# CHICKPEA NITROGEN FIXATION, RHIZOSPHERE NUTRIENT CONCENTRATION AND CONTRIBUTION OF RESIDUAL NITROGEN TO IMPROVE MAIZE PRODUCTION IN RESPONSE TO BIOCHAR APPLICATION IN THREE DIFFERENT SOIL TYPES

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## DECLARATION

I, Siphiwe Gloria Lusiba, hereby declare that this thesis for Doctor of Philosophy (Ph.D.) in Agriculture submitted at the University of Venda is my own, original work and has not been submitted previously for any degree at this or any other University. All references have been duly acknowledged.

Candidate: S.G Lusiba Date: June 2021



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## DEDICATION

To my parents: Mr Zephania and Mrs Ruth Lusiba, to my siblings: Sfiso, Ntokozo, Lindokuhle, Ayanda

To my beloved daughter Uthando Precious Mahlangu

Never forgetting my lovely friend, Busisiwe Vilakazi

May you never stop imagining, and may the sky be not your limit

with love and gratitude.

*Imagination is more important than knowledge. For knowledge is limited, whereas imagination embraces the entire world, stimulating progress, giving birth to evolution. If we knew what it was, we were doing, it would not be called research, would it? (Albert Einstein*).



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# LIST OF ABBREVIATIONS

| | |
|---|---|
| B | Boron |
| BNF | Biological Nitrogen Fixation |
| C | Carbon |
| $Ca^{2+}$ | Calcium Ion |
| $CaC_2$ | Calcium Carbon |
| CEC | Cation Exchange Capacity |
| $CO_2$ | Carbon dioxide |
| $CH_4$ | Methane |
| C/N | Carbon-to-Nitrogen Ratio |
| DM | Dry Matter |
| DNA | Deoxyribonucleic Acid |
| DAE | Days After Emergence |
| EBC | European Biochar Certificate |
| EC | Electrical Conductivity |
| Fe | Iron |
| g | Grams |
| GHG | Greenhouse Gases |
| H | Hydrogen |
| H/C | Hydrogen-to-Carbon ratio |
| HCl | Hydrochloric Acid |
| IBI | International Biochar Initiative |
| K | Potassium |
| KCl | Potassium Chloride |
| kg | Kilograms |
| kg/ha | Kilograms per Hectare |
| $Mg^{2+}$ | Magnesium Ion |
| Mn | Manganese |
| Mo | Molybdenum |
| N | Nitrogen |
| $NH_3$ | Ammonia |
| $NH_4^+$-N | Ammonium-nitrogen |
| $NO_3^-$-N | Nitrate-Nitrogen |
| $N_2$ | Nitrogen Gas |
| $N_2O$ | Nitrous Oxide |



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| | |
|---|---|
| OM | Organic Matter |
| O/C | Oxygen-to-Carbon Ratio |
| P | Phosphorus |
| PCR | Polymerase Chain Reaction |
| SOC | Soil Organic Carbon |
| S | Sulfur |
| TGA | Thermogravimetric Analysis |
| t/ha | Tons per hectare |
| TC | Total Carbon |
| TN | Total Nitrogen |
| WHC | Water Holding Capacity |
| WUE | Water-use efficiency |
| w/w | Weight per weight |
| Zn | Zinc |



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## ABSTRACT

Soil degradation is a major challenge affecting agricultural production around the world in the twenty-first century. Alternative approaches including the use of biochar and the introduction of legumes that will fix nitrogen though the process of biological nitrogen fixation (BNF) are essential for improving soil quality of current low productive soils, thus increasing crop yields, and maintaining food security while conserving the environment. To address this problem, three experiments were conducted in this study. First, two locally produced biochar from poultry litter and acacia feedstocks were assessed whether they qualify as 'b*iochar*' for use as soil amendment according to the international biochar bodies. Secondly, a pot experiment was then conducted to investigate the potential impact of poultry litter biochar (PLB) and acacia biochar (ACB) to improve rhizospheric soil nutrient availability, bacteria abundance and diversity, chickpea growth and total nitrogen fixation in three contrasting soil types. Thirdly, another pot experiment was conducted to determine the contribution of residual N from biochar and N-fixed by chickpea to the following maize crop grown in three contrasting soil type.

The treatments consisted of three soil types classified as Fernwood [Arenosol]; Pinedene [Gleyic Acrisol]; Griffin [Helvic Acrisol], sampled from three different smallholder farmers at Dopeni village, Limpopo Province. The two biochars [PLB and ACB] were applied at four application rates of [0% (control), 0.5, 1 and 2% w/w]. The treatments were arranged in a completely randomized design and replicated four times. For the first experiment, chickpea (*Cicer arietinum*) desi cultivar was inoculated and grown for 65 days in soils with uniformly applied P at 60 kg P/kg in all pots and water maintained at 60% field capacity. For the second experiment, maize (*Zea mays*) was grown for 95 days in the same soil as chickpea and biochar treatments as well as after harvesting maize that was used as a reference crop.

Biochars made from poultry litter and acacia feedstocks meet the International Biochar Initiative (IBI) and European Biochar Certificate (EBC) requirements and qualifies as biochar for use as a soil amendment. Both biochars had C content greater than 50%, with H/C and O/C ratios less than 0.6 and 0.4, respectively, indicating that both biochars are stable for C sequestration and can remain in the soil for about 1000 years. Because poultry litter biochar (PLB) contained more nutrients than acacia biochar (ACB), PLB improved rhizospheric pH, CEC, and nutrient concentration (N, P, K, and Ca) when applied at 0.5-2% in the Griffin and Pinedene soils, resulting in higher biomass production and nutrient uptake of chickpea. In addition, when 2% PLB was applied to those two soils, bacteria capable of fixing N, especially those from the phylum Proteobacteria, were more abundant. Thus, chickpea grown in these soils and at these PLB rates derived more N from the atmosphere, fixed more N, and

