Any further nutrients applied will constitute

an environmental hazard. Then compare it with soil from a semi-arid region with low organic carbon and other macro nutrients. In this soil from a semi-arid region, application of manure or inorganic fertilizer will promote the fertility of the soil and plants productivity with little or no environmental consequences. Therefore, accurate knowledge of the soil chemical properties is needed to guide the choice of soil fertilization.

Finally, the natural approach of using animal or compost manure to supplement soil nutrients, planting of cover crops to sustain a community of active growing microbial population in the rhizospheric soil and the application of chitin or bird feathers to the soil in order to prime microbial production of chitinase are ecofriendly and natural ways of creating a conducive and healthy soil for proper plant growth and productivity (Oka, 2010, Mehta et al., 2014, Cretoiu et al., 2013). These approaches when adopted will ensure proper soil fertility management.

4.8 Conclusion

Integration is the key in the attainment of a stable and functional ecosystem and soil fertilization practices is not an exception. Among the different fertilization regimes currently in use, a combination of organic (manure) and/or inorganic fertilizer will help to increase the diversity of microbes and their community size in the soil. The application of organic manure will not only improve soil fertility, microbial diversity and function, and soil health, but it will also neutralize the acidification and salinization effects associated with prolong use of high quantity of inorganic fertilizer by commercial farmers. However, to achieve a balance nutrient and soil micro-ecosystem, agriculturists must factor in the plants nutrient requirements, and soil physicochemical properties of the soil to decide the best fertilization regime to adopt for growing crops.

CHAPTER FIVE

Effects of Inorganic and Organic treatments on the Microbial Community of Maize Rhizosphere by a Shotgun Metagenomics approach

Abstract

The main drivers of biogeochemical cycling of nutrients, plant growth promotion, and disease suppression are microbes. Organic manure and inorganic fertilizer increase soil quality and plant productivity. In this study, we explored shotgun metagenomics study to investigate how maize (*Zea mays everta*) rhizosphere microbial communities diversity are shaped following the application of both compost manure (8 tons/ha and 4 tons/ha) and inorganic fertilizer (120 kg/ha and 60 kg/ha). The taxonomic analysis of the soil revealed that regardless of the fertilization regimes, *Proteobacteria* and *Bacteroidetes* are distributed across all the samples, and in varying populations. Higher quantities of organic manure (8 tons/ha) and lower nitrogen fertilizer (60 kg/ha) doses as well as the untreated control supports the selection and enrichment of *Proteobacteria and Actinobacteria*, while lower quantities of organic compost manure boost the population of *Bacteroidetes*. On the other hand, *Firmicutes* were most abundant in low organic manure (4 tons/ha) and higher inorganic (120 kg/ha) fertilized soil. Fungi and viruses were selected and enriched by higher (8 tons/ha) and lower (4 tons/ha) compost manure doses, while archaea were mostly supported by higher (120 kg/ha) doses of inorganic fertilizers and high (8 tons/ha) compost manure treatments. Therefore, comprehending the effects of compost and chemical fertilizers (NPK – 20 % Nitrogen, 7 % Phosphorus, 3 % Potassium) on the community structure, dynamics and abundance of rhizosphere microbiome will help in the manipulation of soil microbial community to increase microbial diversity in the agroecosystem.

5.1 INTRODUCTION

The global need to increase food crop production has resulted in the constant subjection of the soil to a wide range of disturbances ranging from tillage, use of organopesticides and plant cultivation to soil fertilization. These practices change the ecological balance of the soil by influencing nutrient

availability, physical as well as chemical properties of the soil. These perturbations have many effects on the microbiota abundance, viability, and composition. They can make the soil-dwelling microbes to become functionally redundant, metabolically active or resilient to the changing conditions of the soil environment (Altieri, 1999, Allison and Martiny, 2008, Babalola et al., 2007).

Conventional intensive soil fertilization systems often depend more on chemical fertilizer application than organic manure. The extensive application of chemical fertilizers hampers crop nutrient uptake, reduces soil quality, and causes environmental hazards like eutrophication, greenhouse gas emissions (Hartmann et al., 2015, Ding et al., 2014, Zhu et al., 2016). Due to the environmental concerns associated with chemical fertilizer and the need to achieve a sustainable agriculture, current researchers are now evaluating the merits of either substituting it with organic manure or combining both organic manure and chemical fertilizers in soil nutrient enrichment for the promotion of a balanced soil microbial ecosystem (Bhattacharyya et al., 2008, Tejada et al., 2008, Babalola et al., 2009).

In the past, regardless of experimental sites and climatic condition, microbial community studies have shown that the diversity of bacteria as well as other microbes are increased in soil fertilized with organic manure, as a result of nutrient enrichment of the soil by the manure (Hamm et al., 2016, Chávez-Romero et al., 2016). Chaudhry et al. (2012) demonstrated in a study involving a comparison of soil fertilization with organic manure (compost) and chemical fertilizer, that the former enhanced certain bacterial phyla populations better than the latter, as a result of improved soil carbon and nitrogen contents.

The biodiversity of soil microbes is increased by soil fertilization, nevertheless, plants regulate rhizosphere microbial communities through root exudation in the form of rhizodeposition, temperature and moisture control (Denef et al., 2009). *Zea mays L*. (Maize), an important food crop and animal feed, is a crop grown in Africa and in particular North West Province of South Africa, amongst other nations, and has an influence on the selection, enrichment and sustenance of

rhizosphere microbial community (Ranum et al., 2014). These microbes perform crucial roles in the biogeochemical cycling processes in the soil as well as in disease suppression.

Shotgun metagenomics approach, in the past has been used to evaluate and comprehend microbial taxonomic diversity and functions in the soil, sediments, composts and water samples (Mendes et al., 2014, Andreote et al., 2012, Martins et al., 2013, Meneghine et al., 2017, Babalola, 2010). In this study, we aimed to use a shotgun metagenomics study to investigate the influence of inorganic and organic fertilizers on the taxonomic abundance, diversity and structure of maize rhizosphere microbial community and to ascertain whether there is any differences between the control and fertilizers in the selection and enrichment of microbial community in the soil.

5.2 Materials and methods

5.2.1 Soil sampling

The five soil samples used were sourced from North-West University's Molelwane farm planted with maize (25º 47' 24.17604" S, 25º 37' 9.08328" E; 25º 47' 29.97048" S, 25º 37' 8.62428" E; 25º 47' 23.9604" S, 25º 37' 8.43348" E; 25º 47' 23.82252' S, 25º 37' 8.30064" E; 25º 47' 24.11844" S, 25º 37' 8.18148" E; altitude: 1012 m), with a temperature range of 22 - 35ºC and an annual average rainfall of 450 mm (Mokoboki and Sebola 2017). The soil type is sandy loam soil. And the chemical composition of the compost manure are N = 20045.3 (g/kg), P = (1.0 g/kg), K = 12.3 (g/kg), pH = 7.1. The age of the manure compost or stabilization period of the compost prior to its use was 16 weeks. A sampling distance of 15 cm (minimum) and 50 cm (maximum) in 3 sampling spots/plot were used. Sampling was done in October 2018 at 7 weeks after the germination of the maize seed. The experimental plots were treated with 120 kg and 60 kg of NPK fertilizer (N/ha) and with community-based compost manure at 8tons and 4 tons/ha respectively. The maize (Mid-altitude variety of maize – *Zea mays everta*) planting distance was 15 cm × 20 cm. The rhizosphere of soil samples was collected using auger at 0–15 cm depth, 2 cm away from the growing maize plant at 7 weeks after germination. The sampling area was split into 3 plots for 60 kg N/ha and 3 plots for 120

kg N/ha. The control and the compost treatments were split into 3 plots each. Soil samples were taken from 9 plants (3 from each replicate/treatments). Therefore, a total of 15 samples were collected (3 replicates × 5 treatments). From each sub-replicated plot of the treatments, nine sub-samples distributed to cover the entire plots were gathered together into composite samples. Each of the five treatments was made up of five composite samples, each containing nine subsamples. The soil samples were put in a plastic bag covered with ice and conveyed to the laboratory, plant and root debris were sieved (with 2-mm sieve) and preserved at −20 °C for metagenomics shotgun analysis. The physicochemical properties of the soil before planting and fertilization as well as the compost manure after 16 weeks stabilization period were analyzed through following the standard basic soil chemical analysis protocols described by Motsara and Roy (2008) and the results are contained in Table 5.1.

Table 5. 1: Physicochemical properties of the soil prior to planting, and fertilization

Soil property	Value
Physical characteristics	
% Sand	80
% Silt	5
% Clay	15
Chemical properties	
pH (1:2.5 water)	8.2
Total Nitrogen (mg/kg)	300
Total Bray 1 Phosphorus (mg/kg)	100.5
Total potassium (mg/kg)	205
Total calcium (mg/kg)	388
Total magnesium (mg/kg)	162
Total sodium (mg/kg)	5
% carbon	0.36
S – Value (sum of extractable Ca, Mg, K and Na) (cmol(+)/kg)	4.59
% calcium	48.0
% magnessium	33.2
% potassium	18.3
% sodium	0.5

Extractable acidity (me %)	0.03

5.2.2 Metagenomics DNA isolation and shotgun sequencing

A PowerSoil community DNA isolation kit from Mo Bio Laboratories, Inc. Carlsbad, were used to isolate microbial community DNA from 0.25 g of the soil samples following the manufacturer's instruction. The Nextera DNA Flex library preparation kit (Illumina Incorporation) was used to prepare the libraries following the protocols in the manufacturer's manual. The initial DNA concentrations were determined using the Qubit® dsDNA HS Assay Kit of Life Technologies, Carlsbad, California, United States. A total of 50 ng extracted DNA were used for the libraries preparation. The samples passed through simultaneous fragmentation and adapter sequences were added. During the 6 cycles of PCR, the adapters and the unique indices were introduced into the samples. After preparation, the libraries concentration were measured using the Qubit® dsDNA HS Assay Kit from Life Technologies. Also, the library sizes (average) were determined using the Agilent 2100 Bioanalyzer from Agilent Technologies, Santa Clara, California, United States. An equal-molar ratio of the libraries (0.7 nM) were pooled together and sequenced paired-end for 300 cycles with NovaSeq 6000 system machine (Illumina). This sequencing was carried out by Mr DNA of the Molecular Research Laboratory (Texas, USA).

5.2.3 Metagenomics sequence annotation and statistical analysis

The shotgun raw sequences generated from the microbial community DNA were uploaded to the MG-RAST online server (Meyer et al., 2008). This online annotation system pipeline performed many quality controls following their standard protocol in the removal of artificial sequences arising from sequencing process (dereplication), removal of host-specific species-sequences, ambiguous base and read length filtering. The sequences were annotated through blasting using BLAT (BLAST-like alignment tool algorithm) against M5NR databases (Kent, 2002). The taxonomic profile was done by Best Hit at E-value cut off of $1x10^{-5}$, minimum alignment length of 15 base pairs, minimum percentage identity cutoff of 60 based on the RefSeq annotation database sources (Wilke et al., 2012)

using MG-RAST. The distribution of the domains, phyla, class and genus of the taxonomic profile were analyzed only for bacteria because of its dominance in the samples. For fungi and archaea, only phylum levels were considered and viral families were equally analyzed.

5.2.4 Statistical analysis

Canoco software v5 were employed in plotting and data representation for PcoA (principal coordinate Analysis) and principal component analysis (PCA). While the taxonomic abundance heatmap at z-score of -1 to 1 using relative abundance data were generated using heatmapper online tool (www1.heatmapper.ca/expression/). Evenness, Simpson and Shannon diversity index were evaluated for the samples. The diversity-indices were compared across the treatments using a Kruskal-Wallis test, and they were calculated on PAST statistical software (Hammer et al., 2001). Beta diversity was shown by the PCoA (principal coordinate analysis) based on Euclidean distance-matrix and ANOSIM (one-way analysis of similarities) (Clarke and Green, 1988). The principal component analysis (PCA) were used to depict the distribution of taxonomic categories between the maize rhizosphere samples. The sequences were deposited in NCBI SRA database, SRA accession: PRJNA607213

5.3 Results

5.3.1 Fertilizer treatments' effects on microbial community structure in the maize rhizosphere

In order to evaluate the microbial community structure of maize (*Zea mays everta*) plants' rhizosphere at different fertilizers treatments, the generated sequence reads were used in calculating Bray Curtis similarity-matrices and used to carry out PCoA (Principal coordinate analysis). Microbial community could be separated based on the treatments with four major clusters (Figure 5.1). The axis 1 corresponds to 96.12% variation, separating the samples according to the use of organic manure (compost) and inorganic fertilizers. On the left hand side, the samples show those treatments receiving moderate inorganic fertilizer (N1) and high levels of compost manure (Cp8). The low inorganic fertilizer treatments (N1) tend to cluster together with the untreated control (Cn0). The samples at the

right side are those ones that are receiving moderate and high input of compost manure and inorganic fertilizer.

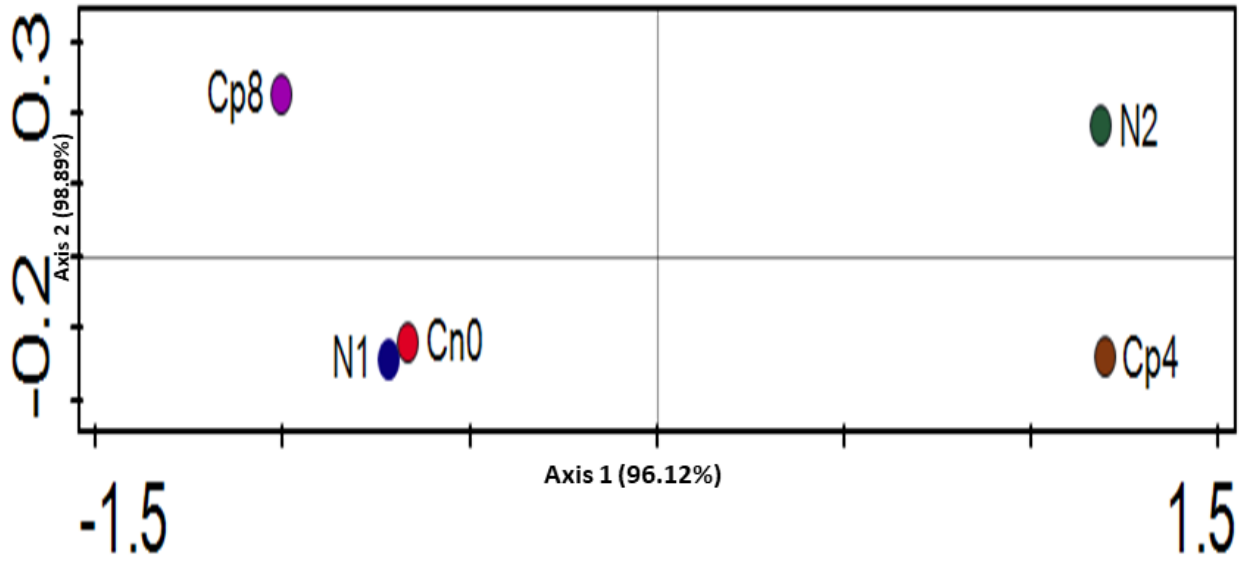


Figure 5. 1: PCoA (Principal Coordinate Analysis) showing the beta diversity between the maize rhizosphere soils at different fertilization regimes. The abbreviation in the figure means, Cp8 (8 tons/ha compost), Cp4 (4 tons/ha compost), N1 (60 kg/ha inorganic fertilizer), N2 (120 kg/ha inorganic fertilizer) and Cn0 (control)

Principal component analysis (PCA) were also generated for the treatments to obtain treatments effects on the bacterial community-structure in particular. There is an appreciable fertilizer effect on the bacterial community-structure at the phylum level (Figure 5.2). The highest dose of compost manure treated soil (8 tons/ha), low inorganic fertilizer (60 kg/ha), and the control exerted maximum selection effects on the bacterial community compared to the high inorganic fertilizers and the medium dose of compost manure treatment. However, the fertilization of the soil with a community-based compost manure resulted in both discrimination and community shifts in the rhizosphere samples of maize plants (*Zea mays everta*).

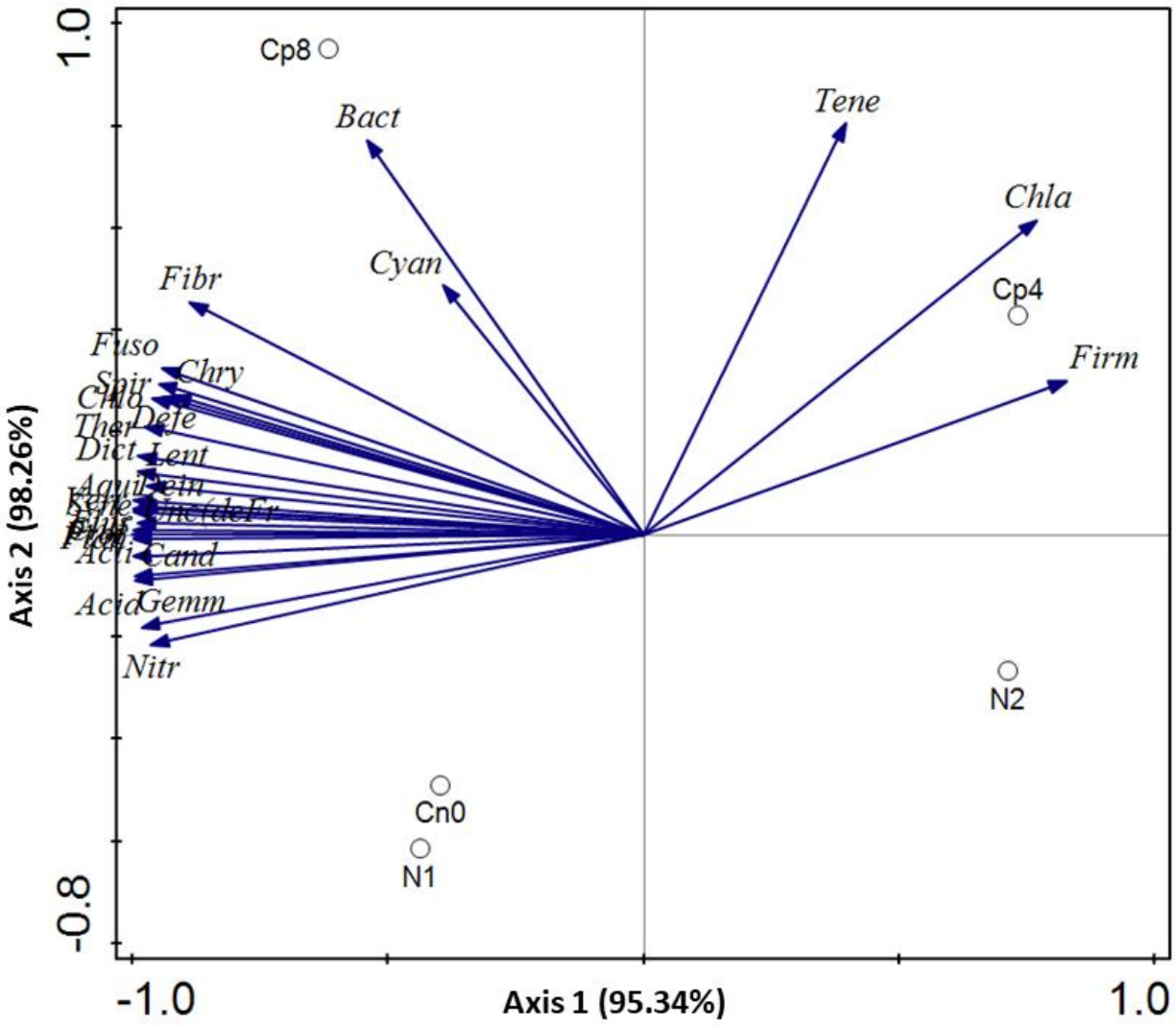


Figure 5. 2: PCA (Principal Component Analysis) of the bacterial community structure associated with maize rhizosphere grown under different fertilization and unfertilized regimes (Compost 8 tons/ha – Cp8, compost 4 tons/ha – Cp4, control – Cn0, 60 kg/ha inorganic fertilizer – N1, 120 kg/ha inorganic fertilizer – N2), showing treatments as the key factors influencing the structural shift and shape of bacterial community at the rhizosphere soil samples. The percentages represents the observed variations.

5.3.2 Comparison of the different rhizospheric soil samples metagenomes

The taxonomic community of the maize rhizospheric soil metagenomes analysis revealed that the samples were dominated by Bacteria (98.2%), with Eukaryota being approximately 1.2%, viruses 2.2%, and Archaea (0.8%) of the total reads in all the samples (Figure 5.3). There was a marked difference in the microbes' composition at the domain-level in the taxa amidst the various fertilizer treatments. The highest was shown in the Bacteria domain. Here, the majority of the sequences were

mapped to the Cp8 (8 tons/hectare compost), N1 (60 kg/hectare inorganic fertilizer), N2 (120 kg/hectare inorganic fertilizer) and Cn0 (unfertilized control), while the Cp4 (4 tons/hectare compost) had considerably lower numbers of sequences ($p<0.05$). Viruses and Eukaryota occur most in low compost (4 tons/ha) manure treated rhizosphere soil, while archaea dominated in 120 kg/ha (N2) inorganic fertilizer treated soil.

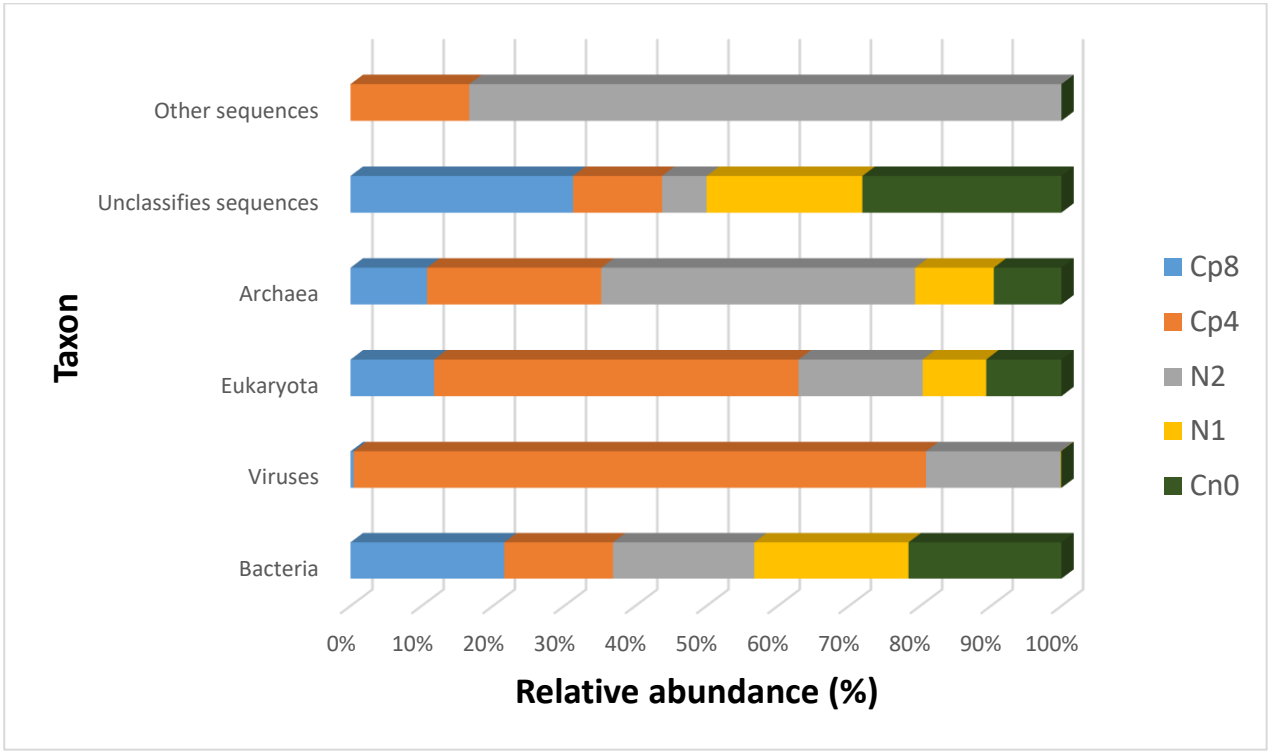


Figure 5. 3: The microbial taxa's relative abundance at the domain level within maize rhizosphere soil samples. The treatment are compost 8 tons/ha (Cp8), compost 4 tons/ha (Cp4), inorganic N - 120 kg N/ha (N2), inorganic N – 60 kg/ha (N1) and control (Cn0).

However, viruses were very low in the control and N1 treatments, but very abundant with the introduction of 4 tons/ha compost manure and a higher quantity of inorganic fertilizer (N2) ($P < 0.05$). However, the viral families observed revealed that Circoviridae, Inoviridae and Microviridae were most abundant in lower organic manure treated rhizospheric soil (Cp4), while Myoviridae, Podoviridae and Siphoviridae were abundant in Cp8 (higher dose of organic manure treated soil) (Figure 5.4).

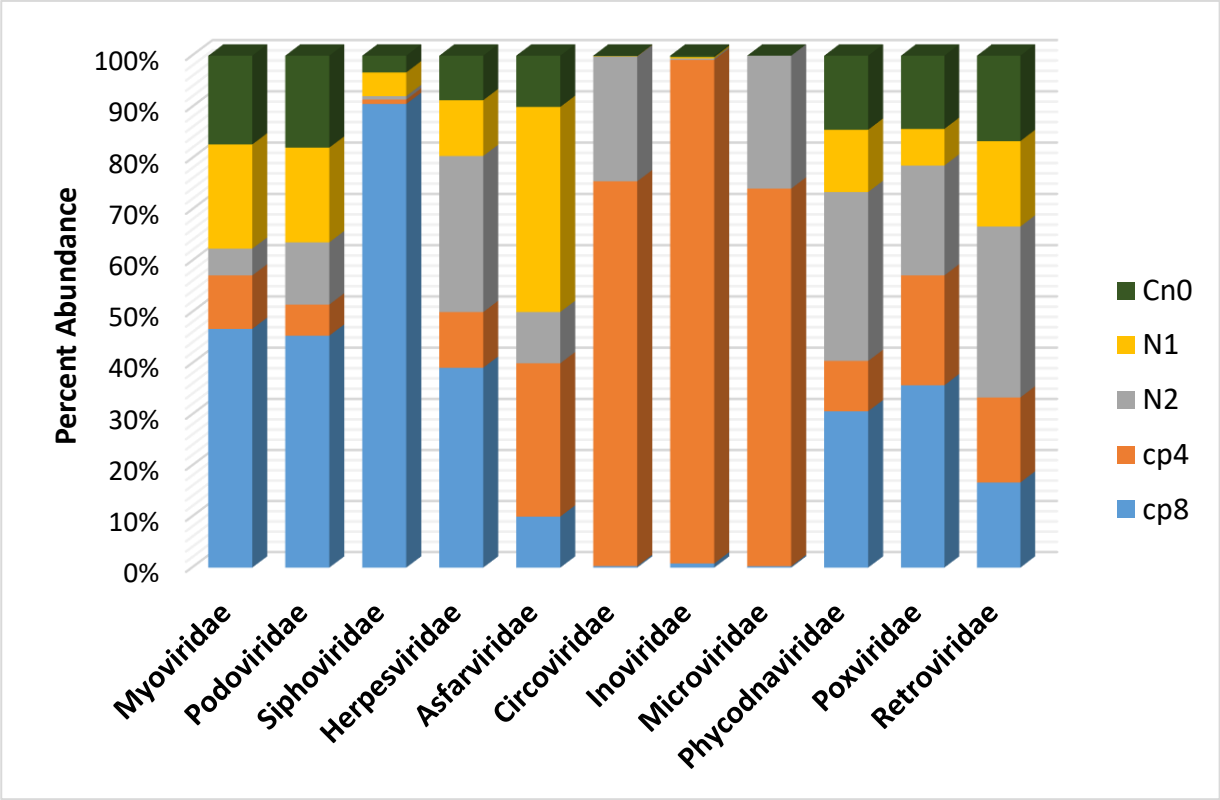


Figure 5. 4: Percent abundance of major viral families present in the maize rhizosphere under the fertilization treatments Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

Eukaryota was higher in Cp8, and Cp4 treatments with *Glomeromycota*, *Ascomycota*, *Basidiomycota* highly abundant in Cp8 treatments. *Blastocladiomycota* and *Chrytridiomycota* were most abundant in Cp4 treatments. Statistical differences were observed regarding to the control, N1, and N2 ($P < 0.05$) treatments (Figure 5.5). Archaea, on the other hand, were most abundant in Cp8 and N2 treatments. Cp8 treatment contains a higher abundance of *Korarchaeota* and *Euryarchaeota*, while *Nanoarchaeota*, *Crenarchaeota*, and *Thaumarchaeota* were most abundant in N2 treated samples (Figure 5.5).

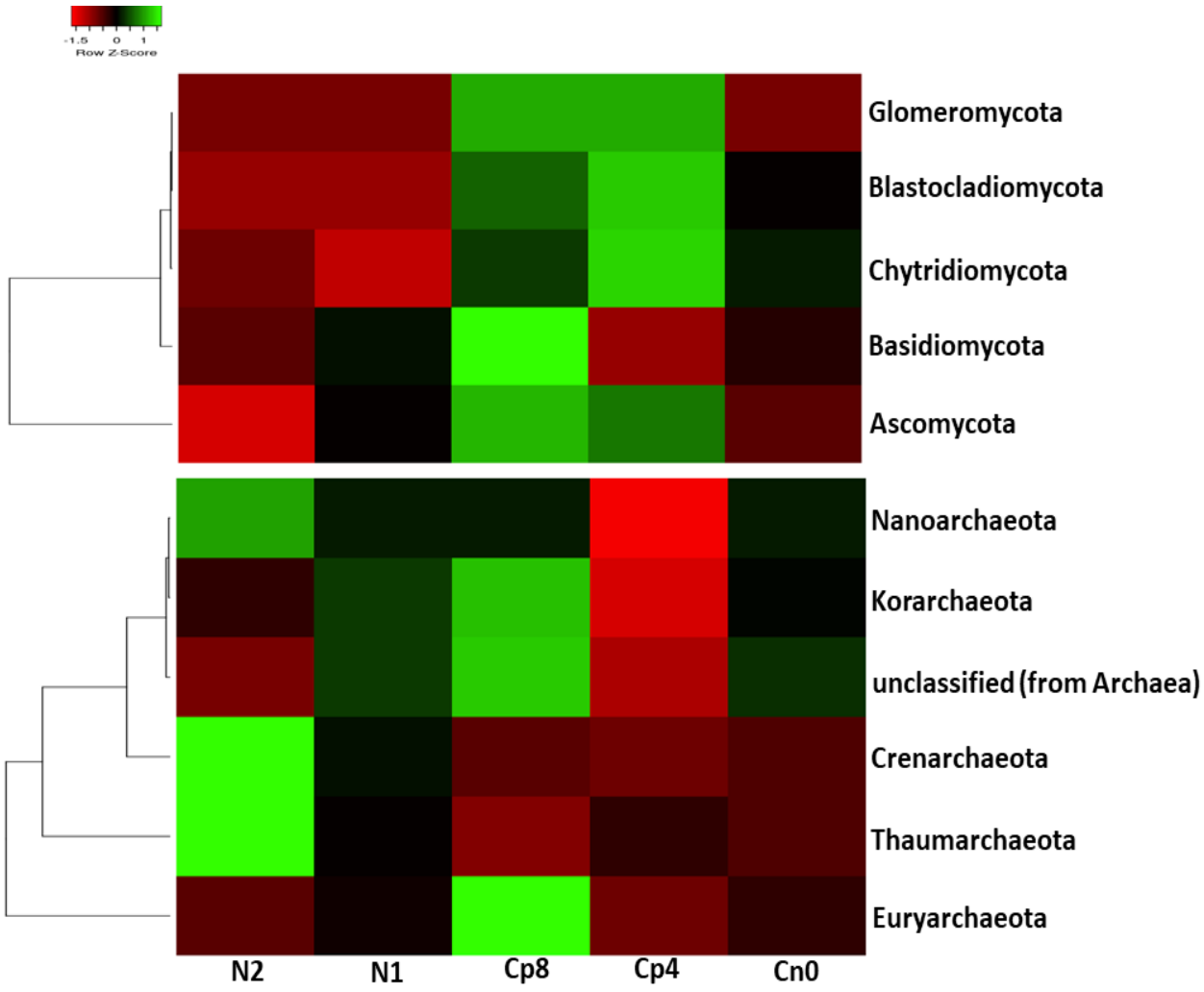


Figure 5. 5: Heatmap representation of the fungal and Archaeal community phylum within the treatments from maize rhizosphere samples Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

Regardless of the fertilization treatment applied, the bacterial reads mapped to *Proteobacteria* and *Actinobacteria* were present across all samples, with highest abundance present in Cp8, N1, and Cn0 (Figure 5.6). *Bacteroidetes* exhibited nearly the same abundance level in all the treatments but were highest in Cp8 sample. On the other hand, *Firmicutes* were most predominant in Cp4 and N2 respectively (Figure 5.6). Within the phylum and class level, *Proteobacteria* and *Alphaproteobacteria* are dominant in Cp8, N1, and Cn0 samples. While *Gammaproteobacteria* predorminates in Cp4 and N2 samples (Figure S5.1). *Actinobacteria* were the second most abundant bacteria in the phylum-level, and were not generally different amidst the fertilizer treatments: Cp8, N1, and Cn0 ($p<0.05$), except for Cp4 and N2 treatments. At the class level, *Bacilli* were most abundant in Cp4 and N2,

while in the other treatments, they were very minute in abundance. *Chloroflexi* were also abundant in the control, Cp8, and N1 samples, while *Verrucomicrobia* predominates in the control and Cp8. *Chlorobia*, however, dominated in the compost treated soils (Cp8 and Cp4) and in the control (Figure 5.5). The most abundant genus was *Bacillus* (with a relative abundance of 49.78% and 79%) belonging to the phylum - *Firmicutes* are present in both Cp4 and N2 samples. In comparison, the abundances between rhizosphere samples revealed that the genera *Streptomyces* and *Conexibacter* were in higher abundance in Cp8, N1, and control samples compared to Cp4 and N2 (Figure S1), while the *Chitinopgaga* are in greater abundance in Cp4 and N2. The next statistically significant genus present in this study is *Nocardiodes*, abundant in soil treated with a lower quantity of inorganic fertilizer (N1). Moreover, *Candidatus Solibacter* were also present in N1 and control samples. Also observed were *Saccharomonospora* and *Sorangium* in Cp8 treatment only compared to others (Table S5.1).

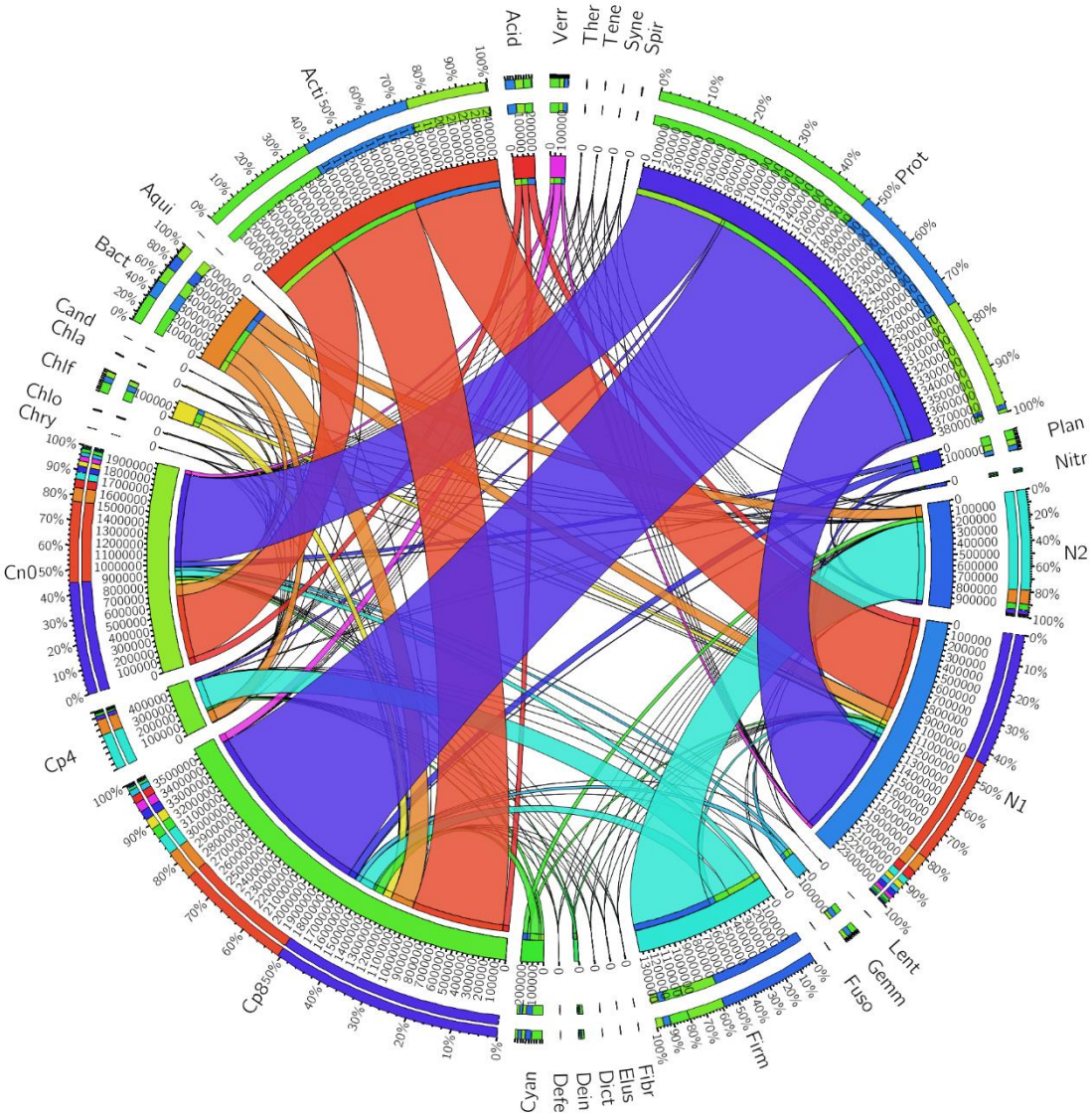


Figure 5. 6: The abundance of bacterial phyla across the treatments from the maize rhizosphere soil samples. Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer). The bacteria abbreviations are presented as follows: Acid (*Acidobacteria*), Acti (*Actinobacteria*), Aqui (*Aquificae*), Bact (*Bacteroidetes*), Cand (*Candidatus Poribacteria*), Chla (*Chlamydiae*), Chlo (*Chlorobi*), Chlf (*Chloroflexi*), Chry (*Chrysiogenetes*), Cyan (*Cyanobacteria*), Defe (*Deferribacteres*), Dein (*Deinococcus-Thermus*), Dict (*Dictyoglomi*), Elus (*Elusimicrobia*), Fibr (*Fibrobacteres*), Firm (*Firmicutes*), Fuso (*Fusobacteria*), Gemm (*Gemmatimonadetes*), Lent (*Lentisphaerae*), Nitr (*Nitrospirae*), Plan (*Planctomycetes*), Prot (*Proteobacteria*), Spir (*Spirochaetes*), Syne (*Synergistetes*), Tene (*Tenericutes*), Ther (*Thermotogae*), Verr (*Verrucomicrobia*)

5.3.3 Diversity (alpha and beta) of the bacteria in the treated rhizospheric soil samples

The Simpson, evenness and Shannon diversity indices showed an insignificant difference ($P > 0.05$) between N1, Cp8, and Cn0, but differed with N2 and Cp4 treated rhizosphere soil samples (Figure 5.7). The differences in the diversity between the rhizosphere samples were significant ($P = 0.001$) under Kruskal Wallis test. However, analysis of similarity (ANOSIM) were significant for the samples (with P value of 0.01 and R value of 0.55).

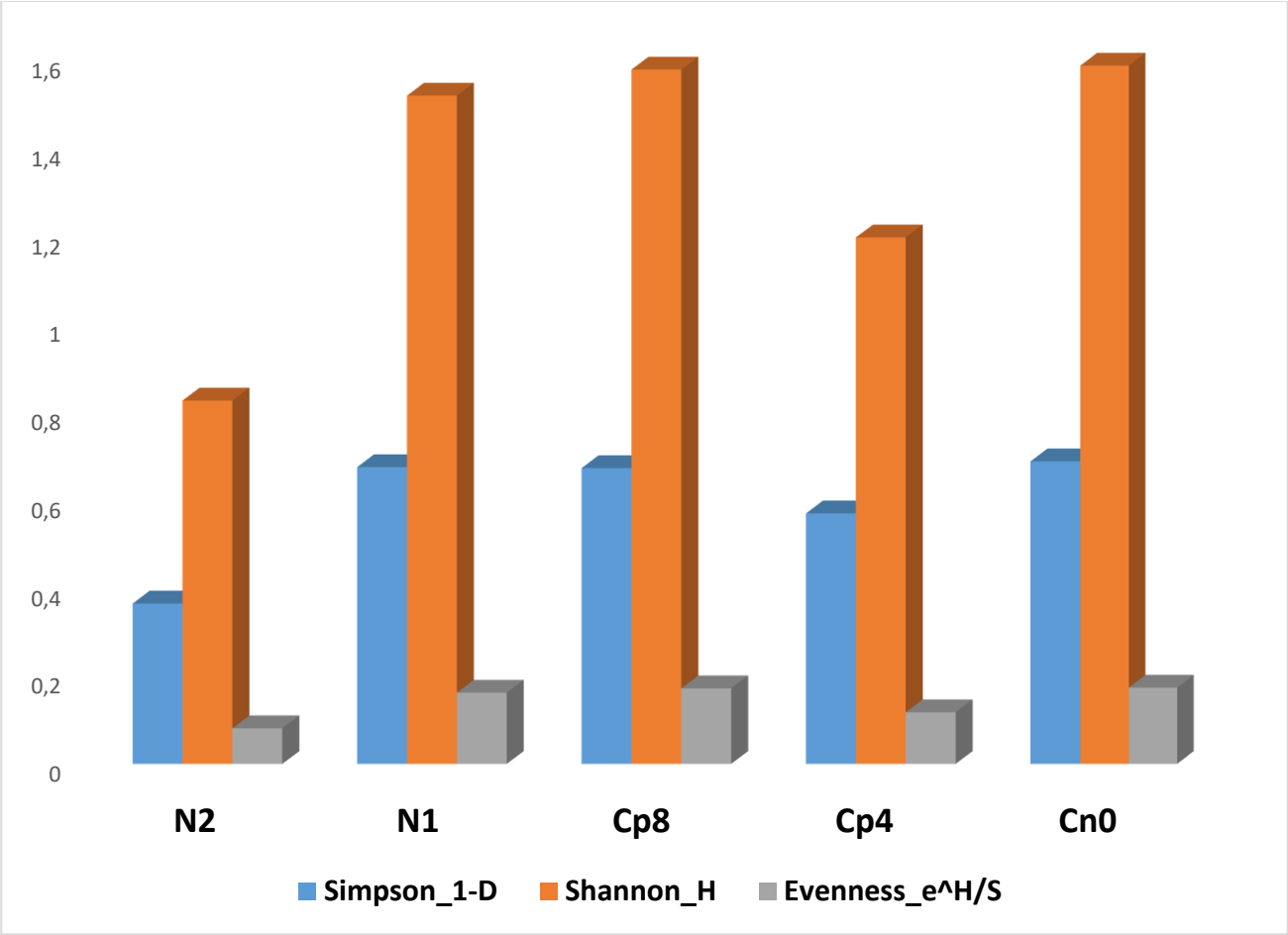


Figure 5. 7: Diversity indices for bacterial phylum within the maize rhizosphere treated with organic and inorganic fertilizer as well as under control condition Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

5.4 Discussion

Soil fertilization is an age-long agricultural practice aimed at increasing soil fertility for maximum crop performance and yield. Recently, researchers have focused on unravelling the impact of the

fertilization regimes on the microbial community in the soil. In this study, we examined the response of maize viral, bacterial, fungi and archaeal communities to compost and inorganic fertilization regimes. The results showed that fertilizer treatments influenced the maize rhizosphere microbial community. The influence of fertilization regimes on the bulk soil microbes has been known (Chen et al., 2016, Francioli et al., 2016). Until now, however, very little was known of the effects of fertilizers on maize microbes' rhizosphere community. Analysis of the fertilizer treated soils showed a marked shift in the microbes' community structure at the rhizosphere, but it is still unclear whether this temporal selection is caused by the maize plants selective pressure or the fertilizers themselves.

The eight tons per hectare compost treated rhizosphere soil and the control samples were similar, indicating that organic manure provided the most stable community, and this is in agreement with Tkacz et al., (2015) as well as the maize plant selection effect in the control, which could be attributed to chemical signaling compounds produced by the plants' roots (Neal et al., 2012). The relationship between the microbial community enrichment effects of organic manure and maize plants are both capable of increasing the soil organic carbon. Maize performs this by rhizodeposition (Philippot et al., 2013) and organic manure by soil nutrient enrichment (Ai et al., 2015). It is also proposed that a small quantity of microbes are likely to be introduced into the soil by the maize seeds (endophytes). A recent study has demonstrated that the number of bacteria as well as their diversity, are higher at the rhizosphere than in the inner tissues of maize plants (Pereira et al., 2011). Amendment of rhizosphere soil with compost manure showed the highest richness and diversity of viruses, bacteria, fungi and archaea, followed by a low quantity of inorganic fertilizer and the control samples. To what extent the compost supplied the soil with microbes is still unclear. Therefore, caution should be applied in comparing inorganic fertilized soil with compost amended soils. Our study suggests that there exists an inversely proportional relationship between inorganic fertilizers and the diversity and abundance of the microbial community at the rhizosphere. The least observable abundance and diversity were found in samples treated with higher inorganic fertilizer and low compost manure treated soil and are in concurrence with other studies (Zeng et al., 2016a, Campbell et al., 2010).

Microbial communities (Bacteria, Viruses, Fungi and Archaea) in the rhizosphere soil treated with compost manure, however, were more distinct from higher inorganic fertilizer treated soil, and this agrees with studies done by Sun et al., (2004) and Ai et al., (2015). Surprisingly, soil treated with a medium quantity of inorganic fertilizer possesses nearly the same effect in the promotion of similar microbial population and structure as the higher dose compost and the control soil. This implies that moderate soil fertilization will have a low or mild alteration of soil chemical properties (pH) (Zhang et al., 2017), while supplying the required nutrients for the plants and promoting microbial growth and metabolism. In this way, maize plants could be able to exert proper rhizosphere effects on the surrounding soil-dwelling microbes. In this study, there is a shift in trend as well as anomaly observed. The low dose of compost amendment has the same effects on the rhizosphere microbial community as do the higher inorganic fertilizers. The mechanism behind the former is still unclear, but the latter could be attributed to negative interference on plant-microbes interaction (Wei et al., 2013, Grillo et al., 2016), soil acidification (Griffiths et al., 2011), reduced microbial respiration, and loss of biodiversity (Wallenstein et al., 2006, Xu et al., 2012, Ramirez et al., 2012). At the genus level for bacteria, these two treatments supported the abundance of *Bacillus* and *Chitinophaga* at the rhizosphere of maize. These organisms are spore formers and highly resistant to environmental stress (Sangkhobol and Skerman, 1981, Kuramae et al., 2012).

5.5 Conclusion

This study brings to light the effects of soil fertilization with community-based compost manure and inorganic NPK (20:7:3) fertilizer on the maize microbial community and, is in agreement with previous studies, revealing that higher levels of inorganic fertilizer treatments affect drastically the microbial community structure and abundance in the agricultural soil. Lower doses of nitrogen fertilizer and higher compost manure application boosted the richness, abundance and diversity of the bacteria, fungi and archaea, thereby enhancing the attainment of a stable community at the rhizosphere. Surprisingly, the effects of a higher dose of inorganic fertilizer were the same as the lower dose of the compost treated soil, except for the enrichment of the archaeal community by the

higher inorganic fertilizer treatment and fungal as well as viral community by the lower compost manure. While the former could be understood, the latter remains unclear and requires further studies. The control shows that maize plants have very significant effects in the selection and enrichment of soil microbes' community. Therefore, to achieve maximum results in promoting plant health, productivity and the microbial community, an integrated fertilizer approach of using 8 tons per hectare compost and 60 kg per hectare inorganic fertilizer could be recommended.

CHAPTER SIX

Shotgun metagenomics Evaluation of the Functional Diversity of Bacterial Communities in the Maize Rhizosphere Grown in a Semi-Arid Region of South Africa

Abstract

Accurate knowledge of bacterial metabolic processes are essential for comprehending and manipulating the agroecosystem for proper nutrient management and crop production. In this study, we focused on evaluating the functional diversity of the bacterial community associated to the rhizosphere of maize treated with organic and inorganic fertilizers. We hypothesized that the treatments would significantly affect the functional diversity of soil bacteria in the maize rhizosphere as well as the bacteria communities. Shotgun metagenomics (Illumina) sequencing were used to analyze samples from compost manure, chemical fertilizer treated, and control soils. And based on the similarity of the influence of the treatments on the rhizosphere bacteria and their functions within the communities, we clustered them in groups of statistical significance. Our results showed that the relative abundance of 21 functional basic categories differed significantly ($P < 0.05$) between the group 1 treatments (Cp8, N1 and Cn0) and group 2 (N2 and Cp4) soil samples. This is in agreement with the beta diversity of the functional categories between the two groups. However within the groups, there are no significant differences observed, particularly in group 1. At genus level, *Capnocytophaga* and *Porphyromonas* were dominant in Cp4 (4 tons/ha compost manure) sample, *Actinoplanes*, *Saccharomonospora*, and *Thermobifida* were predominant in Cp8 (8 tons/ha compost manure) treated soil. *Bacillus* and *Granulicatella* were most abundant in high inorganic fertilizer treated soil (N2 – 120 kg/ha). And N1 (60 kg/ha inorganic fertilizer) treated soil supported bacteria belonging to *Norcardiodes* and *Mycobacterium*, while *Arthrobacter*, *Micromonospora* and *Xanthomonas* were predominant in the control sample (Cn0). The bacterial functional category enrichment capacity - at the high dose compost and low dose chemical fertilizer - is nearly the same as that of the control. It is equally observed in the bacteria genus present in the samples at Cp8, Cp4, N1 and Cn0. This implies that the observed changes were fertilizer-dosage dependent. The high

observed relative abundance of unknown functional category and plant-prokaryote DOE project (a miscellaneous SEED subsystem category – identified genes involved in plant-prokaryotes interactions by the Department of Energy project in USA) suggest there may be numerous beneficial novel genes that could be explored in regards to promoting adaptability, yield, disease suppression, and performance of maize plants in a sustainable agriculture under proper fertilization management.

6.1 Introduction

The changes in succession and structure of plants and microbes in the soil are dependent on the feedback processes between the plants and the soil (Herrera Paredes and Lebeis 2016). The abiotic environment in the soil can be altered by plants and will have a direct effect on the structure and diversity of soil dwelling microbial communities (Frouz et al. 2016). On the other hand, microbes influence the composition, productivity as well as survival of the plant communities by processes involving nutrient cycling, disease suppression, plant hormone production, organic matter decomposition, and priming of immunity in the plants as well as enhancing their tolerance to abiotic stress (Enebe and Babalola 2018; Enebe and Babalola 2019; van der Putten et al. 2016).