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accumulated more N and C in the shoot, but was less water use efficient. Furthermore, maize grown in 1% residual PLB treatments produced more biomass and accumulated more N and other nutrients than maize grown with 0.5-2% residual ACB treatments. However, when grown after chickpea harvest in residual PLB treatments of 2% in the Griffin and Pinedene soils, maize produced greater biomass and accumulated more N and other nutrients. Application of PLB and ACB at 0.5% in the Fernwood soil was ideal to improve rhizospheric nutrient availability, the abundance of bacteria communities [from the phylum Proteobacteria, Acidobacteria, and Firmicutes which are important for C and N cycling and bioremediation], as well as growth, BNF, and C accumulation of chickpea, including maize growth and nutrient uptake in monocropping or in rotation with chickpea. The greatest variation in relative abundance of bacteria communities and growth of chickpea was due to the substantial change in soil pH and rhizospheric nutrient availability such as N, P, K, and Mg, while biomass production and N accumulation were largely attributed to the improved BNF and C accumulation of chickpea in the clay textured soils. The increased growth and nutrient uptake of the following maize crop in the Griffin and Pinedene soils was attributed to high N inputs through BNF and biochar mineralisation, whereas the variation in bacteria communities, chickpea and maize performance in the Fernwood soil was due to the change in rhizospheric soil pH, P, and K.

The findings of this study conclude that biochar made from poultry litter is recommended for use as a soil amendment to improve nutrients and soil quality, although caution should be taken when applied at higher rates (40 t/ha) as it may immobilize N or result in high release of toxic elements. Furthermore, incorporating chickpea into existing maize cropping systems of smallholder farmers and using biochar made from poultry litter will help reduce nitrogen input costs by adding residual nitrogen from biochar mineralisation and through BNF, improving soil quality and maize production. On the other hand, biochar made from acacia feedstock will be excellent for use to improve soil organic carbon and water adsorption, but it should be applied months before planting or supplemented with high organic N and P materials when used as a soil amendment to improve nutrient availability, as it may temporary fix N and P in the soil. When using poultry litter or acacia biochar on a poorly buffered loamy sand soil like the Fernwood, care should be taken to avoid over liming, which can cause nutrient deficiency and negatively affect nutrient uptake. Moreover, to improve chickpea and maize performance in sandy textured, highly leached soils like the Fernwood, biochar should be applied regularly or combined with organic materials to improve soil organic carbon to allow the soil to retain and release nutrients for plant uptake.

Keywords: acacia, biochar, chickpea, nitrogen fixation, poultry litter, soil types



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# CHAPTER 1

# GENERAL BACKGROUND

## 1.1 Introduction

Long-term agricultural sustainability and meeting future food demands have become a burning issue, and can be considered as a major challenge in the twenty-first century. This is because global agriculture must meet the growing world population's food and industrial demands while also protecting the environment (Agegnehu et al., 2017). The global population was 7.35 billion in 2015, but it is expected to reach 9 to 10 billion by 2050 due to continuous growth. To meet global food demand by 2050, global food production should rise by more than 70% from current levels (Grafton et al., 2015, McKeon, 2015). As a result, current arable lands are intensively utilized to meet the growing global demand for food, despite the constraints of limited resources and a changing climate (Agegnehu et al., 2017). This has resulted in overexploitation of arable land with little or excessive use of inorganic fertilizers, resulting in soil degradation and posing a threat to crop production sustainability (Adhikari et al., 2018).

The benefits of allocating more land to agriculture will not outweigh the negative environmental consequences of future land degradation. Instead, increasing yield from currently cultivated lands with low productivity is a more promising approach to ensuring food security (Adhikari et al., 2018, Agegnehu et al., 2017). To ensure food supply for the world's growing population, sustainable agricultural intensification, or increasing productivity per unit land area, is required. Biochar, a by-product of the pyrolysis process, is widely used as a soil amendment and has been evaluated as a means of improving soil quality around the world. The use of biochar has gained popularity as a strategy for long-term carbon storage and climate change mitigation by reducing $CO_2$ concentrations in the atmosphere (Lehmann and Joseph, 2009a). Biochar can also reduce greenhouse gas emissions in the soil, such as nitrous oxide ($N_2O$) and methane ($CH_4$), by trapping these gases in pores (Clough et al., 2013) potentially reducing the ever-increasing global warming.

Biochar's ability to improve soil fertility is linked to its potential to increase pH in acid soils, improve soil structure and functional diversity of soil microorganisms, improve soil organic carbon and nutrients as well as its water retention capabilities, especially in tropical soils, as reported in Terra Preta soils (Lehmann and Joseph, 2009a). Biochar's potential to improve soil physical and chemical properties as well as crop production has been extensively researched, primarily in China (Zhang et al., 2012, Zhao et al., 2014) and Europe (Atkinson et al., 2010, Baronti et al., 2014). Only a few studies, however, have been conducted in African

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countries, including South Africa (Aghoghovwia, 2018, Lusiba et al., 2017, Lusiba et al., 2018, Sika and Hardie, 2014).

Biochar application and its impact on soil microbes and their associated soil processes, as well as its effect on biological nitrogen fixation (BNF) in semi-arid soils, have received little attention in literature. Due to differences in soil type, feedstock source, and application rate, the few studies conducted have found contradictory results in the response of soil microbe abundance and activity following biochar application (Bruun et al., 2011, Castaldi et al., 2011, Khodadad et al., 2011). Furthermore, only a few studies have shown that applying biochar to soil can improve soil quality by increasing BNF by legumes (Mia et al., 2014, Ogawa and Okimori, 2010, Rondon et al., 2007). Despite the information available from previous studies, the explanations for improved BNF with biochar application have remained inconclusive. In the Netherlands, for example, (Giller, 2001) reported a decrease in BNF with biochar addition due to high Ca, P, and low micronutrients in the studied soil.

Biochar increased BNF in temperate soils, due to high availability of Bo and Mo, as well as high Ca and P in Brazil and Colombia (Lehmann et al., 2003, Rondon et al.,2007). Other proposed explanations for biochar's positive effects on BNF include increased soil pH (Ogawa and Okimori, 2010), increased soil available phosphorus (Tagoe et al., 2008, Rondon et al., 2007), and inorganic nitrogen immobilisation (Nelissen et al., 2012, Rondon et al., 2007). In addition, its effects on BNF varied with application rates (Khodadad et al., 2011). As shown by previous studies, increasing the rates of biochar increased BNF (Mia et al., 2014, Ogawa and Okimori, 2010, Rondon et al., 2007). However, there is still a lack of evidence that biochar application can increase bacteria abundance and thus improve nodulation and BNF in South African semi-arid soils. Moreover, published literature show that the effect of biochar on BNF has been studied on other legumes but not on chickpea.

The contradictory reports on biochar and BNF as indicated by (Giller, 2001, Lehmann et al., 2003, Rondon et al., 2007) have resulted in a lack of information on how biochar influences soil microbial biodiversity and activity, as well as plant root behaviour in relation to nutrient availability and uptake by plants. Furthermore, there is limited evidence that fixed nitrogen from biochar application can contribute to subsequent crops such as maize (highly dependent on nitrogen for growth and yield) which is the most commonly grown crop in semi-arid areas. Consequently, a comprehensive study to determine the potential impact of biochar on soil nutrient availability, bacteria abundance and diversity, chickpea nodulation and BNF, and the benefit of N-fixed by chickpea to subsequent crop such as maize is vital. The purpose of this study was to determine the impact of biochar derived from poultry litter and acacia feedstocks on soil nutrient availability, nodulation and biological nitrogen fixation by chickpea, as well as



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the contribution of N-fixed by chickpea to subsequent maize performance in semi-arid soils varying in texture and fertility status in Limpopo Province, South Africa.

### 1.2 Problem Statement

Most smallholder farmers, particularly in the North-Eastern region of South Africa, face significant challenges in maintaining crop yields and meeting their individual and market food demands due to declining soil quality and high inorganic fertilizer prices. Farmers in this region practice continuous cropping with little or no organic or inorganic fertilizer inputs, resulting in acidic and degraded soils with low nutrient availability (particulary nitrogen). Cheaper nitrogen sources, such as incoprating legumes that fix nitrogen through biological nitrogen fixation (BNF), and alternative strategies, such as the use of biochar as a soil amendment, are essential to improve soil quality, yield, and food security while also conserving the environment.

Even though biochar has been shown to increase BNF by legumes in temperate and tropical soils from elsewhere, biochar application did not affect chickpea nodulation in soils varying in texture in South Africa (Lusiba, 2015). In addition, the main explanations underlying biochar application in the soil on BNF are unclear and not fully understood. To make conclusive recommendations, it is important to understand the main reasons why biochar application affects BNF, as well as how nitrogen-fixed in conjunction with biochar application benefits subsequent crops such as maize. Furthermore, biochar use as a soil amendment was recently implemented in South Africa, and it is still under investigation.

Biochar research in South Africa has so far been conducted using commercially produced biochars from various feedstocks such as; *Acacia mearnsii* and *Eucalyptus* (Lusiba et al., 2017, Lusiba et al., 2018), Pinus (Sika and Hardie, 2014), black wattle and vineyard prunings (Uras et al., 2012), and sugarcane bagasse (Carrier et al., 2012), as well as on maize stover, grape pip, grape skin, pine wood, rubber tyre, and sugarcane pith (Aghoghovwia, 2018). The types of biochar used in those studies were not evaluated or characterized physically or chemically to determine their potential as a soil amendment, apart from those investigated by (Aghoghovwia, 2018). Biochar properties must be evaluated to classify the pyrolyzed product as '*biochar*' as recommended by the IBI or EBC guidelines. Moreover, the use of poultry litter biochar as a soil amendment is still limited in literature, and the efficacy of biochar derived from acacia and poultry litter feedstock on BNF are unknown.



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### 1.3 Justification

Earlier research demonstrated the potential effect of biochar derived from *Acacia nilotica (L) Delile and Eucalyptus obliqua (L)* feedstocks on soil physical and chemical properties, as well as chickpea growth performance in sandy and clay textured soils (Lusiba, 2015). The results showed that biochar application has the potential to improve soil properties of acid, low-nutrient sandy textured soil than moderate fertile clay textured soil. Interestingly, despite the high C/N ratio, biochar application increased soil total N, which translated to improved chickpea performance in clay textured soil, especially when phosphorus was not a limiting factor. Biochar is a carbonaceous, porous material with a large surface area that improves soil aggregation and CEC, consequently improving soil fertility and plant growth.

The liming potential of biochar, which is due to the formation of carbonates and alkaline elements, contributed to the buffering capacity of acid soils, thereby improving nutrient retention and availability. Despite the positive effects of biochar on soil properties and pH, chickpea nodulation was not affected in both soils with biochar application with or without phosphorus fertilizer addition, due to insufficient populations of effective native rhizobia. Thus, the question arose as to whether biochar can enhance chickpea nodulation and BNF if phosphorus and water are not limited, and if chickpea is inoculated to increase native rhizobia species.

To better understand this phenomenon, the current study assessed the potential of two locally produced biochars derived from different feedstocks (poultry litter and acacia) under similar pyrolysis temperature with varying properties. The biochars were evaluated to determine their potential to improve soil nutrient availability thereby increasing the abundance of bacteria involved in carbon and nitrogen cycling, as well as chickpea nodulation and biological nitrogen fixation in three contrasting soils varying in texture and fertility status. Also, assessed whether residual nitrogen added by chickpea through fixation in conjunction with residual N from biochar mineralisation can improve maize performance in crop rotation systems.

The use of biochar as a soil amendment and the incorporation of chickpea into the existing predominantly maize cropping systems in most arid and semi-arid areas in the North-Eastern region of South Africa could help alleviate the supply of nitrogen through BNF to meet crop nutrient requirements and improve soil quality while conserving the environment. Biological nitrogen fixation is essential in low-input systems, particularly in arid and semi-arid regions where nitrogen fertilizer inputs and availability are limited. BNF is the cheapest and most efficient way for smallholder farmers, especially those who are financially constrained, to maintain long-term soil fertility and crop yield. Furthermore, incorporating nitrogen-fixing



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legumes such as chickpea into existing crop rotations with cereals can provide an additional source of income for smallholder farmers while also restoring soil nitrogen, improving soil productivity and crop yield. Biochar application in the soil has several benefits, including improving soil quality and crop productivity by improving soil properties. Biochar could also help to mitigate climate change by reducing carbon dioxide and nitrous oxide emissions in the atmosphere via carbon sequestration, thereby lowering global warming.