The rhizosphere is a dynamic ecological niche where microbial community structures are shaped by the presence of exudates from the plant roots, physicochemical attributes of the soil, and other environmental/abiotic conditions. The nutrients excreted by plants such as sugars, secondary metabolites, minerals, and carbohydrates serve as carbon and nutrient sources for the soil borne microbes and, depending on the quality as well as the quantity of the exudates, will influence the degree of the biological activities and the microbial communities that will be prevalent or dominant in the rhizosphere (Philippot et al. 2013). The rhizosphere is the highest point of resources exchange within the biosphere (Mendes et al. 2013; Yu and Hochholdinger 2018).

Maize (*Zea mays*) is a major staple food and hence requires a detailed investigation of its rhizo-microbiome. This has been done in the past using pyrosequencing techniques by Li et al. (2014) and Dohrmann et al. (2013). The maize rhizosphere has been found to possess heritable communities that

are consistent with the plants as observed by Peiffer and Ley (2013) and Walters et al. (2018). Also known is the fact that the seeds of maize excrete a number of amino acids, organic acids, and sugar molecules during their vegetative growth, which influence the selection and attraction of microbes to the nutrient-rich zone compared to the unplanted bulk soil (Alori and Babalola 2018; Nunes da Rocha et al. 2009; Vílchez et al. 2000). However, the influence of soil fertilization at various doses using organic and inorganic fertilizers on the functional genes category present in the maize rhizosphere has not been well studied and understood. Biodiversity could be lost following prolonged use of chemical fertilizer in agricultural soils (Yin et al. 2010), while organic manure or compost enhances bacterial diversity and functions (Chaudhry et al. 2012).

Soil microbial functional diversity is paramount in understanding the ecological processes and functions such as nutrient cycles, plant beneficial effects, growth promotion, and conversion of carbon substances into carbon dioxide (Kaiser et al. 2016; Lahiri et al. 2018; Uzoh et al. 2018; Zhu et al. 2017). In this study, therefore, we aimed to evaluate the functional diversity of the bacterial community in the rhizospheric soil of fertilized maize. We hypothesize that organic fertilizer (compost) will significantly increase the functional diversity of bacteria and bacterial communities within the rhizosphere of maize plants than the inorganic fertilizer. We also hypothesize that the rhizosphere effect of maize plants will be significant enough to increase the diversity of bacterial functional genes and the metabolic profile in the soil compared to the fertilized soils. To test these hypotheses, we used shotgun metagenomics sequencing of the community DNA present in the rhizospheric soil samples to enable us to understand the predominant functional microbial biodiversity present in the treated and untreated soils.

6.2 Materials and methods

6.2.1 Study site and soil sampling

The site has an annual average rainfall of 450 mm (Mokoboki and Sebola 2017). The experimental design and soil sampling were carried out as described in chapter 5 above. The collected soil samples

were put in a sterile plastic bag inside a box containing ice and transported to the laboratory. Plant and root debris were sieved out using a sieve with 2 mm pore size and the samples were stored at minus20ºC for metagenomic shotgun analysis. The physicochemical properties of the soil before planting and fertilization were analysed according to the standard basic soil chemical analysis protocols described by Motsara and Roy (2008).

6.2.2 Community DNA extraction and sequencing

The microbial community DNA was extracted from composite soils containing three replicates using the PowerSoil DNA isolation kit from MO Bio Laboratories, Inc. according to the manufacturer's protocol. The extracted DNA were processed through a shotgun metagenomics sequencing procedure at Mr DNA lab in Texas, United States of America (MR DNA, USA). The quantity of the DNA for the analysis was evaluated using the Qubit® dsDNA HS Assay Kit, Carlsbad, California, United States ("Life Technologies"). Then, following the manufacturer's user guide, the DNA libraries were prepared using the Nextera DNA Flex library preparation kit (Illumina Inc.). A total of 50 ng DNA from each sample were used in preparing the libraries. The samples passed through fragmentation processes in the presence of an added adapter sequence. Adapters, however, are used during the 6 cycles of a limited PCR-cycle in which unique indices was introduced into the sample. To measure the final libraries' concentration obtained, Qubit® dsDNA HS Assay Kit from Life Technologies were used. This was followed by the determination of the average library size by the use of the instrument Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, California, United States). DNA libraries were then combined together in an equalmolar ratio of 0.7 nM, and followed by 300 cycles of sequencing at paired ends using the NovaSeq 6000 system (Illumina).

6.2.3 Annotation and Analysis of the shotgun metagenomics data

The raw reads were uploaded to Metagenomics Rapid Annotation using Subsystem Technology also known as MG-RAST (Meyer et al. 2008). The uploaded sequences were passed through a series of quality control steps to remove artificial sequences, host specific-species sequences, ambiguous

bases, and followed by a read length filtering. The quality control was followed by annotation using the BLAST-like alignment tool (BLAT algorithms) (Kent 2002) against M5NR database that offer a non-redundant combination of many databases (Wilke et al. 2012). SEED is a name gotten from FIG SEED (Fellowship for Interpretation of Genomes) and information from the Diamond Age (a novel by Niel Stephenson). It is a way of organizing gene families into hierarchies of functions. While a subsystem is a group of genes that perform a particular biological function (functional role) either as a class of proteins, metabolic pathway/channels or complex cellular structure like ribosome. Therefore, an arranged functional roles that are similar to each other is called SEED Subsystem. It has level 1 (specific functions), level 2 (metabolic functions), level 3 (metabolic pathway) and level 4 (the function - genes). SEED is a database for gene and genome annotation (Overbeek et al. 2004; Overbeek et al. 2005). The categories of functions were also performed by SEED Subsystem at levels 1, 2, and 3. The e-value cut off was 1e-5, min. alignment length of 15 bps, and percent identity of 60 were set parameters during bacteria and functional category assignment. Sequences that failed to be annotated were discarded and were not analyzed. Since the entire sequences were comprised of 99 percent (approximately) bacteria, we focused on these and put away sequences gotten from archaea, viruses, and eukaryotes. The data were normalized within the MG-RAST system to reduce experimental error and the various functional tables were put together for each level. For statistical purposes, we retained the unclassified reads. Then the abundances were converted to percentages (relative abundance) and were used for statistical analysis. Evenness, Simpson and Shannon diversity indices were calculated and compared between the treatments using the Kruskal – Wallis test and were all done in PAST v3.2 (Hammer et al. 2001). Beta diversity was put together using PCoA (principal coordinate analysis) based on Euclidean distance-matrix. ANOSIM (Analysis of similarities) helped us to test the community composition between the treatments (Overbeek et al. 2005). PCA (principal component analysis) on the basis of Euclidean distance matrix, showed how the functional categories were spread out between the fertilized maize rhizospheric soils. Canoco v5 was used to analyze and plot PCoA and PCA analysis, while the heatmap was done using

www1.heatmapper.ca/expression/. The sequences are available at NCBI SRA dataset, SRA accession: PRJNA607213.

6.3 Results

6.3.1 The sequenced dataset

A combined total sequencing reads counts of 50727026 were gotten from the five-rhizosphere soil samples of maize plants with a combined average length of 173 bps. Following the sequence annotation at MG-RAST, the generated reads at pre and post quality control levels as well as predicted proteins with known and unknown functions together with the GC contents for the analysed rhizosphere soil samples are contained in Table S6.1.

6.3.2 Soil and Compost Chemical Analysis

The soil chemical parameters are: pH 4.97, phosphorus 10.5 mg/kg, nitrogen 377 mg/kg, potassium 285 mg/kg, calcium 388 mg/kg, magnesium 162 mg/kg, organic carbon 0.36 %, and the physical composition was sand 80%, silt 5% and clay 15%. While, the chemical composition of the compost manure are N = 20045.3 (g/kg), P = (1.0 g/kg), K = 12.3 (g/kg), pH = 7.1.

6.3.3 Associated Rhizosphere soil functional analysis

At SEED subsystem one gene annotation hierarchy, in this experiment, we observed 28 (twenty-eight) bacterial related functional categories in all the treatments but with varying relative abundances. Based on the observed effects of the treatment on the functions, they can be grouped into two: Group 1 – (Cp8, N1, and Cn0) and Group 2 – (Cp4 and N2). The acronyms stands for – Cp8 (treatment for 8 tons/ha compost), N1 (60 kg/ha inorganic fertilizer), Cn0 (control), Cp4 (4 tons/ha compost) and N2 (120 kg/ha inorganic fertilizer treatment). Out of the twenty-eight (28) functional categories, the results showed that 21 differed significantly ($p < 0.05$) between the two groups. While within the group 1, there is no significant difference ($P > 0.05$) between the treatments that make up the group for the observed 21 functions, in group 2, SR (Stress Response), NN (Nucleosides and

Nucleotides), DS (Dormancy and Sporulation), and CWC (Cell Wall and Capsule) did not differ at all (Figure 6.1 and Table S6.1). The most abundant functional category in chemically fertilized soil (N2) are Sulfur Metabolism (Sme), Protein Metabolism (PrM), Regulation and Cell signaling (RCS), and Miscellaneous (Mis). The sequences belonging to Phosphorus Metabolism (PM) were more abundant in compost manure treated rhizosphere soil (Cp8) (Figure 6.1.) and were significantly different from other treatments. Regulation and Cell signaling (RCS) was the only function that remained the same in abundance across high and low compost treatments (Cp8 and Cp4) as well as in low chemical fertilizer treatment (N1), and the control (Cn0). The richness of the functions were much higher in treatments of group 1 than in group 2. This factor is the rationale behind the clear separation between the group 1 treatments and group 2 by the Principal Component Analysis. The heatmap analysis (Figure 6.1) clearly agrees with the results contained in the PCA (Principal Component Analysis) (Figure 6.2), with both analyses depicting that different fertilizer dose treatments had a remarkable effect on the prevailing functions in the agricultural soil. The principal component analysis showed how the functional-categories were distributed across the various maize rhizosphere soil treatments (Figure 6.2). The score plot of the PCA showed that Cp4 and N2 grouped to the right on the graph along axis 1, while the other 3 rhizosphere soil samples closely cluster on the left axis 2, which represent 93.71 % of the total variation and axis 2 accounted for 99.77 % of the total variations.

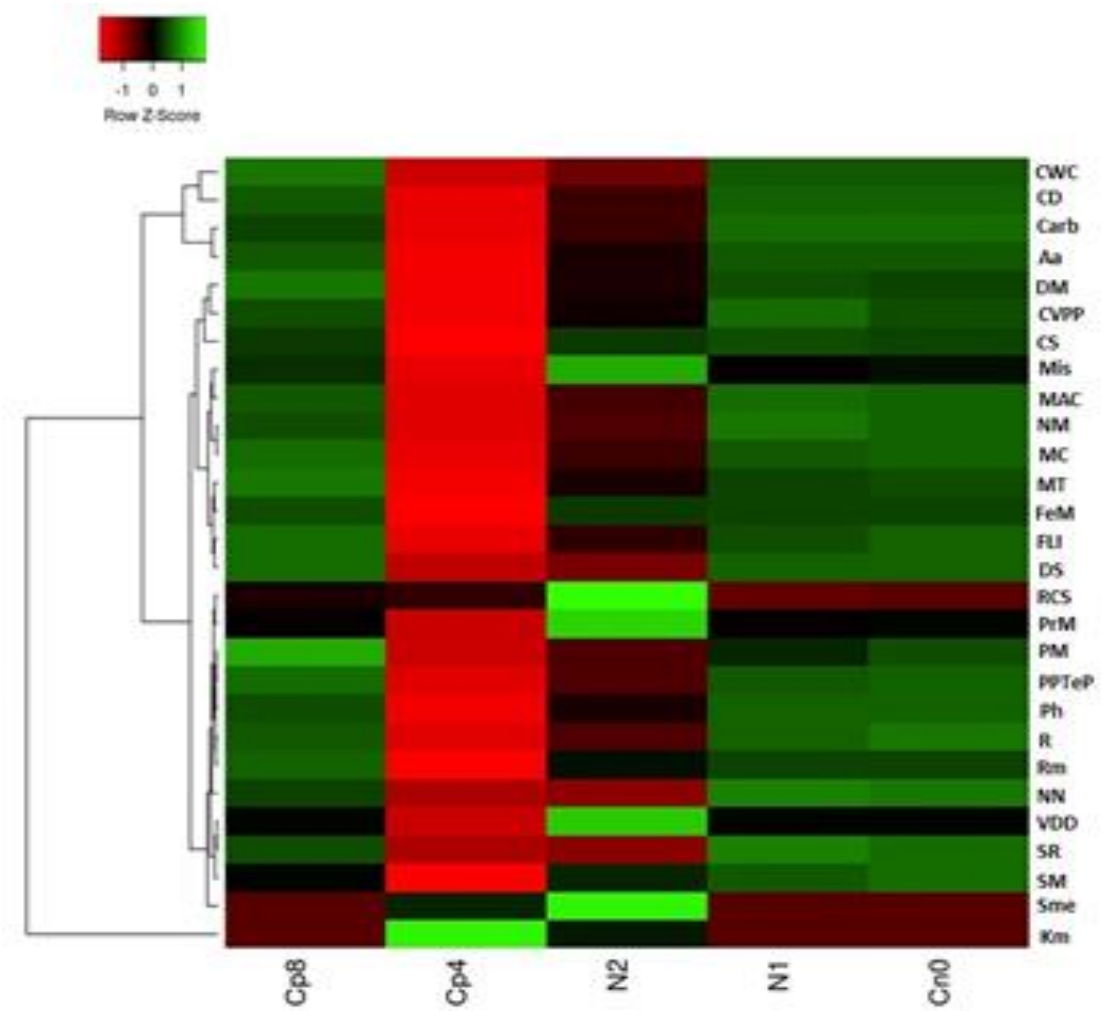


Figure 6. 1: Relative abundance of major metabolisms within the rhizosphere soil samples of maize treated with varying quantities of organic and inorganic fertilizers. Cn0 (control), N1 (60 kg/ha NPK), N2 (120 kg/ha NPK), Cp8 (8 tons/ha compost), and Cp4 (4 tons/ha compost manure). The scale depicts colour saturation gradients on the basis of z-score relative abundance. The abbreviations are Amino Acids and Derivatives (Aa), Carbohydrates (Carb), Cell Division and Cell Cycle (CD), Cell Wall and Capsule (CWC), Clustering-based subsystems (CS), Cofactors, Vitamins, Prosthetic Groups, Pigments (CVPP), DNA Metabolism (DM), Dormancy and Sporulation (DS), Fatty Acids, Lipids, and Isoprenoids (FLI), Iron acquisition and metabolism (FeM), Membrane Transport (MT), Metabolism of Aromatic Compounds (MAC), Miscellaneous (Mis), Motility and Chemotaxis (MC), Nitrogen Metabolism (NM), Nucleosides and Nucleotides (NN), Phages, Prophages, Transposable elements, Plasmids (PPTeP), Phosphorus Metabolism (PM), Photosynthesis (Ph), Potassium metabolism (Km), Protein Metabolism (PrM), RNA Metabolism (Rm), Regulation and Cell signaling (RCS), Respiration (R), Secondary Metabolism (SM), Stress Response (SR), Sulfur Metabolism (Sme), Virulence, Disease and Defense (VDD).

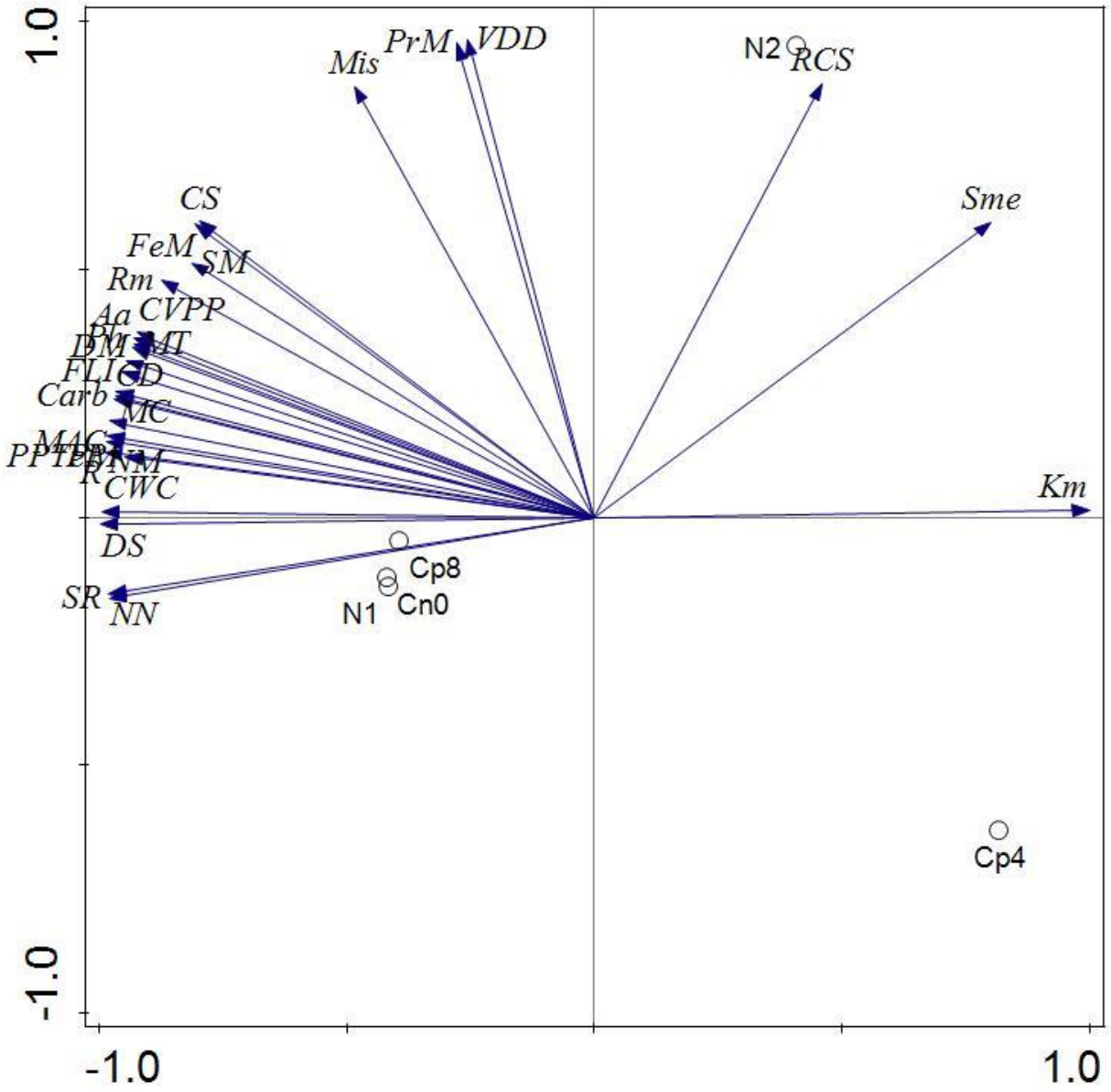


Figure 6. 2: The Principal component analysis (PCA) of the functional genes analysis of the bacterial metagenomes with the strength of influence of each metabolic process represented by the length of the vector. The axis 1 and 2 clearly explained 93.71 and 99.77 percentage variations. The abbreviations are the same as above in Figure 6.1 Cn0 (control), N1 (60 kg/ha NPK), N2 (120 kg/ha NPK), Cp8 (8 tons/ha compost), and Cp4 (4 tons/ha compost manure)

A hierarchical gene annotation at Subsystem level 2, showed that the functions that were unknown were the most abundant in all the treatments. The relative abundances of this particular functions are 21.5 % (Cp8), 12.83 % (Cp4), 20.29 % (N2), 21.27 % (N1) and 21.32 % (Cn0) respectively. The next

most abundant functions are Plant-Prokaryote DOE project – Cp8 (5.75 %), Cp4 (3.46 %), N2 (5.67 %), N1 (5.87 %), and Cn0 (5.86 %). This was followed by Protein biosynthesis. The relative abundance were 5.24 % (Cp8), 3.24 % (Cp4), 3.59 % (N2), 5.0 % (N1), and 5.86 % (Cn0) (Figure 6.3).

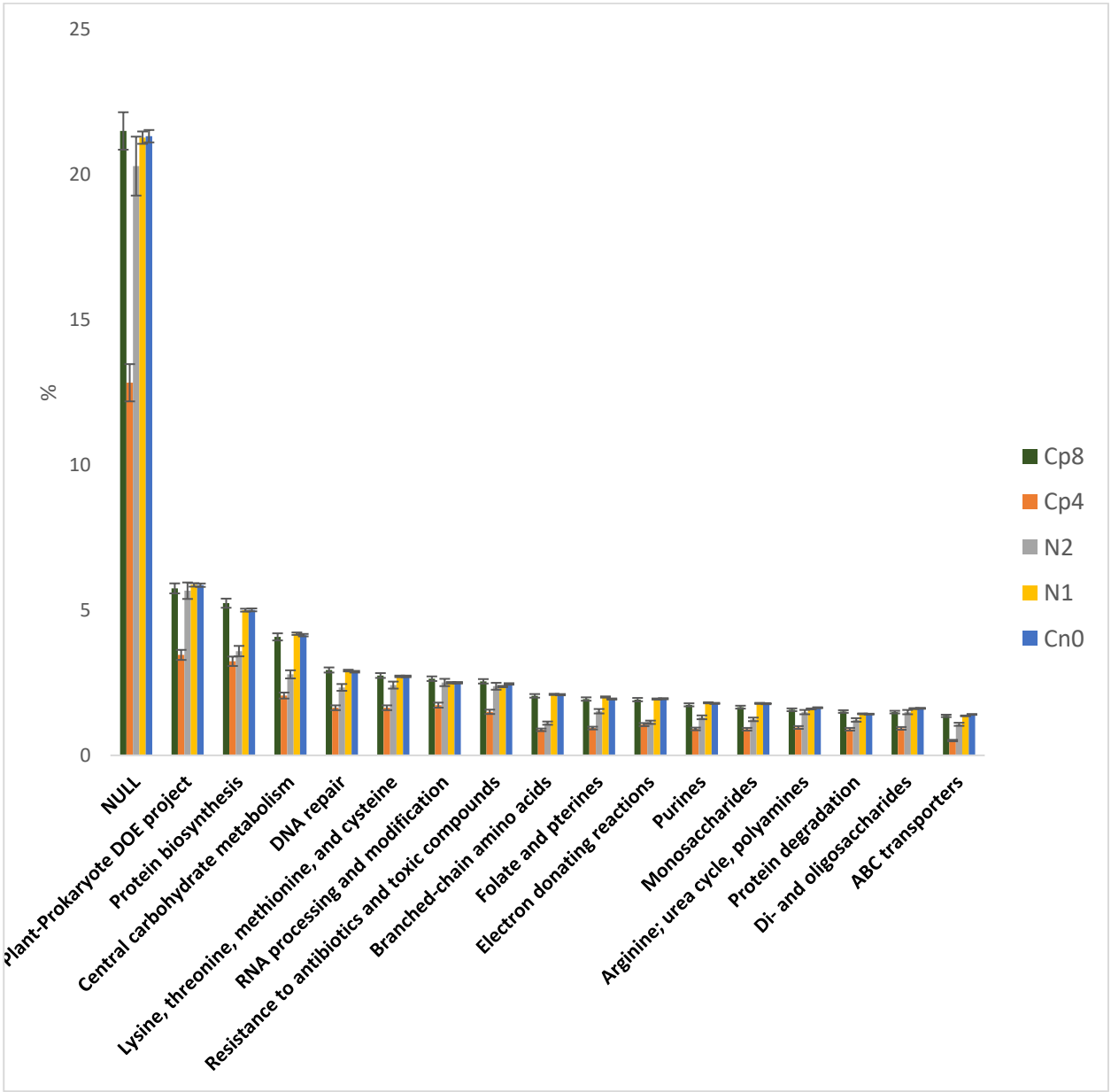


Figure 6. 3: Functional category based on SEED subsystem level 2 classification from the rhizospheric soil samples of maize plants Cn0 (control), N1 (60 kg/ha NPK), N2 (120 kg/ha NPK), Cp8 (8 tons/ha compost), and Cp4 (4 tons/ha compost manure)

At SEED Subsystem 3, there is no significant difference (P>0.05) between treatments N2, Cn0, and Cp8 as well as between treatments Cp4 and N1, but there was a significant difference (p<0.05) across the two groups (Figure 6.4). Also, metabolic pathways involving serine glyoxylate cycle, respiration complex 1, methionine biosynthesis, phosphorus metabolism, and sugar utilization in Thermotogales were the most abundant microbial metabolic pathways dominant in 8 tons/ha compost manure treated soil (Cp8), high dose 120 kg/ha chemical fertilized soil (N2), and the control (Cn0), while the least expressed is ammonia assimilation (Figure 6.4).

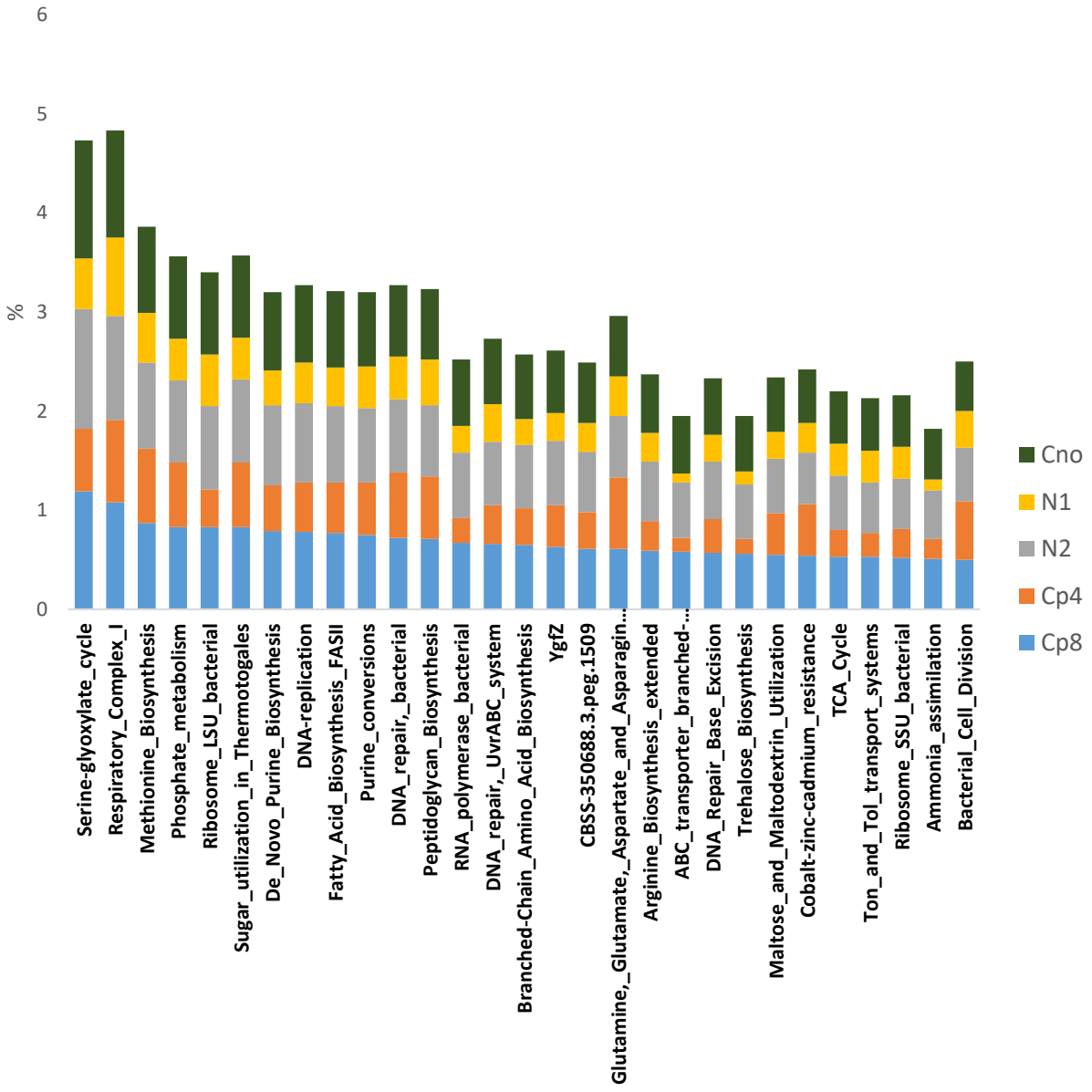


Figure 6. 4: Relative abundance of metabolic pathways at functional category level 3 for maize rhizosphere soil sample under fertilization treatments Cn0 (control), N1 (60 kg/ha NPK), N2 (120 kg/ha NPK), Cp8 (8 tons/ha compost), and Cp4 (4 tons/ha compost manure)

6.3.5 Rhizosphere Bacterial communities

The analysed soil samples were dominated by 98.2 percent bacteria. At genus level, *Capnocytophaga, Flexibacter, Streptococcus, Veillonella* and *Porphyromonas* were dominant in Cp4 sample. The Cp8 sample contain more of *Actinoplanes, Exiguobacterium, Actinomadura, Saccharomonospora, Thermobifida* and *Clostridium*. *Bacillus* and *Granulicatella* were dominant in N2 sample. N1 samples also supported *Nocardiodes*, *Mycobacterium* and *Bacillus*. While, *Arthrobacter*, *Micromonospora* and *Xanthomonas* were predominant in the control sample (Cn0) (Figure 6.5).

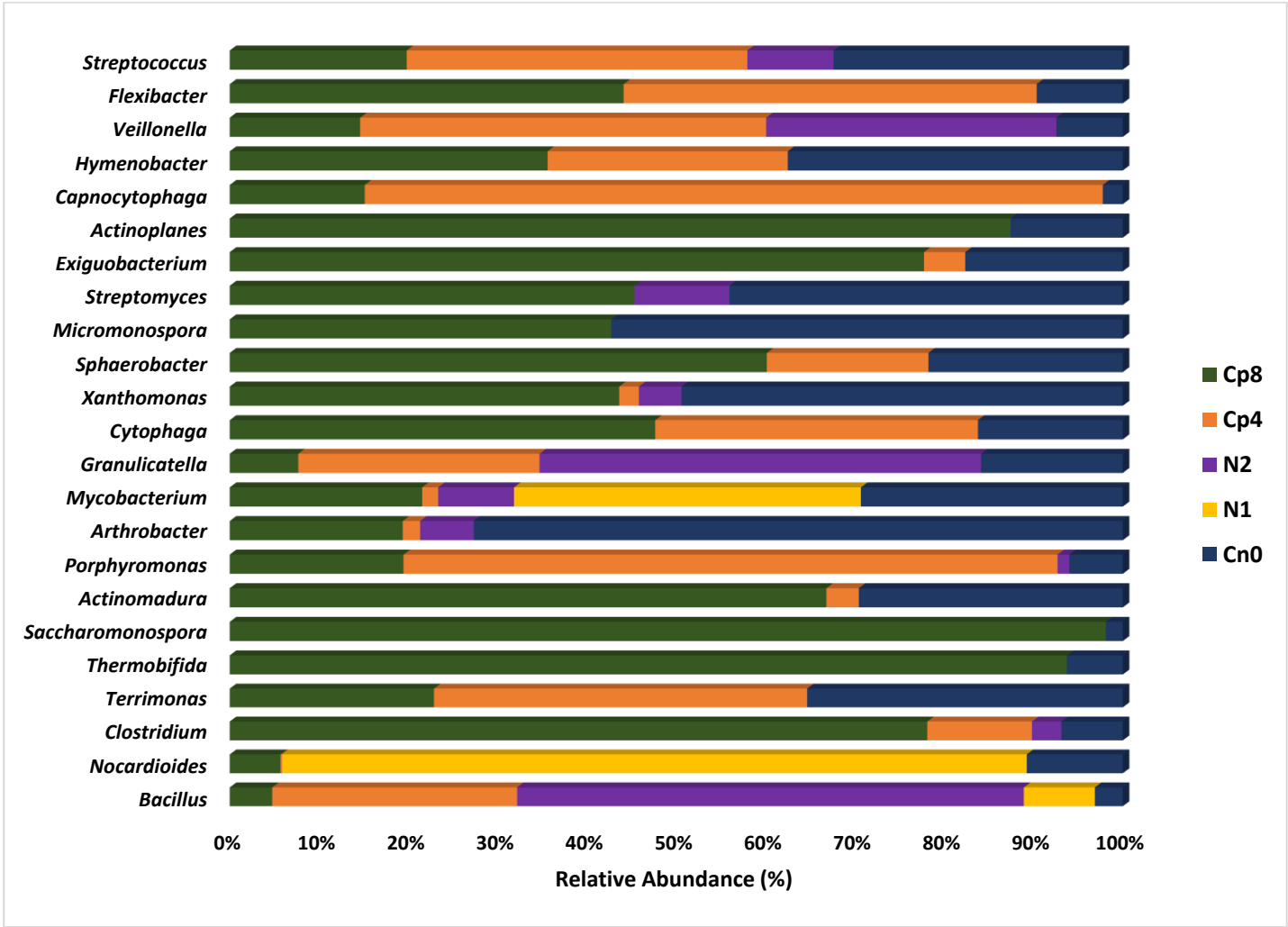


Figure 6.5. Relative abundance of bacterial genus dominant in the maize rhizosphere soil samples under fertilized and unfertilized conditions Cn0 (control), N1 (60 kg/ha NPK), N2 (120 kg/ha NPK), Cp8 (8 tons/ha compost), and Cp4 (4 tons/ha compost manure)

6.3.6 The diversity (alpha and beta) of the functional categories and the bacterial communities

At SEED Subsystem level 1, the functional diversities were calculated using Evenness, Simpson, and Shannon indexes and the differences were statistically insignificant (p = 0.99) among the treatments. Also, the level of differences in the diversity between the treatments based on Kruskal-Wallis test, were not significant (p = 0.60). The differences in beta diversity were statistically significant (ANOSIM, R value = 0.55; p value = 0.01) and the visualization of treatments according to their annotation relative abundances is done by PCoA (Principal Coordinate Analysis) (Figure 6.6). The diversity indexes analysis for the bacterial genus (Figure 6.7) showed that there were statistically significant difference with P value of 1.98×10^{-8} for the bacterial genus present in the samples.

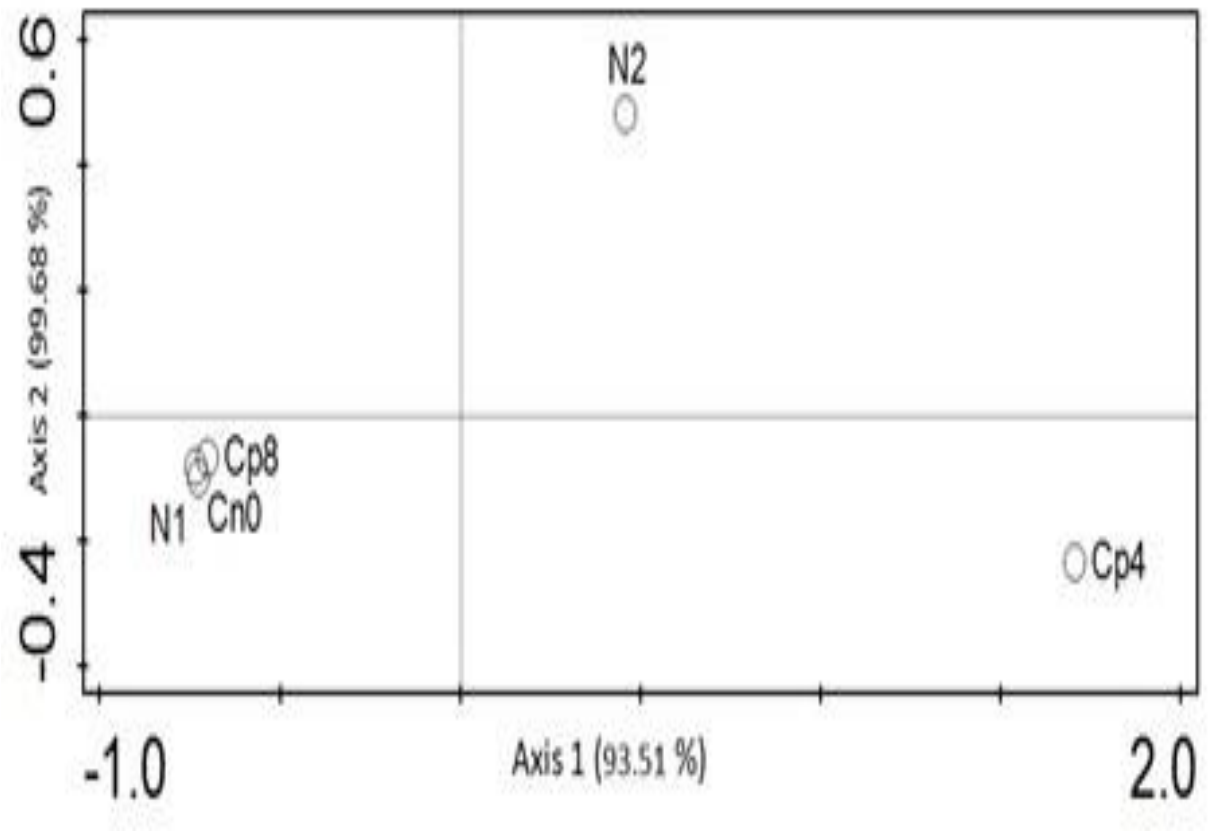


Figure 6. 5: PCoA of the functional category under subsystem level one obtained from the organic, inorganic and unfertilized rhizosphere soil samples of maize plants Cn0 (control), N1 (60 kg/ha NPK), N2 (120 kg/ha NPK), Cp8 (8 tons/ha compost), and Cp4 (4 tons/ha compost manure)

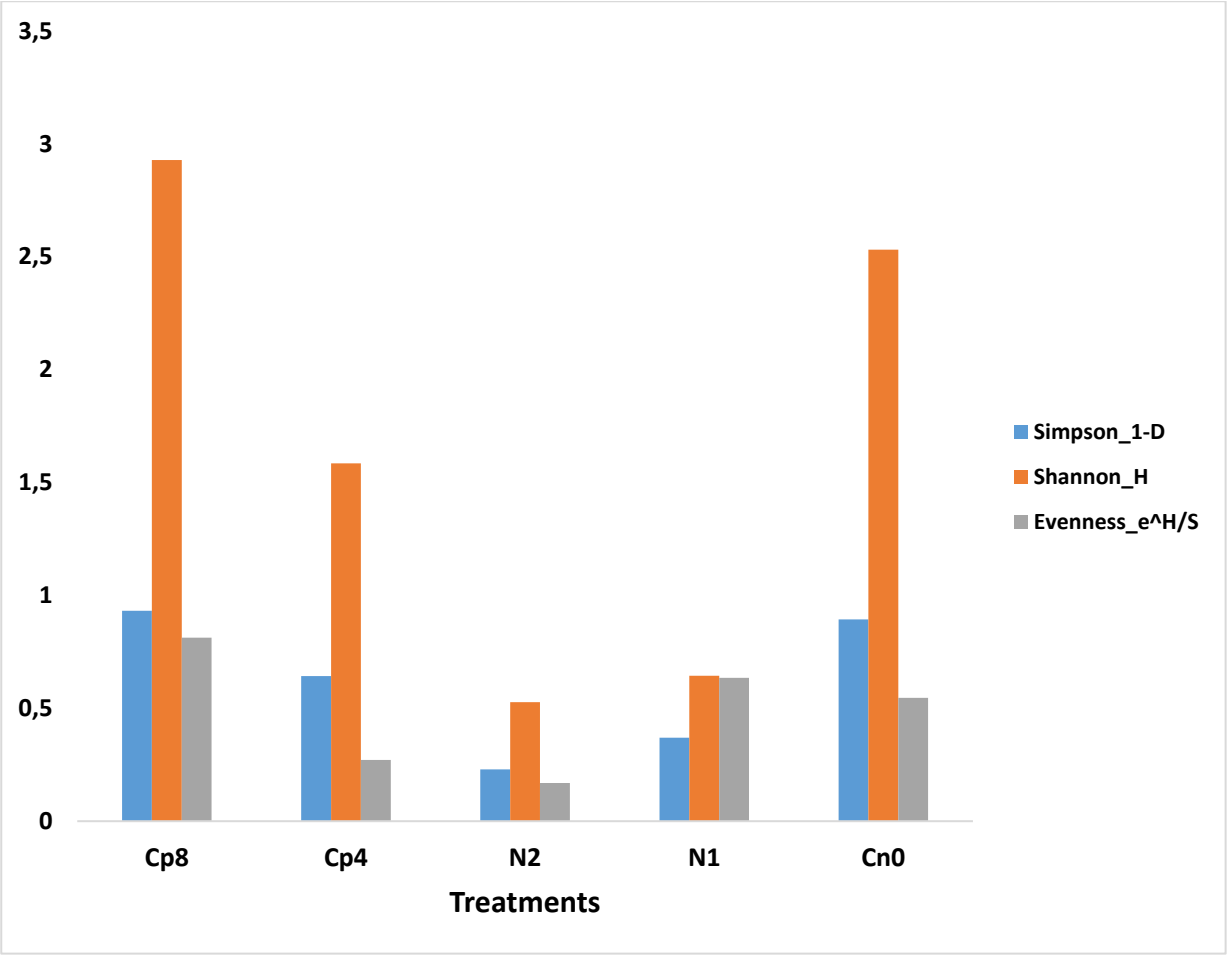


Figure 6.7. Diversity indices for bacterial genus within the maize rhizosphere treated with organic and inorganic fertilizer as well as under control condition. Cn0 (control), N1 (60 kg/ha NPK), N2 (120 kg/ha NPK), Cp8 (8 tons/ha compost), and Cp4 (4 tons/ha compost manure)

6.4 Discussion

In this experimental study, we investigated the effects of two contrasting treatments, chemical and compost fertilizer on the bacterial functional diversity prevalent in the maize rhizosphere. The results showed that soil treatments with fertilizers (organic and inorganic at different doses) had major influences on bacterial functional diversity and the bacterial communities. To effectively relate bacterial community structures to ecological functions, accurate knowledge of the genes involved in biogeochemical cycling is needed (Torsvik and Øvreås 2002). The metabolic pathways are structurally arranged in hierarchy, each bearing essential genes for a particular role or assignment by the SEED assembly into Subsystems and comprises of both catabolic and anabolic pathways (Enagbonma and Babalola 2019; Overbeek et al. 2005). The functional categories within the

treatments did not differ significantly ($p > 0.05$) (Figure 6.4). In addition, there were separations ($R = 0.55$) between the fertilized soils of group 1 (containing N1, Cn0, and Cp8) and group 2 (N2 and Cp4) based on the PCoA plot. This explains the variations observed among bacterial functional communities (Figure 6.6).

The observed variation was tested using ANOSIM (analysis of similarity) and it was found that the functional categories in the maize rhizosphere varied significantly ($P = 0.01$). Hypothetically, each treatment ought to have a distinguishing and remarkable metabolic profile but the PCA - Principal Component Analysis (Figure 6.2) showed that the variation only existed between group 1 and 2 and not within the group (group 1 in particular). We could see in Figure 6.2, which metabolisms are essential for the bacteria in each treatment. For instance, functions involved in RCS (Regulation and cell signaling), PrM (Protein metabolism), VDD (Virulence, Disease, and Defense), and Sme (Sulfur metabolism) placed the bacteria present in high inorganic fertilized rhizosphere soil - labelled N2 (120 kg/ha inorganic fertilizer), apart from the bacteria functions found within Cp8 (8 tons/ha compost manure), Cp4 (4 ton/ha compost), N1 (60 kg/ha inorganic fertilizer), and Cn0 (control). This is in agreement with (Shen et al. 2016; Shen et al. 2011) who found that high doses of inorganic nitrogen fertilizer decreased the functional diversity of microbes in the soil but did not change the microbial community's structure during the fertilization period.

Out of the 28 functional categories, with the exception of Mis (Miscellaneous), RCS (Regulation and cell signaling), PrM (Protein metabolism), and SM (Secondary Metabolism), 21 functions are almost the same between the Cp8, N1, and Cn0 samples. This could likely be that the rhizosphere effects engineered by maize plants are effective in enriching the abundance of soil microbial community and functions in nearly the same proportion as high levels of organic manure and lower quantity of nitrogen fertilizer treatments since there were no differences between the treatments (Cp8, N1) and the control. It has been postulated that root exudate composition, though it may vary from one plant to another, affects the growth and the relative abundance of functional genes and microbes in the soil

(Adegboye and Babalola 2012; Aira et al. 2010; Somers et al. 2004). This could be explained by rhizodeposition (Nihorimbere et al. 2011).

Soil fertilization and tillage could create variation in the environment, generally disturbing soil microecosystems as well as genome stability in the perturbed soil (Mendes et al. 2015). However, microbes present in agricultural soil could enrich the greater abundant genes associated with protein biosynthesis, central carbohydrate metabolism, DNA repair, and plant – prokaryote DOE project to enable them to cope with environmental disturbance and stress as seen in Figure 6.3. This agrees with most of the sequences relating to serine glyoxylate cycle, respiration complex 1, methionine biosynthesis, phosphate metabolism, and sugar utilization (Figure 6.4) which were abundant in Cn0, N2, and Cp8. Also, unknown functions regarded as functionally coupled genes (Castañeda and Barbosa 2017) were precisely the most obvious abundant functional category in this experiment. These unknown functions possessed by bacteria as seen in SEED Subsystem functional category level 2 are underused (Figure 6.3) and proper understanding of the roles of these functions/genes could facilitate proper management of soil nutrient in achieving a sustainable agriculture and promoting biodiversity.

6.5 Conclusion

Our study has shown that bacterial functional diversity in the maize rhizosphere is influenced by the interactions between fertilizer types and doses used. To a greater extent, the rhizosphere soil of the control enriched the bacterial functional genes to nearly the same proportion as the organic (high dose) and inorganic (low dose) fertilizers. We report that the use of compost and inorganic fertilizers affects the functional gene diversity only on a dosage level and not on the type used. Therefore to achieve a remarkable enrichment of bacterial functional diversity in an agricultural soil, we recommend the application of a high dose of compost or organic manure and/or low dose of chemical fertilizer, so that the soil ecosystem functions will be sustained. However, a mixture of the two doses

in an integrated fertilization practice could possibly help in the attainment of a sustainable agriculture and nutrient management.

CHAPTER SEVEN

Soil Fertilization effects on the Abundance and Distribution of Carbon and Nitrogen Cycling Genes in the Maize Rhizosphere

Abstract

Soil microbes perform important functions in nitrogen and carbon cycling in the biosphere. Microbial community in the rhizosphere enhance plants' health and promote nutrient turnover and cycling in the soil. In this study, we evaluated the fundamental effects of soil fertilization with organic (compost manure) and inorganic fertilizer on the abundances and distribution of carbon and nitrogen cycling genes within the rhizospheric regions of maize plants. Our results showed that maize plants through rhizosphere effects in the control samples selected and enriched the same functional genes *glnA, gltB, gudB* involved in nitrogen cycle as do higher compost (Cp8) and lower inorganic (N1) fertilizer treatments. This observation was significantly different from those of higher doses of inorganic fertilizer (N2) and lower compost manure (Cp4) treated soil. Only alpha amylase encoding genes were selectively enriched by lower compost and higher inorganic fertilized soil. The other treatments only selected peculiar carbon cycling genes in the rhizosphere of maize. Also, *Actinomycetales* are selected by high compost, low inorganic fertilizer and the control. While, *Bacillales* were promoted by low compost and higher inorganic fertilizer and this indicated that only microbes capable of tolerating the stress of higher doses of inorganic fertilizer will thrive under such conditions. Therefore, soil fertilization lowers nitrogen gas emission, but increases carbon dioxide evolution in the agricultural soil.

7.1 Introduction

There is a need to profile microbial community structure and functions for the attainment of a sustainable agriculture because of the roles they play in facilitating the biogeochemical cycling of nutrients such as carbon, phosphorus, nitrogen, sulphur and metals (Falkowski et al., 2008). Soil microbes are genetically diverse and plants exert influences on them. These influences vary from

plant to plant and act through root exudation and modification of soil environmental conditions (water, minerals and temperature) (Dini-Andreote and Van Elsas, 2013, Denef et al., 2009). The rhizosphere microbial communities can be altered by the specific genotype of the plants growing in the soil (Aira et al., 2010, Lawal and Babalola, 2014), which may support microbial biomass formation and metabolic activities that will be inherent in the soil. These interactions at the rhizosphere generally control important biogeochemical cycling involved in carbon cycle, emission of greenhouse gases and cycling of other nutrients.

However, fertilization processes and methods have been implicated in altering and shaping the microbes' community and their biological functions in the soil. Studies examining the metabolic potential of microbiomes present at the rhizosphere of crops like grapevine, soybean, wheat, cucumber and barley (Zarraonaindia et al., 2015, Mendes et al., 2014, Ofek-Lalzar et al., 2014, Bulgarelli et al., 2015) have revealed that a consortium of genes involved in chemotaxis, stress tolerance, nutrient cycling, and growth promotion of plants are in abundance in the rhizosphere. Until now, the separation of plant rhizosphere effects (for maize) from fertilization, on the enrichment of functional genes in the soil is still unclear. However, little is known concerning whether fertilizers have greater effects on microbial genes abundance and activities in the soil, or whether the plants shape and decide which genes should be expressed more in a fertilized soil, and if so, how do they influence nutrient cycling in the agricultural soil.

Meanwhile, inorganic fertilizer application to farms drastically affects plant nutrient uptake, increases greenhouse gas emission and causes eutrophication. This leads to the need for the use of organic fertilizer or manure derived from plant materials and animal droppings, which are not only cost effective but also have the merit for improving microbial biomass formation and activities (Ding et al., 2014, Zhu et al., 2016, Bhattacharyya et al., 2008, Tejada et al., 2008, Bumunang et al., 2013) in the fertilized farmland. Organic fertilizers and manure increase the activities and abundance of soil microbes more than inorganic fertilizers, as reported by Zhang et al., (2012) and Chu et al., (2007).

In this experiment, we investigated the fundamental effects of organic and inorganic fertilizers on the structure and abundance of bacterial functional genes in the maize rhizosphere. The key objectives of this study are: (i) to evaluate how inorganic and organic fertilizers at different dosages affect the functional bacterial genes (involved in carbon and nitrogen cycling) from the maize rhizosphere, and (ii) to determine if the maize plant rhizosphere exerts the same functional genes enrichment effects as do the fertilizers used, particularly for carbon and nitrogen cycling genes.

7.2 Materials and methods

7.2.1 Site, samples collection and DNA extraction

The rhizospheric soil samples were collected from an agricultural site located in the Molelwane – a semi-arid savanna climate, near Mafikeng, in the North West Province of South Africa (Figure 7.1) (25º 47' 24.17604" S, 25º 37' 9.08328" E; 25º 47' 29.97048" S, 25º 37' 8.62428" E; 25º 47' 23.9604" S, 25º 37' 8.43348" E; 25º 47' 23.82252' S, 25º 37' 8.30064" E; 25º 47' 24.11844" S, 25º 37' 8.18148" E; altitude: 1012 m), with a temperature range of 22 - 35ºC and an annual average rainfall of 450 mm (Mokoboki and Sebola 2017). The soil type is sandy loam soil. A sampling distance of 15 cm (minimum) and 50 cm (maximum) in 3 sampling spots/plot were used. The chemical composition of the compost manure are N = 20045.3 (g/kg), P = (1.0 g/kg), K = 12.3 (g/kg), pH = 7.1. While the soil physicochemical analysis results are contained in Table 5.1 above. The experimental design pattern and the sample collection procedure were performed as described elsewhere in chapter 5. The collected samples were put in a sterile plastic bag inside a box containing ice and transported to the laboratory. Plant and root debris were sieved out using a sieve with 2 mm pore size and the samples were stored at -20ºC for metagenomic shotgun analysis. The physicochemical properties of the soil before planting and fertilization as well as the compost manure after the stabilization period of 16 weeks were analysed according to the standard basic soil chemical analysis protocols described by Motsara and Roy (2008). This was followed by extraction of total community DNA from the collected rhizospheric soil samples with the aid of a PowerSoil DNA isolation kit from MoBio Laboratories, Incorporation in USA, following the producer's instruction.