## 1.4 Aim and objectives of the study

### 1.4.1 Aim of the study

This study has two aims;

- To assess the impact of locally produced biochar from poultry litter and acacia on selected rhizospheric macro-and micronutrient concentration, bacteria abundance and diversity, as well as growth, symbiotic performance and nutritional benefit to chickpea in three contrasting soils.
- To assess the contribution of N-fixed by chickpea and residual N from biochar to the subsequent maize crop in three contrasting soil types.

### 1.4.2. Specific objectives

- To characterize the physical and chemical properties of poultry litter and acacia biochar
- To assess the effect of poultry litter and acacia biochar on rhizospheric nutrient concentration, and nutrient utilization by chickpea.
- To determine the impact of biochar derived from two different feedstocks of poultry litter and acacia on bacteria community composition and diversity.
- To investigate the response of BNF, C accumulation and water-use efficiency of chickpea to poultry litter and acacia biochar application.
- To determine the impact of residual N from poultry litter or acacia biochar mineralisation and N-fixed by chickpea on growth and nutrient uptake of maize.

## 1.5 Hypotheses

There were four hypotheses for this study and these includes:

- Biochar made from local feedstocks of poultry litter and acacia using different pyrolysis conditions but similar pyrolysis temperature is of good quality.
- Poultry litter and acacia biochar have different properties and nutrient composition, when applied to the soil, both have the potential to enhance soil nutrient availability



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and utilization by chickpea, but the effect will vary depending on the biochar type, application rate and soil type.

- Poultry litter and acacia biochar applied at different rates can be used to improve soil nutrient status and the abundance and diversity of bacteria species responsible for C and N cycling, resulting in increased nodulation and BNF of chickpea grown in three different soil types varying in texture and fertility status.
- Maize grown in soils with residual biochar and high total N-fixed by chickpea will have higher biomass and N accumulation, which will increase as the biochar application rate is increased, though this will vary depending on the type of soil and biochar applied.

### 1.6 Research questions

- Does biochar made from poultry litter and acacia feedstocks under similar pyrolysis temperature of good quality according to the IBI and EBC guidelines?
- Can poultry litter and acacia biochar applied at different rates in soils varying in texture, nutrient status, and mineralogy improve soil nutrient availability and utilization by chickpea?
- Is there a difference in bacteria abundance and diversity after biochar application in different soils? At what biochar application rate is bacteria abundance/diversity highest?
- Can poultry litter and acacia biochar be used as a soil amendment to improve nodulation and biological nitrogen fixation (BNF) of chickpea? Will the effect vary depending on the amount of biochar applied in different soil types, and what are the main reasons for the improved BNF?
- Does residual N from biochar mineralisation combined with N-fixed by chickpea, affect maize growth and nutrient uptake in different soil types?

### 1.7 Significance of this study

- Biochar as a soil amendment is a relatively new concept in South Africa and throughout Africa. The purpose of this research is to add to the existing scientific knowledge and understanding on using biochar made from poultry litter and acacia as a soil amendment to improve degraded, and acid soils found in the North-Eastern region of Limpopo Province.
- The findings of this study can be used to add to the current commercial biochars and compile recommendations for biochars produced in South Africa in accordance with international biochar organizations' guidelines, which provide a clear definition of biochar as well as criteria for evaluating biochar properties before any pyrolyzed



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organic material can qualify as biochar for use as a soil amendment and carbon sequestration.

- The research will add to existing knowledge of how biochar acts as a liming agent and nutrient releaser, enhancing biological nitrogen fixation by legumes and subsequent cereal growth in poorly managed soils. This knowledge will aid in determining whether incorporating biochar and chickpea into existing cropping systems is another alternative approach for improving soil fertility and crop production while also providing smallholder farmers and commercial farmers with higher economic returns on inputs.
- Biochar made from poultry litter and *Acacia mearnsii* feedstocks could be used as an environmentally and economically viable waste management solution in South Africa, while also improving soil fertility and reducing greenhouse gas emissions.
- The use of biochar in South Africa can lead to collaboration between researchers, engineers, and industries to develop technologies such as pyrolysis plants, which can create business and employment opportunities as well as generate electricity due to high amount of energy released during pyrolysis.

### 1.8 Structure of thesis

There are eight chapters in this thesis. A brief introduction on biochar and BNF is provided in Chapter 1. This chapter also discusses the study's objectives, justification and significance. Chapter 2- provides an overview of the fundamental literature for this study. The five objectives that make up this study are discussed in chapter 3 to 7. Chapter 8- is divided into two sections, the first section summarizes the study's overall findings, while the second section focuses on recommendations for future research in this area of research and conclusion.

- Chapter 3: reported the physical and chemical properties of biochar derived from poultry litter and acacia feedstocks. Following the IBI and EBC guidelines. The differences of the biochars in terms of proximate analysis, elemental composition, and physical and chemical properties were discussed.
- Chapter 4: A pot experiment was conducted to understand whether biochar could improve rhizospheric soil nutrient concentration and utilization of selected nutrients by chickpea grown in three different soil types.
- Chapter 5: After chickpea harvesting, rhizospheric soil samples were analysed using molecular techniques to determine the short-term effect of biochar application from different feedstocks on the bacteria community composition and diversity of three different soil types.



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- Chapter 6: The natural $^{15}N$ abundance technique was used to estimate BNF, C accumulation and water-use efficiency ($\delta^{13}C$) of chickpea in response to biochar application.
- Chapter 7: Demonstrate the residual benefit of biochar and N-fixed by chickpea on maize growth, N accumulation and other nutrient uptake in soils varying in texture.