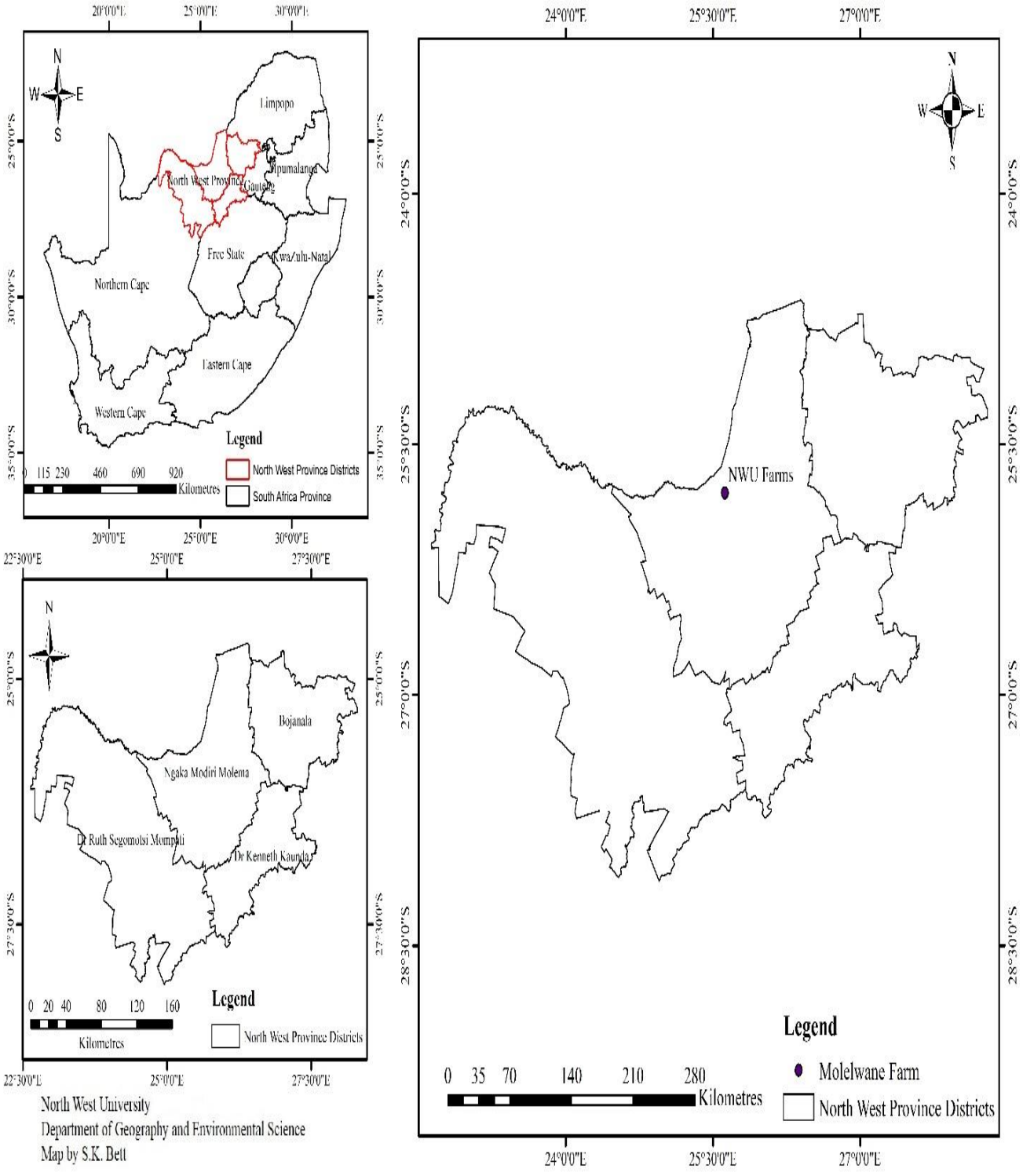


Figure 7. 1: The map of the agricultural sampling site at the Molelwane, North-West University farm, Mafikeng, South Africa

7.2.2 Sequencing

DNA (deoxyribonucleic acid) concentrations were examined with the aid of Qubit® dsDNA HS Assay Kit from Life Technologies, Carlsbad, California, United States. The deoxyribonucleic acid libraries were prepared by the use of Nextera DNA Flex library preparation kit (Illumina Incorporation.) in accordance with the procedure from the manufacturer. A total of 50 nanogram of the DNA molecules from each samples were taken for the libraries preparation. This was followed by fragmentation alongside adapter sequences addition. The adapter molecules were used during PCR cycles together with the addition of unique indices into the samples. The final concentration of the libraries generated were quantified with Qubit® dsDNA HS Assay Kit from Life Technologies. The average sizes of the libraries were determined with the use of analytical machine - Agilent 2100 Bioanalyzer (from Agilent Technologies). DNA libraries were combined together into an equal-molar ratio of 0.7 nM. The pooled DNA were then sequenced paired end for 300 cycles using the machine - NovaSeq 6000 system (Illumina). This was done at the Mr DNA molecular research laboratory in Texas, USA.

7.2.3 Sequence processing, annotation and statistical analysis

The raw metagenome reads were uploaded into MG-RAST where a series of quality control processes were carried out (Meyer et al., 2008). The preprocessing of the reads involved the removal of artificial sequences, host specific sequences and other ambiguous base pairs. This was followed by gene annotation using BLAT algorithm (Kent, 2002), and M5NR database (Wilke et al., 2012). The taxonomy and protein coding genes annotation were executed by blasting at M5NR and SEED Subsystem level – function. The BlastX was used for the hit at an e –value cut off of 10^{-5}, min. alignment length 15 base pairs, and percentage identity of 60. Unannotated sequences were not subjected to any further evaluation/analysis. Also applied was the MG-RAST normalization tool to enable us to cut down on the possible experimental error effect from the work. Nitrogen and carbon cycling genes were curated manually from the total gene file gotten from the blasting result of the SEED Subsystem database, level – function. The sequences were used for statistical analysis and the

bacteria composition, nitrogen and carbon cycling gene variances were evaluated using one-way ANOVA at p - value less than 0.05. The abundance and distribution of bacterial communities at order level was visualized in bar graphical representation using Microsoft Excel software. The online software - Circos (http://circos.ca/) was employed in plotting the graph of nitrogen cycling genes, while the heatmapper (www1.heatmapper.ca/expression/) was used in drawing a heatmap diagram for carbon cycling genes. Evenness, Simpson and Shannon diversity indexes were determined for the rhizospheric samples and contrasted amongst the treatments using Kruskal-Wallis test. The beta diversity was ascertained using PCoA (principal co-ordinate analysis) on the basis of Euclidean distance-matrix and ANOSIM (analysis of similarity) through 9999 permutations. These analyses were carried out on PAST version 3.20 analytical software (Hammer et al. 2001) and Principal Co-ordinate Analysis and principal component analysis using the CANOCO v,5 software from Microcomputer Power, Ithaca, New York. The sequences were deposited on NCBI SRA database, SRA accession: PRJNA607213

7.3 Results

7.3.1 Distribution of Bacteria within the treatments

Analysis of the metagenomes using SEED Subsystem database showed that *Actinomycetales, Bacillales, Sphingobacteriales, Cytophagales, Lactobacillales, Bacteroidales, Flavobacteriales* and *Rhizobiales* were the dominant bacterial orders within the fertilized and the unfertilized soil. However, *Bacillales* were the most abundant group in the high dose inorganic (N2) fertilized soil and low compost (Cp4) manure fertilized soil (Figure 7.2). Also *Actinomycetales* were most abundant in low dose inorganic (N1) fertilized soil, the control (Cn0), and the high dose compost (Cp8) manure fertilized maize rhizosphere soil. The bacterial order relative abundance was not significant (P = 0.94) among the treated and control soils.

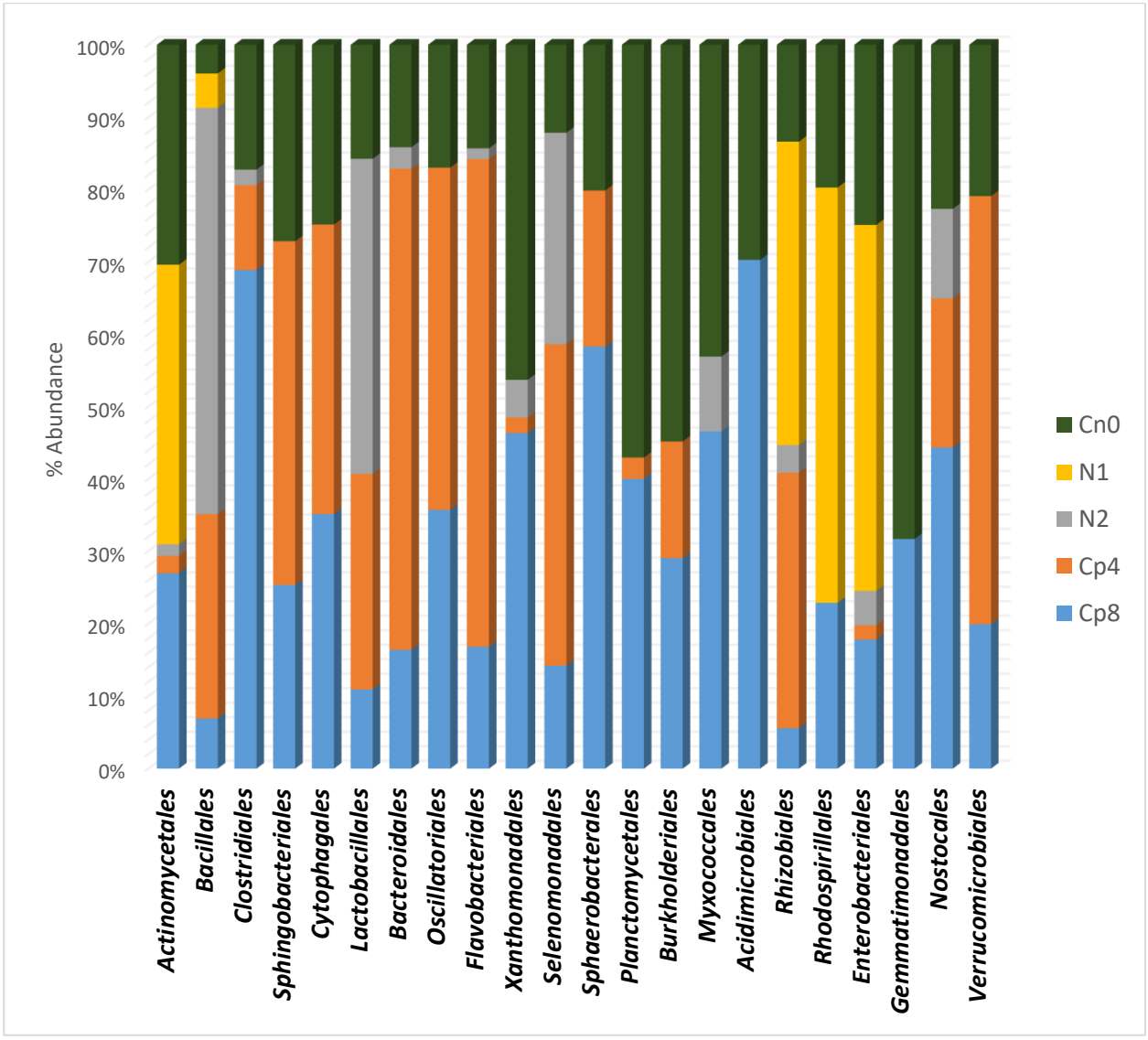


Figure 7. 2: Relative abundance of obvious bacteria orders present in the rhizosphere soil treated samples Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

7.3.2 Nitrogen cycling genes observed within the treated and untreated soils

Of the 4993 genes found within the samples, 37 of them were the most abundant gene families present in all the treatments. These genes (*glnA, gltB, gudB, nifA, nirB, ureC* and others) were involved in nitrogen fixation, nitrification, denitrification, assimilatory nitrogen reduction, dissimilatory nitrogen reduction to ammonium ions, and ammonification (Figure 7.3). The relative abundance of the genes differed significantly ($p < 0.05$) between the treatments and control. Diversity indices – Shannon, Simpson and evenness (Table S7.1) were employed to understand the alpha diversity of the nitrogen

cycling genes. These diversity indices show that there were significant differences (Kruskal – Wallis, P-value = $6.87x10^{-17}$) in alpha diversity of the nitrogen cycling genes (Table S7.1). Moreover, the analysis of similarity (ANOSIM) indicated that there was a very significant difference (P-value = 0.01 and R-value = 0.55) for the beta diversity, that is, the diversity that exists across the fertilized soils and the control as shown by the principal co-ordinate analysis - PCoA (Figure 7.4). Also, the Principal component analysis was conducted to represent how the nitrogen cycling genes were distributed across the treatments and the control samples (Figure 7.5).

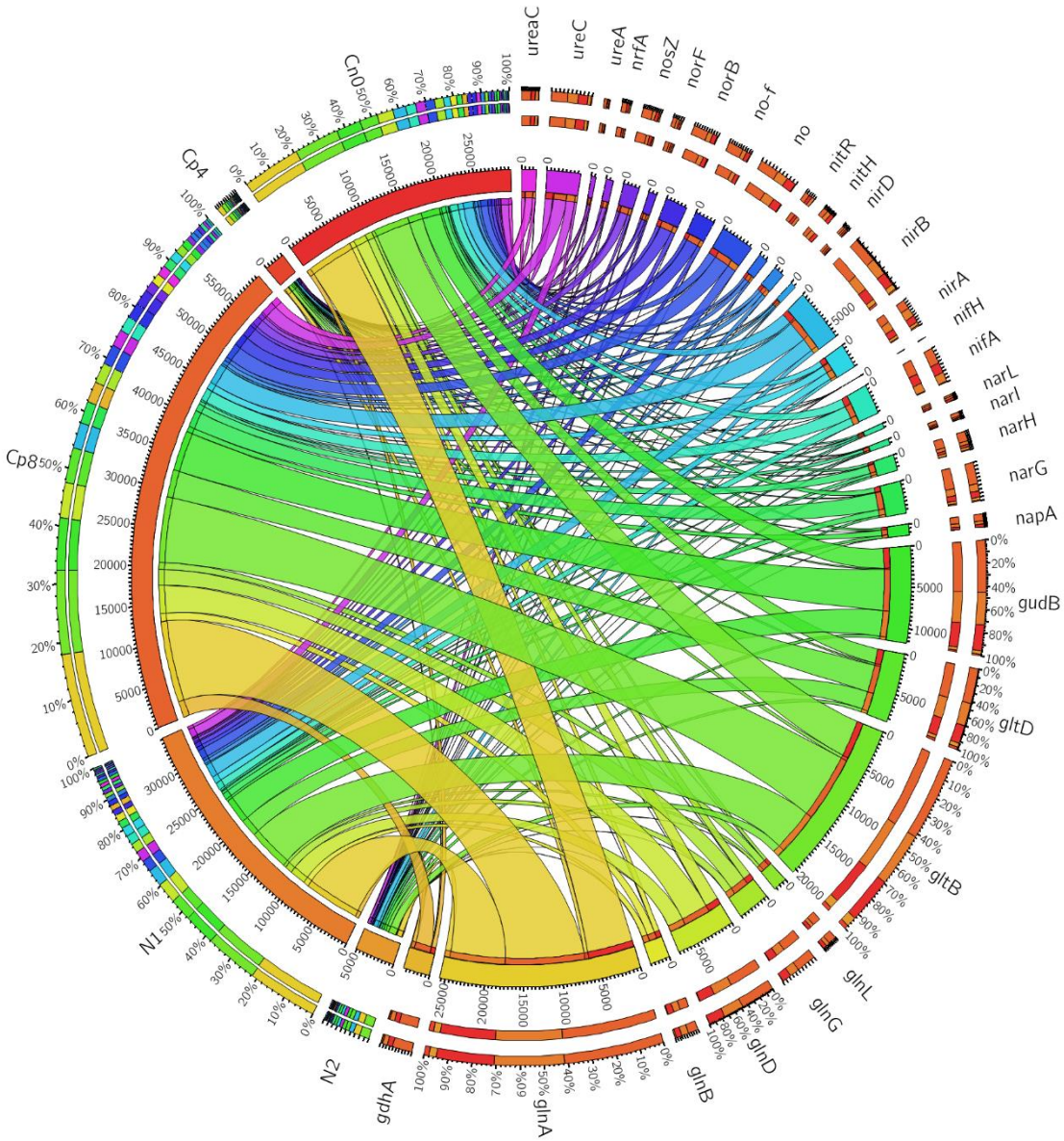


Figure 7. 3: Nitrogen cycling genes found in maize rhizosphere soil under fertilizer treatments plotted with an online software - Circos. Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer). The meaning of the gene abbreviations are (*glnA*) glutamine synthetase, (*gltB*) glutamate synthase (NADPH/NADH) large chain, (*gudB*) glutamate dehydrogenase, (*glnD*) uridylyltransferase, (*gltD*) glutamate synthase (NADPH/NADH) small chain, (*nirB*) nitrite reductase (NAD(P)H) large subunit, (*narG*) nitrate reductase 1, alpha subunit, (*gdhA*) glutamate dehydrogenase (NADP+), (*glnG*) two-component system, nitrogen regulation response regulator GlnG, (no) nitrate reductase catalytic subunit [EC:1.7.99.4], (*ureC*) urease subunit alpha, (*nifA*) Nif-specific regulatory protein, (no-F) nitrite reductase (NO-forming), (*norB*) nitric oxide reductase subunit B, (*nosZ*) nitrous-oxide reductase, (*glnB*) nitrogen regulatory protein P-II 1, (*ureaC*) urea carboxylase, (*narH*) nitrate reductase 1, beta subunit, (*nirA*) ferredoxin-nitrite reductase, (*glnL*) two-component system, nitrogen regulation sensor histidine kinase, (*napA*) periplasmic nitrate reductase, (*nrfA*) cytochrome c-552, (*nitH*) nitrile hydratase, (*nitR*) nitrate reductase (NADH), (*norF*) nitric-oxide reductase, (*narI*) nitrate reductase 1, gamma subunit, (urea) urease subunit gamma, (*nirD*) nitrite reductase (NAD(P)H) small

subunit, (*narL*) two-component system, nitrate/nitrite response regulator, (*nifH*) nitrogenase iron protein NifH.

Note: no, ureaC, nitH, no-f and nitR are our own abbreviation to contain it within the circos plot.

These genes have no specific symbol or abbreviations representing them.

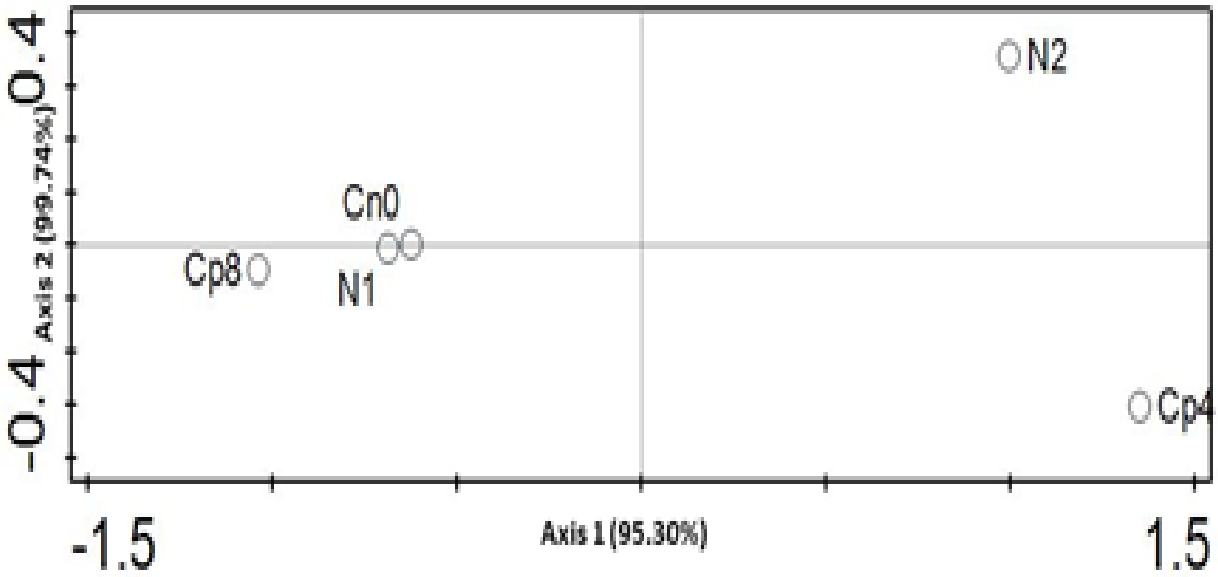


Figure 7. 4: Principal coordinate analysis (PCoA) of the functional nitrogen cycling genes obtained from the fertilized and unfertilized maize rhizosphere soil samples Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

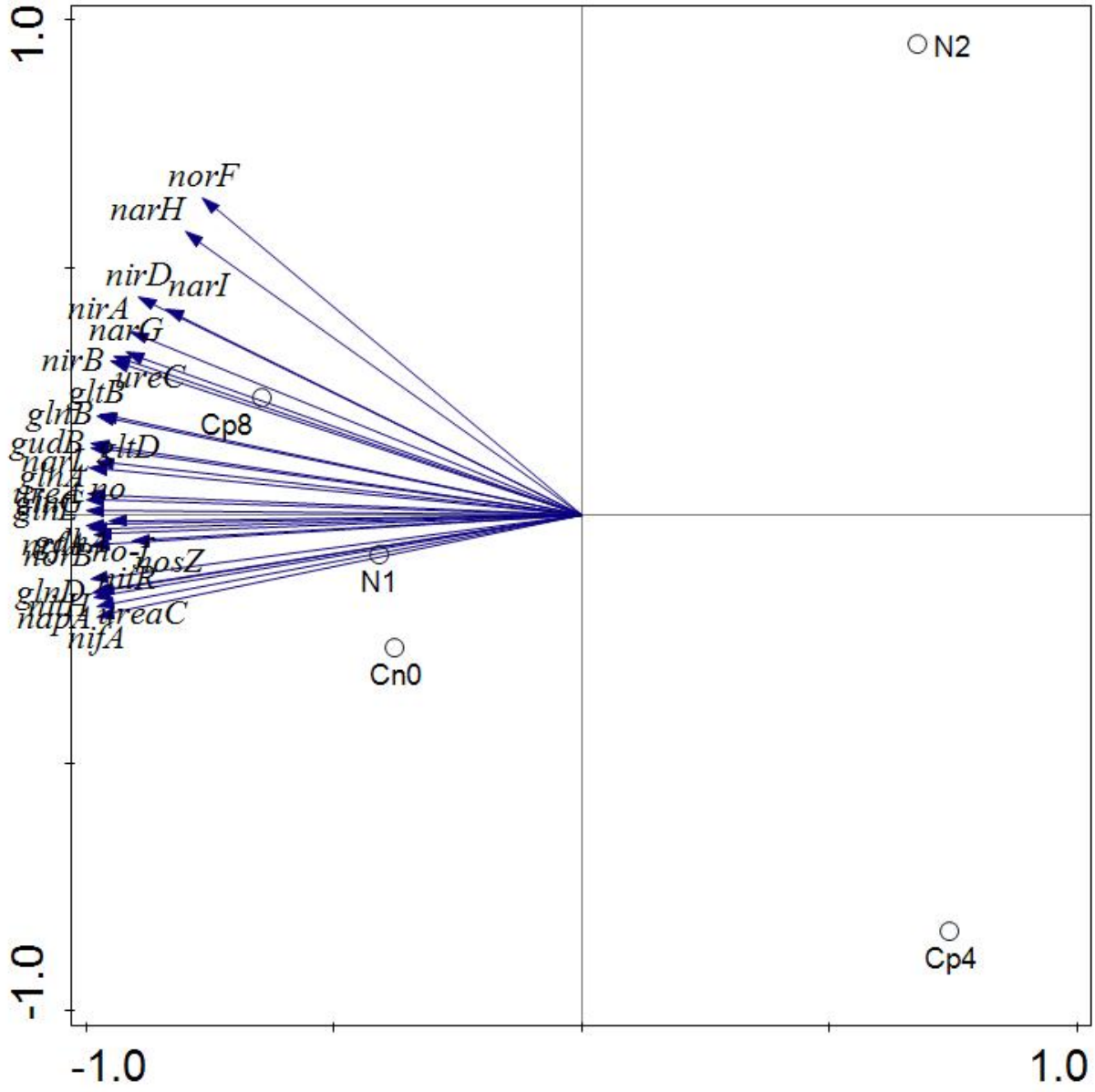


Figure 7. 5: Principal component analysis of the nitrogen cycling genes obtained under fertilization and unfertilized maize rhizosphere soil samples. Axis 1 and Axis 2 explains 94.89% and 98.63% variations. The abbreviated symbols are explained in Figure 7.3. Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

7.3.3 Carbon cycling genes under the treatments and control samples

A total of 39 carbon cycling genes were found to be dominant across all the treatments. They were involved in cellulose, hemicellulose, starch, carbohydrate, cello-oligosaccharides and lignin degradation. None of the carbon fixation genes were found in the treatments and the control

rhizosphere soil samples. The abundance of carbon cycling genes differed significantly (P<0.05) between the samples (Figure 7.6). Simpson, Shannon and evenness indexes were used to show the alpha diversity of the carbon cycling genes in the treatments and the control, and they indicated that there were no significant differences (P-value = 0.89) in the carbon cycling genes alpha diversity (Table S7.2). There were significant differences as depicted by ANOSIM (P-value = 0.01; R value = 0.55) in beta diversity of the carbon cycling genes and this is in agreement with the Principal coordinate analysis (Figure S7.1). Principal Component analysis were performed to show the distribution of the carbon cycling genes across the rhizosphere soil samples under investigation (Figure 7.7).

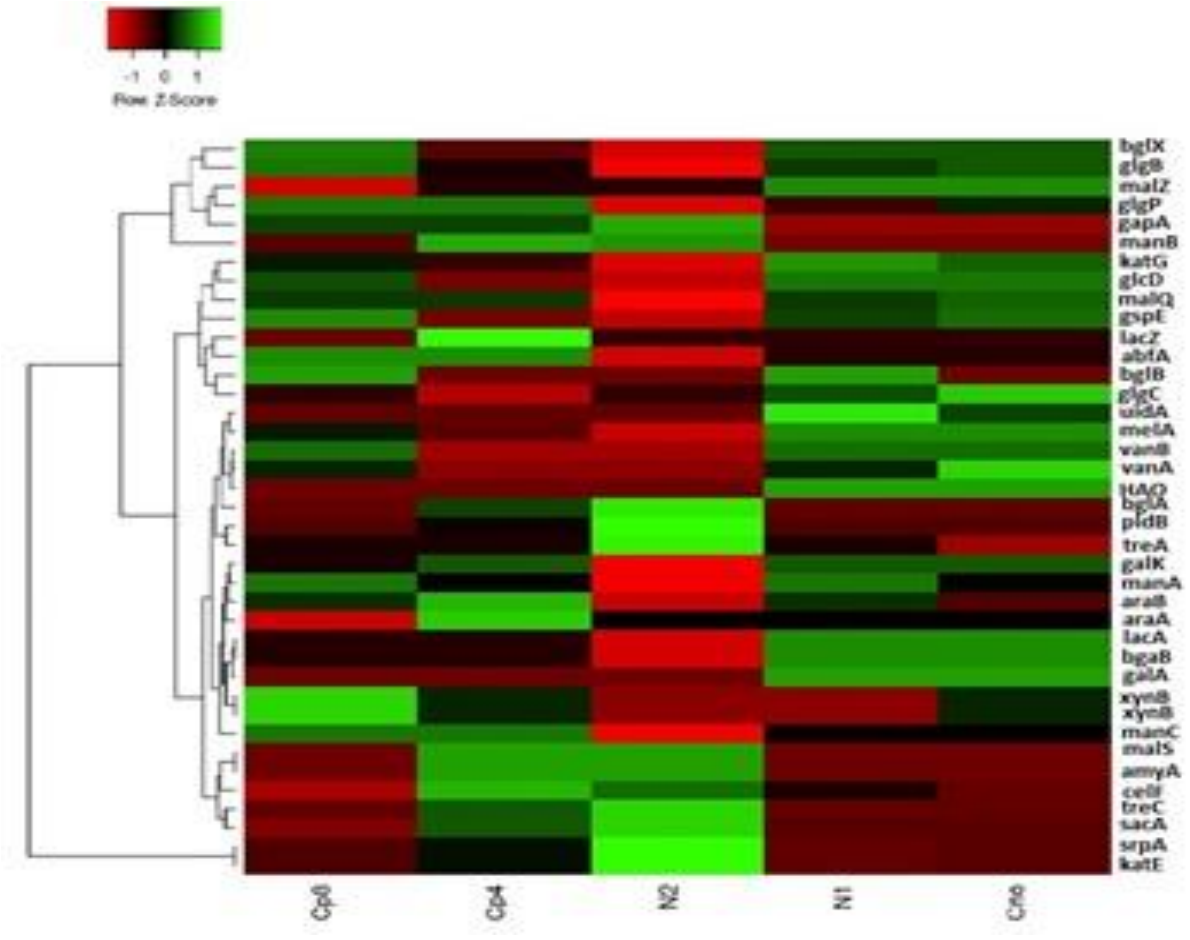


Figure 7. 6: Relative abundance of carbon cycling genes present at maize rhizosphere under fertilization treatments. Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer). The abbreviations meaning are (*amyA*) alpha-amylase, (*glgB*) 1,4-alpha-glucan branching enzyme, (*bglX*) beta-glucosidase, (*glgP*) starch phosphorylase, (*manB*) phosphomannomutase, (*gapA*) glyceraldehyde 3-phosphate dehydrogenase, (*malZ*) alpha-glucosidase, (*malQ*) 4-alpha-glucanotransferase, (*gspE*) general secretion pathway protein E, (*katE*) catalase, (*glgC*) glucose-1-phosphate adenylyltransferase,

(*bglB*) beta-glucosidase, (*manC*) mannose-1-phosphate guanylyltransferase, (*manA*) mannose-6-phosphate isomerase, (*lacZ*) beta-galactosidase, (*xynB*) xylan 1,4-beta-xylosidase, (*galK*) galactokinase, (*araB*) L-ribulokinase, (*araA*) L-arabinose isomerase, (*lacA*) beta-galactosidase, (*glcD*) glycolate oxidase, (*vanA*) vanillate monooxygenase, (*vanB*) vanillate monooxygenase, (*srpA*) catalase, (HAO) (S)-2-hydroxy-acid oxidase, (*treA*) alpha,alpha-trehalase, (*sacA*) beta-fructofuranosidase, (*treC*) trehalose-6-phosphate hydrolase, (*xynB*) xylan 1,4-beta-xylosidase, (*pldB*) lysophospholipase, (*uidA*) beta-glucuronidase, (*bgaB*) beta-galactosidase, (*abfA*) alpha-N-arabinofuranosidase, (*galA*) alpha-galactosidase, (*melA*) alpha-galactosidase, (*malS*) alpha-amylase, (*bglB*) beta-glucosidase, (*bglA*) 6-phospho-beta-glucosidase, (*celF*) 6-phospho-beta-glucosidase, (*katG*) catalase-peroxidase

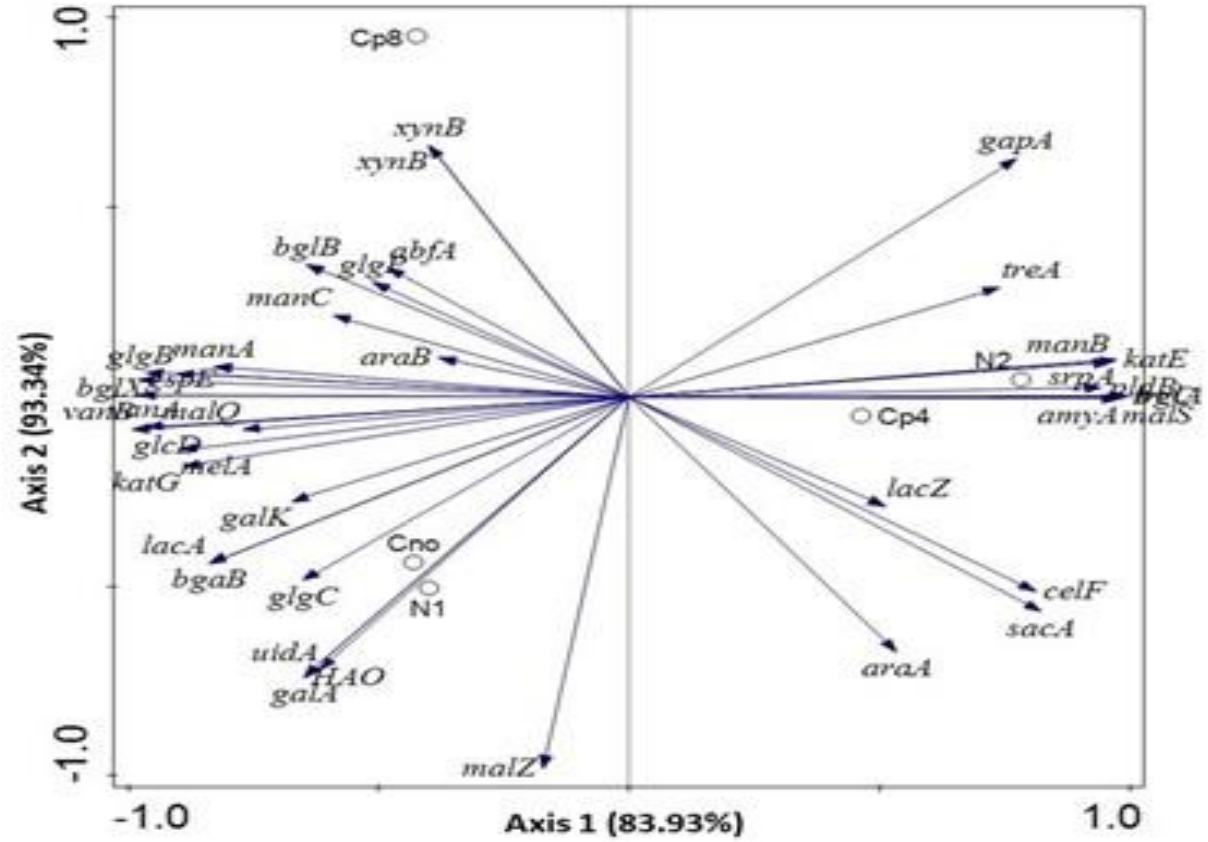


Figure 7. 7: PCA (Principal component analysis) of carbon cycling genes obtained under organic, inorganic and unfertilized rhizosphere soil samples from maize plants Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

7.4 Discussion

Microbes are the agents that facilitate the transition and transformation of nitrogen and carbon containing compounds from one form to another and soil fertilization significantly affects the microbial community (Lammel et al., 2015) and their activities in the cycling of these nutrients. A

few studies in the past have investigated the impact of organic compost manure and inorganic fertilizers on the abundance of the functional genes, but none has considered whether this is influenced by the rhizosphere effect of the plants or the fertilization effects. In this study, we evaluated the relative abundance of maize rhizosphere bacterial functional genes implicated in nitrogen and carbon cycles under organic and inorganic fertilization. We discovered that the application of high doses of compost manure, low inorganic fertilizer and untreated control had more significant impacts on relative abundance of the bacterial community and functional genes responsible for nitrogen and carbon cycle, than, the addition of a lower quantity of compost manure and higher doses of inorganic fertilizer.

The nutrient cycling processes performed by soil-borne microbes like nitrification, denitrification, ammonification, nitrogen fixation, mineralization, carbon degradation, carbon fixation etc. involve a series of enzymatic catalyzed steps and are performed by a number of interrelated-microbial groups in the soil. The abundance of gene families involved in these processes could influence the rates of biogeochemical cycling of these nutrients under fertilization treatments. In our study, there exist an abundance of gene families involved in the nitrogen cycle such as *gln* (glutamine synthetase), *gud* (glutamate dehydrogenase), *nir* (nitrite reductase), *glt* (glutamate synthase), no (nitrate reductase), *ureC* (encoding urease alpha subunit for ammonification) in the maize rhizosphere under fertilized and unfertilized treatments (Figure 7.3).

The by-product of nitrification, which is nitrate (NO_3^{2-}), is the key substrate for the denitrification process, therefore, the abundance of the genes implicated in denitrification significantly contributes to the abundance of the community of nitrogen cycling microbes. The nitrite reductase gene families (*nirB*, nitrite reductase (no-f), *nirA*, *nirD*) detected within the samples, which are responsible for catalyzing the conversion of nitrate to gaseous nitrogen (Zumft, 1997, Butterbach-Bahl et al., 2013), were significantly lower in abundance and unaffected by the various treatments applied (Figure 7.2). Denitrification is the basic avenue for nitrogen loss in agricultural soil (Zhu et al., 2018, Igiehon et al., 2018). Other genes involved in the denitrification process are *nar*, *nap* encode enzymes that

reduces NO_2^- to ammonium ions as well as *nrfA* which serves as marker genes for the identification of microbes that mediate these processes of denitrification (Welsh et al., 2014, Simon, 2002). Surprisingly, nitrogen fixing gene (nifH) was detected in the samples at a very smaller concentration. This implies that the population of microbes possessing the nitrogenase enzyme coding genes in the soil are few compared to other non-nitrogen fixing microbes.

Nitrate reduction is done by nitrate reductase enzymes encoded by *napA*, *narG*, and other *nar* gene families (Sánchez and Minamisawa, 2018, Vaccaro et al., 2016). Obviously, the quantity of carbon compound present in the soil (electron donors) in relation to electron acceptors (nitrates) has a resultant effects on the emission of N_2O during denitrification processes (Richardson et al., 2009, Felgate et al., 2012, Amoo et al., 2017) and as observed in Figure 7.2, in relation to the treatments and maize growth phase of 7 weeks after germination, the rhizosphere effect of the plants greatly affected the abundance of denitrifying functional genes from the maize rhizosphere. Thus, both organic and inorganic fertilization could supported very little emission of nitrogenous gases from the soil. The rhizosphere of maize plants harbours a unique community of nitrifying bacteria as well as possessing rich functional genes that include *nifH* (nitrogen fixation), *gdh*, *ureC* (for ammonification), *amoA*, *hao* (implicated in nitrification) and *narG*, *nirS*, *nirK*, *norB*, *nosZ* (denitrification) when compared to the bulk soil (Cheneby et al., 2004, Li et al., 2014c, Ai et al., 2013, Wang et al., 2017).

On the other hand, gene families involved in ammonia or ammonium assimilation to form glutamine and glutamate like *glnA*, *gltB*, *gudB*, *gltD* are highly abundant in the higher dose compost (Cp8), control (Cn0) and lower inorganic fertilized (N1) rhizosphere soil samples. Gene *glnA* encoding enzyme glutamine synthetase which catalyze the biosynthesis of glutamine from glutamate substrate is the most abundant nitrogen cycling gene observed (Figure 7.3). Also *gltB* (glutamate synthase) is the second most abundant and *gdhA* (glutamate dehydrogenase) catalyzes the assimilation of ammonium by 2-oxoglutarate to form glutamate. Therefore, glutamine synthetase, glutamate synthase, and glutamate dehydrogenase are the central nitrogen metabolic facilitators for ammonium assimilation in the bacteria cells. Studies have shown that intracellular glutamine concentration serves

as an indicator of the quantity of environmentally available nitrogen and are often responsible for microbial growth rate modulation (Ikeda et al., 1996). The quantity of glutamine is usually lower than glutamate at every nitrogen availability, but gradually increases as the external nitrogen source becomes scarce or limited (Ikeda et al., 1996). This is evident with the high level of abundant glutamine synthetase encoding genes and glutamate synthase functional genes observed in our studies.

However, soil microbes perform critical roles in organic carbon cycling and its fixation (Liang and Balser, 2012). The structure of soil microbes community regulate both the changes in soil-carbon pool and the pattern of such changes (Billings and Ziegler, 2008). Our studies revealed that the carbon cycling genes involved in carbon degradation differed significantly under the various fertilization treatments. This cycle, though complex, is a very important metabolic processes in biogeochemical cycling of nutrients (Rui et al., 2015, Babalola, 2014). The result shows that the abundance of functional genes in soil bacterial community at the rhizosphere could predict the activity of carbon degrading enzymes in the rhizosphere. This is in agreement with Trivedi et al., (2016). The relative abundance of carbon cycling genes *xynB* (in Cp8), *lacZ* (Cp4), *bglA*, *pldB*, *trpA* (N2), *uidA* (N1) and *glgC*, *vanA* (Cn0) were significantly different in the observed treatments (Figure 7.6), but the functional genes *malS* and *amyA* were most abundant in treatment Cp4 and N2 (Figure 7.7). The screen metagenomes showed genes involved in cellulose, carbohydrate, hemi-cellulose, lignin and simple sugars degradation (Figure 7.7). Therefore, the fertilization treatments do not promote a common carbon cycling gene, rather each treatments selects a unique set of carbon degradation genes. To our understanding as observed in our studies, neither the fertilization treatments nor the maize plants enrich and support carbon fixation genes rather they promote carbon degradation ones. This implies that agricultural activities of soil fertilization could contribute to the remarkable production of greenhouse gases such as carbon dioxide from the agricultural soils.

7.5 Conclusion

The relative abundance of bacterial functional marker genes obtained in our study describes the processes peculiar to the biogeochemical cycling of nitrogen and carbon in the soil. This gives a better understanding on how soil fertilization with organic and inorganic fertilizers affects the cycling of these nutrients at the rhizosphere of maize plants. We showed that lower quantity of chemical fertilizer, higher dose of compost and no fertilization (control) promoted nitrogen cycling genes, particularly those involved in ammonium assimilation, while they aided the selection of only specific carbon degradation genes that are peculiar to each treatment. This suggest that soil fertilization lowers nitrogen gas emission but increases carbon dioxide evolution in agricultural soil. Also *Actinomycetales* are selected by high compost, low inorganic fertilizer, and control, while *Bacillales* are promoted by low compost and higher inorganic fertilizer. This indicated that only microbes capable of tolerating the stress of higher doses of inorganic fertilizer will thrive under such conditions.

CHAPTER EIGHT

Shotgun metagenomics Assessment of the Effects of Soil Fertilization on Antimicrobial Synthesizing, Siderophores and Chemotaxis Genes for Induction of Disease Suppressive Soil in the Maize Rhizosphere

Abstract

Soil fertility is a function of the level of organic substances present in the soil and it influences the activities of soil borne microbes, plant growth performance, and a host of other beneficial ecological functions. In this metagenomics study, we evaluated the response of maize microbial functional gene diversity involved in chemotaxis, antibiotics, siderophores and antifungals production within the rhizosphere of maize plants under compost, inorganic fertilizer and unfertilized conditions. The results showed that fertilization treatments at higher compost manure and lower inorganic fertilizer doses as well as maize plants itself in the unfertilized soil through rhizosphere effects share similar influences on the abundance of chemotaxis, siderophores, antifungal and antibiotics synthesizing genes present in the samples, while, higher doses of inorganic fertilizer (120 kg/ha) and lower compost manure (4 tons/ha) treatments significantly repressed these genes. The implication is, for a disease suppressive soil to be achieved, soil fertilization with high doses of compost manure (8 tons/ha) as well as lower inorganic fertilizer (60 kg/ha) should be used to enrich soil fertility and boost the abundance of chemotaxis and disease suppressive genes in the soil. Maize crops also should be planted sole or intercropped with other crops to enhance the rhizosphere effect of these plants in promoting the expression and abundance of these beneficial genes in the soil.

8.1 Introduction

The movement of microbes from one point in the environment to another as a result of potential difference in nutrients or useful chemicals gradients is termed chemotaxis. Chemotaxis has many physiological roles such as improving access to growth nutrients as well as in initiation of infection. This movement is ATP (energy) dependent for efficient movement of flagella and other locomotary

structures of the microbes in response to the chemical gradients stimuli. Chemotaxis is a survival mechanism of microbes in either positively going towards or moving away from the source of the stimulus. The priming of the chemosensory pathways by the signaling molecules facilitates chemotaxis and this begins when the chemical molecules bind to the receptors which form complexes with *cheA* (a histidine kinase) and *cheW* (an adaptor protein). The primed *cheA* will result in autophosphorylation on histidine residue with a corresponding trans-phosphorylation of *cheY* (a primary response regulator) which binds to flagella motor to initiate chemotaxis movement (Parkinson et al., 2015, Sourjik and Wingreen, 2012). Other facilitators of chemosensory pathways are *cheR* (methyltransferase), *cheB* (methylesterase) etc. (Wuichet and Zhulin 2010). Often, bacteria perform energy taxis in response to migrating to suitable environments that support their metabolic activities (Schweinitzer and Josenhans, 2010) as observed in the rhizosphere of organic manure fertilized maize rhizosphere soil.

Agricultural intensification necessitates the use of chemical fertilizer in boosting soil fertility. This inorganic fertilizer on prolonged application to the soil causes a range of detrimental effects such as eutrophication, greenhouse gases emission, reduction in plants nutrient uptake, and toxicity on soil microbes (Zhu et al., 2016). The inorganic fertilizer associated drawbacks prompted the need for soil fertilization with compost, plant/crop residues, animal dungs and poultry droppings. These organic substances are not only cost effective, but also are microbiological and environmentally friendly (Tejada et al., 2008, Bhattacharyya et al., 2008, Ajilogba et al., 2013). The nutrients which could serve as inducers to microbial chemoreceptors causes the improvement in the expression of chemotaxis genes and migration of soil borne microbes to the nutrient source. Often this microbial migration is towards the rhizosphere. The organic substances given off by the plant roots' serve as growth enhancing nutrients for the microbes. The rhizosphere is a spot of high metabolic activities and assembly of a vibrant and unique consortium of microbial communities which actively participate in biogeochemical cycling, plant hormone production as well as antagonistic antimicrobial chemicals secretion. Although, both beneficial and pathogenic microbes are equally attracted to the

rhizosphere, the beneficial ones help to check the activities of the phytopathogens, and hence, sustain the health of the plants (Mendes et al., 2013, Marschner et al., 2004, Babalola and Glick, 2012).

Nevertheless, the cascade effect of plant exudates, fertilizer (organic and inorganic) on the priming of chemotaxis genes and attraction of microbial communities to the rhizosphere leads to the promotion and establishment of a disease suppressive soil. This disease suppressive soil operates on a natural principle of the survival of the fittest and competition for dominance. It breeds a stiff microbial competition at the rhizosphere and, depending on the fate of the competitive outcome between the pathogens and the beneficial microbes, determines the chances for development and sustenance of health or death of the plants (Enebe and Babalola, 2019, Adegboye and Babalola, 2015). For instance, the pathogen *Streptomyces* sp. capable of producing thaxtomic substances which aid its infection and initiation of scab disease condition in potato plant is directly controlled by the beneficial bacteria *Pseudomonas fluorescens* LBUM223. This inhibition effect on the *Streptomyces* sp. is as a result of the antimicrobial substance (Phenazine – 1 – carboxylic acid) produced by *P. fluorescens* LBUM223 which interferes with the proper cellular function of the pathogen and so results in its inhibition (Arseneault et al., 2015). Antimicrobial substances like 2,4-diacetylphloroglucinol, hydrogen cyanide, chitinase, phenazines, organic acids as well as iron chelating substances (siderophores) are responsible for the induction and maintenance of a disease suppressive soil (Latz et al., 2012, Raaijmakers and Mazzola, 2012, Ajayi et al., 2016, Adegboye and Babalola, 2012). In this study, we evaluated the functional genes profile abundance involved in chemotaxis and antimicrobial/siderophore producing substances from the maize rhizosphere under organic, inorganic and untreated control using shotgun metagenomics study. Therefore, we hypothesize that both organic compost at higher dose and low inorganic fertilizer application will not differ from those of the untreated control in the enrichment of chemotaxis genes and antimicrobial producing genes within the maize rhizosphere. These treatments will enhance the development of a disease suppressive and healthy soil.

8.2 Materials and Methods

8.2.1 Site description, samples collection and DNA extraction

The soil samples were collected from Molelwane, North-West University's agricultural farm located in a semi-arid region, near Mafikeng, in North-West Province, South Africa The sampling site co-ordinates and the site description are the same as in chapter 5 above. The soil type is sandy loam soil. And the chemical composition of the compost manure are N = 20045.3 (g/kg), P = (1.0 g/kg), K = 12.3 (g/kg), pH = 7.1. The age of the manure compost or stabilization period of the compost prior to its use was 16 weeks. A sampling distance of 15 cm (minimum) and 50 cm (maximum) in 3 sampling spots/plot were used. Sampling was done in October 2018 at 7 weeks after the germination of the maize seed. The experimental plots were treated with 120 kg and 60 kg of NPK fertilizer (N/ha) and with community-based compost manure at 8tons and 4 tons/ha respectively. The maize (Mid-altitude variety of maize – *Zea mays everta*) planting distance was 15 cm × 20 cm. The rhizosphere of soil samples was collected using auger at 0–15 cm depth, 2 cm away from the growing maize plant at 7 weeks after germination. The sampling area was split into 3 plots for 60 kg N/ha and 3 plots for 120 kg N/ha. The control and the compost treatments were split into 3 plots each. Soil samples were taken from 9 plants (3 from each replicate/treatments). Therefore, a total of 15 samples were collected (3 replicates × 5 treatments). From each sub-replicated plot of the treatments, nine sub-samples distributed to cover the entire plots were gathered together into composite samples. Each of the five treatments was made up of five composite samples, each containing nine subsamples. The soil samples were put in a plastic bag covered with ice and conveyed to the laboratory, plant and root debris were sieved (with 2-mm sieve) and preserved at −20 °C for metagenomics shotgun analysis. The physicochemical properties of the soil before planting and fertilization as well as the compost manure after 16 weeks stabilization period were analyzed through following the standard basic soil chemical analysis protocols described by Motsara and Roy (2008). And the result is contained in Table 5.1 in chapter 5 above. This was followed by extraction of total microbial community DNA

from the rhizospheric soil samples with PowerSoil DNA isolation kit from MoBio Laboratories, Incorporation by following the manufacturer's guide.

8.2.2 Sequencing of the community DNA

DNA concentrations were examined with the aid of Qubit® dsDNA HS Assay Kit from Life Technologies, Carlsbad, California, United States. The deoxyribonucleic acid libraries were prepared by the use of Nextera DNA Flex library preparation kit (Illumina Incorporation.) in accordance with the procedure from the manufacturer. A total of 50 nanogram of the DNA molecules from each sample were taken for the libraries preparation. Then, this was followed by fragmentation alongside with adapter sequences addition. The adapter molecules were used during PCR cycles together with the addition of unique indices into the samples. The final concentration of the libraries generated were quantified with Qubit® dsDNA HS Assay Kit from Life Technologies. The library's average sizes were determined with the use of analytical machine - Agilent 2100 Bioanalyzer (from Agilent Technologies). DNA libraries were combined together into an equalmolar ratios of 0.7 nM. The pooled DNA were then sequenced paired end for 300 cycles using the machine - NovaSeq 6000 system (Illumina). This was done at the Mr DNA molecular research laboratory in Texas, USA.