### 1.9 Publication and conference presentations from this study

- Lusiba Sg, Odhiambo Jjo, Adeleke R, Maseko St. 2021. The potential of biochar to enhance concentration and utilization of selected macro and micro nutrients for chickpea (*Cicer arietinum L)* grown in three contrasting soil. Rhizosphere, 17, p: 100289.
- Biochar derived from poultry litter as an alternative soil amendment to improve nutrient availability of three different soil types in the Vhembe district. Presented at Combined Congress, University of Free State, 22-25 January 2019. Won the best paper award from the Soil Science Society of South Africa.
- Effects of biochars produced from different feedstocks on soil bulk density, nodulation and growth of chickpea grown in different soil types. Presented at Combined Congress, University of Free State, 22-25 January 2018.

### 1.10 Manuscripts under review by journals

- Short-term impacts of biochar application from different feedstocks on bacteria community composition and abundance of three contrasting soil. Submitted as a short communication and under review by Applied Soil Ecology Journal.
- Biological N fixation, C accumulation and water-use efficiency ($\delta^{13}C$) of chickpea in three different soil types: response to addition of biochar from poultry litter and acacia. Under review by Soil Science and Plant Nutrition Journal.
- Residual biochar and N fixation by chickpea influences growth and N uptake of maize in soils varying in texture. Under review by Archives of Agronomy and Soil science.



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# CHAPTER 2

# LITERATURE REVIEW

## 2.1 History of biochar

The origin, production and application of biochar is linked to the ancient Terra Preta soils (Hortic Anthrosols) of the Amazon region, where dark highly fertile soils where created using slash-and- char techniques (Lehmann and Joseph, 2009a). Earlier studies on Terra Preta soils has revealed the effects of biochar as an excellent soil amendment for soil fertility and sustainability. The Terra Preta soils were characterized as being highly productive due to high carbon content, pH, base cations, exchangeable cation, and available P concentrations (Sohi et al., 2010). These characteristics made researchers and farmers around the world to recognize that biochar has properties like the Terra-Preta soils. Thereafter, extensive work has been done on the use of biochar as an emerging cost-effective and environmentally friendly tool with multiple functions of sequestering carbon, improving soil fertility and remediating contaminated soil (Yu et al., 2019).

## 2.2 Defining biochar

Biochar is a term that has recently been coined by scientists. The term “char” refers to the residue left over after organic and inorganic materials have disintegrated. Biochar and charcoal are often used interchangeably, but their end uses distinguish them. Biochar is used in soil for carbon sequestration and environmental management, whereas charcoal is used to produce fuel and energy (Ahmad et al., 2014, Břendová et al., 2017). Earlier researchers defined biochar as ‘‘*a black carbon (C)-rich and stable product produced during thermochemical processing of biomass in an oxygen-limited environment with a relatively low temperature of less than 700* °C (Lehmann and Joseph, 2009a). Biochar, according to (Verheijen et al., 2010) is *“biomass that has been pyrolyzed in a zero or low oxygen environment applied to soil at a specific site that is expected to sustainably sequester C and concurrently improve soil functions under current and future management, while avoiding short- and long-term detrimental effects to the wider environment, as well as human and animal health”.* The International Biochar Initiative (IBI) collaborated with interested parties from all over the world to create a biochar definition. They defined biochar as "*a solid material obtained from the thermochemical conversion of biomass in an oxygen-limited environment*" (IBI, 2015). Recently, researchers are defining biochar as “*a solid, highly porous, carbon-rich material produced by pyrolysis from various feedstocks ranging from lignocelluloses to manure that are pyrolyzed at different temperatures ranging from 200 °C to 700 °C with the*



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*goal of sequestering carbon and simultaneously improving soil qualities*" (Fan et al., 2020, Inyang et al., 2016, Wang et al., 2015). These definitions are either directly or indirectly related to biochar production and its application as a soil amendment.

### 2.3 Biochar production

Biochar is produced by thermochemical decomposition of biomass at temperatures of 200–900 $^{o}$C in the presence of little or no oxygen, which is commonly known as pyrolysis. Pyrolysis is generally divided into fast, intermediate and slow and gasification depending on the residence time, temperature, moisture, pressure and vapour (Ahmad et al., 2014). Fast pyrolysis with a very short residence time of less than 2 seconds is often used to produce bio-oil from biomass heated at 425–900°C to yield about 75% bio-oil (Ahmad et al., 2014). Slow and intermediate pyrolysis involves heating biomass at 350–550 °C in the absence of oxygen with a residence time of few minutes to several hours or even days. Slow pyrolysis results in a lower yield of liquid fuel and a higher yield of biochar compared to other thermal chemical processes (Yu et al., 2019). It is generally referred to as the conventional way of synthesizing biochar with an approximate yield of 25 to 35% (Ahmad et al., 2014). During gasification, biomass is converted into gases rich in carbon monoxide and hydrogen by reacting the biomass at high temperature above 700 °C in a controlled oxygen environment and/or steam. The resulting gas mixture is known as synthetic gas or syngas (Inyang et al., 2016).

#### 2.3.1 Types of Feedstock

Woody and non-woody biomass are the two types of biomass considered for biochar production. Woody biomass is primarily made up of forestry and tree residues that are low in moisture, low in ash, high in calorific value, high in bulk density, and low in porosity (Jafri et al., 2018). Non-woody biomass, on the other hand, includes agricultural crops and residues, animal waste, industrial and agro-industrial waste and is characterized by high moisture, high ash content, lower calorific value, low bulk density, and higher volume (Jafri et al., 2018). Rice husk, maize straw, wheat straw, animal litter, agricultural residues, logging and wood processing residues, algae, municipal solid waste, livestock/poultry waste, and wastewater/sewage sludge are some of the common feedstock types used in literature for biochar production. These biomasses contain a variety of organic and inorganic compounds, including hemicellulose, cellulose, lignin, fats, phytosterols, and phenolics, as well as inorganic compounds (N, P, S, Si, K, Na, and other trace minerals), all of which have an impact on the final structure and properties of the biochar produced (Yuan et al., 2019).



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### 2.3.2 Pyrolysis temperature

Pre-pyrolysis, main-pyrolysis, and formation of carbonaceous soil products are the three stages of the biochar production process (Lee et al., 2017). The first stage (from room temperature to 200 $^{o}$C) involves evaporation of moisture and light volatiles. Cárdenas-Aguiar et al. (2017) found that as moisture evaporates, bonds are broken, resulting in the formation of hydroperoxide, –COOH, and –CO groups. The second stage (between 200 and 500 $^{o}$C) involves rapid devolatilization and decomposition of hemicelluloses and cellulose. The last stage (above 500 $^{o}$C) involves degradation of lignin and other organic matter with stronger chemical bonds. Changes in the structure and physicochemical properties of biochar are strongly correlated with pyrolysis temperature (Lee et al., 2017).

## 2.4 Biochar properties

Biochar properties are influenced by factors such as biomass feedstock, pyrolysis temperature, pyrolysis residence time, and pyrolysis pressure (Diatta et al., 2020). Bulk density, surface and pore-size, pore volume, and moisture content are physical properties which mainly dependent on feedstock type, while pH, EC, total C and total N, P, exchangeable cations, CEC, and selected trace elements are chemical properties (Zn, Fe, Pb, Cd, Cu, Ni, Hg, Cr) which largely depend on feedstock type and pyrolysis temperature (Lee et al., 2017). Different biochar types have different physicochemical properties that can be characterized and used as a guide for biochar application as a soil amendment. Since pyrolysis temperature and feedstock type are the two most important factors affecting biochar properties. In the sections below, the effects of feedstock type and pyrolysis temperature on biochar properties are briefly discussed.

### 2.4.1 Physical properties of biochar

**Brunauer-Emmet-Teller (BET)-Specific Surface Area (BET-SSA), pore volume and pore size**- These are the most important physical properties of biochar, and they are affected by the type of feedstock and temperature. Tag et al. (2016) found that biochars made from pyrolysis of orange pomace and vine pruning at 500°C and 600 °C had extremely low BET-SSA of 1.2 $m^2$/g and pore size of 8.1 µm, respectively. At 550 °C, H El-Gamal et al. (2017) found that sugarcane-biochar had a larger pore size of 0.1 µm and an BET-SSA of 185.6 $m^2$/g than rice husks-biochar, which had an BET-SSA of 154.7 $m^2$/g and a total pore volume of 0.1 µm. The BET-SSA of biochar varies with feedstock type but increases with increasing pyrolysis temperature because pore-blocking substances are driven off or thermally cracked, increasing the externally accessible surface area (Tomczyk et al., 2020)**.** Through the progressive degradation of cellulose and lignin, as well as the release of volatile matter and the creation



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of more pores, pyrolysis temperature may increase the BET-SSA and pore volumes (Yu et al., 2019).

Furthermore, feedstocks containing aromatic lignin cores, as well as aliphatic alkyls and ester groups, produce biochars with higher BET-SSA when pyrolyzed at higher temperatures (Tomczyk et al., 2020). Biochars with a low ash content, on the other hand, can have a low BET-SSA and pore volume. For instance, biochars made from cotton seed hull (4.7 $m^2/g$) (Uchimiya et al., 2010), poultry litter (2-3.2 $m^2/g$) (Song and Guo, 2012), and dairy manures (13.0 $m^2/g$) (Cao and Harris, 2010) all had low BET-SSA and ash content. Ahmad et al. (2014) concluded that biochars made from animal litter and solid waste feedstocks have lower BET-SSA than biochars made from crop residues. Deformation, cracking, or blockage of micropores in biochars are all factors that contribute to low BET-SSA (Panahi et al., 2020). The total pore volume increases as the pyrolysis temperature rises, and it is closely related to the BET-SA area. By increasing the pyrolysis temperature from 450 to 700 °C, regardless of the feedstock type, the tendency for smaller size particles to form in biochar is increased (Panahi et al., 2020).

### 2.4.2 Chemical properties of biochar

**Volatile matter (VM), Fixed carbon (FC), ash content and moisture content-** These properties are frequently used to estimate the C recalcitrant/stability of biochar. Both VM and FC are used to determine the labile and recalcitrant biochar fractions (Enders et al., 2012). The VM is referred to as the labile fraction because it is more easily degraded by soil microorganisms (Keiluweit et al., 2010). The non-combustible mineral fraction is known as ash content, and it is related to the liming value of biochar. The main goal of moisture determination is to establish a consistent dry weight. Woody biomass contains more cellulose, hemicellulose, and lignin than herbaceous, grassy biomass, crop residues, or animal waste, which affects the ash content, FC, and VM of biochar (Kloss et al., 2012). The presence of more lignin in plant biomass has been linked to increased carbonization, biochar FC and ash content (Sohi et al., 2010, Wang et al., 2015). H El-Gamal et al. (2017) found that biochar made from rice husk had a higher ash content and a higher lignin content than sugarcane bagasse, which had a higher cellulose and hemicellulose content. Tag et al. (2016) reported a decrease in VM due to higher FC in lignocellulosic biochars (56.6 to 72.3%) and waste-biochars (25.4-57.0 %) at 250 to 600 °C. Biochars derived from woody biomass had a lower ash content of less than 7.0% compared to non-wood-derived biochars with ash content greater than 50.0% (Mukome et al., 2013), because manure and grass biochars contain silica from soil contamination, resulting in high ash content and low VM.



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Biochars made from saw dust, rice husk, poultry litter, and paper sludge, on the other hand, had higher VM at 350 $^{o}$C, and all biochar had higher FC, ash content, and lower VM when the pyrolysis temperature was increased from 350 °C to 650 °C (Pariyar et al., 2020). As the temperature rises, hydroxyl groups dehydrate and cellulose and lignin thermally degrade, resulting in biochar with higher FC, ash, and lower VM (Zhao et al., 2017). The VM of a biochar has an impact on its stability, N availability, plant growth, and sorption capacity (Aller, 2016). The formation of ash is caused by inorganic minerals that remain after the decomposition of H, O, and C in biomass (Zhao et al., 2017). More polymerization leads to a more condensed carbon structure in the biochar, resulting in increased carbon content ranging from 62.2 to 92.4% with an increase in pyrolysis temperature (Lehmann and Joseph, 2009a). The higher the degree of aromatic structure formation, the more resistant the biochar is to microbial degradation (Keiluweit et al., 2010).