8.2.3 Sequence processing, annotation and statistical analysis

The raw metagenomic reads were uploaded to MG-RAST where quality control processes were performed on the reads (Meyer et al., 2008). The preprocessing of the reads involved the removal of artificial reads, host specific sequences and other ambiguous base pairs. This was followed by gene annotation using BLAT algorithm (Kent, 2002) and M5NR database (Wilke et al., 2012). The protein coding genes annotation were executed by blasting through M5NR as well as SEED Subsystem level – function. The BlastX was used to perform hit at an e –value cutoff of 10E-5, minimum alignment length of 15 base pairs, and percentage identity of 60%. The unannotated sequences were not subjected to further evaluation or analysis. Also applied was the MG-RAST normalization tool to enable us to cut down on the possible experimental error from the experimental work. The chemotaxis and disease suppressive genes involved in antibiotics, antifungi, nematicide and siderophores were

curated manually from the total gene files gotten from the SEED Subsystem database, level – function. The sequences were used for statistical analysis and the chemotaxis, disease suppressive genes variances were evaluated using one-way analysis of variance at p-value less than 0.05. The abundance and distribution of disease suppressive genes were visualized in a bar chart representation using Microsoft Excel. The online software – Circos, (http://circos.ca/) was employed in plotting the chart of chemotaxis genes. While, the Evenness, Simpson and Shannon diversity indexes were determined for the samples and also contrasted amongst the treatments using Kruskal-Wallis test. The beta diversity was ascertained using PCoA (principal coordinate analysis) on the basis of Euclidean distance-matrix and ANOSIM (analysis of similarity) through 9999 permutations. These analyses were carried out using PAST version 3.20 software (Hammer et al. 2001) and PCoA and principal component analysis (PCA) using CANOCO 5v (Microcomputer Power, Ithaca, NY). The sequences are deposited on NCBI SRA database, SRA accession: PRJNA607213

8.3 Results

8.3.1 Metagenomics dataset

The sequence reads obtained from the samples clearly show the species richness and justify the sampling efforts (Figure 8.1). The sequence reads pre-post quality control were 5,558,478 (N2), 9,687,815 (N1), 12,070,719 (Cp4), 7,834,687 (Cn0), 15,575,330 (Cp8), with mean GC percent of 43 ± 11%, 64 ± 12%, 49 ± 11%, 64 ± 11%, 63 ± 12% for N2, N1, Cp4, Cn0, and Cp8 respectively. After post quality control process, the sequences that remained are 2,892,203, 8,198,530, 2,945,816, 6,780,803, 13,083,355 sequence reads from N2, N1, Cp4, Cn0, and Cp8 with predicted proteins of known functions comprises of 1,603,127 (61.86%) (Cp4), 5,410,912 (45.25%) (Cp8), 2,834,072 (44.49%) (Cn0), 1,812,036 (69.99%) (N2), 3,418,368 (45.11%) (N1) sequence reads, while, 969,988 (37.43%) (Cp4), 6,519,307 (54.52%) (Cp8), 3,523,113 (55.31%) (Cn0), 764,206 (29.52%) (N2), 4,144,168 (54.69%) (N1) were sequences with predicted proteins of unknown functions. The post quality control mean GC percent are contained in Table S6.1.

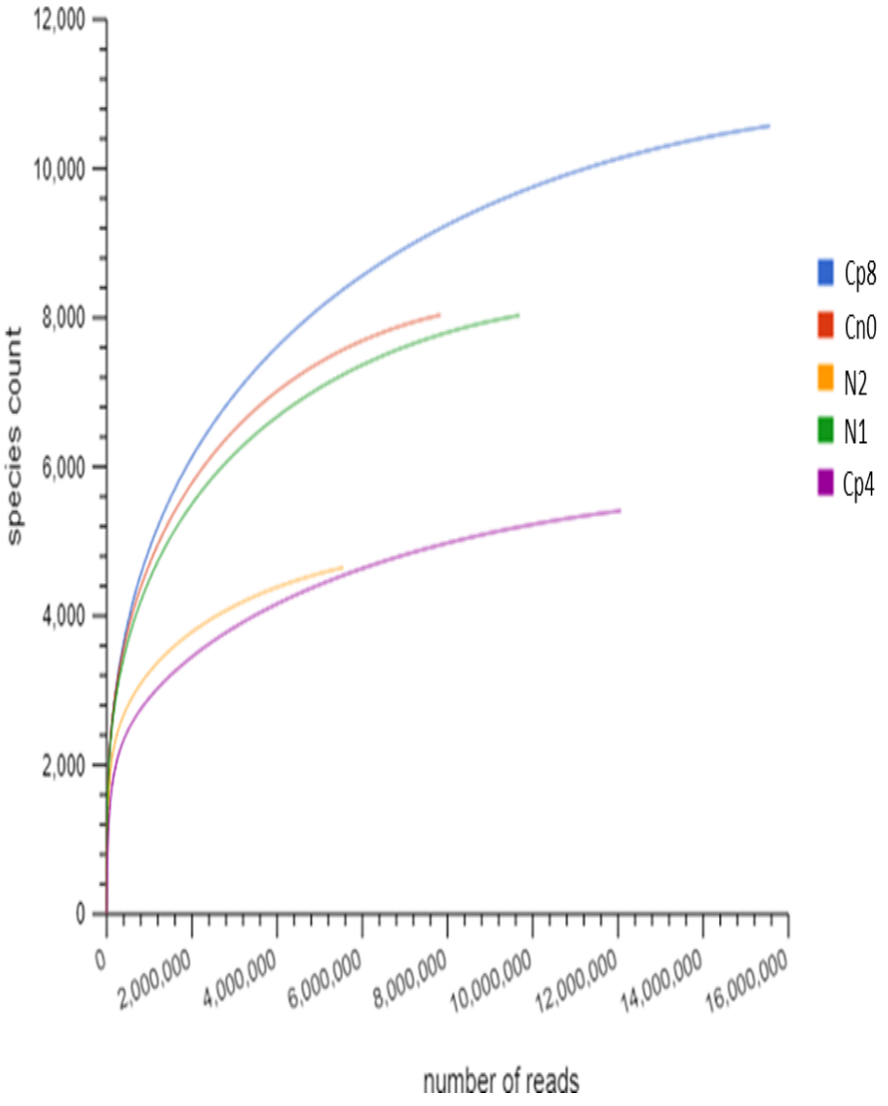


Figure 8. 1: The rarefaction curve depicting the species richness within the maize rhizosphere soil samples under fertilization and unfertilized treatments condition Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

8.3.2 Chemotaxis functional genes at the maize rhizosphere

The metagenomics sequences from the maize rhizosphere soil samples under the treatments containing the chemotaxis genes differ significantly ($P < 0.05$) amongst the treatments (Figure 8.2). The abundance of these genes also differed from one another significantly ($P<0.05$). Of the chemotaxis genes identified, *cheBR* fusion proteins involved in signal transduction of the two component systems, followed by *mcp* (methyl accepting chemotaxis protein), *cheA* (Histidine kinase), *cheB* (methyl esterase), *cheR* (methyl transferase), *mot* B and A (chemotaxis proteins *motB* and *motA*), *cheY* (response regulator) as well as *cheW* (coupling protein) were the most abundant chemotaxis genes present in the sequenced samples from Cp8, N1 and Cn0.

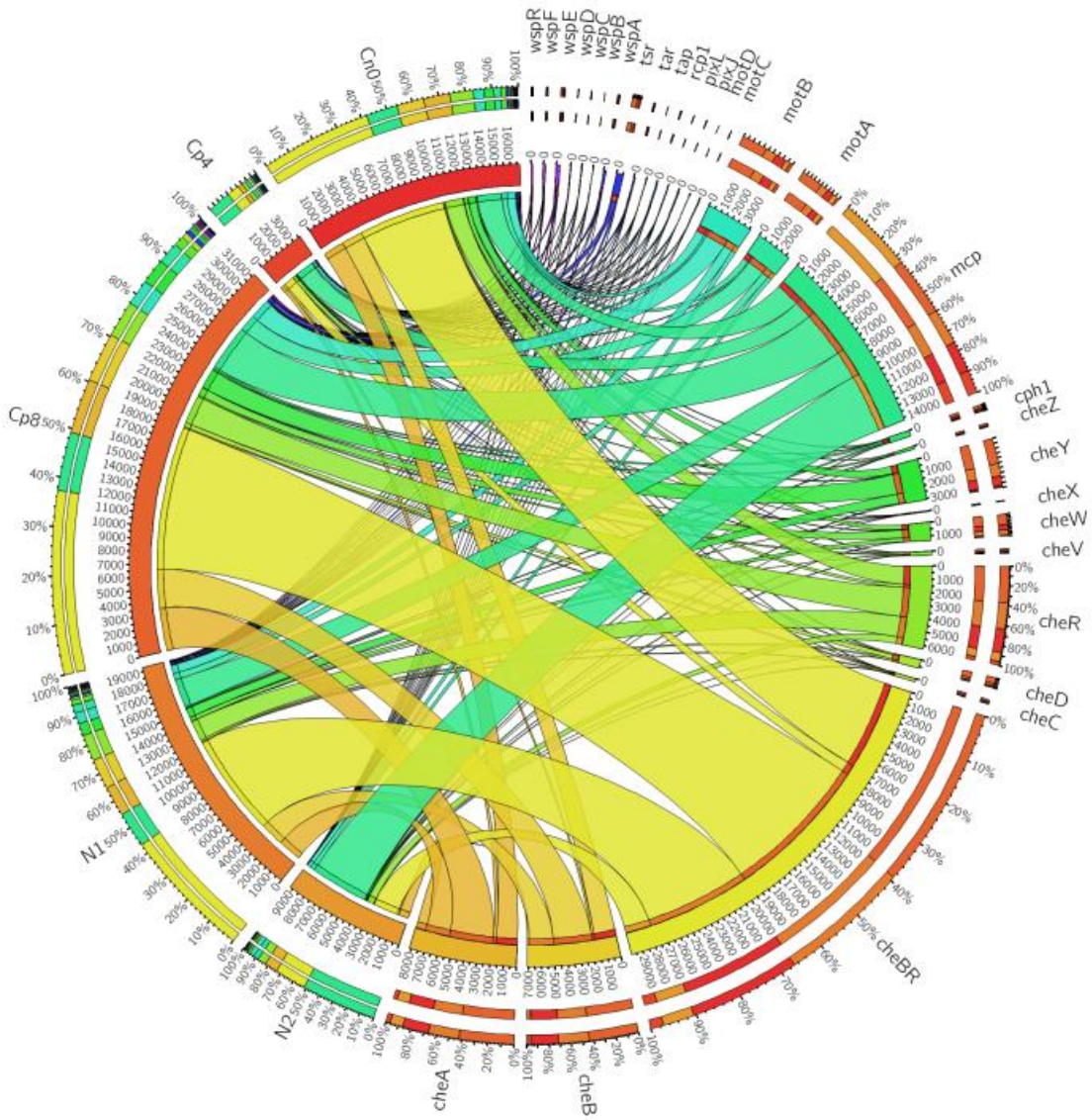


Figure 8. 2: The distribution of chemotaxis genes in maize rhizosphere soil samples under fertilization and control conditions. Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer). The gene symbols are depicted as follows: (*cheBR*) two-component system, chemotaxis family, CheB/CheR fusion protein, (*mcp*) methyl-accepting chemotaxis protein, (*cheA*) two-component system, chemotaxis family, sensor kinase CheA, (*cheB*) two-component system, chemotaxis family, response regulator CheB, (*cheR*) chemotaxis protein methyltransferase CheR, (*motB*) chemotaxis protein MotB, (*cheY*) two-component system, chemotaxis family, response regulator CheY, (*motA*) chemotaxis protein MotA, (*cheW*) purine-binding chemotaxis protein CheW, (*cheD*) chemotaxis protein CheD, (*tsr*) methyl-accepting chemotaxis protein I, serine sensor receptor, (*cph1*) two-component system, chemotaxis family, sensor kinase Cph1, (*cheV*) two-component system, chemotaxis family, response regulator CheV, (*cheZ*) chemotaxis protein CheZ, (*cheC*) chemotaxis protein CheC, (*wspE*) two-component system, chemotaxis family, sensor histidine kinase and response regulator WspE, (*tar*) methyl-accepting chemotaxis protein II, aspartate sensor receptor, (*wspR*) two-component system, chemotaxis family, response regulator WspR, (*wspF*) two-component system, chemotaxis family, response regulator WspF, (*cheX*) chemotaxis protein CheX, (*rcp1*) two-component system,

chemotaxis family, response regulator Rcp1, (*wspA*) methyl-accepting chemotaxis protein WspA, (*motC*) chemotaxis protein MotC, (*wspC*) chemotaxis protein methyltransferase WspC, (*pixJ*) methyl-accepting chemotaxis protein PixJ, (*wspD*) chemotaxis-related protein WspD, (*pixL*) two-component system, chemotaxis family, sensor histidine kinase and response regulator PixL, (*motD*) chemotaxis protein MotD, (*tap*) methyl-accepting chemotaxis protein IV, peptide sensor receptor, (*wspB*) chemotaxis-related protein WspB

8.3.3 Antimicrobial and siderophore genes contributing to disease suppressive soil

Under treatments and untreated fertilization, the metagenomics sequences containing antimicrobial – siderophore genes implicated in the development of a disease suppressive soil are *prnC* (FADH2 O2 – dependent halogenase II), *bceB* (bacitracin transport system permease protein), *cefD* (isopenicillin N epimerase), *irp1* (yersiniabactin nonribosomal protein), *mbtC* (mycobactin polyketide synthetase), *aveA* (type 1 polyketide synthase AVES), *mbtD* (mycobactin polyketide synthase) and chitin deacetylase. They are very abundant in the soil as well as differed significantly ($P < 0.05$) (Figure 8.3).

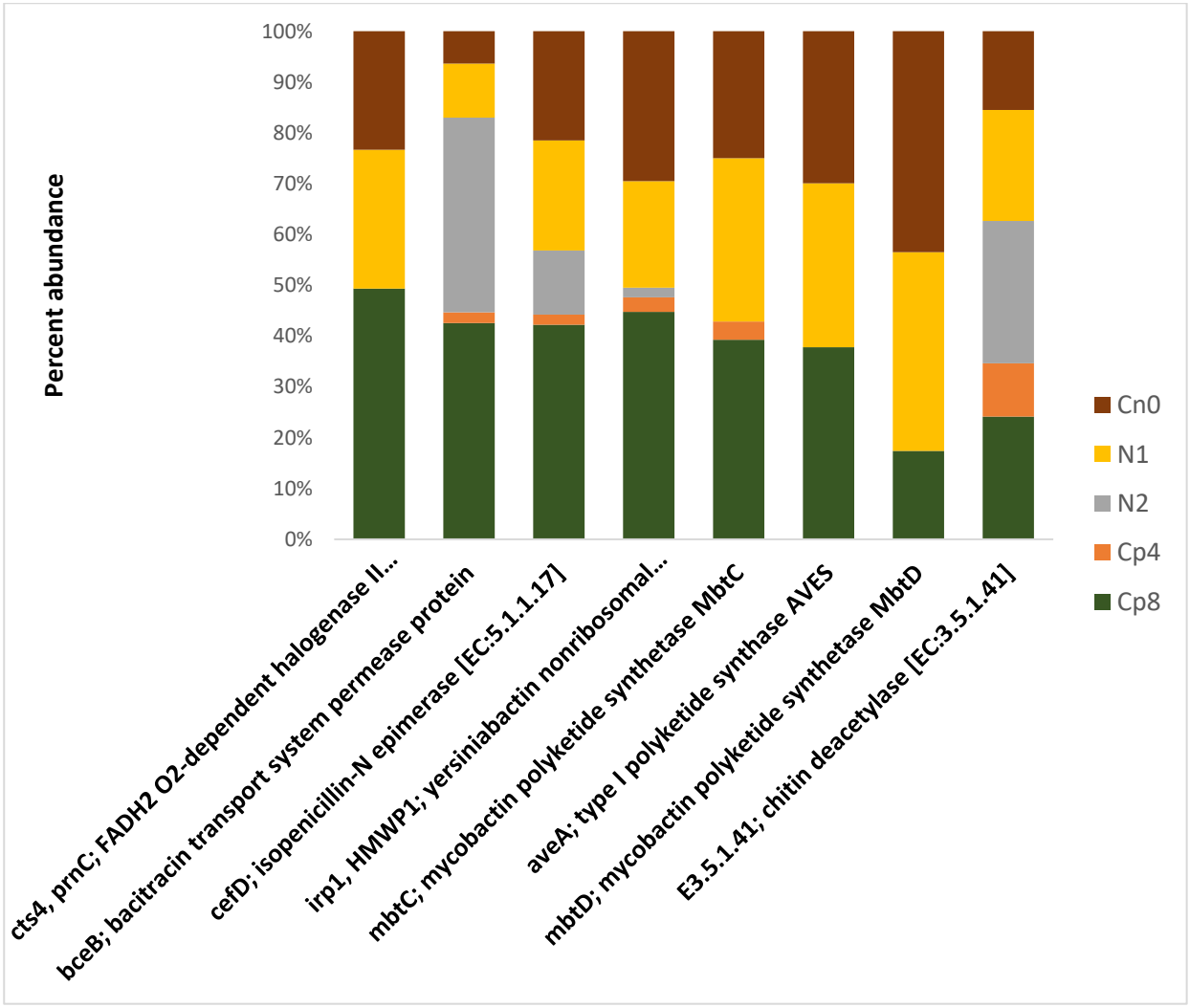


Figure 8. 3: The percentage distribution of antimicrobial facilitating and siderophores producing genes within the rhizosphere soil of maize treated with organic, inorganic fertilizers and control treatments Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

8.3.4 Diversity (alpha and beta) estimation of the chemotaxis and the disease suppressive genes

The diversity indices were depicted by Shannon, Simpson and evenness for alpha diversity of the chemotaxis genes. Shannon, Simpson and evenness diversity indexes clearly showed that there were significant differences (P-value = 0.00057) for the chemotaxis functional genes alpha diversity. Moreover, there was a significant difference in the beta diversity (ANOSIM, P-value of 0.01 and R value = 0.55), that is, the diversity between the unfertilized and fertilized rhizosphere soils (Figure 8.4) and depicted by the PCoA – principal coordinate analysis (Figure 8.5). Also, the alpha diversity

indices based on Kruskal Wallis test for disease suppressive genes differed significantly (P value = 0.016), while beta diversity for the same genes under analysis of similarity test (ANOSIM) differed significantly (ANOSIM, R = 0.55, P value = 0.01) across the samples. Also, the Principal component analysis (PCA) was performed to show the distribution of chemotaxis genes between the treatments and the control samples (Figure 8.6).

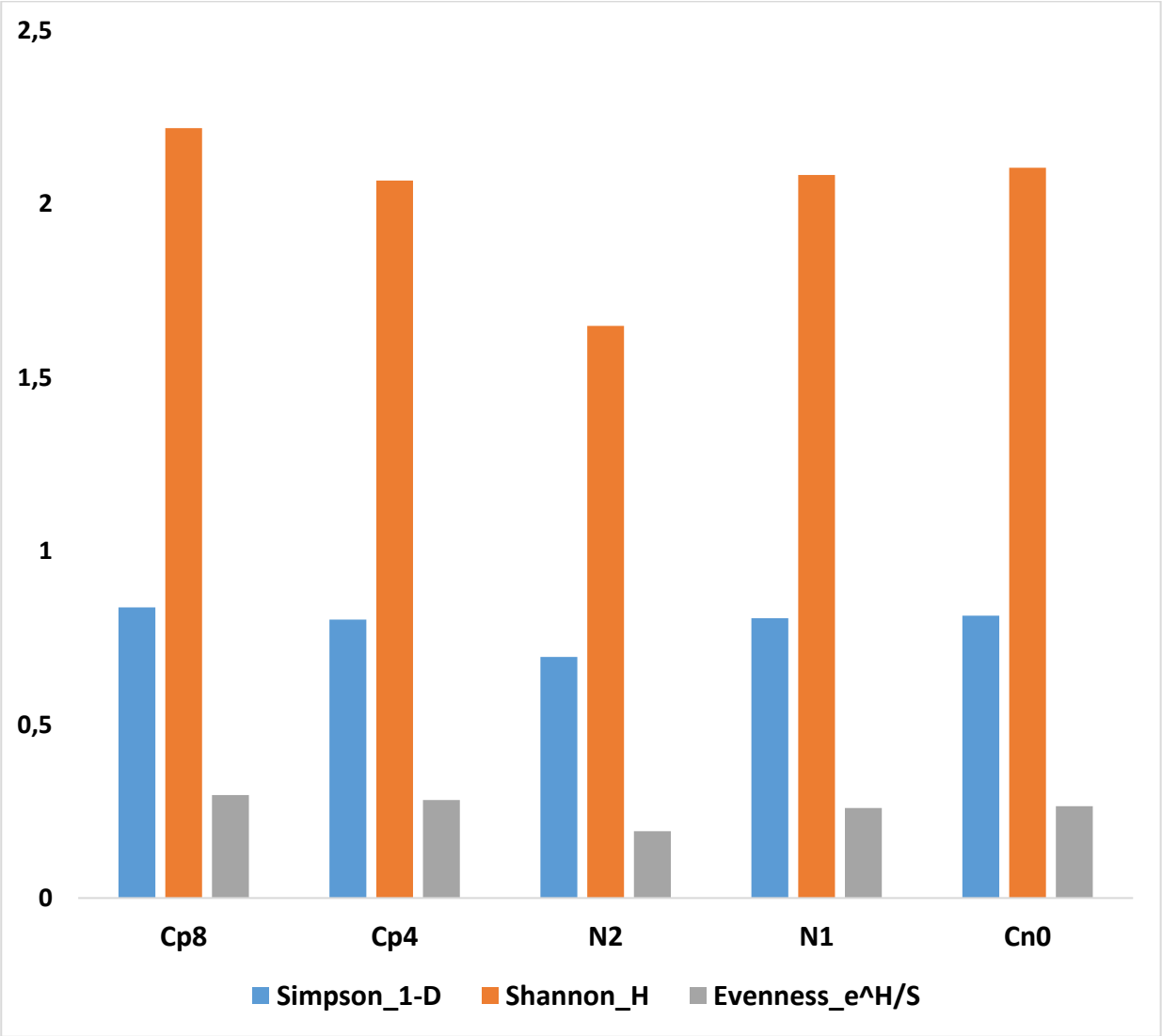


Figure 8. 4: Diversity indexes of chemotaxis genes within the fertilized and unfertilized soils from maize rhizosphere Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

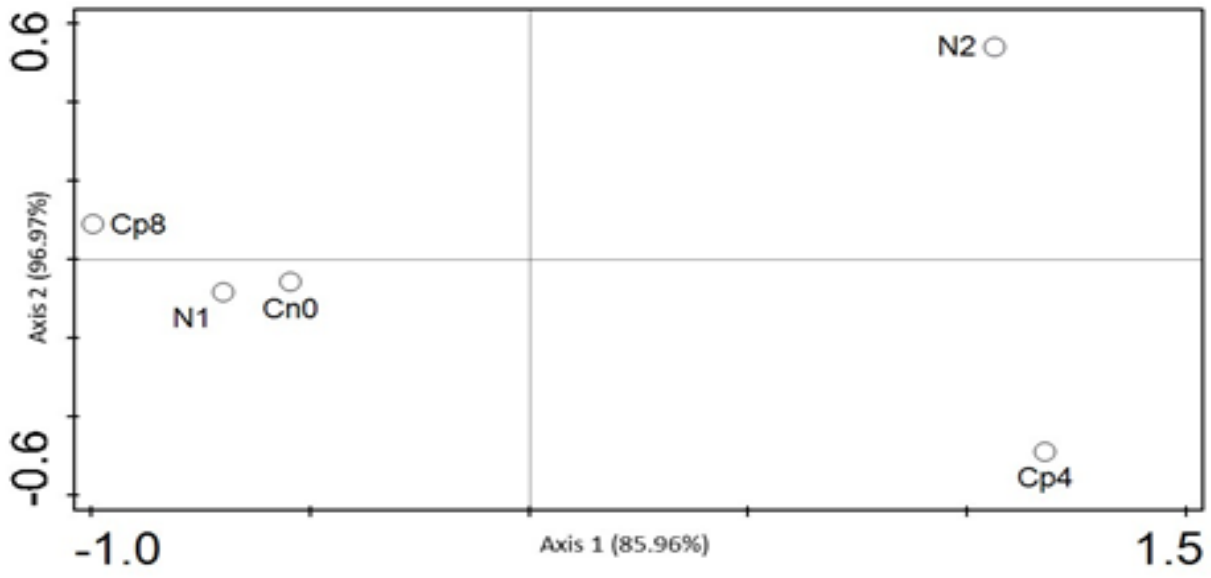


Figure 8. 5: PCoA analysis showing the beta diversity of chemotaxis genes within the fertilized and unfertilized maize rhizosphere soil samples Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

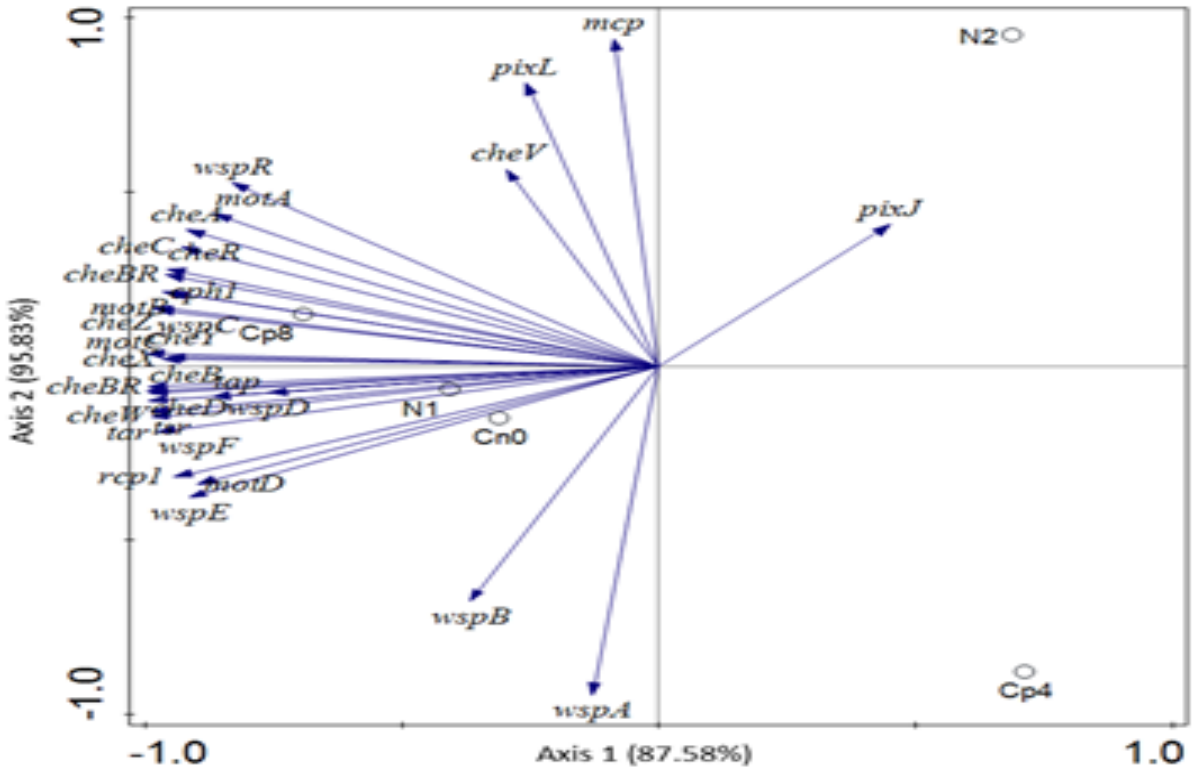


Figure 8. 6: PCA – principal component analysis of the chemotaxis genes abundance within the maize rhizosphere treated with organic, inorganic fertilizer and untreated control Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

8.4 Discussion

Soil fertility is a function of the level of organic substances present in the soil and it influences the activities of soil borne microbes, plant growth performance, and a host of other beneficial ecological functions (Marschner et al., 2003, Lee et al., 2009). The microbes present in the rhizosphere of plants have been implicated in plant nutrient uptake, growth hormones production, scavenging for parasitic pathogens, competing for nutrients with pathogens, induction of systemic and acquired resistance by the plants to pathogens, enabling plants to tolerate abiotic stress and perform biogeochemical cycling processes (Enebe and Babalola, 2019, Enebe and Babalola, 2018, Pieterse et al., 2014, Berendsen et al., 2012).

In this metagenomics study, however, we evaluated the response of maize microbial functional genes diversity involved in chemotaxis, antibiotics, siderophores and antifungals production within the rhizosphere of maize plants under compost, inorganic fertilizer and unfertilized conditions. The results clearly show that fertilization treatments at higher compost manure and lower inorganic fertilizer doses as well as maize plants itself in the unfertilized soil through rhizosphere effects shared similar influence on the abundance of chemotaxis, siderophores, antifungal and antibiotics synthesizing genes present in the samples.

Maize rhizospheric soil treated with compost at a concentration of 8 tons per hectare showed the highest chemotaxis, antibiotics, siderophores and antifungal genes richness, followed by the lower inorganic fertilized and the control soils. It is not yet clearly understood the extent to which the compost manure contributes to the soil enrichment of the chemotaxis and disease suppressive genes in the soil, making it difficult to arrive at a definite conclusion when comparing organic and inorganic fertilizer influence on the bacterial motility and disease suppression in the soil. However, organic manure has been shown to stimulate the antagonistic levels of bacterial community in the soil which in turn enhances disease suppression of soil borne microbial pathogens (Perez et al., 2008, Huang et al., 2006, Ahmad et al., 2012). Also, there exists an inversely proportional relation between inorganic fertilizer application and the diversity as well as richness of microbes, which explains the rationale

behind the observed abundance of these genes at the rhizosphere treated with lower inorganic fertilizer (Zeng et al., 2016a). However, the genes involved in chemotaxis and disease suppression were also enriched by the maize plants. It could be assumed that through rhizodeposition, the plants were able to attract a consortium of specialized microbes (Philippot et al., 2013) or through the production of signaling chemical compounds such as strigolactones capable of attracting arbuscular mycorrhizal fungi (Bouwmeester et al., 2007) or *Pseudomonas* attractant compound such as benzoxazinoids (Neal et al., 2012) and a host of other chemicals that boost the richness and diversity of the rhizosphere microbial population and their corresponding chemotactic and disease suppressive genes (Figures 8.2 and 8.3).

The observed decrease in the abundance of the chemotaxis genes (Figure 8.2) at a higher dose of inorganic fertilizer could be explained by the indirect soil pH modification effects (Zhang et al., 2017) and the roots' functional and structural adjustment in response to the fertilizer's acidification effects that may result in a change of the rhizosphere microbial population, activities and their colonization rate (Geisseler and Scow, 2014).

Our results suggested that the chemotaxis genes *mcp* (methyl-accepting chemotaxis protein) which is a chemoreceptor and *cheBR* (*cheB*/*cheR* fusion protein), *cheA* (histidine kinase), *cheB* (methyl esterase), *cheR* (methyl transferase), *motB* and A (chemotaxis protein *motB*, and *MotA*), *cheY* (response regulator), *cheW* (coupling protein) are cytoplasmic proteins (Sourjik, 2004, Porter et al., 2011, Wuichet and Zhulin, 2010, Stader et al., 1986) which were the most abundant in the high compost, lower inorganic fertilizer and the control. They facilitate the motility of the microbes within the soil rhizosphere environment. It is known that the process of chemotaxis involved in flagellar rotation is ATP (adenosine triphosphate) and electron transport dependent (Zhao et al., 2014b), which explains why a higher dose of compost with abundant nutrient supply efficiency could influence the chemotaxis activities of the rhizosphere microbes above other treatments. However, the maize plants as well as the low dose of inorganic fertilizers also enriched these chemotaxis genes. Their abundance and expression could be proportional to the presence of ligand molecules capable of binding to the

chemoreceptors which are as follows: phosphate ions, phytohormones, sugars, amino acids, oxygen molecules, hydrocarbon molecules, quorum sensing signaling molecules etc. (Matilla and Krell, 2017, Antunez-Lamas et al., 2009, Hegde et al., 2011, Wu et al., 2000, Esuola et al., 2016). Although, these molecules could attract both pathogenic and beneficial microbes, the observed disease suppressive genes present in the treatments (Figure 8.3) have shown that compost manure (cp8), inorganic fertilizer (N1) and maize plant (Cn0) are capable of attracting disease suppressive microbes which could facilitate the development of a disease suppressive soil.

The observed siderophores genes present in the rhizosphere soil treated with the compost and inorganic fertilizer and the control are *mbtC*, *mbtD* (mycobactin polyketide synthases) and *irp1* (yersiniabactin nonribosomal protein). Siderophores are chemical compounds with high affinity for iron molecules which are used by microbes to scavenge iron and create environments of iron deficiency. This iron deficient environment impairs microbial DNA synthesis and respiration which requires iron molecules for it to function effectively (Ahmadi et al., 2015, Perry et al., 1999). Alongside siderophore genes abundance, antibiotics and antifungal genes (*prnC, cefD, aveA*) were abundant in the samples. *aveA* genes are responsible for the production of avermectin (a nematicide) capable of inhibiting nematodes (Ikeda et al., 1999). *prnC* (FADH2-dependent halogenase II) responsible for antifungal pyrrolnitrin production (van Pee et al. 2003). *cefD* (isopenicillin N epimerase) involved in penicillin production (Kovacevic et al., 1990).

This study clearly demonstrated the influence of compost manure at higher treatment, low inorganic fertilizer and maize plants without any fertilization on the microbial chemotaxis genes abundance as well as disease suppressive genes. Therefore, understanding of the effects of fertilizer and maize plants on the enrichment of chemotaxis genes and functional genes involved in disease suppression will be useful in the actualization of a sustainable agriculture through the manipulation of the soil microbiomes.

8.5 Conclusion

This study clearly shows the effects of fertilization on the abundance of chemotaxis and disease suppressive genes in the maize rhizosphere. Our results revealed that treating an agricultural soil with high doses of compost manure derived from plant materials and domestic waste and low inorganic fertilizer separately will lead to the enrichment of the rhizosphere with microbes encoding chemotaxis, antibiotics, siderophores, antifungal, and nematicides synthesizing genes. Maize plants on the other hand, have been proven to exert significant rhizosphere effects (as observed in the control) in attracting and enriching the rhizosphere with beneficial functional microbial genes as well as microbes. Therefore, to achieve a healthy soil, we recommend fertilizing soil with either compost manure alone or in combination with low inorganic fertilizer, as both will synergistically combine their effects in promoting the development of a disease suppressive soil. This notwithstanding, intercropping of maize plants with a disease susceptible crop in a disease conducive soil could be a good alternative in attracting beneficial microbes to combat the invasiveness of the pathogens and hence the achievement of a sustainable agriculture.

CHAPTER NINE

The Influence of Soil Fertilization on the Distribution and Diversity of Phosphorus Cycling Genes from Maize Rhizosphere using Shotgun Metagenomics

Abstract

Biogeochemical cycling of phosphorus in the agro-ecosystem is mediated by soil microbes. These microbes regulate the availability of phosphorus in the soil. Little is known about the response of functional traits of phosphorus cycling microbes to soil fertilization with compost manure (derived from domestic waste and plant materials) and inorganic nitrogen fertilizer at high and low treatment doses. We used a metagenomics investigation study to understand the changes in the abundance and distribution of microbial phosphorus cycling genes in the agricultural farmlands receiving inorganic fertilizers (120 kg N/hectare, 60 kg N/hectare), compost manure (8 tons/ha, 4 tons/ha) and the control. Soil fertilization with compost and low quantity of inorganic nitrogen fertilizer have nearly the same effects as the rhizosphere effects of maize plants in promoting the abundance of genes involved in phosphorus cycle. Genes such as *ppk* involved in polyphosphate formation and *pstSABC* (for phosphate transportation) are highly enriched in these treatments. These genes facilitate phosphorus immobilization. At high dose of inorganic fertilizer (N2) application and low compost manure (Cp4) treatments, the nitrogen cycling genes were repressed and the relative abundance decreased. The bacterial families *Bacillaceae* and *Carnobacteriaceae* were very abundant in the high inorganic fertilizer (N2) treated soil. While *Pseudonocardiaceae, Clostridiaceae, Cytophagaceae, Micromonosporaceae, Thermomonosporaceae, Nocardiopsaceae, Sphaerobacteraceae, Thermoactinomycetaceae, Planococcaceae, Intrasporangiaceae, Opitutaceae, Acidimicrobiaceae, Frankiaceae* were more abundant in compost treated soil (Cp8). *Pyrenophora*, *Talaromyces*, and *Trichophyton* fungi were observed to be more in 8 tons/ha compost manure (Cp8) and *Methanosarcina, Methanobrevibacter, Methanoculleus,* and *Methanosphaera* archaea have the highest percentage occurrence in Cp8 (8 tons/ha compost). Also, at 120 kg/ha inorganic fertilizer (N2) treatment, *Cenarchaeum, Candidatus Nitrososphaera* and *Nitrosopumilus* were most abundant

in the treated rhizosphere soils. Our findings have brought to light the basis for the manipulation of rhizosphere microbial communities and their genes to improve the availability of phosphorus as well as enhance phosphorus cycle regulation in the agro-ecosystems.

9.1 Introduction

Driven by the need to increase crop yield, farmers worldwide have resorted to employing either organic or inorganic fertilizers to boost soil fertility. Phosphorus is an essential nutrients for plants and soil dwelling microbes. To source for phosphorus, phosphate bearing rocks are used in the production of inorganic fertilizers and their source of supply is gradually depleting, making their availability over a long period of time a major concern. Although fertilizers containing phosphate are being added to the soil, its loss through runoff generally has serious consequences to the water environment (Sharpley et al., 2013, Elser and Bennett, 2011, Stutter et al., 2015).

Nevertheless, the reactivity of phosphate ions through redox reaction in the soil makes its availability very limited, and only a minute quantity is accessible to plants for absorption. The rest is either immobilized or leached away (Mishima et al., 2006, Dahlgren et al., 2004, Tak et al., 2012) causing phosphorus starvation to both microbes and plants. To remedy the situation and increase bioavailability of phosphorus, microbes are equipped with the necessary enzyme synthesizing genes that participate in the biogeochemical cycling of phosphorus in the soil (Bergkemper et al., 2016, Arumugam et al.) and the products of these genes enable them to assimilate and trap phosphorus within their biomass. These genes are *gcd* (quinoprotein glucose dehydrogenase) involved in inorganic phosphate solubilization, *phoD, phoA phoB* (alkaline phosphatase), *appA* (phytase), *phnF, phnE, phnD, phnA* (Carbon-phosphate lyase multi- enzyme complex) implicated in organic phosphate mineralization, *pstS*, *pstA*, *pstC*, *pstB* (phosphate-specific transport systems) for phosphorus transport and uptake, *glpQ* (glycerophosphoryldiester phosphodiesterase) etc. (Rodríguez et al., 2006, Hsieh and Wanner, 2010, Richardson and Simpson, 2011). Microbes secrete organic acids (pyruvic acid,

gluconic acid etc.) that facilitate the solubilization of phosphate minerals and these organisms are termed phosphate solubilizing bacteria or fungi. The generated acids are derived from organic carbon compounds via the tricarboxylic acid cycle and there is a close relation between carbon utilization by microbes and phosphorus availability. Thus phosphate dynamics influence the distributions and diversities of phosphate solubilizing microorganisms in the soil (Patel et al., 2008, Oubrie et al., 1999, Zeng et al., 2016b, Mander et al., 2012). Phosphorus exists in soil in the form of phosphodiesters, phosphomonoesters or phytates (in the organic forms) and as metallophosphates in the insoluble inorganic forms (Shen et al., 2011a).

Application of fertilizers, either organic or inorganic, has a direct influence on the microbial community present in the soil through the nutrients it provides, or indirectly via the adjustment of the soil physicochemical properties like the pH. These changes in soil nitrogen and organic carbon contribute to the dynamics in the activities of soil microbes and nutrient cycling processes and to a greater extent influence the microbial gene expression (Zhang et al., 2017, Marschner et al., 2003). This agricultural associated process causes perturbation and changes the diversity, function and distribution of microbes in the agroecosystem. Hence, organic fertilizer boosts the abundance, activities and functions of soil dwelling microbes, unlike inorganic fertilizers whose effectiveness is little (Zhu et al., 2017, Zhang et al., 2012, Olanrewaju et al., 2019).

In this study, we investigated the effect of soil fertilization on the distribution and abundance of phosphorus cycling genes at the rhizosphere of maize plants using shotgun metagenomics. The objectives of the study are (i) to examine how fertilization with compost and inorganic fertilizers affect the distribution and abundance of phosphorus cycling genes within the maize rhizosphere, and (ii) to determine if the untreated control could have an equal effect on the abundance and distribution of these genes within the rhizosphere.

9.2 Experimental procedure

9.2.1 Samples collection and microbial DNA extraction

Soil samples were collected from North-West University's agricultural farm, situated in Mafikeng, North West Province of South Africa. The geo-coordinate of the sampling site are described in chapter 5. The soil type is sandy loam soil. A sampling distance of 15 cm (minimum) and 50 cm (maximum) in 3 sampling spots/plot were used. The chemical composition of the compost manure are N = 20045.3 (g/kg), P = (1.0 g/kg), K = 12.3 (g/kg), pH = 7.1. The age of the manure compost or stabilization period of the compost prior to its use was 16 weeks. The experimental methods and procedure for sample collection were described elsewhere in chapter 5. And the result for soil physicochemical analysis were contained in Table 5.1. The collected samples were put in a sterile plastic bag inside a box containing ice and transported to the laboratory. Plant and root debris were sieved out using a sieve with 2 mm pore size and the samples were stored at -20ºC for metagenomic shotgun analysis. The physicochemical properties of the soil before planting and fertilization as well as the compost manure after the stabilization period of 16 weeks were analysed according to the standard basic soil chemical analysis protocols described by Motsara and Roy (2008). This was followed by extraction of total microbial community DNA from the rhizospheric soil samples with PowerSoil DNA isolation kit from MoBio Laboratories, Incorporation in USA by following manufacturer's guide.

9.2.2 Library preparation and Sequencing of DNA

The community nucleic acid concentration extracted for the rhizosphere soil samples using PowerSoil kit were examined with the aid of Qubit® dsDNA HS Assay Kit from Life Technologies, Carlsbad, California, United States. And the deoxyribonucleic acid libraries were prepared by the use of Nextera DNA Flex library preparation kit (Illumina Incorporation.) in accordance with the procedure from the manufacturer. A total of 50 nanogram of the DNA molecules from each samples were taken for the libraries preparation. Then, this was followed by fragmentation alongside with adapter sequences addition. The adapter molecules were used during PCR cycles together with the addition of unique

indices into the samples. The final concentration of the libraries generated were quantified with Qubit® dsDNA HS Assay Kit from Life Technologies. The library's average sizes were determined with the use of analytical machine - Agilent 2100 Bioanalyzer (from Agilent Technologies). DNA libraries were combined together into an equalmolar ratios of 0.7 nM. The pooled DNA were then sequenced paired end for 300 cycles using the machine - NovaSeq 6000 system (Illumina). This was done at the Mr DNA molecular research laboratory in USA.

9.2.3 Metagenomic Sequence analysis

The raw metagenomic reads were uploaded to MG-RAST were quality control processes were performed on the reads (Meyer et al., 2008). The preprocessing of the reads involved the removal of artificial reads, host specific sequences and other ambiguous base pairs. This was followed by gene annotation using BLAT algorithm (Kent, 2002) and M5NR database (Wilke et al., 2012). The protein coding-genes annotation were executed by M5NR database and SEED Subsystem level – function and the bacterial families were generated through blasting the sequences on RefSeq database. The BlastX was used to perform hit at an e –value cutoff (10E-5), minimum alignment length (15 base pairs), and percentage identity (60%). The unannotated sequences were not subjected to further evaluation or analysis. Also applied was the MG-RAST normalization tool to enable us cut down on the possible experimental error. The phosphorus cycling genes were curated manually from the total gene file obtained from the SEED Subsystem database, level – function.

9.2.4 Statistical analysis

The sequences gotten were used for statistical analysis. The phosphorus cycling genes were evaluated statistically using one-way ANOVA - analysis of variance at p-value of less than 0.05. The abundance and distribution of bacterial families were visualized and analyzed using heatmapper (www1.heatmapper.ca/expression/). The online software - Circos was employed in plotting the chart of phosphorus cycling genes while the Evenness, Simpson and Shannon diversity indexes were determined for the rhizospheric samples and contrasted amongst the treatments using Kruskal-Wallis test. Also, the beta diversity was ascertained using PCoA on the basis of Euclidean distance-matrix

and ANOSIM (analysis of similarity) via 9999 permutations. These analysis were carried out with PAST version 3.20 software (Hammer et al. 2001) and Principal Co-ordinate Analysis and principal component analysis (PCA) using CANOCO 5v (Microcomputer Power, Ithaca, NY). The sequences are deposited on NCBI SRA database, SRA accession: PRJNA607213

9.3 Results

9.3.1 Treatments effects on the distribution and relative abundance of bacterial, percent fungal and archaeal communities

The bacterial families present in the samples using RefSeq database were *Bacillaceae* and *Carnobacteriaceae* which were very abundant in the higher inorganic fertilizer (N2) treated soil, while *Pseudonocardiaceae, Clostridiaceae, Cytophagaceae, Micromonosporaceae, Thermomonosporaceae, Nocardiopsaceae, Sphaerobacteraceae, Thermoactinomycetaceae, Planococcaceae, Intrasporangiaceae, Opitutaceae, Acidimicrobiaceae, Frankiaceae* were more abundant in compost treated soil (Cp8). *Micrococcaceae* and *Planctomycetaceae* were the most abundant in the untreated control. *Nocardioidaceae*, *Microbacteriaceae***,** *Mycobacteriaceae* and *Enterobacteriaceae* were the most abundant in lower inorganic fertilized soil (N1) and *Porphyromonadaceae* and *Flavobacteriaceae* was highly abundant in lower compost treated sample (Cp4) (Figure 9.1). There was a significant difference in the relative abundance of these bacterial families ($p = 8.381 \times 10^{-11}$) within the fertilized and unfertilized maize rhizosphere soil samples.

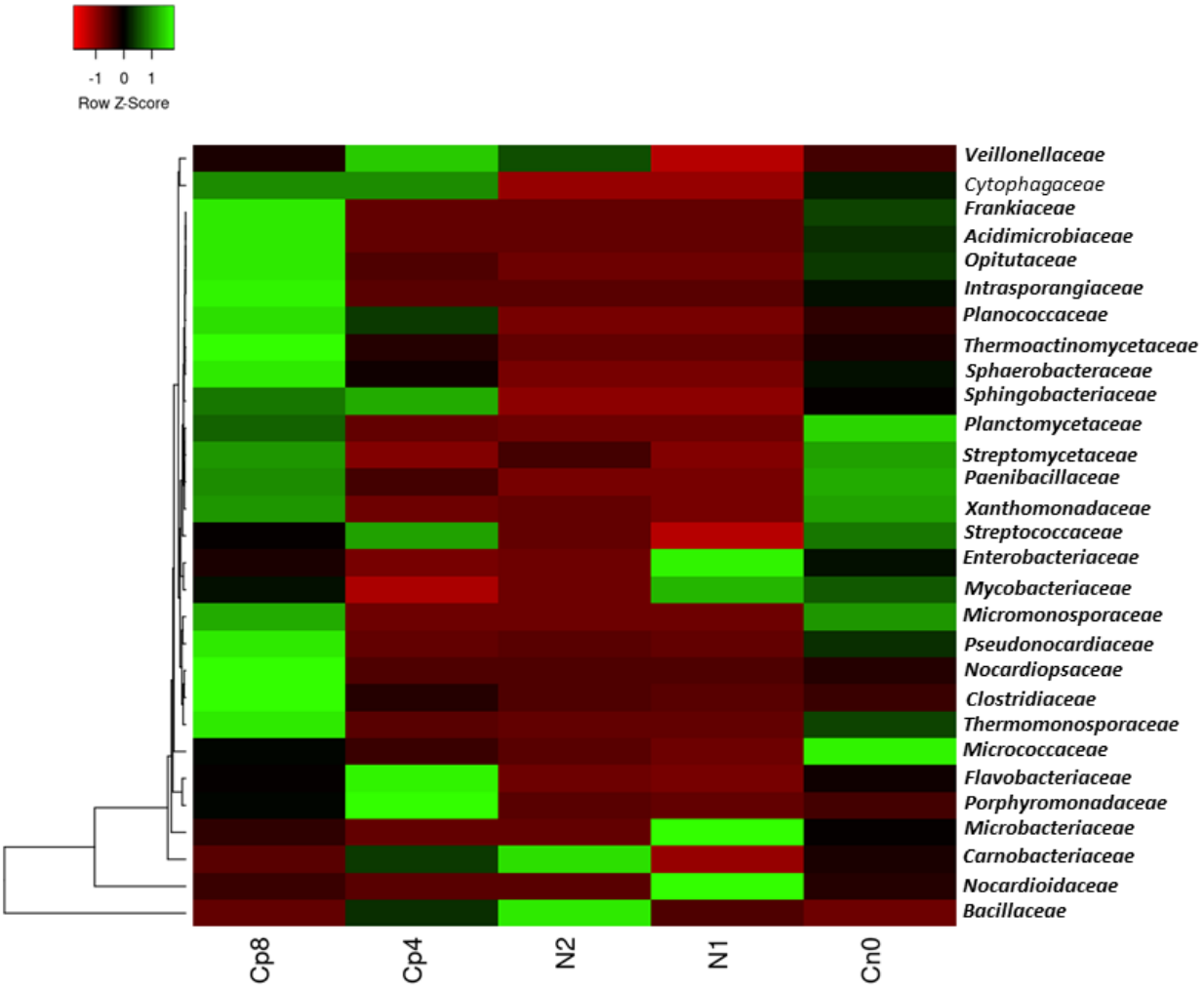


Figure 9. 1: Relative abundance of dominant bacteria families in the maize rhizosphere samples under fertilization and unfertilized soil treatments Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer)

At genus level, the percentage of *Pyrenophora*, *Talaromyces*, and *Trichophyton* fungi were observed to be more in 8 tons/ha compost manure (Cp8), while, *Fusarium* was dominant in 120 kg/ha inorganic fertilizer (N2) treated soil. *Ajellomyces* is the most abundant fungi in the 4 tons/ha compost manure (Cp4) treated soil and *Penicillium* occur more in 60 kg/ha inorganic fertilizer (N1) samples (Figure 9.2). A significant difference of 9.2×10^{-5} (P - value) was observed within the treatments and the control soil samples.