**Elemental composition (C, H, N, S, O, H/C, O/C, C/N ratio)-** The pyrolysis temperature has a greater impact on the element composition than the feedstock type. For example, increasing the pyrolysis temperature increased C content while decreasing H, O, N, and S content of biochars derived from various feedstocks (Ahmad et al., 2014). Tag et al. (2016) found that biochar made from lignocellulosic biomass and poultry litter had higher C (36.6–72.3%) and lower H (1.4-5.7%), as well as higher N (1.3–5.0%), O (8.2–37.4%), and S (0.0-0.80%) when pyrolyzed at 250 to 600 °C. Břendová et al. (2017) found that six biochars pyrolyzed at 400-600 $^{o}$C from wood, grass, wheat, and crop residues had higher C (29-76%), H (1.07-3.50%), N (0.17-4.6%), and O (0.17-4.6%). (8.2-23-9 %). In a recent study, the pyrolysis of biochar derived from saw dust, rice husk, food waste, and poultry litter pyrolyzed at 350-650 $^{o}$C revealed higher C (25.28-62.87%), H (1.61-5.17%), N (0.15-631%), and S (0.0-0.17%) (Pariyar et al., 2020).

These studies show that increasing the pyrolysis temperature resulted in higher C content due to the cleavage and cracking of weak bonds within the biochar, resulting in a decrease in H and O in the biochar, regardless of the feedstock type (Pariyar et al., 2020). Lower molar H/C and O/C ratios result from decreased H and O contents and increased pyrolysis temperature, indicating dehydration and deoxygenation of the biomass. Biochars made at 500 and 600 °C had higher carbon stability, with molar H/C and O/C ratios averaging 0.48 and 0.14, respectively (Tag et al., 2016). The H/C and O/C were 0.03 and 0.23 on average (Břendová et al., 2017) and 0.7 and 0.2 on average (Pariyar et al., 2020). The H/C and O/C ratio is linearly proportional and it is used to determine the stability of biochar in soil environments (Mukome et al., 2013). When applied to soils, biochar with an O/C ratio of less than 0.4 and a H/C ratio of less than 0.6 is thought to be effective as a C sequestration agent. Furthermore, biochar with an O/C ratio greater than 0.6 has a half-life of less than 100 years, while biochar with an



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O/C ratio between 0.2 and 0.6 has a half-life of 100 to 1000 years, and biochar with an O/C ratio greater than 0.2 has a half-life of more than 1000 year (Aller, 2016).

**Total N, C/N and S**- The biomass feedstock is the significant factor determining the N content in biochar, while pyrolysis temperature determines both N and S availability. As shown by Tag et al., 2016) Poultry litter biochar exhibited the highest N content (2.0-5.4%) at lower temperature (250-350 °C), but N and S decreased as temperature increased compared to biochars derived from plant feedstocks. In a similar way, sugarcane straw and poultry litter biochars pyrolyzed at 450-650 °C had the highest N and S content, while sawdust and rice husk biochars had the lowest (Conz et al., 2017). The N content of biochar is largely determined by the biomass feedstock, while pyrolysis temperature determines both N and S availability.

Compared to biochars derived from plant feedstocks, poultry litter biochar had the highest N content (2.0-5.4 %) at lower temperatures (250-350 °C), but N and S decreased as temperature increased Tag et al. (2016). Similarly, as the pyrolyzing temperature (250-650 °C) rises, N content decreases and the highest loss occurs in the manure-based biochar (Břendová et al., 2017, Pariyar et al., 2020). Generally, lignocelluloses-based biochars have relatively lower N content than manure-based biochars, as the manure feedstocks have high protein contents (Pariyar et al., 2020). However, as the pyrolysis temperature rises, both elements become gaseous and volatilize ($N_2O$ or $SO_2$). Furthermore, high-N-content feedstocks, such as poultry manure, may lose N more quickly than C, possibly due to the lack of aromatic C form formation, as evidenced by the lack of fixed carbon at high pyrolysis temperatures (Enders et al., 2012).

The C/N ratio of biochar is determined by the C content and availability of nitrogen in the feedstock, and it determines whether the biochar will mineralise or immobilize N when applied to the soil. Biochar C/N ratios can range from 7 to 400, with higher C/N ratios being linked to higher conversion temperatures because more aromatic groups are formed (Yuan et al., 2019). Břendová et al. (2017) found that C/N of biochars derived from wheat straw was (15.4-16.8) lower than that of wood origin (54.3-491), grass origin (24.7-33.7), and wheat grain (50.1-52.8) due to high C and low N in feedstocks with high lignin and cellulose. Biochars made from woody materials had a C/N ratio nearly four times higher than biochars made from manures (232.3) and three times higher than biochars made from vegetable residues (92.2)(Günal et al., 2019). Biochar with a C/N ratio greater than 30 results in net N immobilisation by microbes (Singh et al., 2010), affecting N availability in soils (EBC 2015). Biochars made from high-C-content feedstocks (woody) had a high C/N ratio (Mukome et al., 2013).



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**pH and Electrical conductivity (EC)**- The pH and EC values are highly dependent on the feedstock used and the temperature at which the pyrolysis takes place. Biochar made from woody feedstocks has a pH range of 4 to 9, whereas livestock/poultry biochar has a pH range of 6 to 12. (Diatta et al., 2020). H El-Gamal et al. (2017) found that the pH and EC values of sugarcane-biochar were 8.6 and 0.7 dS/m, and that of rice husks-biochar were 8.9 and 0.5 dS/m, respectively when pyrolyzed at 550 °C. The pH and EC of eight biochars made from pyrolyzed vegetable waste at 500 °C ranged from 10.5-12.2 and 4.3-18.2 dS/m, while that of woody feedstock ranged from 9.20-10.90 and 2.60-9.30 dS/m, respectively (Günal et al., 2019). Choudhary et al. (2019) discovered that the farm yard manure biochar had the highest pH of 8.5 and EC of 0.6 dS/m of the other two biochars made from sugarcane filter cake and rice husk feedstocks.