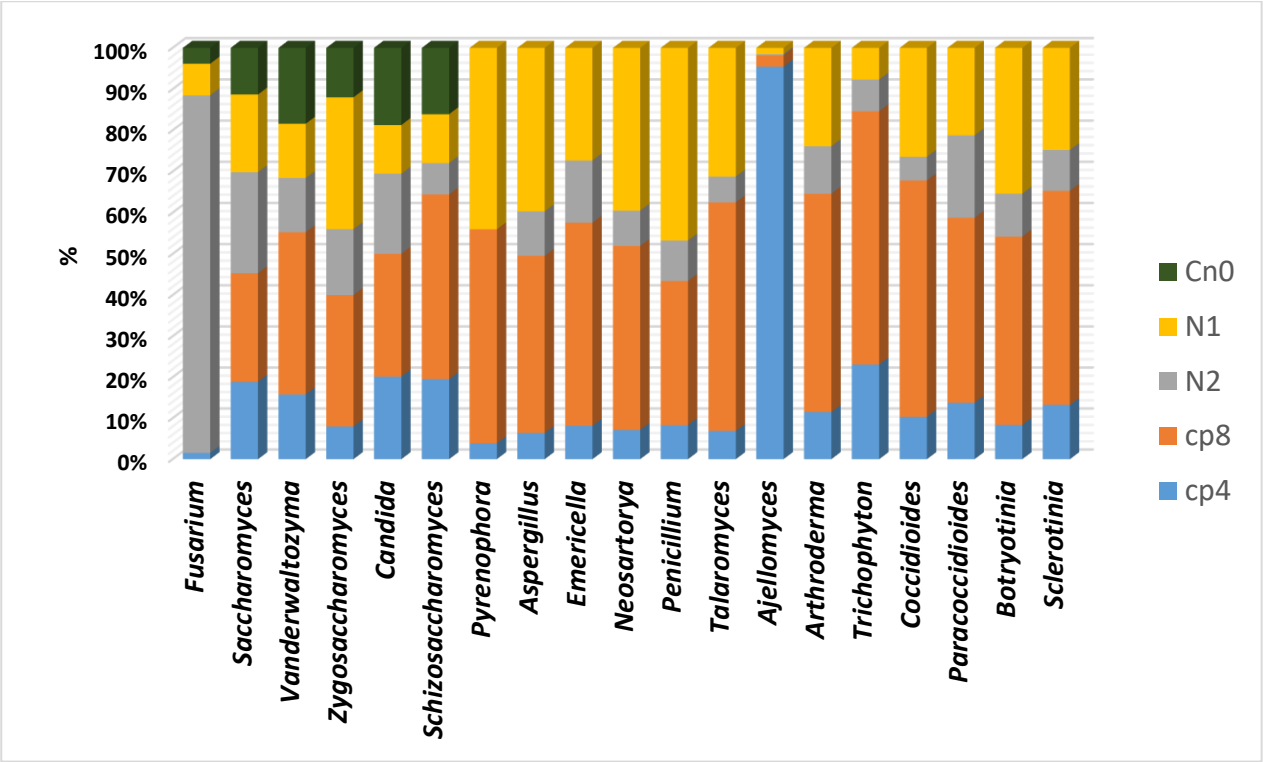


Figure 9. 2: Percent fungal community at genus level present in the maize rhizosphere under fertilization treatments and the control samples. Cn0 (control), N1 (60 kg/ha NPK), N2 (120 kg/ha NPK), Cp8 (8 tons/ha compost) and Cp4 (4 tons/ha compost manure)

However, within the archaeal community present in the soil, *Methanosarcina*, *Methanobrevibacter*, *Methanoculleus*, and *Methanosphaera* have the highest percentage occurrence in Cp8 (8 tons/ha compost). Whereas, at 120 kg/ha inorganic fertilizer (N2) treatment, *Cenarchaeum*, *Candidatus Nitrososphaera* and *Nitrosopumilus* were most abundant in the treated rhizosphere soils. *Haladaptatus* and *Haloarcula* were dominant in 60 kg/ha inorganic fertilized rhizospheric soil (N1). The control sample (Cn0) has *Haloferax* as the most predominant archaea present in the rhizosphere soil of maize. And there was a significant difference in the percent archaeal genus ($P = 3.8 \times 10^{-5}$) within the inorganic fertilizer, compost manure and the control soil samples (Figure 9.3). The number of reads obtained after filtering and the average read length for the phosphorus cycling genes as well as other genes reported are contained in Table S6.1.

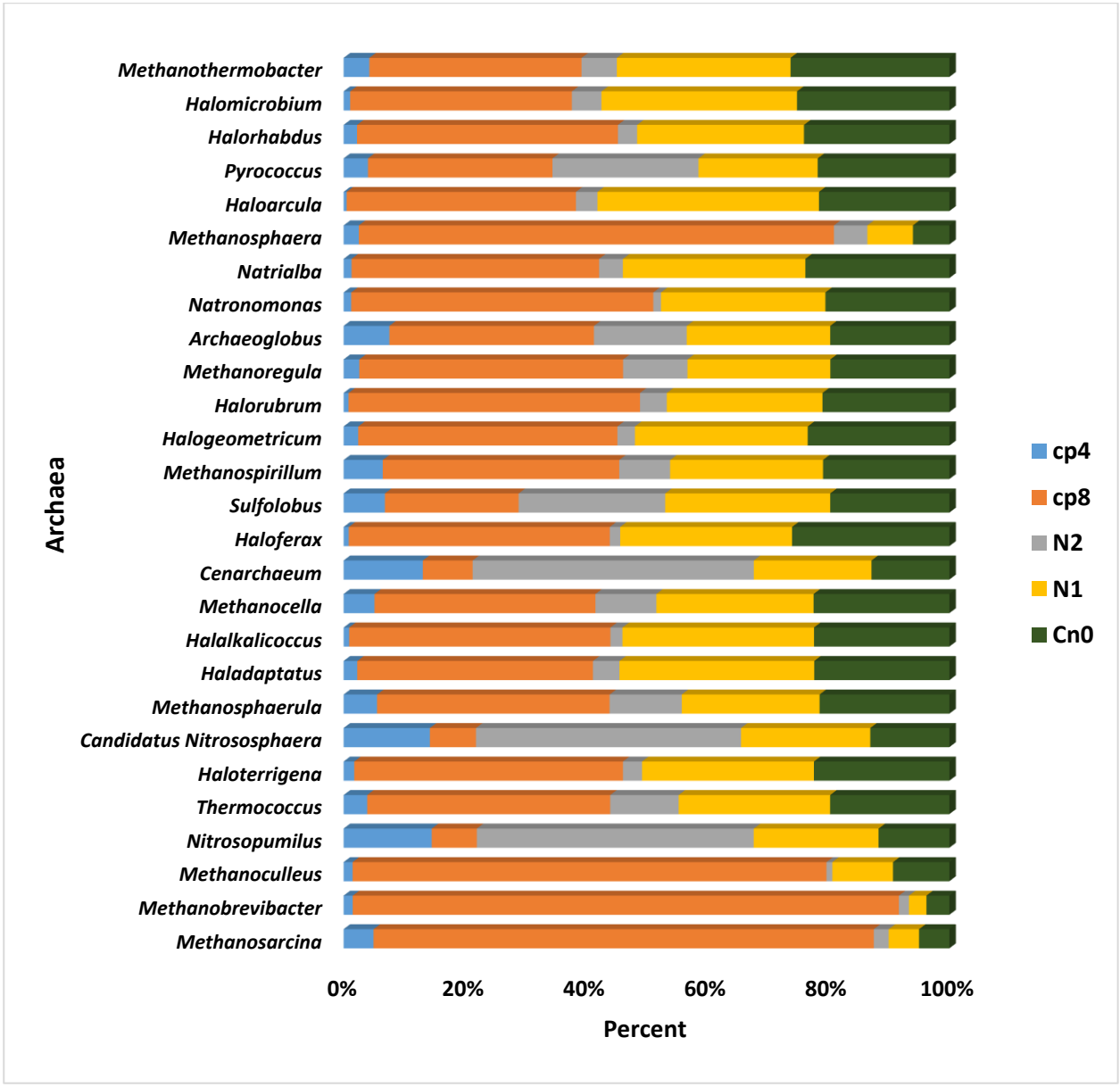


Figure 9. 3: Percent archaeal community at genus level present in the maize rhizosphere soil under fertilized and unfertilized treatments. Cn0 (control), N1 (60 kg/ha NPK), N2 (120 kg/ha NPK), Cp8 (8 tons/ha compost) and Cp4 (4 tons/ha compost manure)

9.3.2 Effects of the treatments on the relative abundance of phosphorus cycling genes

Phosphorus cycling genes abundance differed significantly ($P < 0.05$) among the treatments (Table 9.1). According to Bergkemper et al., (2016), phosphorus cycling genes can be categorized as follows: inorganic phosphate solubilizing genes, phytases, phosphoesterase, phosphonate degradation, phosphate transporters and phosphate starvation regulation genes. The genes that code for the enzymes: inorganic phosphate solubilization were the most abundant in the high compost (Cp8), low

inorganic fertilizer treatments (N1), and control (Cn0) respectively and the least were present in high inorganic fertilized (N2) and low compost manure treated rhizosphere soil (Cp4). Polyphosphate kinase (*ppk*), phosphate transporter coding genes (*pstS, pstC, pstB and pstA*), triosephosphate isomerase (*tpiA*), quinoprotein glucose dehydrogenase (*gcd*), alkaline phosphatase (*phoD*) and phosphate regulon response regulator (for phosphorus starvation regulation) (*PhoB*) were most abundant in the Cp8, N1, and Cn0 treatments. The abundance of phosphorus cycling genes at the rhizosphere of maize plants under fertilization and unfertilized conditions were highly significant ($P < 0.05$). The gene *ugpQ* (glycerophosphoryl diester phosphodiesterase) was abundant in the treatments Cp8, N1, Cn0, and N2, but least abundant in Cp4, implying that the enzyme possesses a high capability for phosphorus mineralization at the maize rhizosphere under fertilization and unfertilized conditions. The microbial enzyme phosphatases, which initiate catalytic hydrolysis of phosphorus to orthophosphate, a form that plants can assimilate, are richly abundant in the rhizosphere soil samples (Cp8, N1, and Cn0) (Figure 9.4).

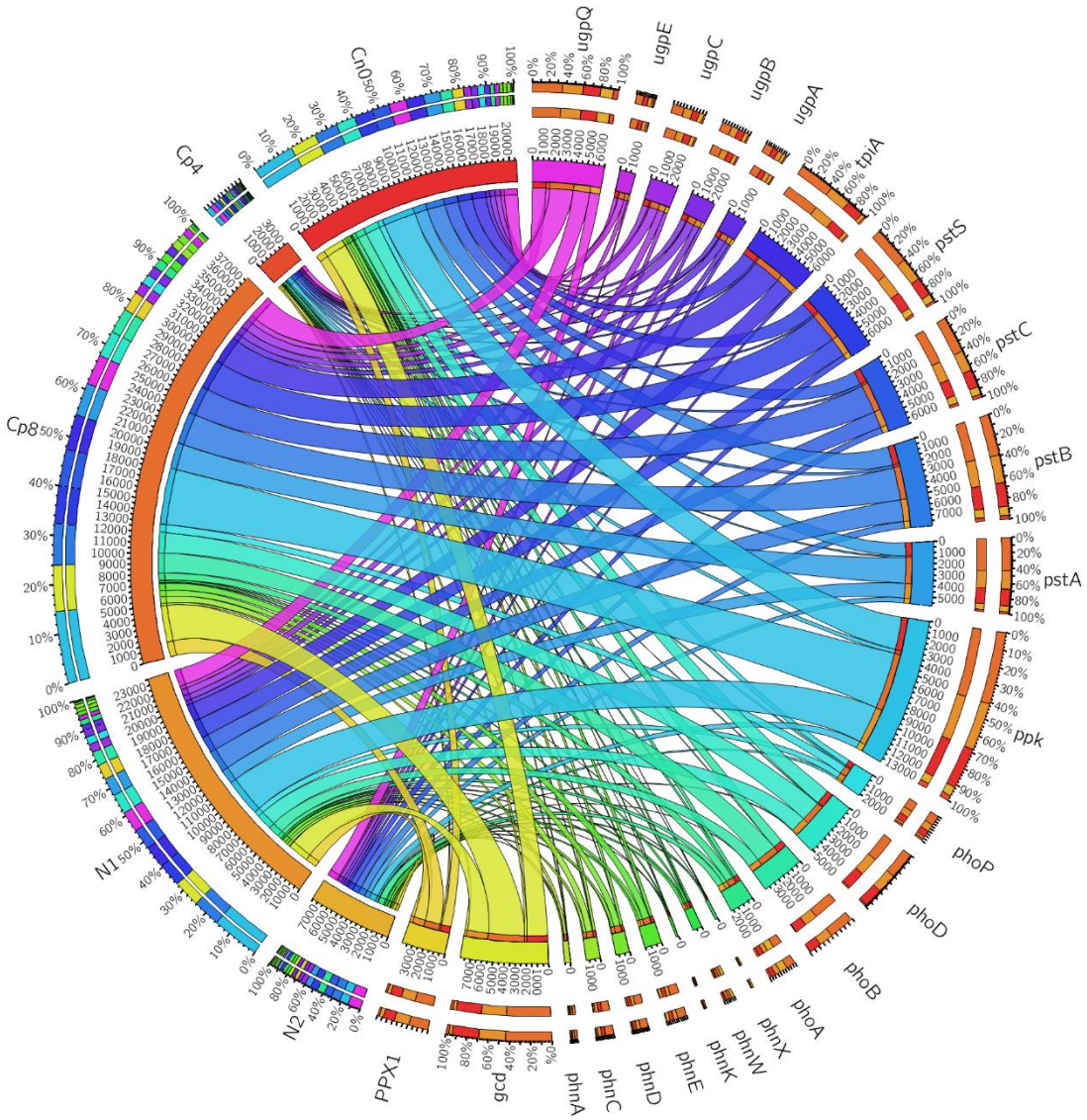


Figure 9. 4: Phosphorous cycling genes in the rhizosphere soil samples from maize plants under fertilization and unfertilized conditions as visualized by circus Cn0 (control), Cp8 (8 tons/ha compost manure), Cp4 (4 tons/ha compost manure), N2 (120 kg/ha inorganic fertilizer) and N1 (60 kg/ha inorganic fertilizer).

The genes depicted as follows: (*phoA*) alkaline phosphatase, (*phoB*) two-component system, OmpR family, phosphate regulon response regulator PhoB, (*phoD*) alkaline phosphatase D, (*phoP*) two-component system, OmpR family, alkaline phosphatase synthesis response regulator PhoP, (*tpiA*) triosephosphate isomerase (TIM), (*pstS*) phosphate transport system substrate-binding protein, (*pstC*) phosphate transport system permease protein, (*pstA*) phosphate transport system permease protein, (*ugpQ*) glycerophosphoryl diester phosphodiesterase, (*phnE*) phosphonate transport system permease

protein, (*phnC*) phosphonate transport system ATP-binding protein, (*phnD*) phosphonate transport system substrate-binding protein, (*phnA*) phosphonoacetate hydrolase, (*phnW*) 2-aminoethylphosphonate-pyruvate transaminase, (*phnX*) phosphonoacetaldehyde hydrolase, (*PPX1*) exopolyphosphatase, (*ppk*) polyphosphate kinase, (*gcd*) quinoprotein glucose dehydrogenase, (*pstB*) phosphate transport system ATP-binding protein, (*ugpA*) sn-glycerol 3-phosphate transport system permease protein, (*ugpB*) sn-glycerol 3-phosphate transport system substrate-binding protein, (*ugpC*) sn-glycerol 3-phosphate transport system ATP-binding protein, (*phnK*) putative phosphonate transport system ATP-binding protein, (*ugpE*) sn-glycerol 3-phosphate transport system permease protein.

The PCA (principal component analysis) of the phosphorus cycling genes shows that most of the genes were clustered around treatment Cp8, N1 and Cn0 (Figure 9.5) validating the observation on the abundance distribution of these genes in Figure 9.4. The alpha diversity of the phosphorus cycling genes within the treatments were shown by the Shannon, Simpson and evenness diversity indices. These indices clearly show that there exist a significant difference (Kruskal Wallis, $P = 9.3 \times 10^{-9}$) in the phosphorus genes alpha diversity (Table 9.1). Analysis of similarities show that there was a significant difference in the beta diversity (ANOSIM, P = 0.01; R value = 0.55) and it is depicted by the principal coordinate analysis, PCoA (Figure 9.6).

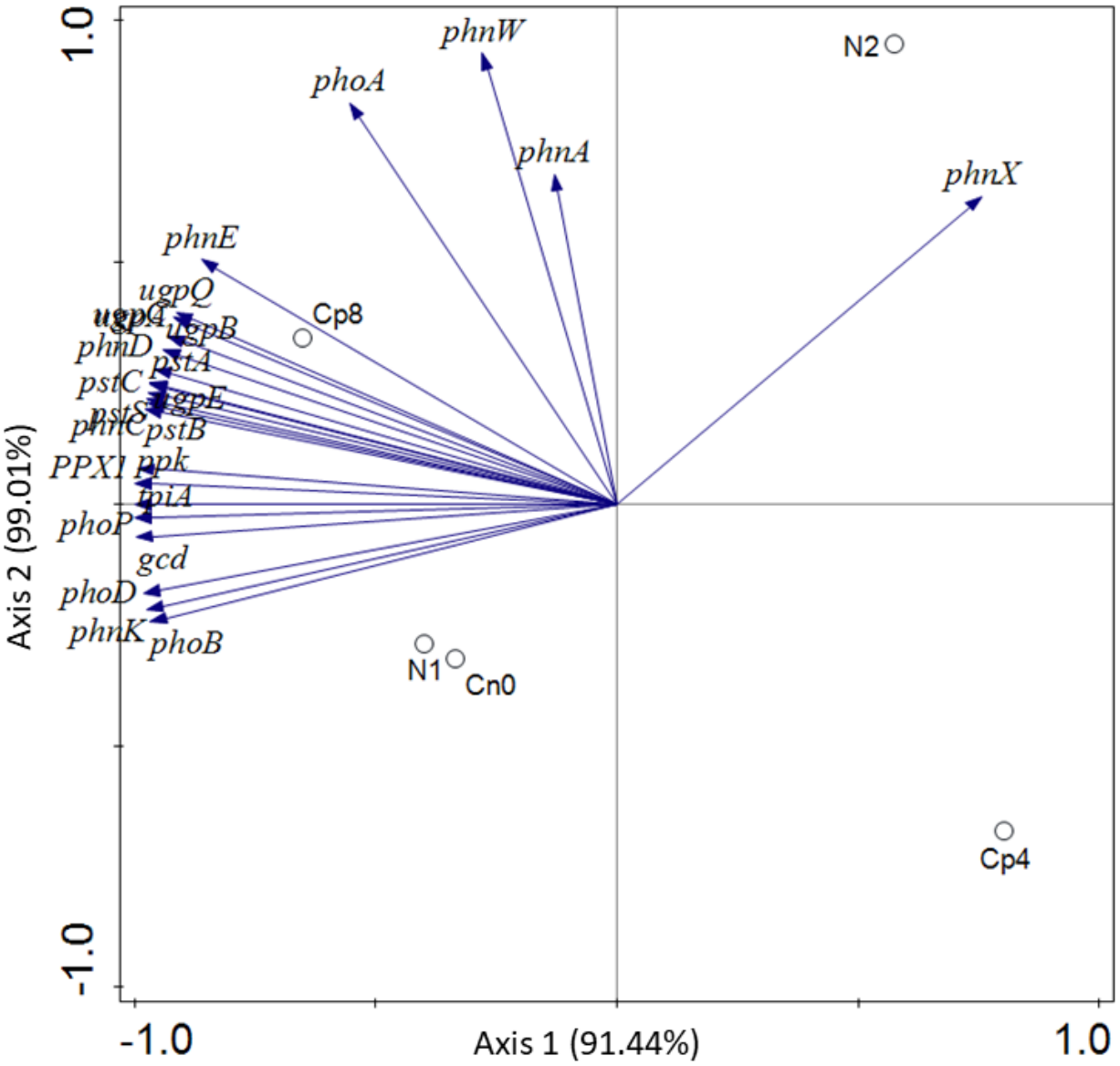


Figure 9. 5: Principal component analysis of the phosphorus cycling genes under fertilization and control treatments within the rhizosphere of maize plants. Cn0 (control), N1 (60 kg/ha NPK), N2 (120 kg/ha NPK), Cp8 (8 tons/ha compost) and Cp4 (4 tons/ha compost manure)

Table 9. 1: Diversity indices of the phosphorus cycling genes within the rhizosphere of maize plants under treatments

Diversity Indices	Cp8	Cp4	N2	N1	Cn0	P value
Simpson_1-D	0.9278	0.9375	0.9338	0.9259	0.9266	9.3×10^{-9}
Shannon_H	2.82	2.933	2.885	2.798	2.811	
Evenness_eH/S	0.699	0.7827	0.7458	0.6836	0.693	

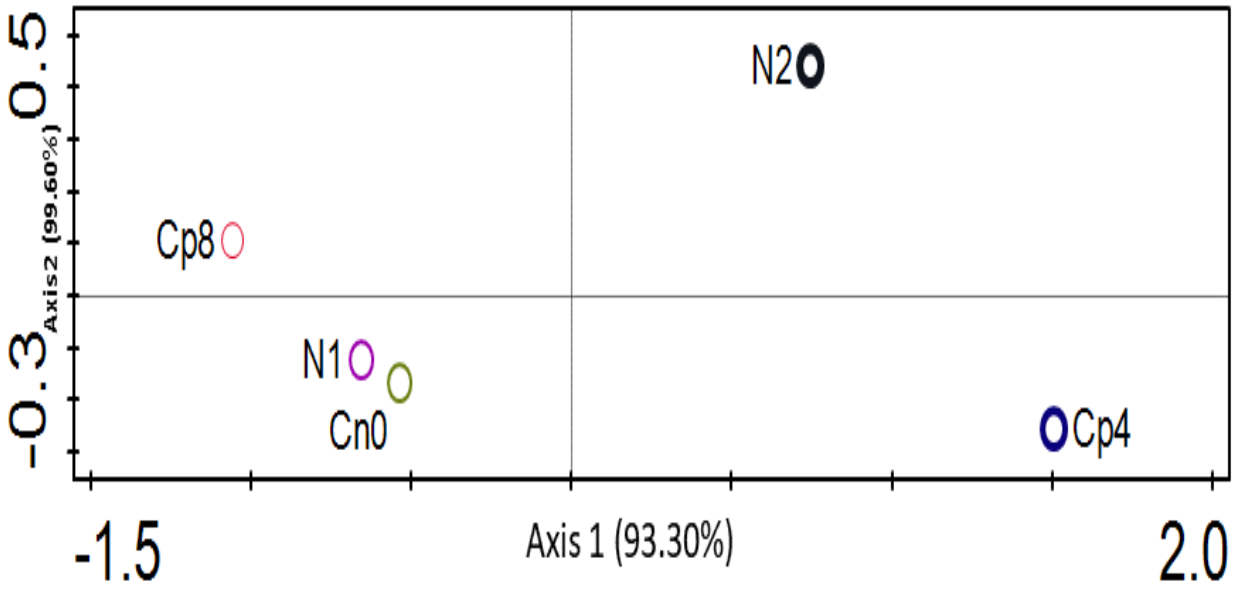


Figure 9. 6: Principal coordinate analysis of the phosphorus cycling genes present at the rhizosphere of maize plants under fertilization and control treatments. Cn0 (control), N1 (60 kg/ha NPK), N2 (120 kg/ha NPK), Cp8 (8 tons/ha compost) and Cp4 (4 tons/ha compost manure)

9.4 Discussion

Microbes in the agricultural soil play an active role in mineralization, assimilation and solubilization of phosphorus containing compounds. In our metagenomics study, we found an abundance of bacteria sequences belonging to *Frankiaceae, Thermoactinomycetaceae, Streptomycetaceae, Paenibacillaceae, Enterobacteriaceae, Norcardioidaceae* amongst others (Figure 9.1), which are plant growth promoting microbes with lots of beneficial functions (Hayat et al., 2010). They possess genes involved in plant growth promotion, of which phosphorus cycling genes are essential components of these genes pool contained in the genome of these organisms. Fungi and archaea equally bear these genes and are among the key players in the biogeochemical cycling of phosphorus. Phosphorus exists in the soil in organic forms as phospholipids, phosphomonoesters, phytates and

phosphodiesters (Condron et al., 2005, Read et al., 2003, Stutter et al., 2012, Tan et al., 2013, Alori et al., 2017b).

Fertilization of soil with organic and inorganic fertilizers has been shown to influence the abundance and distribution of phosphorus cycling genes within the maize rhizosphere. The obtained metagenomes from the compost treated (Cp8 and Cp4), inorganic fertilized (N2 and N1) and control (Cn0) samples have genes encoding for phosphoesterase, inorganic phosphate solubilization, phosphate transport, degradation of phosphonate and starvation sensitive phosphate regulation genes. Our study showed that the genes involved in phosphorus cycling at the maize rhizosphere were in high abundance. Genes implicated in inorganic phosphate solubilization were also high and this indicates that the microbes present at the rhizosphere have the capacity to utilize inorganic phosphorus. Alkaline phosphatase (*phoD*) was positively increased by the compost (high dose), which is in agreement with Fraser et al., (2015) as well as in inorganic fertilizer (low dose), and the control.

However, the principle of nitrogen – phosphorus ratio stoichiometry, which states that increase in nitrogen addition enhances microbial inorganic phosphorus demand (Xiao et al., 2018), was observed in our study to an extent, especially at the low inorganic fertilizer dose (N1 – 60kg N/ha) which increased the abundance of the phosphorus cycling genes, but did not hold true at the very high dose of 120 kg N/ha (N2) treatment. The only gene increased was *ugpQ* (glycerolphosphoryl diester phosphodiesterase), encoding an alkaline phosphatase capable of catalyzing the breakdown of phospholipid (glycerolphosphodiesters) to generate glycerol 3 phosphate and alcohol (Brzoska and Boos, 1988). High dose of inorganic fertilizer does have repressive effects on the abundance of phosphate starvation regulation genes, phosphodiesterase, phosphonate degradation, inorganic phosphate solubilization and phosphorus transport genes (Figure 9.2). This implies that assimilation, solubilization, transformation and transport of phosphorus were impaired by high dose of inorganic nitrogen fertilizer, despite a slight enhancement on the abundance of *ugpQ* genes. This observation could be as a result of inorganic fertilizer associated acidification effects that suppress the viability

and activities of the rhizosphere microbial community (Schroder et al., 2011). To enhance organic phosphorus mineralization by microbes, inorganic nitrogen fertilizer application should be at a quantity suitable to enhance the microbial extraction of phosphorus from the organic compounds (Heuck et al., 2015). Surprisingly, low dose of compost manure have the same repressive effects on the phosphorus cycling genes as do the high dose of inorganic nitrogen fertilizer, therefore further investigation is needed to understand the rationale behind this observation.

Our study also demonstrated that the most abundant phosphorus cycling gene present at the maize rhizosphere under compost (Cp8), inorganic fertilizer (N1) and control (Cn0) treatments is *ppk* (polyphosphate kinase) which catalyzes the polymerization of phosphorus monomers to generate polyphosphate molecules. Polyphosphate molecules serve as energy reservoirs in microbes for biochemical processes involving phosphorylation of biomolecules like sugars, proteins, nucleic acid, proteins, and enhance their survival and growth in the phosphorus scarce environment (Holden, 2015, Rao et al., 2009). The formation of biofilm, sequestration of cations, expression of genes and signaling are among the biological roles of polyphosphate molecules in a microbial cell (Toso et al., 2011). The second most abundant gene family is *pstSBAC* (the high-affinity-phosphate transporters) which facilitate the assimilation of phosphorus from the soil. There is a relationship between the polyphosphate kinase genes abundance and the transporters. For polyphosphate to be formed, phosphate transporters must enhance the acquisition of these phosphate molecules from the environment. Therefore, high abundance of *ppk* genes and *pstSBAC* signifies that there was high microbial capacity for the assimilation of phosphorus in the treated maize rhizosphere. At high inorganic fertilization, the genes implicated in transport, uptake and solubilization of phosphorus were decreased, which is in agreement with the works of Bergkemper et al., (2016) and Ikoyi et al., (2018).

Also, *gcd* (quinoprotein glucose dehydrogenase) was increased in abundance by the high compost, low inorganic nitrogen fertilizer and the control treatments. The enzyme synthesized by this gene is paramount in inorganic phosphate metabolism due to its catalytic conversion of glucose molecules to

gluconic acid using a prosthetic group cofactor, pyrroloquinoline quinone (Sashidhar and Podile, 2010, Khan et al., 2007), thereby regulating as well as enhancing the solubilization of trapped inorganic phosphorus in the soil.

9.5 Conclusion

In summary, soil fertilization with both organic manure (compost derived from domestic waste and plant materials), and low quantities of inorganic nitrogen fertilizer have the same effects as maize plants in promoting the abundance of genes involved in the phosphorus cycle. Genes such as *ppk* involved in polyphosphate formation and *pstSABC* (for phosphate transportation across the cell membrane) are highly enriched in these treatments. These genes facilitate phosphorus assimilation and mobilization. At high dose of inorganic fertilizer application and low compost manure treatments, the phosphorus cycling genes were repressed and their abundance decreased. Our study has brought to light the basis for the manipulation of the rhizosphere microbial communities and their genes to improve availability of phosphorus and in phosphorus cycle regulation in agro-ecosystems.

CHAPTER TEN

Summary and Conclusion

10.1 Final Remarks

Farming is the sure way of increasing food supply and meeting human nutritional needs. The increase in human population has necessitated a corresponding increase in agricultural products output, thereby putting pressure on the existing limited arable land used for crop production. This pressure and perturbation includes soil tillage, continuous cultivation, fertilization, application of pesticides and lots more. Thus agricultural practices as well as the present climate change associated effects (drought) and biotic stresses are the major challenges militating against increase in crop yield. However, they also have enormous effects on the soil microbiomes and tend to affect the efficiency and efficacy of biogeochemical cycling of nutrients. Each of the above pressure inducing activities or conditions come with its own challenges in the achievement of an increased crop yield.

In this study, we evaluated the effects of soil fertilization on the microbial community structure, composition, diversity and function within the rhizosphere of maize plants growing in a tilled farmland under a semi-arid climatic condition. The fertilizers used were inorganic fertilizer (NPK) and a community-based compost manure derived from domestic wastes and plants materials. As clearly evidenced in our study, the fertilizer types and doses applied have significant effects on the rhizosphere microbial community structure, abundance and functions within the maize rhizosphere.

As observed in the study, fertilizing a maize planted soil with 8 tons per hectare compost manure and 60 kg/ha inorganic fertilizer have nearly the same enrichment effects on the bacterial communities in the soil as those of the rhizosphere of maize plants in the untreated control. High dose of inorganic fertilizer selected and enriched the archaeal community, while low compost manure treated rhizosphere soil promoted the dominance of fungi. Also, both 8 tons per hectare and 4 tons per hectare compost treatments enriched viruses in the maize rhizosphere. At the functional level, the dominant microbial functions were affected by the quantity of fertilizers used and not the type applied. High

compost, control as well as low inorganic fertilizers prevailed in promoting the abundance of microbial functions at the maize rhizosphere. Functions such as those involved in the carbon, nitrogen, and phosphorus cycles were supported by these fertilizer doses (high compost, and low inorganic fertilizer) as revealed by the increased abundance of their associated genes.

However, the basic functions involved in the production of antimicrobial substances such as antibiotics, antifungal, nematicides and siderophores as well as enhanced chemotaxis were also boosted by the applied fertilizers for low inorganic and high organic fertilized soils. This is evidenced by the increase in the genes involved in these basic agriculturally beneficial functions that facilitate the development of a disease suppressive soil. Maize plants, on the other hand, exert the same enrichment and selection effects on the structure, function and diversity of the rhizosphere microbes. Studies have shown that maize plants through rhizodeposition support the soil microbial communities and enhance their functions. Surprisingly, high inorganic fertilizer and low compost manure share the same observable reductive effects on the microbial communities present in the soil. Studies have also shown that inorganic fertilizer associated acidification and pH alteration effects interfere with the proper physiological activities of soil microbial communities. However, it is still very unclear how low organic fertilizer (compost manure at 4 tons/ha) could perform the suppressive action on the soil microbial communities at the maize rhizosphere, and thus further detailed study is required.

Based on our general observations, we recommend the co-application of high compost or organic manure with low inorganic fertilizer as an integrative soil nutrient enrichment approach or applying them individually. These nutrients when applied alone or together might synergistically increase soil health, promote microbial diversity and functions and also enhance the development of disease suppressive soil. They might also facilitate plant tolerance to biotic and abiotic stresses due to their effects on the soil microbiome. Therefore, since separate applications benefited greatly the soil microbes in our study, we propose that co-application might lead to the maximum benefits to the yield of plants and the viability of soil microbes. Hence excessive use of inorganic fertilizer should

be avoided to enable the establishment of a stable microbial community and enhance biodiversity in the agricultural soil.

APPENDIX

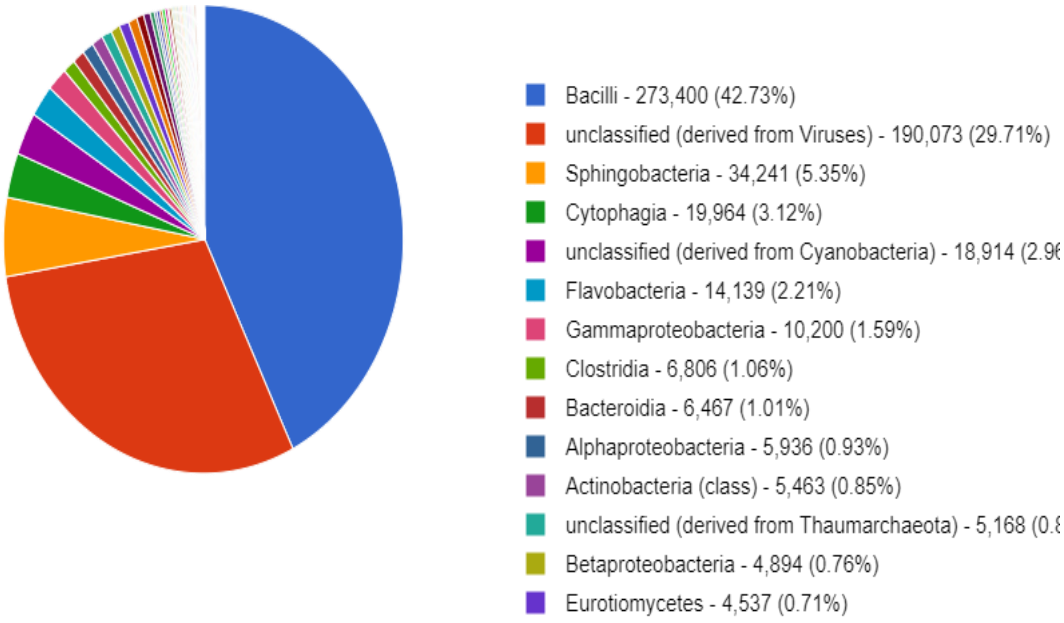
Supplementary

Table S5.1. Diversity indices of the bacterial genus from the rhizosphere soil samples of maize under different fertilization regime

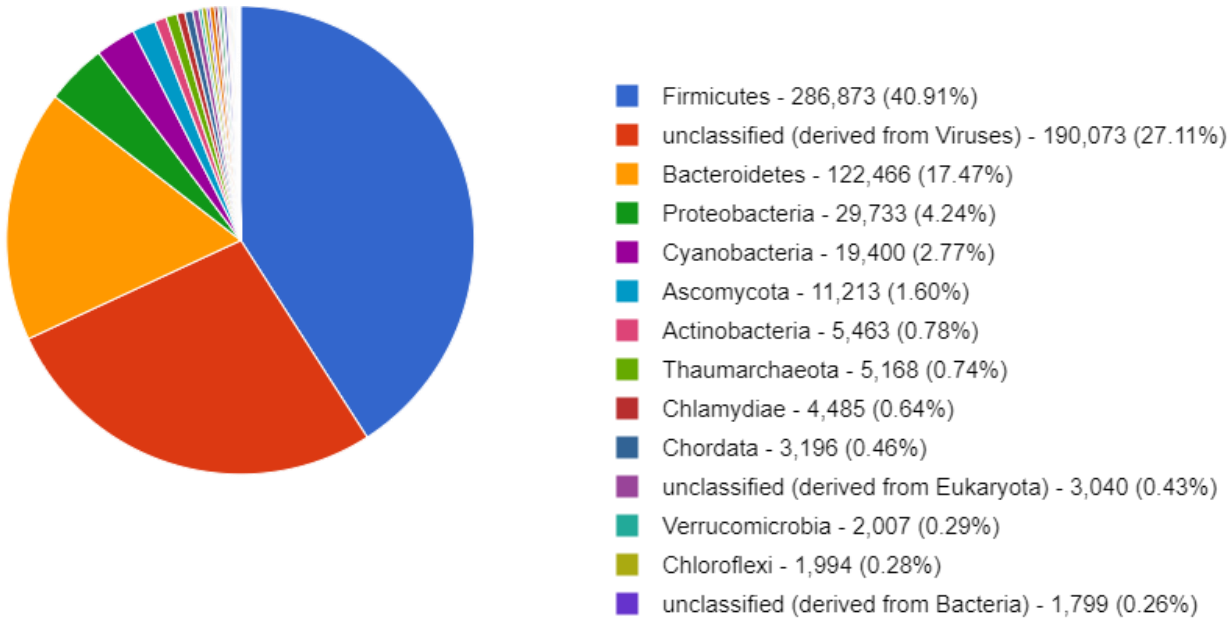
	Shannon_H	Evenness_e^H/S	Chao-1
Cp8	4.223	0.1399	631.2
Cp4	2.93	0.1283	239.3
N2	0.9972	0.1084	51
N1	1.668	0.4079	23.5
Cn0	3.928	0.1456	501.5

Table S6.1 Diversity and evenness estimate of the functional categories of rhizosphere soil samples of maize at Seed Subsystem 1

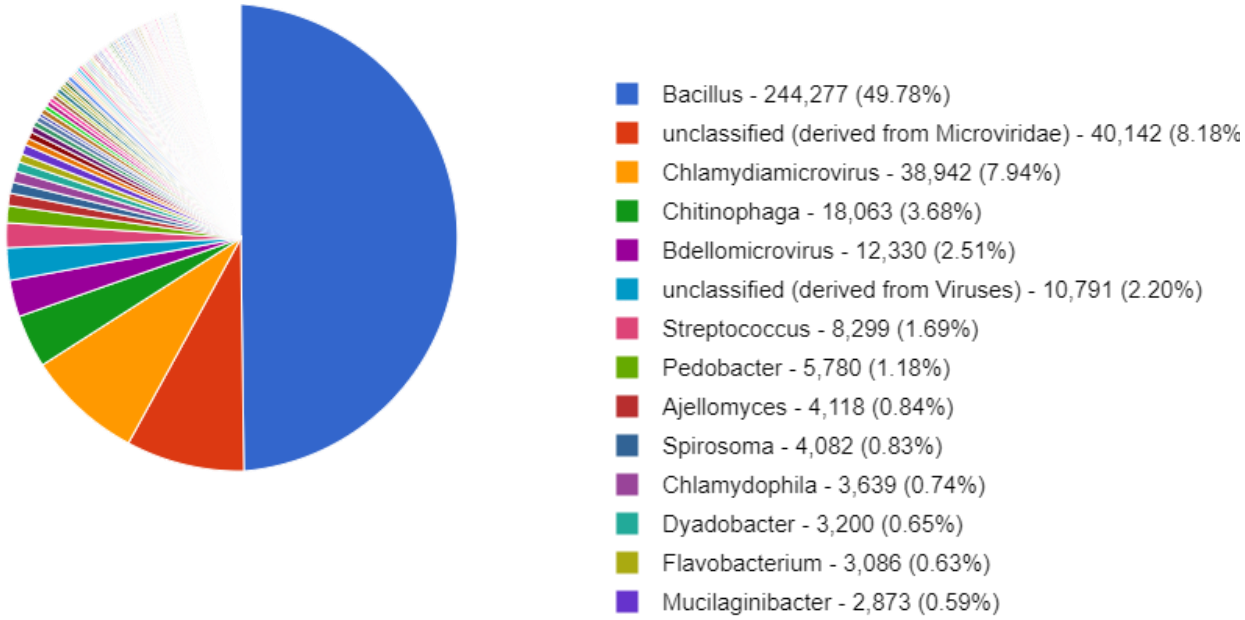
	Cp8	Cp4	N2	N1	Cn0	P-value
Shannon_H	1.123	1.176	1.177	1.06	1.109	0.99
Evenness_e^H/S	0.5126	0.5404	0.5408	0.4813	0.5052	



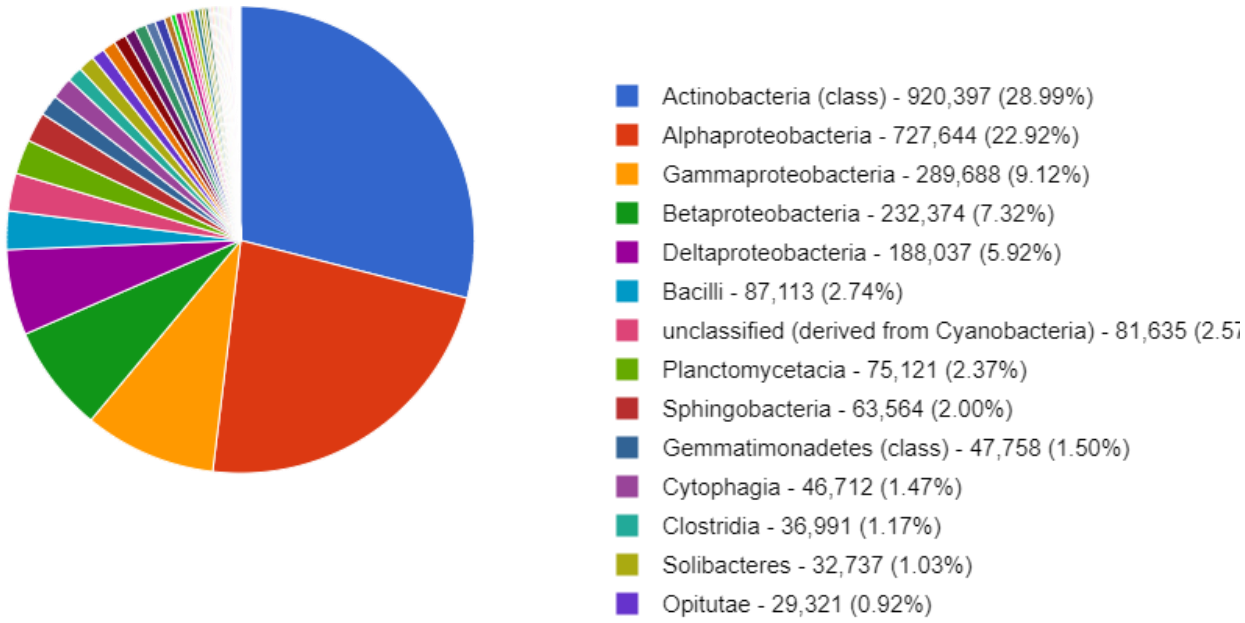
Taxonomic category class for Cp4 – compost 4 tons



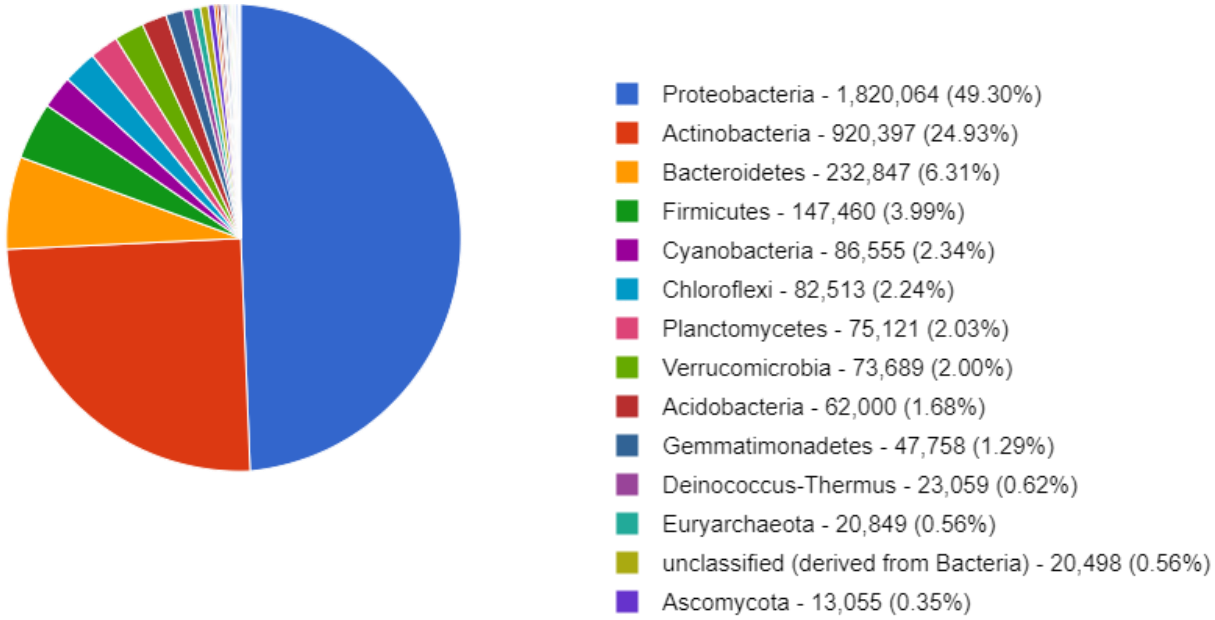
Taxonomic category phylum for Cp4 – compost 4 tons



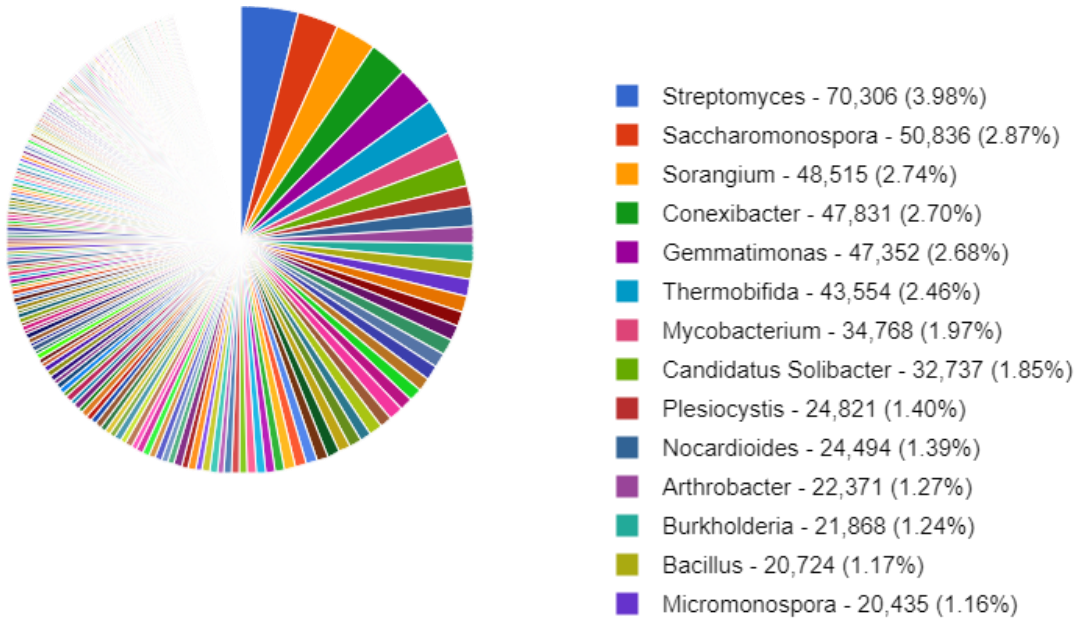
Taxonomic category genus for Cp4 – compost 4 tons



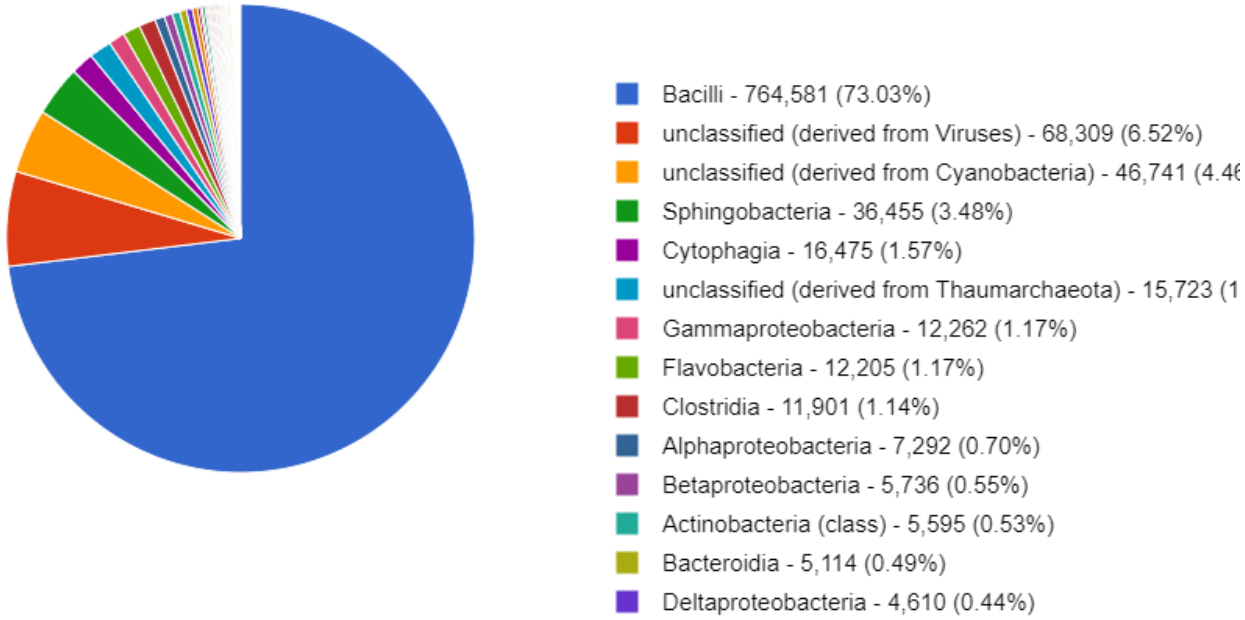
Taxonomic category class for Cp8 – compost 8 tons



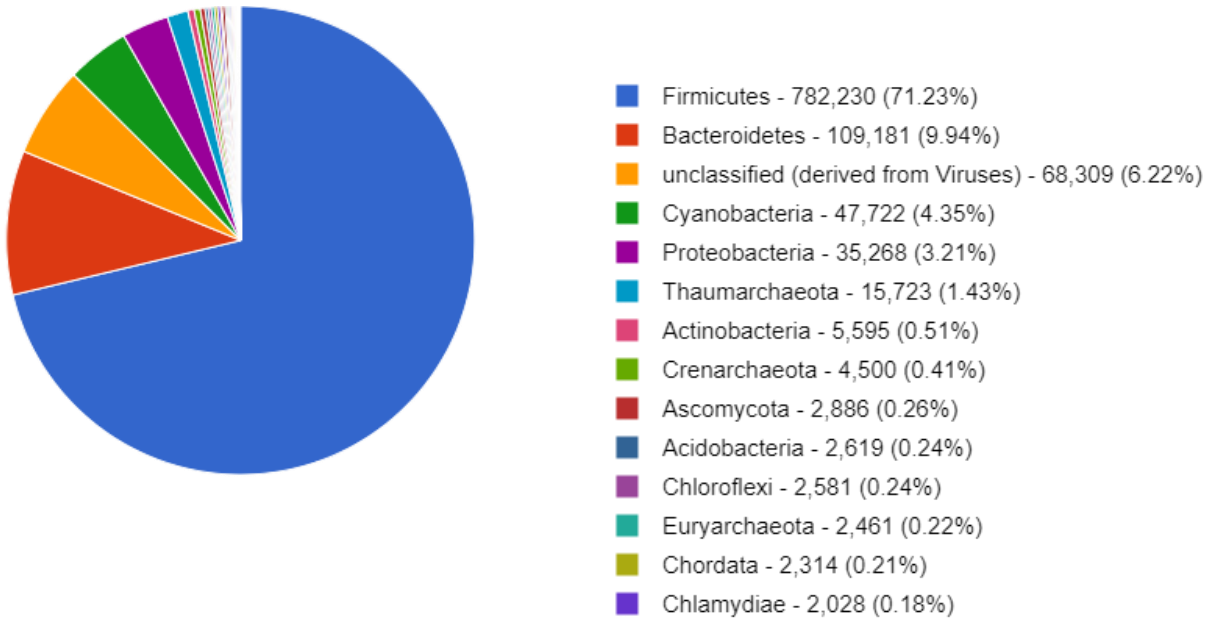
Taxonomic category phylum for Cp8 – compost 8 tons



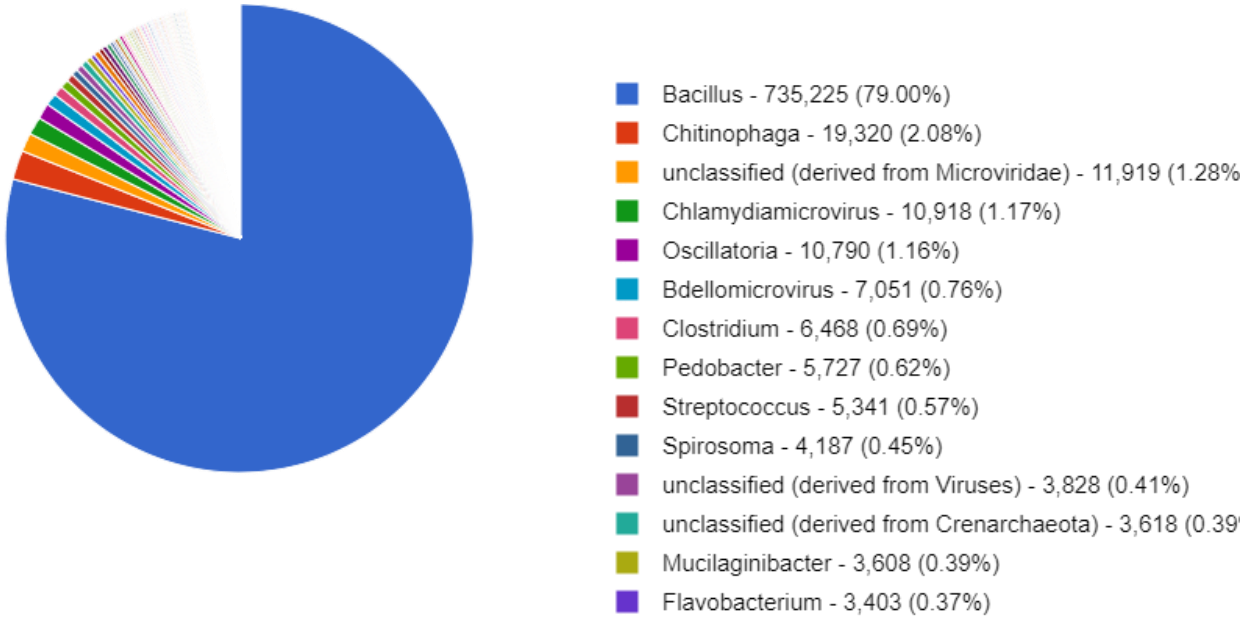
Taxonomic category genus for Cp8 – compost 8 tons



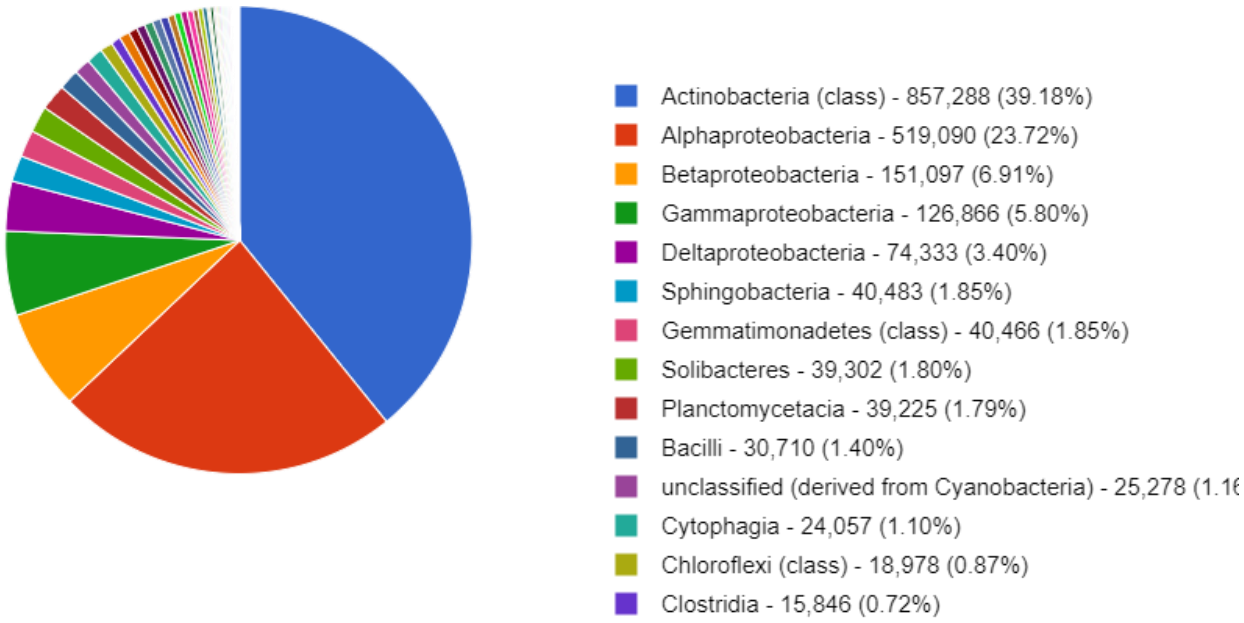
Taxonomic category class for N2 – 120kg N/ha



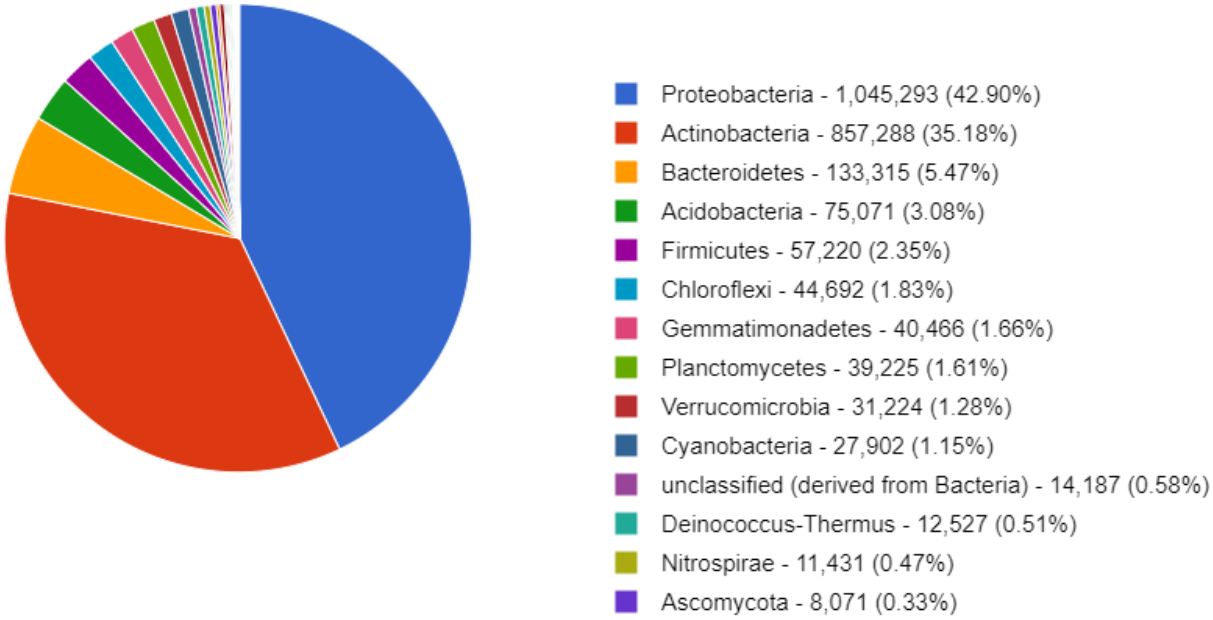
Taxonomic category phylum for N2 – 120kg N/ha



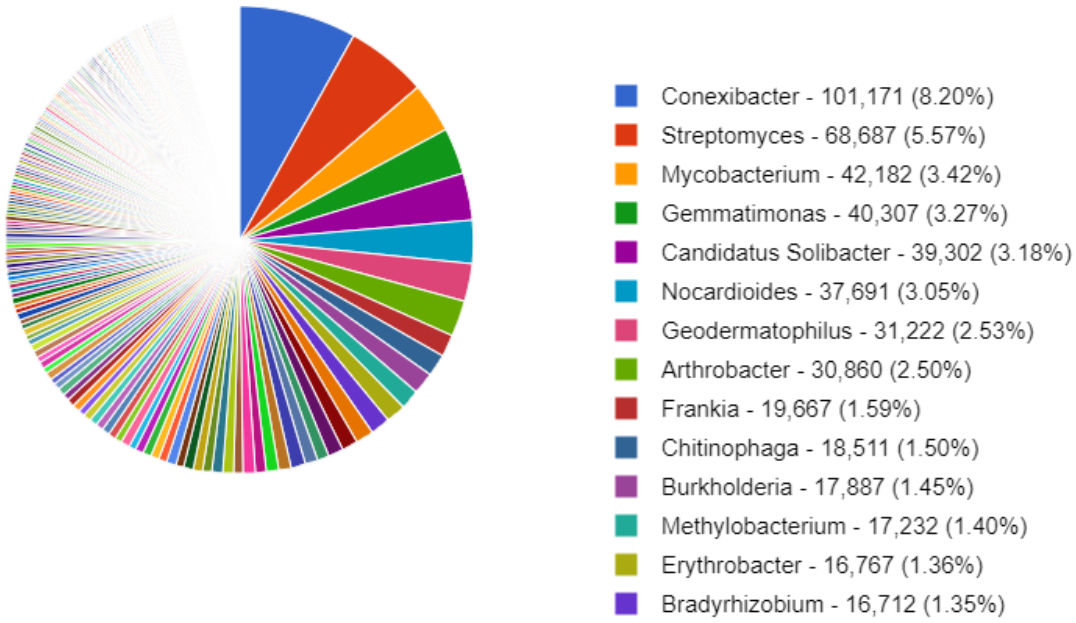
Taxonomic category genus for N2 – 120kg N/ha



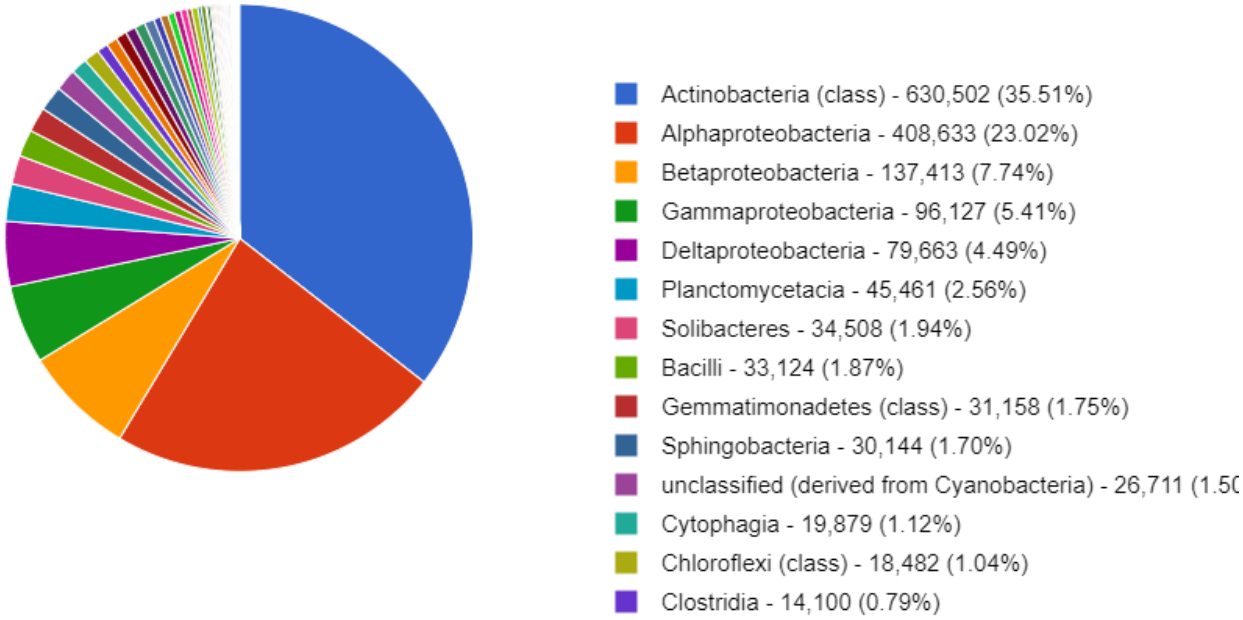
Taxonomic category class for N1 – 60kg N/ha



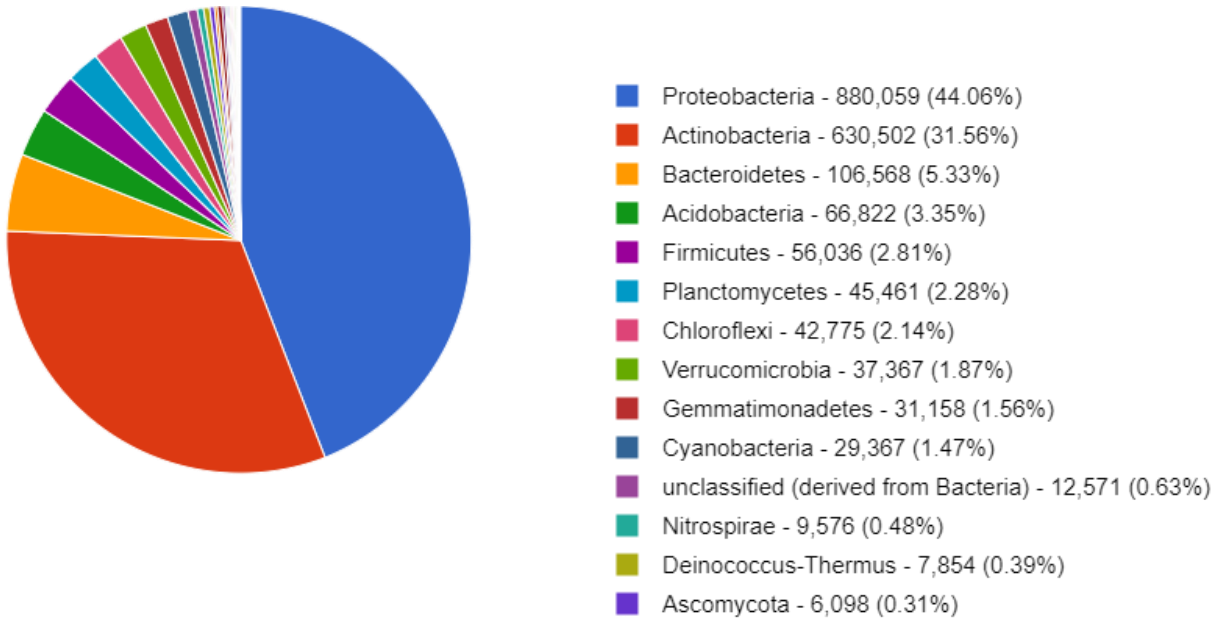
Taxonomic category phylum for N1 – 60kg N/ha



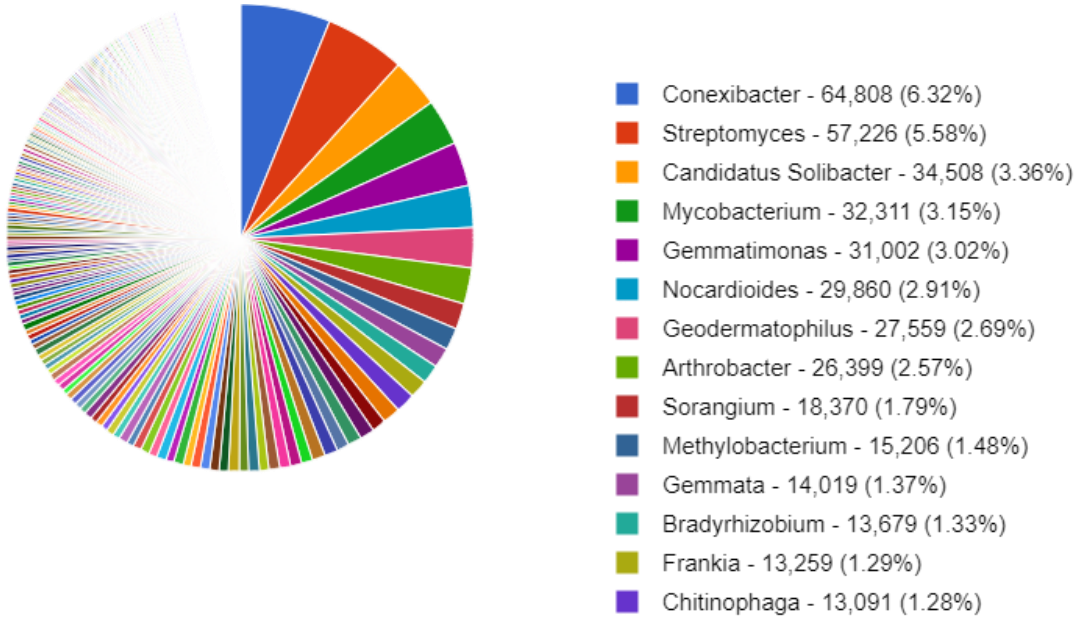
Taxonomic category genus for N1 – 60kg N/ha



Taxonomic category class for Cn0 – control



Taxonomic category phylum for Cn0 – control



Taxonomic category genus for Cn0 – control

Figure S5.1. Taxonomic categories at the class, phylum and genus across all the treatments

Table S6.1 Diversity and evenness estimate of the functional categories of rhizosphere soil samples of maize at Seed Subsystem 1

	Cp8	Cp4	N2	N1	Cn0	P-value
Shannon_H	1.123	1.176	1.177	1.06	1.109	0.99
Evenness_e^H/S	0.5126	0.5404	0.5408	0.4813	0.5052	

Table S7.1. Diversity index for nitrogen cycling genes present in the rhizosphere soil samples

	Cp8	Cp4	N2	N1	Cn0
Simpson_1-D	0.9189	0.9004	0.9017	0.8996	0.9044
Shannon_H	2.885	2.699	2.627	2.744	2.787
Evenness_e^H/S	0.6176	0.5124	0.4771	0.536	0.5598

Table S7.2. Diversity indices of carbon cycling genes

0	Cp8	Cp4	N2	N1	Cno
Simpson_1-D	0.9546	0.9558	0.9206	0.9569	0.9563
Shannon_H	3.27	3.322	3.007	3.327	3.317
Evenness_e^H/S	0.7307	0.7489	0.5617	0.7332	0.7258

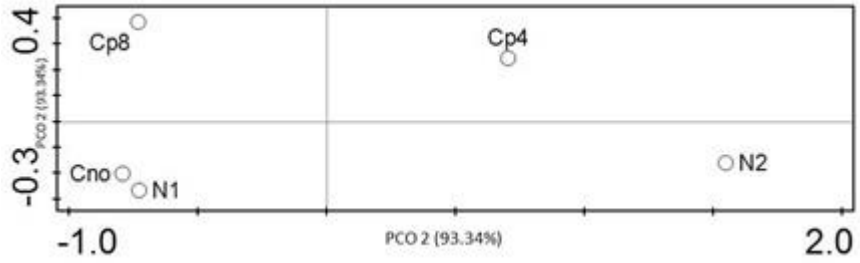


Figure S7.1. The PCoA for the carbon cycling genes abundance in the maize rhizosphere

Table S6.1: Sequence data for the analyzed rhizosphere soil samples under the treatments

	Upload: bp Count	Upload: Sequences Count	Upload: Mean Sequence Length	Upload: Mean GC percent	Artificial Duplicate Reads: Sequence Count	Post QC: bp Count	Post QC: Sequences Count	Post QC: Mean Sequence Length	Post QC: Mean GC percent	Processed: Predicted Protein Features	Processed: Predicted rRNA Features	Alignment: Identified Protein Features	Alignment: Identified rRNA Features
Cp4	**1,939,891,250 bp**	**12,070,719**	**161 ± 73 bp**	**49 ± 11 %**	**8,391,961**	**526,025,382 bp**	**2,945,816**	**179 ± 71 bp**	**44 ± 10 %**	**1,342,578**	**18,398**	**387,527**	**2,577**
Cp8	**2,618,758,280 bp**	**15,575,330**	**168 ± 71 bp**	**63 ± 12 %**	**1,832,282**	**2,270,368,498 bp**	**13,083,355**	**174 ± 67 bp**	**63 ± 10 %**	**11,042,146**	**36,758**	**4,547,525**	**10,755**
N2	**978,960,789 bp**	**5,558,478**	**176 ± 74 bp**	**43 ± 11 %**	**2,399,030**	**553,971,710 bp**	**2,892,203**	**192 ± 71 bp**	**41 ± 9 %**	**1,164,182**	**13,662**	**418,097**	**3,170**
N1	**1,694,792,733 bp**	**9,687,815**	**175 ± 72 bp**	**64 ± 12 %**	**1,078,716**	**1,474,813,072 bp**	**8,198,530**	**180 ± 68 bp**	**65 ± 10 %**	**7,188,585**	**18,817**	**3,057,707**	**6,303**
Cn0	**1,430,407,056 bp**	**7,834,687**	**183 ± 70 bp**	**64 ± 11 %**	**788,921**	**1,257,716,569 bp**	**6,780,803**	**185 ± 67 bp**	**64 ± 10 %**	**6,123,837**	**17,813**	**2,612,059**	**5,936**

REFERENCES

Abd-Alla, M. H., El-Enany, A.-W. E., Nafady, N. A., Khalaf, D. M. & Morsy, F. M. 2014. Synergistic interaction of Rhizobium leguminosarum bv. viciae and arbuscular mycorrhizal fungi as a plant growth promoting biofertilizers for faba bean (*Vicia faba* L.) in alkaline soil. *Microbiological Research,* 169**,** 49-58.

Abujabhah, I. S., Bound, S. A., Doyle, R. & Bowman, J. P. 2016. Effects of biochar and compost amendments on soil physico-chemical properties and the total community within a temperate agricultural soil. *Applied Soil Ecology,* 98**,** 243-253.

Adam, M., Heuer, H. & Hallmann, J. 2014. Bacterial antagonists of fungal pathogens also control root-knot nematodes by induced systemic resistance of tomato plants. *PloS One,* 9.

Adams, D. & Yang, S. 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. *Proceedings of the National Academy of Sciences,* 76**,** 170-174.

Adegboye, M. F. & Babalola, O. O. 2012. Taxonomy and ecology of antibiotic producing actinomycetes. *African Journal of Agricultural Research,* 7**,** 2255-2261.

Adegboye, M. F. & Babalola, O. O. 2015. Evaluation of antibiotic biosynthetic potential of actinomycete isolates to produce antimicrobial agents. *British Microbiology Research Journal,* 243-254.

Afshari-Behbahanizadeh, S., Akbari, G. A., Shahbazi, M. & Alahdadi, I. 2014. Relations between barley root traits and osmotic adjustment under terminal drought stress. *Journal of Agricultural Science,* 6**,** 112.

Ahmad, F., Babalola, O. O., Siddiqui, M. A. 2012. Integrated Approach for Management of Nematodes in Chickpea. *Journal of Pure and Applied Microbiology* 6**,** 1063-1068.

Ahmadi, M. K., Fawaz, S., Jones, C. H., Zhang, G. & Pfeifer, B. A. 2015. Total biosynthesis and diverse applications of the nonribosomal peptide-polyketide siderophore yersiniabactin. *Applied Environmental Microbiology* 81**,** 5290-5298.

Ahn, J.-H., Song, J., Kim, B.-Y., Kim, M.-S., Joa, J.-H. & Weon, H.-Y. 2012. Characterization of the bacterial and archaeal communities in rice field soils subjected to long-term fertilization practices. *Journal of Microbiology,* 50**,** 754-765.

Ai, C., Liang, G., Sun, J., Wang, X., He, P. & Zhou, W. 2013. Different roles of rhizosphere effect and long-term fertilization in the activity and community structure of ammonia oxidizers in a calcareous fluvo-aquic soil. *Soil Biology and Biochemistry,* 57**,** 30-42.

Ai, C., Liang, G., Sun, J., Wang, X., He, P., Zhou, W. & He, X. 2015. Reduced dependence of rhizosphere microbiome on plant-derived carbon in 32-year long-term inorganic and organic fertilized soils. *Soil Biology and Biochemistry,* 80**,** 70-78.

Ai, C., Zhang, S., Zhang, X., Guo, D., Zhou, W. & Huang, S. 2018. Distinct responses of soil bacterial and fungal communities to changes in fertilization regime and crop rotation. *Geoderma,* 319**,** 156-166.

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

Ajayi, A., Onibokun, E., George, F. & Atolagbe, O. 2016. Isolation and characterization of chitinolytic bacteria for chitinase production from the African Catfish, *Clarias gariepinus* (Burchell, 1822). *Research Journal of Microbiology* 11**,** 119-125.

Ajilogba, C. F., Babalola, O. O. & Ahmad, F. 2013. Antagonistic effects of *Bacillus* species in biocontrol of tomato *Fusarium* wilt. *Journal Studies on Ethno-Medicine* 7**,** 205-216.

Akram, W., Anjum, T. & Ali, B. 2016. Phenylacetic acid is ISR determinant produced by *Bacillus fortis* IAGS162, which involves extensive re-modulation in metabolomics of tomato to protect against Fusarium wilt. *Frontiers in Plant Science,* 7**,** 498.

Ali, S., Mir, Z. A., Tyagi, A., Mehari, H., Meena, R. P., Bhat, J. A., Yadav, P., Papalou, P., Rawat, S. & Grover, A. 2017. Overexpression of NPR1 in *Brassica juncea* confers broad spectrum resistance to fungal pathogens. *Frontiers in Plant Science,* 8**,** 1693.

Alkorta, I., Aizpurua, A., Riga, P., Albizu, I., Amézaga, I. & Garbisu, C. 2003. Soil enzyme activities as biological indicators of soil health. *Reviews on Environmental Health,* 18**,** 65-73.

Allison, S. D. & Martiny, J. B. 2008. Resistance, resilience, and redundancy in microbial communities. *Proceedings of the National Academy of Sciences,* 105**,** 11512-11519.

Alori, E. T. & Babalola, O. O. 2018. Microbial inoculants for improving crop quality and human health in Africa. *Frontier in Microbiology,* 9**,** 2213.

Alori, E. T., Dare, M. O. & Babalola, O. O. 2017a. Microbial inoculants for soil quality and plant health. *Sustainable Agriculture Reviews.* Springer.

Alori, E. T., Glick, B. R. & Babalola, O. O. 2017b. Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Frontiers in Microbiology,* 8**,** 971.

Altieri, M. A. 1999. The ecological role of biodiversity in agroecosystems. *Invertebrate biodiversity as bioindicators of sustainable landscapes.* Elsevier.

Amoo, A. E., Babalola, O. O. 2017. Ammonia-oxidizing microorganisms: key players in the promotion of plant growth. *Journal of Soil Science and Plant Nutrition,* 17**,** 935-947.

Andreote, F. D., Jiménez, D. J., Chaves, D., Dias, A. C. F., Luvizotto, D. M., Dini-Andreote, F., Fasanella, C. C., Lopez, M. V., Baena, S. & Taketani, R. G. 2012. The microbiome of Brazilian mangrove sediments as revealed by metagenomics. *PloS One,* 7.

Antunez-Lamas, M., Cabrera, E., Lopez-Solanilla, E., Solano, R., González-Melendi, P., Chico, J. M., Toth, I., Birch, P., Pritchard, L. & Liu, H. 2009. Bacterial chemoattraction towards jasmonate plays a role in the entry of *Dickeya dadantii* through wounded tissues. *Molecular Microbiology,* 74**,** 662-671.

Armada, E., Azcón, R., López-Castillo, O. M., Calvo-Polanco, M. & Ruiz-Lozano, J. M. 2015a. Autochthonous arbuscular mycorrhizal fungi and Bacillus thuringiensis from a degraded Mediterranean area can be used to improve physiological traits and performance of a plant of agronomic interest under drought conditions. *Plant Physiology and Biochemistry,* 90**,** 64-74.

Armada, E., Barea, J.-M., Castillo, P., Roldán, A. & Azcón, R. 2015b. Characterization and management of autochthonous bacterial strains from semiarid soils of Spain and their interactions with fermented agrowastes to improve drought tolerance in native shrub species. *Applied Soil Ecology,* 96**,** 306-318.

Armada, E., Portela, G., Roldán, A. & Azcón, R. 2014. Combined use of beneficial soil microorganism and agrowaste residue to cope with plant water limitation under semiarid conditions. *Geoderma,* 232**,** 640-648.

Arora, M., Kaushik, A., Rani, N. & Kaushik, C. 2010. Effect of cyanobacterial exopolysaccharides on salt stress alleviation and seed germination. *Journal of Environmental Biology,* 31**,** 701-704.

Arseneault, T., Goyer, C. & Filion, M. 2015. *Pseudomonas fluorescens* LBUM223 increases potato yield and reduces common scab symptoms in the field. *Phytopathology,* 105**,** 1311-1317.

Arumugam, K., Renganathan, S., Renganathan, K., Sharma, N. K. & Babalola, O. O. Enhancing the post consumer waste management through vermicomposting along with bioinoculumn.

Asari, S., Ongena, M., Debois, D., De Pauw, E., Chen, K., Bejai, S. & Meijer, J. 2017. Insights into the molecular basis of biocontrol of *Brassica* pathogens by *Bacillus amyloliquefaciens* UCMB5113 lipopeptides. *Annals of Botany,* 120**,** 551-562.

Ashwin, N., Barnabas, E. L., Sundar, A. R., Muthumeena, M., Malathi, P. & Viswanathan, R. 2017. Disease suppressive effects of resistance-inducing agents against red rot of sugarcane. *European Journal of Plant Pathology,* 149**,** 285-297.

Ashwin, N., Barnabas, L., Sundar, A. R., Malathi, P., Viswanathan, R., Masi, A., Agrawal, G. K. & Rakwal, R. 2018. CfPDIP1, a novel secreted protein of *Colletotrichum falcatum*, elicits defense responses in sugarcane and triggers hypersensitive response in tobacco. *Applied Microbiology and Biotechnology,* 102**,** 6001-6021.

Aung, M. M. T., Sarobol, E., Nakasathein, S. & Chai-aree, W. 2011. Differential responses of selected soybean cultivars to drought stress and their drought tolerant attributions. *Kasetsart Journal, Natural Sciences,* 45**,** 571-582.

Avio, L., Castaldini, M., Fabiani, A., Bedini, S., Sbrana, C., Turrini, A. & Giovannetti, M. 2013. Impact of nitrogen fertilization and soil tillage on arbuscular mycorrhizal fungal communities in a Mediterranean agroecosystem. *Soil Biology and Biochemistry,* 67**,** 285-294.

Azziz, G., Monza, J., Etchebehere, C. & Irisarri, P. 2017. *nirS*-and *nirK*-type denitrifier communities are differentially affected by soil type, rice cultivar and water management. *European Journal of Soil Biology,* 78**,** 20-28.

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

Babalola, O. O. 2014. Does nature make provision for backups in the modification of bacterial community structures? *Biotechnology and Genetic Engineering Reviews,* 30**,** 31-48.

Babalola, O. O. & Glick, B. R. 2012. The use of microbial inoculants in African agriculture: current practice and future prospects. *Scientific Research and Essay,* 10**,** 540-549.

Babalola, O. O., Kirby, B. M., Le Roes-Hill, M., Cook, A. E., Cary, S. C., Burton, S. G. & Cowan, D. A. 2009. Phylogenetic analysis of actinobacterial populations associated with Antarctic Dry Valley mineral soils. *Environmental Microbiology,* 11**,** 566-576.

Babalola, O. O., Sanni, A. I., Odhiambo, G. D., Torto, B. J. 2007. Plant growth-promoting rhizobacteria do not pose any deleterious effect on cowpea and detectable amounts of ethylene are produced. *World Journal of Microbiology and Biotechnology,* 23**,** 747-752.

Bakker, M. G., Manter, D. K., Sheflin, A. M., Weir, T. L. & Vivanco, J. M. 2012. Harnessing the rhizosphere microbiome through plant breeding and agricultural management. *Plant and Soil,* 360**,** 1-13.

Bán, R., Baglyas, G., Virányi, F., Barna, B., Posta, K., Kiss, J. & Körösi, K. 2017. The chemical inducer, BTH (benzothiadiazole) and root colonization by mycorrhizal fungi (*Glomus* spp.)

trigger resistance against white rot (*Sclerotinia Sclerotiorum*) in sunflower. *Acta Biologica Hungarica,* 68**,** 50-59.

Barbosa, M. A. M., da Silva Lobato, A. K., Tan, D. K. Y., Viana, G. D. M., Coelho, K. N. N., Barbosa, J. R. S., da Costa, R. C. L., dos Santos Filho, B. G., dos Santos Moraes, M. d. C. H. & de Oliveira Neto, C. F. 2013. *'Bradyrhizobium'improves* nitrogen assimilation, osmotic adjustment and growth in contrasting cowpea cultivars under drought. *Australian Journal of Crop Science,* 7**,** 1983.

Bastida, F., Torres, I., Romero-Trigueros, C., Baldrian, P., Větrovský, T., Bayona, J., Alarcón, J., Hernández, T., García, C. & Nicolás, E. 2017. Combined effects of reduced irrigation and water quality on the soil microbial community of a citrus orchard under semi-arid conditions. *Soil Biology and Biochemistry,* 104**,** 226-237.

Berendsen, R. L., Pieterse, C. M. & Bakker, P. A. 2012. The rhizosphere microbiome and plant health. *Trends in Plant Science,* 17**,** 478-486.

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

Bergkemper, F., Schöler, A., Engel, M., Lang, F., Krüger, J., Schloter, M. & Schulz, S. 2016. Phosphorus depletion in forest soils shapes bacterial communities towards phosphorus recycling systems. *Environmental Microbiology,* 18**,** 1988-2000.

Berthrong, S. T., Yeager, C. M., Gallegos-Graves, L., Steven, B., Eichorst, S. A., Jackson, R. B. & Kuske, C. R. 2014. Nitrogen fertilization has a stronger effect on soil nitrogen-fixing bacterial communities than elevated atmospheric CO2. *Applied and. Environmental Microbiology,* 80**,** 3103-3112.

Bhat, A. 2013. Preserving microbial diversity of soil ecosystem: a key to sustainable productivity. *International Journal of Current Microbiology and Applied Sciences,* 2**,** 85-101.

Bhattacharyya, R., Kundu, S., Prakash, V. & Gupta, H. 2008. Sustainability under combined application of mineral and organic fertilizers in a rainfed soybean–wheat system of the Indian Himalayas. *European Journal of Agronomy,* 28**,** 33-46.

Billings, S. A. & Ziegler, S. E. 2008. Altered patterns of soil carbon substrate usage and heterotrophic respiration in a pine forest with elevated CO2 and N fertilization. *Global Change Biology*, 14**,** 1025-1036.

Bistgani, Z. E., Siadat, S. A., Bakhshandeh, A., Pirbalouti, A. G. & Hashemi, M. 2017. Interactive effects of drought stress and chitosan application on physiological characteristics and essential oil yield of Thymus daenensis Celak. *The Crop Journal,* 5**,** 407-415.

Blainski, J. M. L., da Rocha Neto, A. C., Schimidt, E. C., Voltolini, J. A., Rossi, M. J. & Di Piero, R. M. 2018. Exopolysaccharides from *Lactobacillus plantarum* induce biochemical and physiological alterations in tomato plant against bacterial spot. *Applied Microbiology and Biotechnology*, 102**,** 4741-4753.

Böhm, H., Albert, I., Fan, L., Reinhard, A. & Nürnberger, T. 2014. Immune receptor complexes at the plant cell surface. *Current Opinion in Plant Biology,* 20**,** 47-54.

Bouwmeester, H. J., Roux, C., Lopez-Raez, J. A. & Becard, G. 2007. Rhizosphere communication of plants, parasitic plants and AM fungi. *Trends in Plant Science,* 12**,** 224-230.

Bouzid, S. & Rahmoune, C. 2012. Enhancement of saline water for irrigation of *Phaseolus vulgaris* L. species in presence of molybdenum. *Procedia Engineering,* 33**,** 168-173.

Bresson, J., Varoquaux, F., Bontpart, T., Touraine, B. & Vile, D. 2013. The PGPR strain *Phyllobacterium brassicacearum* STM 196 induces a reproductive delay and physiological changes that result in improved drought tolerance in *Arabidopsis*. *New Phytologist*, 200**,** 558-569.

Brzoska, P. & Boos, W. 1988. Characteristics of a ugp-encoded and phoB-dependent glycerophosphoryl diester phosphodiesterase which is physically dependent on the ugp transport system of *Escherichia coli. Journal of Bacteriology*, 170**,** 4125-4135.

Bu, B., Qiu, D., Zeng, H., Guo, L., Yuan, J. & Yang, X. 2014. A fungal protein elicitor PevD1 induces *Verticillium* wilt resistance in cotton. *Plant Cell Reports,* 33**,** 461-470.

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

Bumunang, E. W., Babalola, O. O. & Barros, E. 2013. Bacterial community profiling in the rhizosphere of field grown GM and non-GM maize.

Butterbach-Bahl, K., Baggs, E. M., Dannenmann, M., Kiese, R. & Zechmeister-Boltenstern, S. 2013. Nitrous oxide emissions from soils: how well do we understand the processes and their controls? *Philosophical Transactions of the Royal Society B: Biological Sciences,* 368**,** 20130122.

Caarls, L., Elberse, J., Awwanah, M., Ludwig, N. R., de Vries, M., Zeilmaker, T., Van Wees, S. C., Schuurink, R. C. & Van den Ackerveken, G. 2017. Arabidopsis JASMONATE-INDUCED OXYGENASES down-regulate plant immunity by hydroxylation and inactivation of the hormone jasmonic acid. *Proceedings of the National Academy of Sciences,* 114**,** 6388-6393.

Calvo-Polanco, M., Sánchez-Romera, B., Aroca, R., Asins, M. J., Declerck, S., Dodd, I. C., Martínez-Andújar, C., Albacete, A. & Ruiz-Lozano, J. M. 2016. Exploring the use of recombinant inbred lines in combination with beneficial microbial inoculants (AM fungus and PGPR) to improve drought stress tolerance in tomato. *Environmental and Experimental Botany,* 131**,** 47-57.

Campbell, B. J., Polson, S. W., Hanson, T. E., Mack, M. C. & Schuur, E. A. 2010. The effect of nutrient deposition on bacterial communities in Arctic tundra soil. *Environmental microbiology*, 12**,** 1842-1854.

Canfora, L., Vendramin, E., Felici, B., Tarricone, L., Florio, A. & Benedetti, A. 2018. Vineyard microbiome variations during different fertilisation practices revealed by 16s rRNA gene sequencing. *Applied Soil Ecology,* 125**,** 71-80.

Cao, H., Bowling, S. A., Gordon, A. S. & Dong, X. 1994. Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. *The Plant Cell,* 6**,** 1583-1592.

Cardinale, M., Ratering, S., Suarez, C., Montoya, A. M. Z., Geissler-Plaum, R. & 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.

Carrara, J. E., Walter, C. A., Hawkins, J. S., Peterjohn, W. T., Averill, C. & Brzostek, E. R. 2018. Interactions among plants, bacteria, and fungi reduce extracellular enzyme activities under long-term N fertilization. *Global Change Biology,* 24**,** 2721-2734.

Castañeda, L. E. & Barbosa, O. 2017. Metagenomic analysis exploring taxonomic and functional diversity of soil microbial communities in Chilean vineyards and surrounding native forests. *PeerJ,* 5**,** e3098.

Cawoy, H., Mariutto, M., Henry, G., Fisher, C., Vasilyeva, N., Thonart, P., Dommes, J. & Ongena, M. 2014. Plant defense stimulation by natural isolates of Bacillus depends on efficient surfactin production. *Molecular Plant-Microbe Interactions,* 27**,** 87-100.

Cecchini, N. M., Steffes, K., Schläppi, M. R., Gifford, A. N. & Greenberg, J. T. 2015. *Arabidopsis* AZI1 family proteins mediate signal mobilization for systemic defence priming. *Nature Communications,* 6**,** 7658.

Cesarano, G., De Filippis, F., La Storia, A., Scala, F. & Bonanomi, G. 2017. Organic amendment type and application frequency affect crop yields, soil fertility and microbiome composition. *Applied Soil Ecology,* 120**,** 254-264.

Chaerun, S. K., Pangesti, N. P., Toyota, K. & Whitman, W. B. 2011. Changes in microbial functional diversity and activity in paddy soils irrigated with industrial wastewaters in Bandung, West Java Province, Indonesia. *Water, Air, & Soil Pollution,* 217**,** 491-502.

Chang, Y. H., Yan, H. Z. & Liou, R. F. 2015. A novel elicitor protein from *Phytophthora parasitica* induces plant basal immunity and systemic acquired resistance. *Molecular Plant Pathology,* 16**,** 123-136.

Chaudhary, D. & Sindhu, S. S. 2017. Amelioration of salt stress in chickpea (*Cicer arietinum* L.) by coinculation of ACC deaminase-containing rhizospheric bacteria with Mesorhizobium strains. *Legume Research-An International Journal,* 40**,** 80-86.

Chaudhry, V., Rehman, A., Mishra, A., Chauhan, P. S. & Nautiyal, C. S. 2012. Changes in bacterial community structure of agricultural land due to long-term organic and chemical amendments. *Microbial Ecology,* 64**,** 450-460.

Chávez-Romero, Y., Navarro-Noya, Y. E., Reynoso-Martínez, S. C., Sarria-Guzmán, Y., Govaerts, B., Verhulst, N., Dendooven, L. & Luna-Guido, M. 2016. 16S metagenomics reveals changes in the soil bacterial community driven by soil organic C, N-fertilizer and tillage-crop residue management. *Soil and Tillage Research,* 159**,** 1-8.

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

Chen, L., Yin, H., Xu, J. & Liu, X. 2011. Enhanced antioxidative responses of a salt-resistant wheat cultivar facilitate its adaptation to salt stress. *African Journal of Biotechnology,* 10**,** 16884-16886.

Chen, L., Zhang, J., Zhao, B., Zhou, G. & Ruan, L. 2015. Bacterial community structure in maize stubble-amended soils with different moisture levels estimated by bar-coded pyrosequencing. *Applied Soil Ecology,* 86**,** 62-70.

Chen, Y., Dong, J., Bennetzen, J. L., Zhong, M., Yang, J., Zhang, J., Li, S., Hao, X., Zhang, Z. & Wang, X. 2017. Integrating transcriptome and microRNA analysis identifies genes and microRNAs for AHO-induced systemic acquired resistance in N. tabacum. *Scientific Reports,* 7**,** 1-13.

Chen, Z., Hou, H., Zheng, Y., Qin, H., Zhu, Y., Wu, J. & Wei, W. 2012. Influence of fertilisation regimes on a nosZ-containing denitrifying community in a rice paddy soil. *Journal of the Science of Food and Agriculture,* 92**,** 1064-1072.

Cheneby, D., Perrez, S., Devroe, C., Hallet, S., Couton, Y., Bizouard, F., Iuretig, G., Germon, J. & Philippot, L. 2004. Denitrifying bacteria in bulk and maize-rhizospheric soil: diversity and N2O-reducing abilities. *Canadian Journal of Microbiology,* 50**,** 469-474.

Chu, H., Lin, X., Fujii, T., Morimoto, S., Yagi, K., Hu, J. & Zhang, J. 2007. Soil microbial biomass, dehydrogenase activity, bacterial community structure in response to long-term fertilizer management. *Soil Biology and Biochemistry,* 39**,** 2971-2976.

Clair, S. B. S. & Lynch, J. P. 2010. The opening of Pandora's Box: climate change impacts on soil fertility and crop nutrition in developing countries. *Plant and Soil,* 335**,** 101-115.

Clark, K. B. 2013. Biotic activity of Ca2+-modulating non-traditional antimicrobial and-viral agents. *Frontiers in Microbiology,* 4**,** 381.

Clarke, K. & Green, R. 1988. Statistical design and analysis for a'biological effects' study. *Marine Ecology Progress Series***,** 213-226.

Condron, L. M., Turner, B. L. & Cade-Menun, B. J. 2005. Chemistry and dynamics of soil organic phosphorus. *Phosphorus: Agriculture and the Environment,* 46**,** 87-121.

Constantino, N., Mastouri, F., Damarwinasis, R., Borrego, E., Moran-Diez, M. E., Kenerley, C. M., Gao, X. & Kolomiets, M. V. 2013. Root-expressed maize lipoxygenase 3 negatively regulates induced systemic resistance to *Colletotrichum graminicola* in shoots. *Frontiers in Plant Science,* 4**,** 510.

Coolon, J. D., Jones, K. L., Todd, T. C., Blair, J. M. & Herman, M. A. 2013. Long-term nitrogen amendment alters the diversity and assemblage of soil bacterial communities in tallgrass prairie. *PloS One,* 8.

Cretoiu, M., Korthals, G., Visser, J. & van Elsas, J. 2013. Chitin amendment raises the suppressiveness of soil towards plant pathogens and modulates the actinobacteriaceal

communities in an experimental agricultural field. *Applied and Environmental Microbiology,* 79**,** 5291-5301.

Creus, C. M., Pereyra, M. A., Casanovas, E. M., Sueldo, R. J. & Barassi, C. A. 2010. Plant growth-promoting effects of rhizobacteria on abiotic stressed plants. *Azospirillum*-Grasses model. *Plant Science and Biotechnology in South America: Focus on Argentina,* 2**,** 49-59.

Crouzet, J., Roland, J., Peeters, E., Trombik, T., Ducos, E., Nader, J. & Boutry, M. 2013. NtPDR1, a plasma membrane ABC transporter from Nicotiana tabacum, is involved in diterpene transport. *Plant molecular biology,* 82**,** 181-192.

Cruz, C., Ramos, A., Babalola, O. O., Kamel, H., Dias, T. & Varma, A. 2017. Soil: do not disturb, mycorrhiza in action. *Mycorrhiza-Function, Diversity, State of the Art.* Springer.

Cui, P., Fan, F., Yin, C., Song, A., Huang, P., Tang, Y., Zhu, P., Peng, C., Li, T. & Wakelin, S. A. 2016. Long-term organic and inorganic fertilization alters temperature sensitivity of potential N2O emissions and associated microbes. *Soil Biology and Biochemistry,* 93**,** 131-141.

D'ALESSANDRO, M., Erb, M., Ton, J., Brandenburg, A., Karlen, D., Zopfi, J. & Turlings, T. C. 2014. Volatiles produced by soil-borne endophytic bacteria increase plant pathogen resistance and affect tritrophic interactions. *Plant, Cell & Environment,* 37**,** 813-826.

Dahlgren, R., Saigusa, M. & Ugolini, F. 2004. The nature, properties and management of volcanic soils. *Advances in Agronomy,* 82**,** 113-182.

Dai, H., Chen, Y., Yang, X., Cui, J. & Sui, P. 2017. The effect of different organic materials amendment on soil bacteria communities in barren sandy loam soil. *Environmental Science and Pollution Research,* 24**,** 24019-24028.

Damam, M., Gaddam, B. & Kausar, R. 2014. Effect of plant growth promoting rhizobacteria (PGPR) on *Coleus forskohlii. International Journal of Current Microbiology and Applied Science,* 3**,** 266-274.

Daquiado, A. R., Kuppusamy, S., Kim, S. Y., Kim, J. H., Yoon, Y.-E., Kim, P. J., Oh, S.-H., Kwak, Y.-S. & Lee, Y. B. 2016. Pyrosequencing analysis of bacterial community diversity in long-term fertilized paddy field soil. *Applied Soil Ecology,* 108**,** 84-91.

Delfini, R., Belgoff, C., Fernández, E., Fabra, A. & Castro, S. 2010. Symbiotic nitrogen fixation and nitrate reduction in two peanut cultivars with different growth habit and branching pattern structures. *Plant Growth Regulation,* 61**,** 153-159.

Denef, K., Roobroeck, D., Wadu, M. C. M., Lootens, P. & Boeckx, P. 2009. Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. *Soil Biology and Biochemistry,* 41**,** 144-153.

Dey, S., Wenig, M., Langen, G., Sharma, S., Kugler, K. G., Knappe, C., Hause, B., Bichlmeier, M., Babaeizad, V. & Imani, J. 2014. Bacteria-triggered systemic immunity in barley is associated with WRKY and ETHYLENE RESPONSIVE FACTORs but not with salicylic acid. *Plant Physiology,* 166**,** 2133-2151.

Ding, J., Jiang, X., Ma, M., Zhou, B., Guan, D., Zhao, B., Zhou, J., Cao, F., Li, L. & Li, J. 2016. Effect of 35 years inorganic fertilizer and manure amendment on structure of bacterial and archaeal communities in black soil of northeast China. *Applied Soil Ecology,* 105**,** 187-195.

Ding, L.-J., An, X.-L., Li, S., Zhang, G.-L. & Zhu, Y.-G. 2014. Nitrogen loss through anaerobic ammonium oxidation coupled to iron reduction from paddy soils in a chronosequence. *Environmental Science & Technology,* 48**,** 10641-10647.

Ding, T., Su, B., Chen, X., Xie, S., Gu, S., Wang, Q., Huang, D. & Jiang, H. 2017. An endophytic bacterial strain isolated from *Eucommia ulmoides* inhibits southern corn leaf blight. *Frontiers in Microbiology,* 8**,** 903.

Dini-Andreote, F. & van Elsas, J. D. 2013. Back to the basics: the need for ecophysiological insights to enhance our understanding of microbial behaviour in the rhizosphere. *Plant and Soil,* 373**,** 1-15.

Dohrmann, A. B., Küting, M., Jünemann, S., Jaenicke, S., Schlüter, A. & Tebbe, C. C. 2013. Importance of rare taxa for bacterial diversity in the rhizosphere of Bt-and conventional maize varieties. *The ISME Journal,* 7**,** 37-49.

Dong, J. G., Fernandez-Maculet, J. C. & Yang, S. F. 1992. Purification and characterization of 1-aminocyclopropane-1-carboxylate oxidase from apple fruit. *Proceedings of the National Academy of Sciences,* 89**,** 9789-9793.

Dos Santos, P. J. C., Savi, D. C., Gomes, R. R., Goulin, E. H., Senkiv, C. D. C., Tanaka, F. A. O., Almeida, Á. M. R., Galli-Terasawa, L., Kava, V. & Glienke, C. 2016. *Diaporthe endophytica* and *D. terebinthifolii* from medicinal plants for biological control of *Phyllosticta citricarpa*. *Microbiological Research,* 186**,** 153-160.

Downie, J. A. 2014. Calcium signals in plant immunity: a spiky issue. *New Phytologist,* 204**,** 733-735.

e Silva, M. C. P., Schloter-Hai, B., Schloter, M., van Elsas, J. D. & Salles, J. F. 2013. Temporal dynamics of abundance and composition of nitrogen-fixing communities across agricultural soils. *PloS One,* 8.

Egamberdieva, D., Jabborova, D. & Hashem, A. 2015a. Pseudomonas induces salinity tolerance in cotton (*Gossypium hirsutum*) and resistance to Fusarium root rot through the modulation of indole-3-acetic acid. *Saudi Journal of Biological Sciences,* 22**,** 773-779.

Egamberdieva, D., Wirth, S., Alqarawi, A. A. & Abd_Allah, E. 2015b. Salt tolerant *Methylobacterium mesophilicum* showed viable colonization abilities in the plant rhizosphere. *Saudi journal of Biological Sciences,* 22**,** 585-590.

Egel, D. S., Kleczewski, N. M., Mumtaz, F. & Foster, R. 2018. Acibenzolar-S-methyl is associated with yield reduction when used for managing bacterial wilt (*Erwinia tracheiphila*) in cantaloupe. *Crop Protection,* 109**,** 136-141.

Ekinci, M., Turan, M., Yildirim, E., Güneş, A., Kotan, R. & Dursun, A. 2014. Effect of plant growth promoting rhizobacteria on growth, nutrient, organic acid, amino acid and hormone content

of cauliflower (*Brassica oleracea L. var. botrytis*) transplants. *Acta Sci Pol Hortorum Cultus,* 13**,** 71-85.

El-Borollosy, A. M. & Oraby, M. M. 2012. Induced systemic resistance against Cucumber mosaic cucumovirus and promotion of cucumber growth by some plant growth-promoting rhizobacteria. *Annals of Agricultural Sciences,* 57**,** 91-97.

El-Hamied, S. A. A. 2014. Effect of multi-ingredient of Bokashi on productivity of mandarin trees and soil properties under saline water irrigation. *IOSR Journal of Agriculture and Vetenary Science (IOSR-JAVS),* 7**,** 79-87.

El-Shetehy, M., Wang, C., Shine, M., Yu, K., Kachroo, A. & Kachroo, P. 2015. Nitric oxide and reactive oxygen species are required for systemic acquired resistance in plants. *Plant Signaling & Behavior,* 10**,** e998544.

Elser, J. & Bennett, E. 2011. A broken biogeochemical cycle. *Nature,* 478**,** 29-31.

Enagbonma, B. J. & Babalola, O. O. 2019. Potentials of termite mound soil bacteria in ecosystem engineering for sustainable agriculture. 69**,** 211-219.

Enebe, M. C. & Babalola, O. O. 2018. The influence of plant growth-promoting rhizobacteria in plant tolerance to abiotic stress: a survival strategy. *Applied Microbiology and Biotechnology,* 102**,** 7821-7835.

Enebe, M. C. & Babalola, O. O. 2019. The impact of microbes in the orchestration of plants' resistance to biotic stress: a disease management approach. *Applied Microbiology and Biotechnology,* 103**,** 9-25.

Entwistle, E. M., Zak, D. R. & Edwards, I. P. 2013. Long-term experimental nitrogen deposition alters the composition of the active fungal community in the forest floor. *Soil Science Society of America Journal,* 77**,** 1648-1658.

Eo, J. & Park, K.-C. 2016. Long-term effects of imbalanced fertilization on the composition and diversity of soil bacterial community. *Agriculture, Ecosystems & Environment,* 231**,** 176-182.

Esuola, C. O., Babalola, O. O., Heine, T., Schwabe, R., Schlömann, M. & Tischler, D. 2016. Identification and characterization of a FAD-dependent putrescine N-hydroxylase (GorA) from *Gordonia rubripertincta* CWB2. *Journal of Molecular Catalysis B: Enzymatic,* 134**,** 378-389.

Falkowski, P. G., Fenchel, T. & Delong, E. F. 2008. The microbial engines that drive Earth's biogeochemical cycles. *Science,* 320**,** 1034-1039.

Fan, F., Li, Z., Wakelin, S. A., Yu, W. & Liang, Y. 2012. Mineral fertilizer alters cellulolytic community structure and suppresses soil cellobiohydrolase activity in a long-term fertilization experiment. *Soil Biology and Biochemistry,* 55**,** 70-77.

Farace, G., Fernandez, O., Jacquens, L., Coutte, F., Krier, F., Jacques, P., Clément, C., Barka, E. A., Jacquard, C. & Dorey, S. 2015. Cyclic lipopeptides from *Bacillus subtilis* activate distinct patterns of defence responses in grapevine. *Molecular Plant Pathology,* 16**,** 177-187.

Fashola, M. O., Jeme, V.-M. N. & Babalola, O. O. 2015. Diversity of acidophilic bacteria and archaea and their roles in bioremediation of acid mine drainage. *Microbiology Research Journal International***,** 443-456.

Fatima, S. & Anjum, T. 2017. Identification of a potential ISR determinant from *Pseudomonas aeruginosa* PM12 against *Fusarium* wilt in tomato. *Frontiers in Plant Science,* 8**,** 848.

Felgate, H., Giannopoulos, G., Sullivan, M. J., Gates, A. J., Clarke, T. A., Baggs, E., Rowley, G. & Richardson, D. J. 2012. The impact of copper, nitrate and carbon status on the emission of nitrous oxide by two species of bacteria with biochemically distinct denitrification pathways. *Environmental Microbiology,* 14**,** 1788-1800.

Fernández-Crespo, E., Scalschi, L., Llorens, E., García-Agustín, P. & Camañes, G. 2015. NH4+ protects tomato plants against *Pseudomonas syringae* by activation of systemic acquired acclimation. *Journal of Experimental Botany,* 66**,** 6777-6790.

Ferraz, H. B., Bertolucci, P. H. F., Pereira, J. S., Lima, J. G. C. & Andrade, L. A. F. d. 1988. Chronic exposure to the fungicide maneb may produce symptoms and signs of CNS manganese intoxication. *Neurology,* 38**,** 550-550.

Ferreira, E. d. B., Martins, L., Xavier, G. R. & Rumjanek, N. G. 2011. Nodulation and grain yield by cowpea (Vigna unguiculata L. Walp.) inoculated with rhizobia isolates. *Revista Caatinga,* 24**,** 27-35.

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

Figueredo, M. S., Tonelli, M. L., Ibáñez, F., Morla, F., Cerioni, G., del Carmen Tordable, M. & Fabra, A. 2017. Induced systemic resistance and symbiotic performance of peanut plants challenged with fungal pathogens and co-inoculated with the biocontrol agent *Bacillus* sp. CHEP5 and *Bradyrhizobium* sp. SEMIA6144. *Microbiological Research,* 197**,** 65-73.

Francioli, D., Schulz, E., Lentendu, G., Wubet, T., Buscot, F. & Reitz, T. 2016. Mineral vs. organic amendments: microbial community structure, activity and abundance of agriculturally relevant microbes are driven by long-term fertilization strategies. *Frontiers in Microbiology,* 7**,** 1446.

Fraser, T. D., Lynch, D. H., Bent, E., Entz, M. H. & Dunfield, K. E. 2015. Soil bacterial *phoD* gene abundance and expression in response to applied phosphorus and long-term management. *Soil Biology and Biochemistry,* 88**,** 137-147.

Frouz, J., Toyota, A., Mudrák, O., Jílková, V., Filipová, A. & Cajthaml, T. 2016. Effects of soil substrate quality, microbial diversity and community composition on the plant community during primary succession. *Soil Biology and Biochemistry*, 99**,** 75-84.

Fujita, M., Kusajima, M., Okumura, Y., Nakajima, M., Minamisawa, K. & Nakashita, H. 2017. Effects of colonization of a bacterial endophyte, *Azospirillum* sp. B510, on disease resistance in tomato. *Bioscience, Biotechnology, and Biochemistry,* 81**,** 1657-1662.

Fukami, J., Ollero, F. J., Megías, M. & Hungria, M. 2017. Phytohormones and induction of plant-stress tolerance and defense genes by seed and foliar inoculation with *Azospirillum brasilense* cells and metabolites promote maize growth. *AMB Express,* 7**,** 153.

Fukasawa-Akada, T., Kung, S.-d. & Watson, J. C. 1996. Phenylalanine ammonia-lyase gene structure, expression, and evolution in Nicotiana. *Plant Molecular Biology,* 30**,** 711-722.

Gao, Y., Tian, Y., Liang, X. & Gao, L. 2015. Effects of single-root-grafting, double-root-grafting and compost application on microbial properties of rhizosphere soils in Chinese protected cucumber (*Cucumis sativus* L.) production systems. *Scientia Horticulturae,* 186**,** 190-200.

García, J. E., Maroniche, G., Creus, C., Suárez-Rodríguez, R., Ramirez-Trujillo, J. A. & Groppa, M. D. 2017. In vitro PGPR properties and osmotic tolerance of different *Azospirillum* native strains and their effects on growth of maize under drought stress. *Microbiological Research,* 202**,** 21-29.

Geisseler, D. & Scow, K. M. 2014. Long-term effects of mineral fertilizers on soil microorganisms–A review. *Soil Biology and Biochemistry,* 75**,** 54-63.

Gelfand, I. & Robertson, G. P. 2015. A reassessment of the contribution of soybean biological nitrogen fixation to reactive N in the environment. *Biogeochemistry,* 123**,** 175-184.

Ghazalibiglar, H., Hampton, J. G., de Jong, E. v. Z. & Holyoake, A. 2016. Is induced systemic resistance the mechanism for control of black rot in Brassica oleracea by a *Paenibacillus* sp.? *Biological Control,* 92**,** 195-201.

Giguere, A. T., Taylor, A. E., Myrold, D. D. & Bottomley, P. J. 2015. Nitrification responses of soil ammonia-oxidizing archaea and bacteria to ammonium concentrations. *Soil Science Society of America Journal,* 79**,** 1366-1374.

Glaeser, S. P., Imani, J., Alabid, I., Guo, H., Kumar, N., Kämpfer, P., Hardt, M., Blom, J., Goesmann, A. & Rothballer, M. 2016. Non-pathogenic *Rhizobium radiobacter* F4 deploys plant beneficial activity independent of its host *Piriformospora indica*. *The ISME Journal,* 10**,** 871-884.

Glaser, B., Haumaier, L., Guggenberger, G., Zech, W., 2001. The “Terra Preta” phenomenon: a model for sustainable agriculture in the humic tropics. Berlin Die Naturwiss. Nature Science 88 (2), 37–41.

Glick, B. R., Cheng, Z., Czarny, J. & Duan, J. 2007. Promotion of plant growth by ACC deaminase-producing soil bacteria. *New Perspectives and Approaches in Plant Growth-Promoting Rhizobacteria Research.* Springer.

Gond, S. K., Bergen, M. S., Torres, M. S. & White Jr, J. F. 2015. Endophytic *Bacillus* spp. produce antifungal lipopeptides and induce host defence gene expression in maize. *Microbiological Research,* 172**,** 79-87.

Gong, C., Liu, Y., Liu, S.-y., Cheng, M.-z., Zhang, Y., Wang, R.-h., Chen, H.-y., Li, J.-f., Chen, X.-l. & Wang, A.-x. 2017. Analysis of *Clonostachys rosea*-induced resistance to grey mould disease and identification of the key proteins induced in tomato fruit. *Postharvest Biology and Technology,* 123**,** 83-93.

Gonthier, D. J., Ennis, K. K., Farinas, S., Hsieh, H.-Y., Iverson, A. L., Batáry, P., Rudolphi, J., Tscharntke, T., Cardinale, B. J. & Perfecto, I. 2014. Biodiversity conservation in agriculture requires a multi-scale approach. *Proceedings of the Royal Society B: Biological Sciences,* 281**,** 20141358.

Goswami, D., Dhandhukia, P., Patel, P. & Thakker, J. N. 2014. Screening of PGPR from saline desert of Kutch: growth promotion in *Arachis hypogea* by *Bacillus licheniformis* A2. *Microbiological Research,* 169**,** 66-75.

Griffiths, R. I., Thomson, B. C., James, P., Bell, T., Bailey, M. & Whiteley, A. S. 2011. The bacterial biogeography of British soils. *Environmental Microbiology,* 13**,** 1642-1654.

Grillo, M. A., Stinchcombe, J. R. & Heath, K. D. 2016. Nitrogen addition does not influence pre-infection partner choice in the legume–rhizobium symbiosis. *American Journal of Botany,* 103**,** 1763-1770.

Group, S. C. W. 1991. Soil classification: a taxonomic system for South Africa. *Memoirs on the Agricultural Natural Resources of South Africa,* 15**,** 1-262.

Gruau, C., Trotel-Aziz, P., Villaume, S., Rabenoelina, F., Clément, C., Baillieul, F. & Aziz, A. 2015. *Pseudomonas fluorescens* PTA-CT2 triggers local and systemic immune response against Botrytis cinerea in grapevine. *Molecular Plant-Microbe Interactions,* 28**,** 1117-1129.

Gujral, M. S., Agrawal, P., Khetmalas, M. B. & Pandey, R. 2013. Colonization and plant growth promotion of Sorghum seedlings by endorhizospheric Serratia sp. *Acta Biologica Indica,* 2**,** 343-352.

Haas, J. C., Street, N. R., Sjödin, A., Lee, N. M., Högberg, M. N., Näsholm, T. & Hurry, V. 2018. Microbial community response to growing season and plant nutrient optimisation in a boreal Norway spruce forest. *Soil Biology and Biochemistry,* 125**,** 197-209.

Haidar, R., Roudet, J., Bonnard, O., Dufour, M. C., Corio-Costet, M. F., Fert, M., Gautier, T., Deschamps, A. & Fermaud, M. 2016. Screening and modes of action of antagonistic bacteria to control the fungal pathogen *Phaeomoniella chlamydospora* involved in grapevine trunk diseases. *Microbiological Research,* 192**,** 172-184.

Hamm, A. C., Tenuta, M., Krause, D. O., Ominski, K. H., Tkachuk, V. L. & Flaten, D. N. 2016. Bacterial communities of an agricultural soil amended with solid pig and dairy manures, and urea fertilizer. *Applied Soil Ecology,* 103**,** 61-71.

Hammer, Ø., Harper, D. A. & Ryan, P. D. 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica,* 4**,** 9.

Hammerschmidt, R. 1999. Induced disease resistance: how do induced plants stop pathogens? : Elsevier.

Hammerschmidt, R., Nuckles, E. & Kuć, J. 1982. Association of enhanced peroxidase activity with induced systemic resistance of cucumber to *Colletotrichum lagenarium*. *Physiological Plant Pathology,* 20**,** 73-82.

Han, Y., Luo, Y., Qin, S., Xi, L., Wan, B. & Du, L. 2014. Induction of systemic resistance against tobacco mosaic virus by Ningnanmycin in tobacco. *Pesticide Biochemistry and Physiology,* 111**,** 14-18.

Hariprasad, P., Chandrashekar, S., Singh, S. B. & Niranjana, S. 2014. Mechanisms of plant growth promotion and disease suppression by *Pseudomonas aeruginosa* strain 2apa. *Journal of Basic Microbiology,* 54**,** 792-801.

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

Hasanah, Y. & Rahmawat, N. Soybean production under drought stress with application of *Bradyrhizobium japonicum* induced by genistein. Proceedings of The Annual International Conference, Syiah Kuala University-Life Sciences & Engineering Chapter, 2012.

Hayat, R., Ali, S., Amara, U., Khalid, R. & Ahmed, I. 2010. Soil beneficial bacteria and their role in plant growth promotion: a review. *Annals of Microbiology,* 60**,** 579-598.

He, L., Bi, Y., Zhao, J., Pittelkow, C. M., Zhao, X., Wang, S. & Xing, G. 2018. Population and community structure shifts of ammonia oxidizers after four-year successive biochar application to agricultural acidic and alkaline soils. *Science of the Total Environment,* 619**,** 1105-1115.

Heath, M. C. 1998. Apoptosis, programmed cell death and the hypersensitive response. *European Journal of Plant Pathology,* 104**,** 117-124.

Hegde, M., Englert, D. L., Schrock, S., Cohn, W. B., Vogt, C., Wood, T. K., Manson, M. D. & Jayaraman, A. 2011. Chemotaxis to the quorum-sensing signal AI-2 requires the Tsr chemoreceptor and the periplasmic LsrB AI-2-binding protein. *Journal of bacteriology,* 193**,** 768-773.

Heidari, M. & Golpayegani, A. 2012. Effects of water stress and inoculation with plant growth promoting rhizobacteria (PGPR) on antioxidant status and photosynthetic pigments in basil (*Ocimum basilicum* L.). *Journal of the Saudi Society of Agricultural Sciences,* 11**,** 57-61.

Herrera Paredes, S. & Lebeis, S. L. 2016. Giving back to the community: microbial mechanisms of plant–soil interactions. *Functional Ecology,* 30**,** 1043-1052.

Herrera, S. D., Grossi, C., Zawoznik, M. & Groppa, M. D. 2016. Wheat seeds harbour bacterial endophytes with potential as plant growth promoters and biocontrol agents of *Fusarium graminearum*. *Microbiological Research,* 186**,** 37-43.

Heuck, C., Weig, A. & Spohn, M. 2015. Soil microbial biomass C: N: P stoichiometry and microbial use of organic phosphorus. *Soil Biology and Biochemistry,* 85**,** 119-129.

Holden, D. W. 2015. Persisters unmasked. *Science,* 347**,** 30-32.

Hong, C. E., Kwon, S. Y. & Park, J. M. 2016a. Biocontrol activity of Paenibacillus polymyxa AC-1 against Pseudomonas syringae and its interaction with Arabidopsis thaliana. *Microbiological Research,* 185**,** 13-21.

Hong, S. H., Ham, S. Y., Kim, J. S., Kim, I.-S. & Lee, E. Y. 2016b. Application of sodium polyacrylate and plant growth-promoting bacterium, *Micrococcaceae* HW-2, on the growth of plants cultivated in the rooftop. *International Biodeterioration & Biodegradation,* 113**,** 297-303.

Hong, S. H. & Lee, E. Y. 2017. Phytostabilization of salt accumulated soil using plant and biofertilizers: Field application. *International Biodeterioration & Biodegradation,* 124**,** 188-195.

Honma, M. & Shimomura, T. 1978. Metabolism of 1-aminocyclopropane-1-carboxylic acid. *Agricultural and Biological Chemistry,* 42**,** 1825-1831.

Hosseini, F., Mosaddeghi, M. R. & Dexter, A. R. 2017. Effect of the fungus Piriformospora indica on physiological characteristics and root morphology of wheat under combined drought and mechanical stresses. *Plant Physiology and Biochemistry,* 118**,** 107-120.

Hsieh, Y.-J. & Wanner, B. L. 2010. Global regulation by the seven-component Pi signaling system. *Current Opinion in Microbiology,* 13**,** 198-203.

Hsu, C.-K. & Micallef, S. A. 2017. Plant-mediated restriction of *Salmonella enterica* on tomato and spinach leaves colonized with *Pseudomonas* plant growth-promoting rhizobacteria. *International Journal of Food Microbiology,* 259**,** 1-6.

Hu, Z., Shao, S., Zheng, C., Sun, Z., Shi, J., Yu, J., Qi, Z. & Shi, K. 2018. Induction of systemic resistance in tomato against *Botrytis cinerea* by N-decanoyl-homoserine lactone via jasmonic acid signaling. *Planta,* 247**,** 1217-1227.

Huang, J., Li, H. & Yuan, H. 2006. Effect of organic amendments on *Verticillium* wilt of cotton. *Crop Protection,* 25**,** 1167-1173.

Huot, B., Yao, J., Montgomery, B. L. & He, S. Y. 2014. Growth–defense tradeoffs in plants: a balancing act to optimize fitness. *Molecular Plant,* 7**,** 1267-1287.

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

Igiehon, N. O., 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.

Ikeda, H., Nonomiya, T., Usami, M., Ohta, T. & Ōmura, S. 1999. Organization of the biosynthetic gene cluster for the polyketide anthelmintic macrolide avermectin in *Streptomyces avermitilis*. *Proceedings of the National Academy of Sciences,* 96**,** 9509-9514.

Ikeda, T. P., Shauger, A. E. & Kustu, S. 1996. *Salmonella typhimurium* apparently perceives external nitrogen limitation as internal glutamine limitation. *Journal of Molecular Biology,* 259**,** 589-607.

Ikoyi, I., Fowler, A. & Schmalenberger, A. 2018. One-time phosphate fertilizer application to grassland columns modifies the soil microbiota and limits its role in ecosystem services. *Science of the Total Environment,* 630**,** 849-858.

Imparato, V., Hansen, V., Santos, S. S., Nielsen, T. K., Giagnoni, L., Hauggaard-Nielsen, H., Johansen, A., Renella, G. & Winding, A. 2016. Gasification biochar has limited effects on

functional and structural diversity of soil microbial communities in a temperate agroecosystem. *Soil Biology and Biochemistry,* 99**,** 128-136.

Jia, Z. & Conrad, R. 2009. Bacteria rather than Archaea dominate microbial ammonia oxidation in an agricultural soil. *Environmental Microbiology,* 11**,** 1658-1671.

Jiang, C.-H., Huang, Z.-Y., Xie, P., Gu, C., Li, K., Wang, D.-C., Yu, Y.-Y., Fan, Z.-H., Wang, C.-J. & Wang, Y.-P. 2016. Transcription factors WRKY70 and WRKY11 served as regulators in rhizobacterium *Bacillus cereus* AR156-induced systemic resistance to *Pseudomonas syringae* pv. tomato DC3000 in Arabidopsis. *Journal of Experimental Botany,* 67**,** 157-174.

Joa, J. H., Weon, H. Y., Hyun, H. N., Jeun, Y. C. & Koh, S. W. 2014. Effect of long-term different fertilization on bacterial community structures and diversity in citrus orchard soil of volcanic ash. *Journal of Microbiology,* 52**,** 995-1001.

Jonason, D., Andersson, G. K., Öckinger, E., Rundlöf, M., Smith, H. G. & Bengtsson, J. 2011. Assessing the effect of the time since transition to organic farming on plants and butterflies. *Journal of Applied Ecology,* 48**,** 543-550.

Jureková, Z., Németh-Molnár, K. & Paganová, V. 2011. Physiological responses of six tomato (*Lycopersicon esculentum* Mill.) cultivars to water stress. *Journal of Horticulture and Forestry,* 3**,** 294-300.

Kaiser, K., Wemheuer, B., Korolkow, V., Wemheuer, F., Nacke, H., Schöning, I., Schrumpf, M. & Daniel, R. 2016. Driving forces of soil bacterial community structure, diversity, and function in temperate grasslands and forests. *Scientific Reports,* 6**,** 1-12.

Kamatham, S., Neela, K. B., Pasupulati, A. K., Pallu, R., Singh, S. S. & Gudipalli, P. 2016. Benzoylsalicylic acid isolated from seed coats of *Givotia rottleriformis* induces systemic acquired resistance in tobacco and *Arabidopsis*. *Phytochemistry,* 126**,** 11-22.

Kang, H., Freeman, C. & Chun, J. 2005. N-Acetylglucosaminidase activities in wetlands: a global survey. *Hydrobiologia,* 532**,** 103-110.

Kapoor, R., Gupta, M., Kumar, N. & Kanwar, S. 2017. Analysis of *nhaA* gene from salt tolerant and plant growth promoting *Enterobacter ludwigii*. *Rhizosphere,* 4**,** 62-69.

Karimi, E., Safaie, N., Shams-Baksh, M. & Mahmoudi, B. 2016. *Bacillus amyloliquefaciens* SB14 from rhizosphere alleviates *Rhizoctonia* damping-off disease on sugar beet. *Microbiological Research,* 192**,** 221-230.

Karlidag, H., Yildirim, E., Turan, M., Pehluvan, M. & Donmez, F. 2013. Plant growth-promoting rhizobacteria mitigate deleterious effects of salt stress on strawberry plants (*Fragaria*× *ananassa*). *Hortscience,* 48**,** 563-567.

Kasim, W. A., Gaafar, R. M., Abou-Ali, R. M., Omar, M. N. & Hewait, H. M. 2016. Effect of biofilm forming plant growth promoting rhizobacteria on salinity tolerance in barley. *Annals of Agricultural Sciences,* 61**,** 217-227.

Kasmani, M. B., Samavat, S., Mostafavi, M. & Khalighi, A. 2013. The effect of application of humic acid foliar on biochemical parameters of pistachio under drought stress. *New York Sci J,* 6**,** 26-31.

Kavamura, V. N., Hayat, R., Clark, I. M., Rossmann, M., Mendes, R., Hirsch, P. R. & Mauchline, T. H. 2018. Inorganic nitrogen application affects both taxonomical and predicted functional structure of wheat rhizosphere bacterial communities. *Frontiers in Microbiology,* 9**,** 1074.

Kavamura, V. N., Santos, S. N., da Silva, J. L., Parma, M. M., Ávila, L. A., Visconti, A., Zucchi, T. D., Taketani, R. G., Andreote, F. D. & de Melo, I. S. 2013. Screening of Brazilian cacti rhizobacteria for plant growth promotion under drought. *Microbiological Research,* 168**,** 183-191.

Kennedy, A. C. & Smith, K. 1995. Soil microbial diversity and the sustainability of agricultural soils. *Plant and Soil,* 170**,** 75-86.

Kent, W. J. 2002. BLAT—the BLAST-like alignment tool. *Genome Research,* 12**,** 656-664.

Keyvan, S. 2010. The effects of drought stress on yield, relative water content, proline, soluble carbohydrates and chlorophyll of bread wheat cultivars. *Journal of Animal and Plant Science,* 8**,** 1051-1060.

Khan, A., Zhao, X. Q., Javed, M. T., Khan, K. S., Bano, A., Shen, R. F. & Masood, S. 2016a. *Bacillus pumilus* enhances tolerance in rice (*Oryza sativa* L.) to combined stresses of NaCl and high boron due to limited uptake of Na+. *Environmental and Experimental Botany,* 124**,** 120-129.

Khan, A. L., Waqas, M., Asaf, S., Kamran, M., Shahzad, R., Bilal, S., Khan, M. A., Kang, S.-M., Kim, Y.-H. & Yun, B.-W. 2017. Plant growth-promoting endophyte Sphingomonas sp. LK11 alleviates salinity stress in Solanum pimpinellifolium. *Environmental and Experimental Botany,* 133**,** 58-69.

Khan, M. S., Zaidi, A. & Wani, P. A. 2007. Role of phosphate-solubilizing microorganisms in sustainable agriculture—a review. *Agronomy for Sustainable Development,* 27**,** 29-43.

Khan, Z., Rho, H., Firrincieli, A., Hung, S. H., Luna, V., Masciarelli, O., Kim, S.-H. & Doty, S. L. 2016b. Growth enhancement and drought tolerance of hybrid poplar upon inoculation with endophyte consortia. *Current Plant Biology,* 6**,** 38-47.

Khare, D., Choi, H., Huh, S. U., Bassin, B., Kim, J., Martinoia, E., Sohn, K. H., Paek, K.-H. & Lee, Y. 2017. Arabidopsis ABCG34 contributes to defense against necrotrophic pathogens by mediating the secretion of camalexin. *Proceedings of the National Academy of Sciences,* 114**,** E5712-E5720.

Kim, J.-S., Lee, J., Lee, C.-h., Woo, S. Y., Kang, H., Seo, S.-G. & Kim, S.-H. 2015. Activation of pathogenesis-related genes by the rhizobacterium, *Bacillus* sp. JS, which induces systemic resistance in tobacco plants. *The Plant Pathology Journal,* 31**,** 195.

Klein, A., Keyster, M. & Ludidi, N. 2015. Response of soybean nodules to exogenously applied caffeic acid during NaCl-induced salinity. *South African Journal of Botany,* 96**,** 13-18.

Knelman, J. E., Schmidt, S. K., Lynch, R. C., Darcy, J. L., Castle, S. C., Cleveland, C. C. & Nemergut, D. R. 2014. Nutrient addition dramatically accelerates microbial community succession. *PloS One,* 9.

Kolton, M., Harel, Y. M., Pasternak, Z., Graber, E. R., Elad, Y. & Cytryn, E. 2011. Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. *Applied and Environmental Microbiology,* 77**,** 4924-4930.

Kong, H. G., Shin, T. S., Kim, T. H. & Ryu, C.-M. 2018. Stereoisomers of the bacterial volatile compound 2, 3-Butanediol differently elicit systemic defense responses of pepper against multiple viruses in the field. *Frontiers in Plant Science,* 9**,** 90.

Kong, J., Pei, Z., Du, M., Sun, G. & Zhang, X. 2014. Effects of arbuscular mycorrhizal fungi on the drought resistance of the mining area repair plant Sainfoin. *International Journal of Mining Science and Technology,* 24**,** 485-489.

Kovacevic, S., Tobin, M. B. & Miller, J. R. 1990. The beta-lactam biosynthesis genes for isopenicillin N epimerase and deacetoxycephalosporin C synthetase are expressed from a single transcript in Streptomyces clavuligerus. *Journal of Bacteriology,* 172**,** 3952-3958.

Kremen, C. & Miles, A. 2012. Ecosystem services in biologically diversified versus conventional farming systems: benefits, externalities, and trade-offs. *Ecology and Society,* 17.

Kumar, U., Nayak, A. K., Shahid, M., Gupta, V. V., Panneerselvam, P., Mohanty, S., Kaviraj, M., Kumar, A., Chatterjee, D. & Lal, B. 2018. Continuous application of inorganic and organic fertilizers over 47 years in paddy soil alters the bacterial community structure and its influence on rice production. *Agriculture, Ecosystems & Environment,* 262**,** 65-75.

Kumari, P. & Khanna, V. 2016. Biodiversity of *Pseudomonas* and *Bacillus* possessing both bioantagonistic and plant growth promoting traits in chickpea rhizosphere. *International Journal of Science and Nature,* 7**,** 153-158.

Kuppusamy, S., Daquiado, A. R., Kim, S. Y., Yoon, Y.-E., Kim, J. H., Kim, S. J. & Lee, Y. B. 2018. Agriculturally relevant microbial community structure in long-term fertilized paddy soils as

revealed by phospholipid fatty acid (PLFA) and pyrosequencing analyses. *Archives of Agronomy and Soil Science,* 64**,** 1379-1393.

Kuramae, E. E., Yergeau, E., Wong, L. C., Pijl, A. S., van Veen, J. A. & Kowalchuk, G. A. 2012. Soil characteristics more strongly influence soil bacterial communities than land-use type. *FEMS Microbiology Ecology,* 79**,** 12-24.

Kusajima, M., Okumura, Y., Fujita, M. & Nakashita, H. 2017. Abscisic acid modulates salicylic acid biosynthesis for systemic acquired resistance in tomato. *Bioscience, Biotechnology, and Biochemistry,* 81**,** 1850-1853.

Lahiri, S., Ghosh, D. & Sarkar, D. 2018. Biogeochemical cycling bacteria and nutrient dynamics in waste stabilization pond system. *Wastewater Management Through Aquaculture.* Springer.

Lai, J., Cao, X., Yu, T., Wang, Q., Zhang, Y., Zheng, X. & Lu, H. 2018. Effect of *Cryptococcus laurentii* on inducing disease resistance in cherry tomato fruit with focus on the expression of defense-related genes. *Food Chemistry,* 254**,** 208-216.

Lai, Y.-R., Lin, P.-Y., Chen, C.-Y. & Huang, C.-J. 2016. Feasible management of southern corn leaf blight via induction of systemic resistance by *Bacillus cereus* C1L in combination with reduced use of dithiocarbamate fungicides. *The Plant Pathology Journal,* 32**,** 481.

Lamdan, N.-L., Shalaby, S., Ziv, T., Kenerley, C. M. & Horwitz, B. A. 2015. Secretome of *Trichoderma* interacting with maize roots: role in induced systemic resistance. *Molecular & Cellular Proteomics,* 14**,** 1054-1063.

Lammel, D. R., Nüsslein, K., Tsai, S. M. & Cerri, C. C. 2015. Land use, soil and litter chemistry drive bacterial community structures in samples of the rainforest and Cerrado (Brazilian Savannah) biomes in Southern Amazonia. *European Journal of Soil Biology,* 66**,** 32-39.

Lammerts van Bueren, E., Struik, P. & Jacobsen, E. 2002. Ecological concepts in organic farming and their consequences for an organic crop ideotype. *Netherlands Journal of Agricultural Science (en).*

Lassaletta, L., Billen, G., Grizzetti, B., Anglade, J. & Garnier, J. 2014. 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. *Environmental Research Letters,* 9**,** 105011.

Lastochkina, O., Pusenkova, L., Yuldashev, R., Babaev, M., Garipova, S., Blagova, D. y., Khairullin, R. & Aliniaeifard, S. 2017. Effects of *Bacillus subtilis* on some physiological and biochemical parameters of *Triticum aestivum* L.(wheat) under salinity. *Plant Physiology and Biochemistry,* 121**,** 80-88.

Latif Khan, A., Ahmed Halo, B., Elyassi, A., Ali, S., Al-Hosni, K., Hussain, J., Al-Harrasi, A. & 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.

Latz, E., Eisenhauer, N., Rall, B. C., Allan, E., Roscher, C., Scheu, S. & Jousset, A. 2012. Plant diversity improves protection against soil-borne pathogens by fostering antagonistic bacterial communities. *Journal of Ecology,* 100**,** 597-604.

Lawal, T. E. & Babalola, O. O. 2014. Assessing the associated challenges in the use of animal manure in plant growth. *Journal of Human Ecology,* 48**,** 285-297.

Lazcano, C., Gómez-Brandón, M., Revilla, P. & Domínguez, J. 2013. Short-term effects of organic and inorganic fertilizers on soil microbial community structure and function. *Biology and Fertility of Soils,* 49**,** 723-733.

Lazzarotto, P., Calanca, P., Semenov, M. & Fuhrer, J. 2010. Transient responses to increasing CO2 and climate change in an unfertilized grass–clover sward. *Climate Research,* 41**,** 221-232.

Lee, B. D., Dutta, S., Ryu, H., Yoo, S.-J., Suh, D.-S. & Park, K. 2015. Induction of systemic resistance in Panax ginseng against *Phytophthora cactorum* by native *Bacillus amyloliquefaciens* HK34. *Journal of Ginseng Research,* 39**,** 213-220.

Lee, G., Lee, S.-H., Kim, K. M. & Ryu, C.-M. 2017. Foliar application of the leaf-colonizing yeast *Pseudozyma churashimaensis* elicits systemic defense of pepper against bacterial and viral pathogens. *Scientific Reports,* 7**,** 1-13.

Lee, S. B., Lee, C. H., Jung, K. Y., Do Park, K., Lee, D. & Kim, P. J. 2009. Changes of soil organic carbon and its fractions in relation to soil physical properties in a long-term fertilized paddy. *Soil and Tillage Research,* 104**,** 227-232.

Lei, C., Ma, D., Pu, G., Qiu, X., Du, Z., Wang, H., Li, G., Ye, H. & Liu, B. 2011. Foliar application of chitosan activates artemisinin biosynthesis in *Artemisia annua* L. *Industrial Crops and Products,* 33**,** 176-182.

Li, C., Yan, K., Tang, L., Jia, Z. & Li, Y. 2014a. Change in deep soil microbial communities due to long-term fertilization. *Soil Biology and Biochemistry,* 75**,** 264-272.

Li, H., Lei, P., Pang, X., Li, S., Xu, H., Xu, Z. & Feng, X. 2017. Enhanced tolerance to salt stress in canola (*Brassica napus* L.) seedlings inoculated with the halotolerant *Enterobacter cloacae* HSNJ4. *Applied Soil Ecology,* 119**,** 26-34.

Li, L., Guo, P., Jin, H. & Li, T. 2016. Different proteomics of Ca2+ on SA-induced resistance to *Botrytis cinerea* in tomato. *Horticultural Plant Journal,* 2**,** 154-162.

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

Li, X., Rui, J., Xiong, J., Li, J., He, Z., Zhou, J., Yannarell, A. C. & Mackie, R. I. 2014c. Functional potential of soil microbial communities in the maize rhizosphere. *PloS One,* 9.

Li, Y., Li, Q., Hong, Q., Lin, Y., Mao, W. & Zhou, S. 2018. Reactive oxygen species triggering systemic programmed cell death process via elevation of nuclear calcium ion level in tomatoes resisting tobacco mosaic virus. *Plant Science,* 270**,** 166-175.

Li, Y., Yang, Y., Shen, F., Tian, D., Zeng, Y., Yang, G., Zhang, Y. & Deng, S. 2019. Partitioning biochar properties to elucidate their contributions to bacterial and fungal community composition of purple soil. *Science of the Total Environment,* 648**,** 1333-1341.

Liang, B., Ma, C., Fan, L., Wang, Y. & Yuan, Y. 2018. Soil amendment alters soil physicochemical properties and bacterial community structure of a replanted apple orchard. *Microbiological Research,* 216**,** 1-11.

Liang, C. & Balser, T. C. 2012. Warming and nitrogen deposition lessen microbial residue contribution to soil carbon pool. *Nature Communications,* 3**,** 1-4.

Ling, N., Sun, Y., Ma, J., Guo, J., Zhu, P., Peng, C., Yu, G., Ran, W., Guo, S. & Shen, Q. 2014. Response of the bacterial diversity and soil enzyme activity in particle-size fractions of Mollisol after different fertilization in a long-term experiment. *Biology and Fertility of Soils,* 50**,** 901-911.

Ling, N., Zhu, C., Xue, C., Chen, H., Duan, Y., Peng, C., Guo, S. & Shen, Q. 2016. Insight into how organic amendments can shape the soil microbiome in long-term field experiments as revealed by network analysis. *Soil Biology and Biochemistry,* 99**,** 137-149.

Liu, E., Yan, C., Mei, X., He, W., Bing, S. H., Ding, L., Liu, Q., Liu, S. & Fan, T. 2010. Long-term effect of chemical fertilizer, straw, and manure on soil chemical and biological properties in northwest China. *Geoderma,* 158**,** 173-180.

Liu, J.-J., Williams, H., Li, X. R., Schoettle, A. W., Sniezko, R. A., Murray, M., Zamany, A., Roke, G. & Chen, H. 2017. Profiling methyl jasmonate-responsive transcriptome for understanding induced systemic resistance in whitebark pine (*Pinus albicaulis*). *Plant Molecular Biology,* 95**,** 359-374.

Liu, J., Zhang, X., Wang, H., Hui, X., Wang, Z. & Qiu, W. 2018. Long-term nitrogen fertilization impacts soil fungal and bacterial community structures in a dryland soil of Loess Plateau in China. *Journal of Soils and Sediments,* 18**,** 1632-1640.

Lloret, E., Pascual, J. A., Brodie, E. L., Bouskill, N. J., Insam, H., Juárez, M. F.-D. & Goberna, M. 2016. Sewage sludge addition modifies soil microbial communities and plant performance depending on the sludge stabilization process. *Applied Soil Ecology,* 101**,** 37-46.

Lopez-Gresa, M. P., Lisón, P., Yenush, L., Conejero, V., Rodrigo, I. & Belles, J. M. 2016. Salicylic acid is involved in the basal resistance of tomato plants to citrus exocortis viroid and tomato spotted wilt virus. *PloS One,* 11.

Lotter, D., Valentine, A. J., Van Garderen, E. A. & Tadross, M. 2014. Physiological responses of a fynbos legume, *Aspalathus linearis* to drought stress. *South African Journal of Botany,* 94**,** 218-223.

Lu, F., Liang, X., Lu, H., Li, Q., Chen, Q., Zhang, P., Liu, G., Yan, W., Song, J. & Duan, C. 2017. Overproduction of superoxide dismutase and catalase confers cassava resistance to Tetranychus cinnabarinus. *Scientific Reports,* 7**,** 40179.

Lu, H., Lashari, M. S., Liu, X., Ji, H., Li, L., Zheng, J., Kibue, G. W., Joseph, S. & Pan, G. 2015. Changes in soil microbial community structure and enzyme activity with amendment of biochar-manure compost and pyroligneous solution in a saline soil from Central China. *European Journal of Soil Biology,* 70**,** 67-76.

Lu, L., Han, W., Zhang, J., Wu, Y., Wang, B., Lin, X., Zhu, J., Cai, Z. & Jia, Z. 2012. Nitrification of archaeal ammonia oxidizers in acid soils is supported by hydrolysis of urea. *The ISME Journal,* 6**,** 1978-1984.

Lu, X.-M., Lu, P.-L., Lu, P.-Z. & Zhang, H. 2014. A comparative study of microbial communities in soils amended by manures from pigs fed with organic versus synthetic feeds. *Toxicological & Environmental Chemistry,* 96**,** 426-441.

Lucas, J. A., García-Cristobal, J., Bonilla, A., Ramos, B. & 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.

Luiz, C., Neto, A. R. & Di Piero, R. 2015. Resistance to *Xanthomonas gardneri* in tomato leaves induced by polysaccharides from plant or microbial origin. *Journal of Plant Pathology***,** 119-127.

Ma, M., Zhou, J., Ongena, M., Liu, W., Wei, D., Zhao, B., Guan, D., Jiang, X. & Li, J. 2018. Effect of long-term fertilization strategies on bacterial community composition in a 35-year field experiment of Chinese Mollisols. *AMB Express,* 8**,** 20.

Ma, Y., Rajkumar, M., Zhang, C. & Freitas, H. 2016. Inoculation of *Brassica oxyrrhina* with plant growth promoting bacteria for the improvement of heavy metal phytoremediation under drought conditions. *Journal of Hazardous Materials,* 320**,** 36-44.

Ma, Z., Ongena, M. & Höfte, M. 2017. The cyclic lipopeptide orfamide induces systemic resistance in rice to *Cochliobolus miyabeanus* but not to *Magnaporthe oryzae*. *Plant Cell Reports,* 36**,** 1731-1746.

Majumder, S. P. & Das, A. C. 2016. Phosphate-solubility and phosphatase activity in Gangetic alluvial soil as influenced by organophosphate insecticide residues. *Ecotoxicology and Environmental Safety,* 126**,** 56-61.

Makhalanyane, T. P., Valverde, A., Gunnigle, E., Frossard, A., Ramond, J.-B. & Cowan, D. A. 2015. Microbial ecology of hot desert edaphic systems. *FEMS Microbiology Reviews,* 39**,** 203-221.

Malfanova, N., Lugtenberg, B. & Berg, G. 2013. Bacterial endophytes: who and where, and what are they doing there. *Molecular Microbial Ecology of the Rhizosphere***,** 15-37.

Manaf, H. H. & Zayed, M. S. 2015. Productivity of cowpea as affected by salt stress in presence of endomycorrhizae and *Pseudomonas fluorescens*. *Annals of Agricultural Sciences,* 60**,** 219-226.

Mander, C., Wakelin, S., Young, S., Condron, L. & O'Callaghan, M. 2012. Incidence and diversity of phosphate-solubilising bacteria are linked to phosphorus status in grassland soils. *Soil Biology and Biochemistry,* 44**,** 93-101.

Marschner, P., Crowley, D. & Yang, C. H. 2004. Development of specific rhizosphere bacterial communities in relation to plant species, nutrition and soil type. *Plant and Soil,* 261**,** 199-208.

Marschner, P., Kandeler, E. & Marschner, B. 2003. Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biology and Biochemistry,* 35**,** 453-461.

Marschner, P., Umar, S. & Baumann, K. 2011. The microbial community composition changes rapidly in the early stages of decomposition of wheat residue. *Soil Biology and Biochemistry,* 43**,** 445-451.

Martínez-Hidalgo, P., García, J. M. & Pozo, M. J. 2015. Induced systemic resistance against *Botrytis cinerea* by *Micromonospora* strains isolated from root nodules. *Frontiers in Microbiology,* 6**,** 922.

Martínez-Medina, A., Fernandez, I., Lok, G. B., Pozo, M. J., Pieterse, C. M. & Van Wees, S. C. 2017. Shifting from priming of salicylic acid-to jasmonic acid-regulated defences by *Trichoderma* protects tomato against the root knot nematode *Meloidogyne incognita*. *New Phytologist,* 213**,** 1363-1377.

Martínez, R., Espejo, A., Sierra, M., Ortiz-Bernad, I., Correa, D., Bedmar, E., López-Jurado, M. & Porres, J. M. 2015. Co-inoculation of *Halomonas maura* and *Ensifer meliloti* to improve alfalfa yield in saline soils. *Applied Soil Ecology,* 87**,** 81-86.

Martins, L. F., Antunes, L. P., Pascon, R. C., de Oliveira, J. C. F., Digiampietri, L. A., Barbosa, D., Peixoto, B. M., Vallim, M. A., Viana-Niero, C. & Ostroski, E. H. 2013. Metagenomic analysis of a tropical composting operation at the São Paulo Zoo Park reveals diversity of biomass degradation functions and organisms. *PloS One,* 8.

Masciarelli, O., Llanes, A. & Luna, V. 2014. A new PGPR co-inoculated with *Bradyrhizobium japonicum* enhances soybean nodulation. *Microbiological Research,* 169**,** 609-615.

Matilla, M. A. & Krell, T. 2017. Chemoreceptor-based signal sensing. *Current Opinion in Biotechnology,* 45**,** 8-14.

Mauch, F., Mauch-Mani, B. & Boller, T. 1988. Antifungal hydrolases in pea tissue: II. Inhibition of fungal growth by combinations of chitinase and β-1, 3-glucanase. *Plant Physiology,* 88**,** 936-942.

Meco, G., Bonifati, V., Vanacore, N. & Fabrizio, E. 1994. Parkinsonism after chronic exposure to the fungicide maneb (manganese ethylene-bis-dithiocarbamate). *Scandinavian Journal of Work, Environment & Health***,** 301-305.

Mehta, C., Palni, U., Franke-Whittle, I. & Sharma, A. 2014. Compost: its role, mechanism and impact on reducing soil-borne plant diseases. *Waste Management,* 34**,** 607-622.

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

Mendes, L. W., Tsai, S. M., Navarrete, A. A., De Hollander, M., van Veen, J. A. & Kuramae, E. E. 2015. Soil-borne microbiome: linking diversity to function. *Microbial Ecology,* 70**,** 255-265.

Mendes, R., Garbeva, P. & Raaijmakers, J. M. 2013. The rhizosphere microbiome: significance of plant beneficial, plant pathogenic, and human pathogenic microorganisms. *FEMS Microbiology Reviews,* 37**,** 634-663.

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. & Bakker, P. A. 2011. Deciphering the rhizosphere microbiome for disease-suppressive bacteria. *Science,* 332**,** 1097-1100.

Meneghine, A. K., Nielsen, S., Varani, A. M., Thomas, T. & Alves, L. M. C. 2017. Metagenomic analysis of soil and freshwater from zoo agricultural area with organic fertilization. *PloS One,* 12.

Meng, X., Wang, L., Long, X., Liu, Z., Zhang, Z. & Zed, R. 2012. Influence of nitrogen fertilization on diazotrophic communities in the rhizosphere of the Jerusalem artichoke (*Helianthus tuberosus* L.). *Research in Microbiology,* 163**,** 349-356.

Mengual, C., Roldán, A., Caravaca, F. & Schoebitz, M. 2014a. Advantages of inoculation with immobilized rhizobacteria versus amendment with olive-mill waste in the afforestation of a semiarid area with *Pinus halepensis* Mill. *Ecological Engineering,* 73**,** 1-8.

Mengual, C., Schoebitz, M., Azcón, R. & Roldán, A. 2014b. Microbial inoculants and organic amendment improves plant establishment and soil rehabilitation under semiarid conditions. *Journal of Environmental Management,* 134**,** 1-7.

Meyer, F., Paarmann, D., D'Souza, M., Olson, R., Glass, E. M., Kubal, M., Paczian, T., Rodriguez, A., Stevens, R. & Wilke, A. 2008. The metagenomics RAST server–a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC bioinformatics,* 9**,** 386.

Michelsen, C. F., Pedas, P., Glaring, M. A., Schjoerring, J. K. & Stougaard, P. 2014. Bacterial diversity in Greenlandic soils as affected by potato cropping and inorganic versus organic fertilization. *Polar Biology,* 37**,** 61-71.

Miliute, I., Buzaite, O., Baniulis, D. & Stanys, V. 2015. Bacterial endophytes in agricultural crops and their role in stress tolerance: a review. *Zemdirbyste-Agriculture,* 102**,** 465-478.

Mirza, B. S., Potisap, C., Nüsslein, K., Bohannan, B. J. & Rodrigues, J. L. 2014. Response of free-living nitrogen-fixing microorganisms to land use change in the Amazon rainforest. *Applied Environmental Microbiology,* 80**,** 281-288.

Mishima, S.-i., Taniguchi, S. & Komada, M. 2006. Recent trends in nitrogen and phosphate use and balance on Japanese farmland. *Soil Science and Plant Nutrition,* 52**,** 556-563.

Mishra, A., Morang, P., Deka, M., Kumar, S. N. & Kumar, B. D. 2014. Plant growth-promoting rhizobacterial strain-mediated induced systemic resistance in tea (*Camellia sinensis* (L.) O. Kuntze) through defense-related enzymes against brown root rot and charcoal stump rot. *Applied Biochemistry and Biotechnology,* 174**,** 506-521.

Misra, S., Dixit, V. K., Khan, M. H., Mishra, S. K., Dviwedi, G., Yadav, S., Lehri, A. & Chauhan, P. S. 2017. Exploitation of agro-climatic environment for selection of 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase producing salt tolerant indigenous plant growth promoting rhizobacteria. *Microbiological Research,* 205**,** 25-34.

Mokoboki, K. & Sebola, N. 2017. Chemical composition and feed intake of *Opuntia cladodes* varieties offered to goats. *Journal of Animal &Plant Sciences,* 32**,** 5096-5103.

Molinari, S., Fanelli, E. & Leonetti, P. 2014. Expression of tomato salicylic acid (SA)-responsive pathogenesis-related genes in Mi-1-mediated and SA-induced resistance to root-knot nematodes. *Molecular Plant Pathology,* 15**,** 255-264.

Moustaine, M., Elkahkahi, R., Benbouazza, A., Benkirane, R. & Achbani, E. 2017. Effect of plant growth promoting rhizobacterial (PGPR) inoculation on growth in tomato (*Solanum*

lycopersicum L.) and characterization for direct PGP abilities in Morocco. *International Journal of Environment, Agriculture and Biotechnology,* 2.

Motsara, M. R., & Roy, R. N. 2008 Guide to laboratory establishment for plant nutrient analysis. Food and Agriculture Organization of the United Nations FAO Fertilizer and Plant Nutrition Bulletin No 19. ISBN 978-92-5-105981-4. 204pp

Nair, A., Kolet, S., Thulasiram, H. & Bhargava, S. 2015. Systemic jasmonic acid modulation in mycorrhizal tomato plants and its role in induced resistance against *Alternaria alternata*. *Plant Biology,* 17**,** 625-631.

Nanda, A. K., Andrio, E., Marino, D., Pauly, N. & Dunand, C. 2010. Reactive oxygen species during plant-microorganism early interactions. *Journal of Integrative Plant Biology,* 52**,** 195-204.

Nassar, A. M. & Adss, I. A. 2016. 2, 4-Dichlorophenoxy acetic acid, abscisic acid, and hydrogen peroxide induced resistance-related components against potato early blight (*Alternaria solani, Sorauer*). *Annals of Agricultural Sciences,* 61**,** 15-23.

Naveed, M., Qureshi, M. A., Zahir, Z. A., Hussain, M. B., Sessitsch, A. & Mitter, B. 2015. L-Tryptophan-dependent biosynthesis of indole-3-acetic acid (IAA) improves plant growth and colonization of maize by *Burkholderia phytofirmans* PsJN. *Annals of Microbiology,* 65**,** 1381-1389.

Nawrocka, J., Małolepsza, U., Szymczak, K. & Szczech, M. 2018. Involvement of metabolic components, volatile compounds, PR proteins, and mechanical strengthening in multilayer protection of cucumber plants against *Rhizoctonia solani* activated by *Trichoderma atroviride* TRS25. *Protoplasma,* 255**,** 359-373.

Naznin, H. A., Kiyohara, D., Kimura, M., Miyazawa, M., Shimizu, M. & Hyakumachi, M. 2014. Systemic resistance induced by volatile organic compounds emitted by plant growth-promoting fungi in *Arabidopsis thaliana. PloS One,* 9.

Neal, A. L., Ahmad, S., Gordon-Weeks, R. & Ton, J. 2012. Benzoxazinoids in root exudates of maize attract Pseudomonas putida to the rhizosphere. *PloS One,* 7.

Ng, J. P., Hollister, E. B., González-Chávez, M. d. C. A., Hons, F. M., Zuberer, D. A., Aitkenhead-Peterson, J. A., Loeppert, R. & Gentry, T. J. 2012. Impacts of cropping systems and long-term tillage on soil microbial population levels and community composition in dryland agricultural setting. *ISRN Ecology*, 2012.

Nie, P., Li, X., Wang, S., Guo, J., Zhao, H. & Niu, D. 2017. Induced systemic resistance against *Botrytis cinerea* by *Bacillus cereus* AR156 through a JA/ET-and NPR1-dependent signaling pathway and activates PAMP-triggered immunity in Arabidopsis. *Frontiers in Plant Science,* 8**,** 238.

Nihorimbere, V., Ongena, M., Smargiassi, M. & Thonart, P. 2011. Beneficial effect of the rhizosphere microbial community for plant growth and health. *Biotechnologie, Agronomie, Société et Environnement,* 15**,** 327-337.

Niu, D., Wang, X., Wang, Y., Song, X., Wang, J., Guo, J. & Zhao, H. 2016. Bacillus cereus AR156 activates PAMP-triggered immunity and induces a systemic acquired resistance through a NPR1-and SA-dependent signaling pathway. *Biochemical and Biophysical Research Communications,* 469**,** 120-125.

Nocker, A., Fernández, P. S., Montijn, R. & Schuren, F. 2012. Effect of air drying on bacterial viability: a multiparameter viability assessment. *Journal of Microbiological Methods,* 90**,** 86-95.

Norton, J. M. 2011. Diversity and environmental distribution of ammonia-oxidizing bacteria. *Nitrification.* American Society of Microbiology.

Nunes da Rocha, U., Van Overbeek, L. & Van Elsas, J. D. 2009. Exploration of hitherto-uncultured bacteria from the rhizosphere. *FEMS Microbiology Ecology,* 69**,** 313-328.

O'Hanlon, K. A., Knorr, K., Jørgensen, L. N., Nicolaisen, M. & Boelt, B. 2012. Exploring the potential of symbiotic fungal endophytes in cereal disease suppression. *Biological Control,* 63**,** 69-78.

Ofek-Lalzar, M., Sela, N., Goldman-Voronov, M., Green, S. J., Hadar, Y. & Minz, D. 2014. Niche and host-associated functional signatures of the root surface microbiome. *Nature Communications,* 5**,** 1-9.

Oka, Y. 2010. Mechanisms of nematode suppression by organic soil amendments—a review. *Applied Soil Ecology,* 44**,** 101-115.

Olanrewaju, O. S., Ayangbenro, A. S., Glick, B. R., Babalola, O. O. 2019. Plant health: feedback effect of root exudates-rhizobiome interactions. *Applied Microbiology and Biotechnology,* 103**,** 1155-1166.

Olanrewaju, O. S., Glick, B. R. & Babalola, O. O. 2017. Mechanisms of action of plant growth promoting bacteria. *World Journal of Microbiology and Biotechnology,* 33**,** 197.

Orhan, F. 2016. Alleviation of salt stress by halotolerant and halophilic plant growth-promoting bacteria in wheat (*Triticum aestivum*). *Brazilian Journal of Microbiology,* 47**,** 621-627.

Orr, C. H., James, A., Leifert, C., Cooper, J. M. & Cummings, S. P. 2011. Diversity and activity of free-living nitrogen-fixing bacteria and total bacteria in organic and conventionally managed soils. *Applied and Environmental Microbiology,* 77**,** 911-919.

Ortiz, N., Armada, E., Duque, E., Roldán, A. & Azcón, R. 2015. Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: effectiveness of autochthonous or allochthonous strains. *Journal of Plant Physiology,* 174**,** 87-96.

Oubrie, A., Rozeboom, H. J., Kalk, K. H., Olsthoorn, A. J., Duine, J. A. & Dijkstra, B. W. 1999. Structure and mechanism of soluble quinoprotein glucose dehydrogenase. *The EMBO Journal,* 18**,** 5187-5194.

Ouyang, Y., Norton, J. M., Stark, J. M., Reeve, J. R. & Habteselassie, M. Y. 2016. Ammonia-oxidizing bacteria are more responsive than archaea to nitrogen source in an agricultural soil. *Soil Biology and Biochemistry,* 96**,** 4-15.

Overbeek, R., Disz T., & Stevens, R. 2004 The SEED: a peer-to-peer environment for genome annotation Communication ACM 4746–51

Overbeek, R., Begley, T., Butler, R. M., Choudhuri, J. V., Chuang, H.-Y., Cohoon, M., de Crécy-Lagard, V., Diaz, N., Disz, T. & 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.

Parkinson, J. S., Hazelbauer, G. L. & Falke, J. J. 2015. Signaling and sensory adaptation in *Escherichia coli* chemoreceptors: 2015 update. *Trends in Microbiology,* 23**,** 257-266.

Pascault, N., Ranjard, L., Kaisermann, A., Bachar, D., Christen, R., Terrat, S., Mathieu, O., Lévêque, J., Mougel, C. & Henault, C. 2013. Stimulation of different functional groups of bacteria by various plant residues as a driver of soil priming effect. *Ecosystems,* 16**,** 810-822.

Patel, D. K., Archana, G. & Kumar, G. N. 2008. Variation in the nature of organic acid secretion and mineral phosphate solubilization by *Citrobacter* sp. DHRSS in the presence of different sugars. *Current Microbiology,* 56**,** 168-174.

Patel, S., Jinal, H. & Amaresan, N. 2017. Isolation and characterization of drought resistance bacteria for plant growth promoting properties and their effect on chilli (*Capsicum annuum*) seedling under salt stress. *Biocatalysis and Agricultural Biotechnology,* 12**,** 85-89.

Pathan, S. M., Lee, J. D., Sleper, D., Fritschi, F., Sharp, R., Carter Jr, T., Nelson, R. L., King, C., Schapaugh, W. & Ellersieck, M. 2014. Two soybean plant introductions display slow leaf wilting and reduced yield loss under drought. *Journal of Agronomy and Crop Science,* 200**,** 231-236.

Paulucci, N. S., Gallarato, L. A., Reguera, Y. B., Vicario, J. C., Cesari, A. B., de Lema, M. B. G. & Dardanelli, M. S. 2015. *Arachis hypogaea* PGPR isolated from Argentine soil modifies its lipids components in response to temperature and salinity. *Microbiological Research,* 173**,** 1-9.

Peiffer, J. A. & Ley, R. E. 2013. Exploring the maize rhizosphere microbiome in the field: a glimpse into a highly complex system. *Communicative & Integrative Biology,* 6**,** e25177.

Pereira, P., Ibáñez, F., Rosenblueth, M., Etcheverry, M. & Martínez-Romero, E. 2011. Analysis of the bacterial diversity associated with the roots of maize (*Zea mays* L.) through culture-dependent and culture-independent methods. *ISRN Ecology,* 2011.

Pereira, S. I., Moreira, H., Argyras, K., Castro, P. M. & Marques, A. P. 2016. Promotion of sunflower growth under saline water irrigation by the inoculation of beneficial microorganisms. *Applied Soil Ecology,* 105**,** 36-47.

Pereyra, M., García, P., Colabelli, M., Barassi, C. & Creus, C. 2012. A better water status in wheat seedlings induced by *Azospirillum* under osmotic stress is related to morphological changes in xylem vessels of the coleoptile. *Applied Soil Ecology,* 53**,** 94-97.

Perez, C., Dill-Macky, R. & Kinkel, L. L. 2008. Management of soil microbial communities to enhance populations of *Fusarium graminearum*-antagonists in soil. *Plant and Soil,* 302**,** 53-69.

Perry, R. D., Balbo, P. B., Jones, H. A., Fetherston, J. D. & DeMoll, E. 1999. Yersiniabactin from *Yersinia pestis*: biochemical characterization of the siderophore and its role in iron transport and regulation. *Microbiology,* 145**,** 1181-1190.

Pershina, E., Valkonen, J., Kurki, P., Ivanova, E., Chirak, E., Korvigo, I., Provorov, N. & Andronov, E. 2015. Comparative analysis of prokaryotic communities associated with organic and conventional farming systems. *PloS One,* 10**,** e0145072.

Pezzolla, D., Marconi, G., Turchetti, B., Zadra, C., Agnelli, A., Veronesi, F., Onofri, A., Benucci, G. M. N., Buzzini, P. & Albertini, E. 2015. Influence of exogenous organic matter on prokaryotic and eukaryotic microbiota in an agricultural soil. A multidisciplinary approach. *Soil Biology and Biochemistry,* 82**,** 9-20.

Philippot, L., Raaijmakers, J. M., Lemanceau, P. & Van Der Putten, W. H. 2013. Going back to the roots: the microbial ecology of the rhizosphere. *Nature Reviews Microbiology,* 11**,** 789-799.

Pieterse, C. M., Van der Does, D., Zamioudis, C., Leon-Reyes, A. & Van Wees, S. C. 2012. Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology,* 28**,** 489-521.

Pieterse, C. M., Zamioudis, C., Berendsen, R. L., Weller, D. M., Van Wees, S. C. & Bakker, P. A. 2014. Induced systemic resistance by beneficial microbes. *Annual Review of Phytopathology,* 52**,** 347-375.

Planchamp, C., Glauser, G. & Mauch-Mani, B. 2015. Root inoculation with *Pseudomonas putida* KT2440 induces transcriptional and metabolic changes and systemic resistance in maize plants. *Frontiers in Plant Science,* 5**,** 719.

Porter, S. L., Wadhams, G. H. & Armitage, J. P. 2011. Signal processing in complex chemotaxis pathways. *Nature Reviews Microbiology,* 9**,** 153-165.

Poulsen, P. H., Al-Soud, W. A., Bergmark, L., Magid, J., Hansen, L. H. & Sørensen, S. J. 2013. Effects of fertilization with urban and agricultural organic wastes in a field trial–Prokaryotic diversity investigated by pyrosequencing. *Soil Biology and Biochemistry,* 57**,** 784-793.

Qian, X., Gu, J., Sun, W., Li, Y.-D., Fu, Q.-X., Wang, X.-J. & Gao, H. 2014. Changes in the soil nutrient levels, enzyme activities, microbial community function, and structure during apple orchard maturation. *Applied Soil Ecology,* 77**,** 18-25.

Quiza, L., St-Arnaud, M. & Yergeau, E. 2015. Harnessing phytomicrobiome signaling for rhizosphere microbiome engineering. *Frontiers in Plant Science,* 6**,** 507.

Qurashi, A. W. & 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.

Raaijmakers, J. M. & Mazzola, M. 2012. Diversity and natural functions of antibiotics produced by beneficial and plant pathogenic bacteria. *Annual Review of Phytopathology,* 50**,** 403-424.

Raaijmakers, J. M. & Mazzola, M. 2016. Soil immune responses. *Science,* 352**,** 1392-1393.

Rais, A., Jabeen, Z., Shair, F., Hafeez, F. Y. & Hassan, M. N. 2017. *Bacillus* spp., a bio-control agent enhances the activity of antioxidant defense enzymes in rice against *Pyricularia oryzae*. *PloS One,* 12.

Ramirez, K. S., Craine, J. M. & Fierer, N. 2012. Consistent effects of nitrogen amendments on soil microbial communities and processes across biomes. *Global Change Biology,* 18**,** 1918-1927.

Ramirez, K. S., Lauber, C. L., Knight, R., Bradford, M. A. & Fierer, N. 2010. Consistent effects of nitrogen fertilization on soil bacterial communities in contrasting systems. *Ecology,* 91**,** 3463-3470.

Ranum, P., Peña-Rosas, J. P. & Garcia-Casal, M. N. 2014. Global maize production, utilization, and consumption. *Annals of the New York Academy of Sciences,* 1312**,** 105-112.

Rao, N. N., Gómez-García, M. R. & Kornberg, A. 2009. Inorganic polyphosphate: essential for growth and survival. *Annual Review of Biochemistry,* 78**,** 605-647.

Raut, S. & Borkar, S. 2014. PR-proteins accumulation in tomato plant due to application of resistance inducing chemicals during period of induced resistance against Alternaria leaf blight. *Science International Journal,* 2**,** 72-75.

Raza, W., Yousaf, S. & Rajer, F. U. 2016. Plant growth promoting activity of volatile organic compounds produced by biocontrol strains. *Scientific Letter,* 4**,** 40-43.

Read, D., Bengough, A. G., Gregory, P. J., Crawford, J. W., Robinson, D., Scrimgeour, C., Young, I. M., Zhang, K. & Zhang, X. 2003. Plant roots release phospholipid surfactants that modify the physical and chemical properties of soil. *New Phytologist,* 157**,** 315-326.

Reilly, K., Cullen, E., Lola-Luz, T., Stone, D., Valverde, J., Gaffney, M., Brunton, N., Grant, J. & Griffiths, B. S. 2013. Effect of organic, conventional and mixed cultivation practices on soil microbial community structure and nematode abundance in a cultivated onion crop. *Journal of the Science of Food and Agriculture,* 93**,** 3700-3709.

Richardson, A. E. & Simpson, R. J. 2011. Soil microorganisms mediating phosphorus availability update on microbial phosphorus. *Plant Physiology,* 156**,** 989-996.

Richardson, D., Felgate, H., Watmough, N., Thomson, A. & Baggs, E. 2009. Mitigating release of the potent greenhouse gas N2O from the nitrogen cycle–could enzymic regulation hold the key? *Trends in Biotechnology,* 27**,** 388-397.

Rodríguez, H., Fraga, R., Gonzalez, T. & Bashan, Y. 2006. Genetics of phosphate solubilization and its potential applications for improving plant growth-promoting bacteria. *Plant and Soil,* 287**,** 15-21.

Rousk, J., Bååth, E., Brookes, P. C., Lauber, C. L., Lozupone, C., Caporaso, J. G., Knight, R. & Fierer, N. 2010. Soil bacterial and fungal communities across a pH gradient in an arable soil. *The ISME Journal,* 4**,** 1340-1351.

Rui, J., Li, J., Wang, S., An, J., Liu, W.-t., Lin, Q., Yang, Y., He, Z. & Li, X. 2015. Responses of bacterial communities to simulated climate changes in alpine meadow soil of the Qinghai-Tibet Plateau. *Applied and Environmental Microbiology,* 81**,** 6070-6077.

Ryan, R. P., Germaine, K., Franks, A., Ryan, D. J. & Dowling, D. N. 2008. Bacterial endophytes: recent developments and applications. *FEMS Microbiology Letters,* 278**,** 1-9.

Saakre, M., Baburao, T. M., Salim, A. P., Ffancies, R. M., Achuthan, V. P., Thomas, G. & Sivarajan, S. R. 2017. Identification and characterization of genes responsible for drought tolerance in rice mediated by *Pseudomonas Fluorescens*. *Rice Science,* 24**,** 291-298.

Salas-Marina, M. A., Isordia-Jasso, M. I., Islas-Osuna, M. A., Delgado-Sánchez, P., Jiménez-Bremont, J. F., Rodríguez-Kessler, M., Rosales-Saavedra, M. T., Herrera-Estrella, A. & Casas-Flores, S. 2015. The Epl1 and Sm1 proteins from *Trichoderma atroviride* and *Trichoderma virens* differentially modulate systemic disease resistance against different life style pathogens in *Solanum lycopersicum*. *Frontiers in Plant Science,* 6**,** 77.

Saleem, A. R., Bangash, N., Mahmood, T., Khalid, A., Centritto, M. & Siddique, M. 2015. Rhizobacteria capable of producing ACC deaminase promote growth of velvet bean (*Mucuna pruriens*) under water stress condition.

Sánchez, C. & Minamisawa, K. 2018. Redundant roles of Bradyrhizobium oligotrophicum Cu-type (*NirK*) and cd 1-type (*NirS*) nitrite reductase genes under denitrifying conditions. *FEMS Microbiology Letters,* 365**,** fny015.

Sangkhobol, V. & Skerman, V. 1981. Chitinophaga, a new genus of chitinolytic myxobacteria. *International Journal of Systematic and Evolutionary Microbiology,* 31**,** 285-293.

Santhanam, R., Weinhold, A., Goldberg, J., Oh, Y. & 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.

Sapp, M., Harrison, M., Hany, U., Charlton, A. & Thwaites, R. 2015. Comparing the effect of digestate and chemical fertiliser on soil bacteria. *Applied Soil Ecology,* 86**,** 1-9.

Sapre, S., Gontia-Mishra, I. & Tiwari, S. 2018. *Klebsiella* sp. confers enhanced tolerance to salinity and plant growth promotion in oat seedlings (*Avena sativa*). *Microbiological Research,* 206**,** 25-32.

Saravanakumar, K., Fan, L., Fu, K., Yu, C., Wang, M., Xia, H., Sun, J., Li, Y. & Chen, J. 2016. Cellulase from *Trichoderma harzianum* interacts with roots and triggers induced systemic resistance to foliar disease in maize. *Scientific reports,* 6**,** 35543.

Sarkar, A., Ghosh, P. K., Pramanik, K., Mitra, S., Soren, T., Pandey, S., Mondal, M. H. & Maiti, T. K. 2018. A halotolerant *Enterobacter* sp. displaying ACC deaminase activity promotes rice seedling growth under salt stress. *Research in Microbiology,* 169**,** 20-32.

Sashidhar, B. & Podile, A. R. 2010. Mineral phosphate solubilization by rhizosphere bacteria and scope for manipulation of the direct oxidation pathway involving glucose dehydrogenase. *Journal of Applied Microbiology,* 109**,** 1-12.

Savci, S. 2012. An agricultural pollutant: chemical fertilizer. *International Journal of Environmental Science and Development,* 3**,** 73.

Schoebitz, M., Mengual, C. & Roldán, A. 2014. Combined effects of clay immobilized *Azospirillum brasilense* and *Pantoea dispersa* and organic olive residue on plant performance and soil properties in the revegetation of a semiarid area. *Science of the total environment,* 466**,** 67-73.

Schroder, J. L., Zhang, H., Girma, K., Raun, W. R., Penn, C. J. & Payton, M. E. 2011. Soil acidification from long-term use of nitrogen fertilizers on winter wheat. *Soil Science Society of America Journal,* 75**,** 957-964.

Schweinitzer, T. & Josenhans, C. 2010. Bacterial energy taxis: a global strategy? *Archives of Microbiology,* 192**,** 507-520.

Shabani, G., Ardakani, M., Chaichi, M., Friedel, J. & Khavazi, K. 2015. Effect of different fertilizing treatments on nutrient uptake in annual medic (*Medicago scutellata* cv. Robinson) under irrigated and dry farming systems. *Journal of Agricultural Science and Technology,* 17**,** 299-310.

Shaddad, M., HM, A. E.-S. & Mostafa, D. 2013. Role of gibberellic acid (GA3) in improving salt stress tolerance of two wheat cultivars. *Int J Plant Physiol Biochem,* 5**,** 50-57.

Shah, G., Jan, M., Afreen, M., Anees, M., Rehman, S., Daud, M., Malook, I. & Jamil, M. 2017. Halophilic bacteria mediated phytoremediation of salt-affected soils cultivated with rice. *Journal of Geochemical Exploration,* 174**,** 59-65.

Sharma, C. K., Vishnoi, V. K., Dubey, R. & Maheshwari, D. 2018. A twin rhizospheric bacterial consortium induces systemic resistance to a phytopathogen *Macrophomina phaseolina* in mung bean. *Rhizosphere,* 5**,** 71-75.

Sharma, S. K., Ramesh, A., Sharma, M. P., Joshi, O. P., Govaerts, B., Steenwerth, K. L. & Karlen, D. L. 2010. Microbial community structure and diversity as indicators for evaluating soil quality. *Biodiversity, Biofuels, Agroforestry and Conservation Agriculture.* Springer.

Sharpley, A., Jarvie, H. P., Buda, A., May, L., Spears, B. & Kleinman, P. 2013. Phosphorus legacy: overcoming the effects of past management practices to mitigate future water quality impairment. *Journal of Environmental Quality,* 42**,** 1308-1326.

Shen, J., Yuan, L., Zhang, J., Li, H., Bai, Z., Chen, X., Zhang, W. & Zhang, F. 2011a. Phosphorus dynamics: from soil to plant. *Plant physiology,* 156**,** 997-1005.

Shen, W., Gao, N., Min, J., Shi, W., He, X. & Lin, X. 2016. Influences of past application rates of nitrogen and a catch crop on soil microbial communities between an intensive rotation. *Acta Agriculturae Scandinavica, Section B—Soil & Plant Science,* 66**,** 97-106.

Shen, W., Lin, X., Gao, N., Shi, W., Min, J. & He, X. 2011b. Nitrogen fertilization changes abundance and community composition of ammonia-oxidizing bacteria. *Soil Science Society of America Journal,* 75**,** 2198-2205.

Shine, M., Xiao, X., Kachroo, P. & Kachroo, A. 2019. Signaling mechanisms underlying systemic acquired resistance to microbial pathogens. *Plant Science,* 279**,** 81-86.

Shivakrishna, P., Reddy, K. A. & Rao, D. M. 2018. Effect of PEG-6000 imposed drought stress on RNA content, relative water content (RWC), and chlorophyll content in peanut leaves and roots. *Saudi Journal of Biological Sciences,* 25**,** 285-289.

Simon, J. 2002. Enzymology and bioenergetics of respiratory nitrite ammonification. *FEMS Microbiology Reviews,* 26**,** 285-309.

Singh, H., Verma, A., Ansari, M. W. & Shukla, A. 2014. Physiological response of rice (*Oryza sativa* L.) genotypes to elevated nitrogen applied under field conditions. *Plant Signaling & Behavior,* 9**,** e29015.

Singh, U. B., Malviya, D., Singh, S., Pradhan, J. K., Singh, B. P., Roy, M., Imram, M., Pathak, N., Baisyal, B. & Rai, J. P. 2016. Bio-protective microbial agents from rhizosphere eco-systems trigger plant defense responses provide protection against sheath blight disease in rice (Oryza sativa L.). *Microbiological Research,* 192**,** 300-312.

Sirikantaramas, S., Yamazaki, M. & Saito, K. 2008. Mechanisms of resistance to self-produced toxic secondary metabolites in plants. *Phytochemistry Reviews,* 7**,** 467.

Sobti, S., Belhadj, H. A. & Djaghoubi, A. 2015. Isolation and characterization of the native rhizobia under hyper-salt edaphic conditions in Ouargla (Southeast Algeria). *Energy Procedia,* 74**,** 1434-1439.

Somers, E., Vanderleyden, J. & Srinivasan, M. 2004. Rhizosphere bacterial signalling: a love parade beneath our feet. *Critical Reviews in Microbiology,* 30**,** 205-240.

Song, G. C. & Ryu, C.-M. 2013. Two volatile organic compounds trigger plant self-defense against a bacterial pathogen and a sucking insect in cucumber under open field conditions. *International Journal of Molecular Sciences,* 14**,** 9803-9819.

Song, Y., Chen, D., Lu, K., Sun, Z. & Zeng, R. 2015. Enhanced tomato disease resistance primed by arbuscular mycorrhizal fungus. *Frontiers in Plant Science,* 6**,** 786.

Sourjik, V. 2004. Receptor clustering and signal processing in *E. coli* chemotaxis. *Trends in Microbiology,* 12**,** 569-576.

Sourjik, V. & Wingreen, N. S. 2012. Responding to chemical gradients: bacterial chemotaxis. *Current Opinion in Cell Biology,* 24**,** 262-268.

Spence, C., Alff, E., Johnson, C., Ramos, C., Donofrio, N., Sundaresan, V. & Bais, H. 2014. Natural rice rhizospheric microbes suppress rice blast infections. *BMC Plant Biology,* 14**,** 130.

Stader, J., Matsumura, P., Vacante, D., Dean, G. & Macnab, R. 1986. Nucleotide sequence of the Escherichia coli motB gene and site-limited incorporation of its product into the cytoplasmic membrane. *Journal of bacteriology,* 166**,** 244-252.

Stagnari, F., Perpetuini, G., Tofalo, R., Campanelli, G., Leteo, F., Della Vella, U., Schirone, M., Suzzi, G. & Pisante, M. 2014. Long-term impact of farm management and crops on soil microorganisms assessed by combined DGGE and PLFA analyses. *Frontiers in microbiology,* 5**,** 644.

Stajković-Srbinović, O., Delić, D., Kuzmanović, D., Protić, N., Rasulić, N. & Knežević-Vukčević, J. 2014. Growth and nutrient uptake in oat and barley plants as affected by rhizobacteria. *Romanian Biotechnology Letter,* 19**,** 9429-9436.

Stamler, R. A., Holguin, O., Dungan, B., Schaub, T., Sanogo, S., Goldberg, N. & Randall, J. J. 2015. BABA and *Phytophthora nicotianae* induce resistance to *Phytophthora capsici* in chile pepper (*Capsicum annuum*). *PloS One,* 10.

Stangarlin, J. R., Kuhn, O. J., Toledo, M. V., Portz, R. L. & Pascholati, S. 2011. A defesa vegetal contra fitopatógenos. *Scientia Agraria Paranaensis,* 10**,** 18.

Staudinger, C., Mehmeti-Tershani, V., Gil-Quintana, E., Gonzalez, E. M., Hofhansl, F., Bachmann, G. & Wienkoop, S. 2016. Evidence for a rhizobia-induced drought stress response strategy in Medicago truncatula. *Journal of Proteomics,* 136**,** 202-213.

Stringlis, I. A., Proietti, S., Hickman, R., Van Verk, M. C., Zamioudis, C. & Pieterse, C. M. J. T. P. J. 2018. Root transcriptional dynamics induced by beneficial rhizobacteria and microbial immune elicitors reveal signatures of adaptation to mutualists. 93**,** 166-180.

Stutter, M. I., Shand, C. A., George, T. S., Blackwell, M. S., Bol, R., MacKay, R. L., Richardson, A. E., Condron, L. M., Turner, B. L. & Haygarth, P. M. 2012. Recovering phosphorus from soil: a root solution? : ACS Publications.

Stutter, M. I., Shand, C. A., George, T. S., Blackwell, M. S., Dixon, L., Bol, R., MacKay, R. L., Richardson, A. E., Condron, L. M. & Haygarth, P. M. 2015. Land use and soil factors affecting accumulation of phosphorus species in temperate soils. *Geoderma,* 257**,** 29-39.

Su, F., Villaume, S., Rabenoelina, F., Crouzet, J., Clément, C., Vaillant-Gaveau, N. & Dhondt-Cordelier, S. 2017a. Different *Arabidopsis thaliana* photosynthetic and defense responses to hemibiotrophic pathogen induced by local or distal inoculation of *Burkholderia phytofirmans*. *Photosynthesis Research,* 134**,** 201-214.

Su, P., Tan, X., Li, C., Zhang, D., Cheng, J. e., Zhang, S., Zhou, X., Yan, Q., Peng, J. & Zhang, Z. 2017b. Photosynthetic bacterium *R hodopseudomonas palustris* GJ-22 induces systemic resistance against viruses. *Microbial Biotechnology,* 10**,** 612-624.

Suarez, C., Cardinale, M., Ratering, S., Steffens, D., Jung, S., Montoya, A. M. Z., Geissler-Plaum, R. & Schnell, S. 2015. Plant growth-promoting effects of *Hartmannibacter diazotrophicus* on summer barley (*Hordeum vulgare* L.) under salt stress. *Applied Soil Ecology,* 95**,** 23-30.

Suleiman, A. K. A., Manoeli, L., Boldo, J. T., Pereira, M. G. & Roesch, L. F. W. 2013. Shifts in soil bacterial community after eight years of land-use change. *Systematic and Applied Microbiology,* 36**,** 137-144.

Sun, B., Wang, F., Jiang, Y., Li, Y., Dong, Z., Li, Z. & Zhang, X. X. 2014. A long-term field experiment of soil transplantation demonstrating the role of contemporary geographic separation in shaping soil microbial community structure. *Ecology and Evolution,* 4**,** 1073-1087.

Sun, H., Deng, S. P. & Raun, W. R. 2004. Bacterial community structure and diversity in a century-old manure-treated agroecosystem. *Applied and Environmental Microbiology*, 70**,** 5868-5874.

Sun, R., Zhang, X.-X., Guo, X., Wang, D. & Chu, H. 2015. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. *Soil Biology and Biochemistry,* 88**,** 9-18.

Szymańska, S., Płociniczak, T., Piotrowska-Seget, Z., Złoch, M., Ruppel, S. & Hrynkiewicz, K. 2016. Metabolic potential and community structure of endophytic and rhizosphere bacteria associated with the roots of the halophyte Aster tripolium L. *Microbiological Research,* 182**,** 68-79.

Tada, Y., Spoel, S. H., Pajerowska-Mukhtar, K., Mou, Z., Song, J., Wang, C., Zuo, J. & Dong, X. 2008. Plant immunity requires conformational charges of NPR1 via S-nitrosylation and thioredoxins. *Science,* 321**,** 952-956.

Tahir, H. A. S., Gu, Q., Wu, H., Raza, W., Safdar, A., Huang, Z., Rajer, F. U. & Gao, X. 2017. Effect of volatile compounds produced by Ralstonia solanacearum on plant growth promoting and systemic resistance inducing potential of Bacillus volatiles. *BMC Plant Biology,* 17**,** 133.

Tak, H. I., Ahmad, F., Babalola, O. & Inam, A. 2012. Growth, photosynthesis and yield of chickpea as influenced by urban wastewater and different levels of phosphorus. *International Journal of Plant Research* 2**,** 6-13.

Tak, H. I., Ahmad, F. & Babalola, O. O. 2013. Advances in the application of plant growth-promoting rhizobacteria in phytoremediation of heavy metals. *Reviews of Environmental Contamination and Toxicology Volume 223.* Springer.

Tan, H., Barret, M., Mooij, M. J., Rice, O., Morrissey, J. P., Dobson, A., Griffiths, B. & O'Gara, F. 2013. Long-term phosphorus fertilisation increased the diversity of the total bacterial community and the phoD phosphorus mineraliser group in pasture soils. *Biology and Fertility of Soils,* 49**,** 661-672.

Tao, R., Wakelin, S. A., Liang, Y., Hu, B. & Chu, G. 2018. Nitrous oxide emission and denitrifier communities in drip-irrigated calcareous soil as affected by chemical and organic fertilizers. *Science of the Total Environment,* 612**,** 739-749.

Tardy, V., Chabbi, A., Charrier, X., De Berranger, C., Reignier, T., Dequiedt, S., Faivre-Primot, C., Terrat, S., Ranjard, L. & Maron, P.-A. 2015. Land use history shifts in situ fungal and bacterial successions following wheat straw input into the soil. *PloS One,* 10.

Tayyab, M., Islam, W., Arafat, Y., Pang, Z., Zhang, C., Lin, Y., Waqas, M., Lin, S., Lin, W. & Zhang, H. 2018. Effect of sugarcane straw and goat manure on soil nutrient transformation and bacterial communities. *Sustainability,* 10**,** 2361.

Tejada, M., Gonzalez, J., García-Martínez, A. & Parrado, J. 2008. Effects of different green manures on soil biological properties and maize yield. *Bioresource Technology,* 99**,** 1758-1767.

Thomson, C., Marschner, H. & Römheld, V. 1993. Effect of nitrogen fertilizer form on pH of the bulk soil and rhizosphere, and on the growth, phosphorus, and micronutrient uptake of bean. *Journal of Plant Nutrition,* 16**,** 493-506.

Tiwari, S., Lata, C., Chauhan, P. S. & Nautiyal, C. S. 2016. Pseudomonas putida attunes morphophysiological, biochemical and molecular responses in Cicer arietinum L. during drought stress and recovery. *Plant Physiology and Biochemistry,* 99**,** 108-117.

Tkacz, A., Cheema, J., Chandra, G., Grant, A. & Poole, P. S. 2015. Stability and succession of the rhizosphere microbiota depends upon plant type and soil composition. *The ISME Journal,* 9**,** 2349-2359.

Tonelli, M. L. & Fabra, A. 2014. The biocontrol agent Bacillus sp. CHEP5 primes the defense response against Cercospora sojina. *World Journal of Microbiology and Biotechnology,* 30**,** 2503-2509.

Tonelli, M. L., Magallanes-Noguera, C. & Fabra, A. 2017. Symbiotic performance and induction of systemic resistance against Cercospora sojina in soybean plants co-inoculated with Bacillus sp. CHEP5 and Bradyrhizobium japonicum E109. *Archives of Microbiology,* 199**,** 1283-1291.

Torsvik, V. & Øvreås, L. 2002. Microbial diversity and function in soil: from genes to ecosystems. *Current Opinion in Microbiology,* 5**,** 240-245.

Toso, D. B., Henstra, A. M., Gunsalus, R. P. & Zhou, Z. H. 2011. Structural, mass and elemental analyses of storage granules in methanogenic archaeal cells. *Environmental microbiology,* 13**,** 2587-2599.

Toyota, M., Spencer, D., Sawai-Toyota, S., Jiaqi, W., Zhang, T., Koo, A. J., Howe, G. A. & Gilroy, S. 2018. Glutamate triggers long-distance, calcium-based plant defense signaling. *Science,* 361**,** 1112-1115.

Trivedi, P., Delgado-Baquerizo, M., Trivedi, C., Hu, H., Anderson, I. C., Jeffries, T. C., Zhou, J. & Singh, B. K. 2016. Microbial regulation of the soil carbon cycle: evidence from gene–enzyme relationships. *The ISME Journal,* 10**,** 2593-2604.

Unc, A. & Goss, M. J. 2004. Transport of bacteria from manure and protection of water resources. *Applied Soil Ecology,* 25**,** 1-18.

Uzoh, I. M., & Babalola, O. O. 2018. Rhizosphere biodiversity as a premise for application in bio-economy. *Journal of Agriculture, Ecosystems and Environment* 265**,** 524-534.

Vaccaro, B. J., Thorgersen, M. P., Lancaster, W. A., Price, M. N., Wetmore, K. M., Poole, F. L., Deutschbauer, A., Arkin, A. P. & Adams, M. W. 2016. Determining roles of accessory genes in denitrification by mutant fitness analyses. *Applied and Environmental Microbiology,* 82**,** 51-61.

Vacheron, J., Renoud, S., Muller, D., Babalola, O. O. & Prigent-Combaret, C. 2015. Alleviation of abiotic and biotic stresses in plants by Azospirillum. *Handbook for Azospirillum.* Springer.

van der Bom, F., Nunes, I., Raymond, N. S., Hansen, V., Bonnichsen, L., Magid, J., Nybroe, O. & Jensen, L. S. 2018. Long-term fertilisation form, level and duration affect the diversity, structure and functioning of soil microbial communities in the field. *Soil Biology and Biochemistry,* 122**,** 91-103.

van der Putten, W. H., Bradford, M. A., Pernilla Brinkman, E., van de Voorde, T. F. & Veen, G. 2016. Where, when and how plant–soil feedback matters in a changing world. *Functional Ecology,* 30**,** 1109-1121.

Van Lelyveld, L. & Brodrick, H. 1975. Enzymic responses of avocado leaves to Phytophthora rootrot. *Agroplantae*.

Vanitha, S. C., Niranjana, S. R. & Umesha, S. 2009. Role of phenylalanine ammonia lyase and polyphenol oxidase in host resistance to bacterial wilt of tomato. *Journal of Phytopathology,* 157**,** 552-557.

Velivelli, S. L., Lojan, P., Cranenbrouck, S., de Boulois, H. D., Suarez, J. P., Declerck, S., Franco, J. & Prestwich, B. D. 2015. The induction of Ethylene response factor 3 (ERF3) in potato as a result of co-inoculation with Pseudomonas sp. R41805 and Rhizophagus irregularis MUCL 41833–a possible role in plant defense. *Plant Signaling & Behavior,* 10**,** e988076.

Vílchez, S., Manzanera, M. & Ramos, J. L. 2000. Control of Expression of DivergentPseudomonas putida put Promoters for Proline Catabolism. *Applied and Environmental Microbiology,* 66**,** 5221-5225.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S. L. & Lorito, M. 2008. Trichoderma–plant–pathogen interactions. *Soil Biology and Biochemistry,* 40**,** 1-10.

Vitti, A., La Monaca, E., Sofo, A., Scopa, A., Cuypers, A. & Nuzzaci, M. 2015. Beneficial effects of Trichodermaharzianum T-22 in tomato seedlings infected by Cucumber mosaic virus (CMV). *BioControl,* 60**,** 135-147.

Vladimir, R. 2012. The influence of Rhizobacteria and phosphorus supply on nitrogen and phosphorus contents in soybean under insufficiency moisture of soil. *Lucrări Ştiinţifice,* 55**,** 59-62.

Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M. & SkZ, A. 2016a. Enhancement of drought stress tolerance in crops by plant growth promoting rhizobacteria. *Microbiological Research,* 184**,** 13-24.

Vurukonda, S. S. K. P., Vardharajula, S., Shrivastava, M. & SkZ, A. 2016b. Multifunctional Pseudomonas putida strain FBKV2 from arid rhizosphere soil and its growth promotional effects on maize under drought stress. *Rhizosphere,* 1**,** 4-13.

Wagg, C., Bender, S. F., Widmer, F. & van der Heijden, M. G. 2014. Soil biodiversity and soil community composition determine ecosystem multifunctionality. *Proceedings of the National Academy of Sciences,* 111**,** 5266-5270.

Wallenstein, M. D., McNulty, S., Fernandez, I. J., Boggs, J. & Schlesinger, W. H. 2006. Nitrogen fertilization decreases forest soil fungal and bacterial biomass in three long-term experiments. *Forest Ecology and Management,* 222**,** 459-468.

Walley, J. W., Shen, Z., McReynolds, M. R., Schmelz, E. A. & Briggs, S. P. 2018. Fungal-induced protein hyperacetylation in maize identified by acetylome profiling. *Proceedings of the National Academy of Sciences,* 115**,** 210-215.

Walters, W. A., Jin, Z., Youngblut, N., Wallace, J. G., Sutter, J., Zhang, W., González-Peña, A., Peiffer, J., Koren, O. & Shi, Q. 2018. Large-scale replicated field study of maize rhizosphere identifies heritable microbes. *Proceedings of the National Academy of Sciences,* 115**,** 7368-7373.

Wang, C., Zheng, M., Song, W., Wen, S., Wang, B., Zhu, C. & Shen, R. 2017. Impact of 25 years of inorganic fertilization on diazotrophic abundance and community structure in an acidic soil in southern China. *Soil Biology and Biochemistry,* 113**,** 240-249.

Wang, J., Bao, J., Su, J., Li, X., Chen, G. & Ma, X. 2015. Impact of inorganic nitrogen additions on microbes in biological soil crusts. *Soil Biology and Biochemistry,* 88**,** 303-313.

Wang, N., Liu, M., Guo, L., Yang, X. & Qiu, D. 2016. A novel protein elicitor (PeBA1) from Bacillus amyloliquefaciens NC6 induces systemic resistance in tobacco. *International Journal of Biological Sciences,* 12**,** 757.

Wang, S., Kurepa, J., Hashimoto, T. & Smalle, J. A. 2011. Salt stress–induced disassembly of Arabidopsis cortical microtubule arrays involves 26S proteasome–dependent degradation of SPIRAL1. *The Plant Cell,* 23**,** 3412-3427.

Wei, C., Yu, Q., Bai, E., Lü, X., Li, Q., Xia, J., Kardol, P., Liang, W., Wang, Z. & Han, X. 2013. Nitrogen deposition weakens plant–microbe interactions in grassland ecosystems. *Global Change Biology,* 19**,** 3688-3697.

Wei, Y., Xu, M., Wu, H., Tu, S., Pan, L. & Tu, K. 2016. Defense response of cherry tomato at different maturity stages to combined treatment of hot air and Cryptococcus laurentii. *Postharvest Biology and Technology,* 117**,** 177-186.

Welsh, A., Chee-Sanford, J. C., Connor, L. M., Löffler, F. E. & Sanford, R. A. 2014. Refined NrfA phylogeny improves PCR-based nrfA gene detection. *Applied and Environmental Microbiology,* 80**,** 2110-2119.

Wertz, S., Leigh, A. K. & Grayston, S. J. 2012. Effects of long-term fertilization of forest soils on potential nitrification and on the abundance and community structure of ammonia oxidizers and nitrite oxidizers. *FEMS Microbiology Ecology,* 79**,** 142-154.

Wilke, A., Harrison, T., Wilkening, J., Field, D., Glass, E. M., Kyrpides, N., Mavrommatis, K. & Meyer, F. 2012. The M5nr: a novel non-redundant database containing protein sequences and annotations from multiple sources and associated tools. *BMC bioinformatics,* 13**,** 141.

Wu, F., Jia, Z., Wang, S., Chang, S. X. & Startsev, A. 2013. Contrasting effects of wheat straw and its biochar on greenhouse gas emissions and enzyme activities in a Chernozemic soil. *Biology and Fertility of Soils,* 49**,** 555-565.

Wu, H., Kato, J., Kuroda, A., Ikeda, T., Takiguchi, N. & Ohtake, H. 2000. Identification and characterization of two chemotactic transducers for inorganic phosphate in Pseudomonas aeruginosa. *Journal of bacteriology,* 182**,** 3400-3404.

Wu, Z., Peng, Y., Guo, L. & Li, C. 2014. Root colonization of encapsulated Klebsiella oxytoca Rs-5 on cotton plants and its promoting growth performance under salinity stress. *European journal of soil biology,* 60**,** 81-87.

Wuichet, K. & Zhulin, I. B. 2010. Origins and diversification of a complex signal transduction system in prokaryotes. *Sci. Signal.,* 3**,** ra50-ra50.

Xiang, X., He, D., He, J.-S., Myrold, D. D. & Chu, H. 2017. Ammonia-oxidizing bacteria rather than archaea respond to short-term urea amendment in an alpine grassland. *Soil Biology and Biochemistry,* 107**,** 218-225.

Xiao, W., Chen, X., Jing, X. & Zhu, B. 2018. A meta-analysis of soil extracellular enzyme activities in response to global change. *Soil Biology and Biochemistry,* 123**,** 21-32.

Xu, G., Fan, X. & Miller, A. J. 2012. Plant nitrogen assimilation and use efficiency. *Annual review of plant biology,* 63**,** 153-182.

Xu, Q., Fan, H., Jiang, Z., Zhou, Z., Yang, L., Mei, F. & Qu, L. 2013. Cell wall degradation and the dynamic changes of Ca 2+ and related enzymes in the developing aerenchyma of wheat (Triticum aestivum L.) under waterlogging. *Acta Biologica Hungarica,* 64**,** 328-340.

Xun, W., Zhao, J., Xue, C., Zhang, G., Ran, W., Wang, B., Shen, Q. & Zhang, R. 2016. Significant alteration of soil bacterial communities and organic carbon decomposition by different long-term fertilization management conditions of extremely low-productivity arable soil in S outh C hina. *Environmental microbiology,* 18**,** 1907-1917.

Yadav, S. K., Singh, S., Singh, H. B. & Sarma, B. K. 2017. Compatible rhizosphere-competent microbial consortium adds value to the nutritional quality in edible parts of chickpea. *Journal of agricultural and food chemistry,* 65**,** 6122-6130.

Yan, Y., Tang, L., Hu, J., Wang, J., Adelakun, T. A., Yang, D., Di, Y., Zhang, Y. & Hao, X. 2018. Munronin O, a potential activator for plant resistance. *Pesticide biochemistry and physiology,* 146**,** 13-18.

Yanni, Y., Zidan, M., Dazzo, F., Rizk, R., Mehesen, A., Abdelfattah, F. & Elsadany, A. 2016. Enhanced symbiotic performance and productivity of drought stressed common bean after inoculation with tolerant native rhizobia in extensive fields. *Agriculture, Ecosystems & Environment,* 232**,** 119-128.

Yin, C., Jones, K. L., Peterson, D. E., Garrett, K. A., Hulbert, S. H. & Paulitz, T. C. 2010. Members of soil bacterial communities sensitive to tillage and crop rotation. *Soil Biology and Biochemistry,* 42**,** 2111-2118.

Yolcu, H., Turan, M., Lithourgidis, A., Çakmakçi, R. & Koc, A. 2011. Effects of plant growth-promoting rhizobacteria and manure on yield and quality characteristics of Italian ryegrass under semi arid conditions. *Australian Journal of Crop Science,* 5**,** 1730.

Yoo, G. & Kang, H. 2012. Effects of biochar addition on greenhouse gas emissions and microbial responses in a short-term laboratory experiment. *Journal of Environmental Quality,* 41**,** 1193-1202.

Yoodee, S., Kobayashi, Y., Songnuan, W., Boonchird, C., Thitamadee, S., Kobayashi, I. & Narangajavana, J. 2018. Phytohormone priming elevates the accumulation of defense-related gene transcripts and enhances bacterial blight disease resistance in cassava. *Plant Physiology and Biochemistry,* 122**,** 65-77.

Yu, C., Fan, L., Gao, J., Wang, M., Wu, Q., Tang, J., Li, Y. & Chen, J. 2015. The platelet-activating factor acetylhydrolase gene derived from Trichoderma harzianum induces maize resistance to Curvularia lunata through the jasmonic acid signaling pathway. *Journal of Environmental Science and Health, Part B,* 50**,** 708-717.

Yu, H., Ling, N., Wang, T., Zhu, C., Wang, Y., Wang, S. & 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.

Yu, P. & Hochholdinger, F. 2018. The Role of Host Genetic Signatures on Root–Microbe Interactions in the Rhizosphere and Endosphere. *Frontiers in plant science,* 9**,** 1896.

Zain, N. A. M. & Ismail, M. R. 2016. Effects of potassium rates and types on growth, leaf gas exchange and biochemical changes in rice (Oryza sativa) planted under cyclic water stress. *Agricultural Water Management,* 164**,** 83-90.

Zamioudis, C. & Pieterse, C. M. 2012. Modulation of host immunity by beneficial microbes. *Molecular Plant-Microbe Interactions,* 25**,** 139-150.

Zarraonaindia, I., Owens, S. M., Weisenhorn, P., West, K., Hampton-Marcell, J., Lax, S., Bokulich, N. A., Mills, D. A., Martin, G. & Taghavi, S. 2015. The soil microbiome influences grapevine-associated microbiota. *MBio,* 6**,** e02527-14.

Zeng, J., Liu, X., Song, L., Lin, X., Zhang, H., Shen, C. & Chu, H. 2016a. Nitrogen fertilization directly affects soil bacterial diversity and indirectly affects bacterial community composition. *Soil Biology and Biochemistry,* 92**,** 41-49.

Zeng, Q., Wu, X. & Wen, X. 2016b. Effects of soluble phosphate on phosphate-solubilizing characteristics and expression of gcd gene in Pseudomonas frederiksbergensis JW-SD2. *Current microbiology,* 72**,** 198-206.

Zeng, W. & He, S. Y. 2010. A prominent role of the flagellin receptor FLAGELLIN-SENSING2 in mediating stomatal response to Pseudomonas syringae pv tomato DC3000 in Arabidopsis. *Plant physiology,* 153**,** 1188-1198.

Zhang, M., Fang, Y., Ji, Y., Jiang, Z. & Wang, L. 2013a. Effects of salt stress on ion content, antioxidant enzymes and protein profile in different tissues of Broussonetia papyrifera. *South African Journal of Botany,* 85**,** 1-9.

Zhang, Q.-C., Shamsi, I. H., Xu, D.-T., Wang, G.-H., Lin, X.-Y., Jilani, G., Hussain, N. & Chaudhry, A. N. 2012. Chemical fertilizer and organic manure inputs in soil exhibit a vice versa pattern of microbial community structure. *Applied Soil Ecology,* 57**,** 1-8.

Zhang, Q., Sun, J., Liu, S. & Wei, Q. 2013b. Manure refinement affects apple rhizosphere bacterial community structure: a study in sandy soil. *PloS one,* 8.

Zhang, Y., Shen, H., He, X., Thomas, B. W., Lupwayi, N. Z., Hao, X., Thomas, M. C. & Shi, X. 2017. Fertilization shapes bacterial community structure by alteration of soil pH. *Frontiers in microbiology,* 8**,** 1325.

Zhang, Y., Yan, X., Guo, H., Zhao, F. & Huang, L. 2018. A novel protein elicitor BAR11 from Saccharothrix yanglingensis Hhs. 015 improves plant resistance to pathogens and interacts with catalases as targets. *Frontiers in microbiology,* 9**,** 700.

Zhao, J., Ni, T., Li, Y., Xiong, W., Ran, W., Shen, B., Shen, Q. & Zhang, R. 2014a. Responses of bacterial communities in arable soils in a rice-wheat cropping system to different fertilizer regimes and sampling times. *PloS one,* 9.

Zhao, X., Norris, S. J. & Liu, J. 2014b. Molecular architecture of the bacterial flagellar motor in cells. *Biochemistry,* 53**,** 4323-4333.

Zheng, B.-X., Hao, X.-L., Ding, K., Zhou, G.-W., Chen, Q.-L., Zhang, J.-B. & Zhu, Y.-G. 2017. Long-term nitrogen fertilization decreased the abundance of inorganic phosphate solubilizing bacteria in an alkaline soil. *Scientific reports,* 7**,** 1-10.

Zheng, X.-y., Zhou, M., Yoo, H., Pruneda-Paz, J. L., Spivey, N. W., Kay, S. A. & Dong, X. 2015. Spatial and temporal regulation of biosynthesis of the plant immune signal salicylic acid. *Proceedings of the National Academy of Sciences,* 112**,** 9166-9173.

Zhong, W., Bian, B., Gao, N., Min, J., Shi, W., Lin, X. & Shen, W. 2016. Nitrogen fertilization induced changes in ammonia oxidation are attributable mostly to bacteria rather than archaea in greenhouse-based high N input vegetable soil. *Soil Biology and Biochemistry,* 93**,** 150-159.

Zhong, W., Gu, T., Wang, W., Zhang, B., Lin, X., Huang, Q. & Shen, W. 2010. The effects of mineral fertilizer and organic manure on soil microbial community and diversity. *Plant and Soil,* 326**,** 511-522.

Zhou, J., Guan, D., Zhou, B., Zhao, B., Ma, M., Qin, J., Jiang, X., Chen, S., Cao, F. & Shen, D. 2015. Influence of 34-years of fertilization on bacterial communities in an intensively cultivated black soil in northeast China. *Soil Biology and Biochemistry,* 90**,** 42-51.

Zhu, F., Qu, L., Hong, X. & Sun, X. 2011. Isolation and characterization of a phosphate-solubilizing halophilic bacterium Kushneria sp. YCWA18 from Daqiao Saltern on the coast of Yellow Sea of China. *Evidence-Based Complementary and Alternative Medicine,* 2011.

Zhu, F., Xi, D.-H., Yuan, S., Xu, F., Zhang, D.-W. & Lin, H.-H. 2014. Salicylic acid and jasmonic acid are essential for systemic resistance against tobacco mosaic virus in Nicotiana benthamiana. *Molecular Plant-Microbe Interactions,* 27**,** 567-577.

Zhu, G., Wang, S., Li, Y., Zhuang, L., Zhao, S., Wang, C., Kuypers, M. M., Jetten, M. S. & Zhu, Y. 2018. Microbial pathways for nitrogen loss in an upland soil. *Environmental microbiology,* 20**,** 1723-1738.

Zhu, L.-x., Xiao, Q., Shen, Y.-f. & Li, S.-q. 2017. Microbial functional diversity responses to 2 years since biochar application in silt-loam soils on the Loess Plateau. *Ecotoxicology and environmental safety,* 144**,** 578-584.

Zhu, Y.-G., Su, J.-Q., Cao, Z., Xue, K., Quensen, J., Guo, G.-X., Yang, Y.-F., Zhou, J., Chu, H.-Y. & Tiedje, J. M. 2016. A buried Neolithic paddy soil reveals loss of microbial functional diversity after modern rice cultivation. *Science bulletin,* 61**,** 1052-1060.

Zumft, W. G. 1997. Cell biology and molecular basis of denitrification. *Microbiol. Mol. Biol. Rev.,* 61**,** 533-616.