The average pH of biochar made from wood is generally 2 units lower than that of other biomasses produced under similar pyrolysis conditions (Tag et al., 2016). The thermal decomposition of hydroxyl bonds and other weak bonds within the biochar structure at high temperatures is thought to be the cause of the pH elevation (Panahi et al., 2020). The formation of carbonates and the contents of alkaline elements (Mg, Na, K, Ca) are positively correlated with the pH and EC values of biochar as the temperature rises (Ding et al., 2016). Furthermore, increases in ash content and oxygen functional groups have been linked to higher pH with increasing temperature during pyrolysis (Zhao et al., 2017). The EC value is a measurement of the total water-soluble ions (salinity) present in biochar, which can have a negative impact on plant growth if it is too high, resulting in reduced water uptake by plant roots and nutrient imbalances (Tag et al., 2016, Uras et al., 2012).

**Cation exchange capacity (CEC)**- indicates biochar's ability to adsorb cations. The CEC of biochar is affected by the composition of exchangeable cations of Mg, Ca, Na, and K, which is dependent on the constituents of the feedstock type and the pyrolysis temperature (Tag et al., 2016). Song and Guo (2012) found that the CEC values of poultry litter biochar pyrolyzed at 300 to 600 $^{o}$C ranged from 29.2 to 51.1 cmol/kg, and that the CEC values decreased as the pyrolysis temperature increased. At 500 $^{o}$C, the CEC of biochars made from pig manure (32.7 cmol/kg) was lower than that of biochars made from chicken manure (81.4 cmol/kg) (Cely et al., 2015)(Cely et al. 2015).

The removal of surface functional groups and the formation of aromatic carbon resulted in a decrease in CEC of the four biochars studied when the pyrolysis temperature was increased from 250 to 600 $^{o}$C (Tag et al., 2016). Pariyar et al. (2020) reported that the CEC of biochar made from paper sludge, saw dust, rice husk, and food waste decreased when heated to 550 $^{o}$C, but the CEC of poultry litter biochar was 51.5 to 67-2 cmol/kg higher than the other



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biochars. The presence of alkali and alkali metals (Mg, Ca, Na, and K) in biomass promote the formation of surface functional groups containing O during pyrolysis, resulting in higher CEC (Cely et al., 2015, Tag et al., 2016).

**Macronutrient (P, Mg, Ca, K, Na) and micronutrient/heavy metals (Fe, Cu, Zn, Mn)-** the concentration of P, K, Ca, Mg, Fe, Cu, Zn, and Mn in biochar generally increases with pyrolysis temperature, but their bioavailability decreases. This is because these elements are incorporated into biochar's highly aromatic structure at higher pyrolysis temperatures. Enders et al. (2012) found that the concentration of P, N, Ca, Mg, K, and Na in biochars varied greatly depending on the feedstock elemental contents, with the poultry manure biochars having high levels of K, Ca, Mg, and Na at 300-600 °C. Even when the temperature was increased from 250 to 600 $^{o}$C, Tag et al. (2016) found that extractable K, Ca, and Mg Na were higher in algae and poultry litter biochar than vine pruning and orange pomace. H El-Gamal et al. (2017) found that sugar cane bagasse biochar had higher concentrations of S, P, K, Na, Cu, Fe, and Zn than rice husk biochar when pyrolyzed at 500 °C, but Fe and Zn concentrations were similar in both biochars. Farm yard manure biochar pyrolyzed at 350 °C, on the other hand, had higher P, Mg, Na, K, Zn, and sugarcane filter cake had higher Cu, Fe, and Mn than rice husk biochar (Choudhary et al., 2019). The constituents of the feedstock type and ash content after pyrolysis were related to the increase in nutrients in all biochars, especially in manure-based biochars. Heavy metal concentration is solely determined by biomass type and pyrolysis peak temperature, with metal concentration increasing as temperature increases. For instance, the concentrations of heavy metals were higher in poultry litter and paper sludge biochars than in plant/agricultural waste biochars when pyrolyzed at 550 to °C (Pariyar et al., 2020). This is because heavy metals are difficult to volatilize because they remain stable even at high temperatures; they either become more concentrated or remain the same. The International Biochar Initiative (IBI 2015) recommended that any pyrolyzed biochar have a Cu concentration of 63 to 1500 mg/kg and a Zn concentration of 200 to 7000 mg/kg, however Fe has no standard threshold because it is not a required element.

### 2.4.3 Biological properties of biochar

Biochar's properties, such as surface area and pore size, provide habitat for microorganisms, while its amendment improves bulk density, pH, and air, water, and nutrient movement within the soil matrix, promoting microbial abundance and activity (Quilliam et al., 2013, Lehmann et al., 2011). Gomez et al. (2014) stated that the porous structure of biochar, as well as its high internal surface area and ability to adsorb soluble organic matter, gases, and inorganic nutrients, are likely to provide a highly suitable habitat for microbes to colonize, grow, and reproduce, particularly for bacteria, actinomycetes, and arbuscular mycorrhizal fungi.



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Biochar micropores can vary in size depending on the feedstock. For example, the diameter of bamboo biochar pores ranges from 0.001 to 1000 µm, while that of wood biochar pores ranges from 10 to 3000 µm; however, bacteria size ranges from 0.3 to 3 µm, fungi 2-80 µm, protozoa 7-30 µm, and nematodes 3-30 µm, so different microbes can be accommodated onto biochar pores (Thies and Rillig, 2012). In addition, biochars provide bacteria with carbon, nutrients, gases, and water, which they use to grow and reproduce.

Biochar application at high rates can cause changes in soil microbial composition, favouring bacterially dominated communities (Gomez et al. 2014; Ippolito et al. 2014). For example, Xu et al. (2016) found that corn straw biochar pyrolyzed at 500 °C and applied at 2 and 4 t/ha increased communities of Proteobacteria, Bacteroidetes and Actinobacteria but decreased communities of Acidobacteria, Chloroflexi and Gemmatimonadetes. On the contrary, the proportion of Actinobacteria decreased as rice straw biochar levels increased, but the proportions of Proteobacteria and Acidobacteria increased (Gao et al., 2017). (Noyce et al., 2015) concluded that biochar (pyrolyzed from sugar maple and white spruce sawdust at 400 °C) addition at 5 t/ha is neither beneficial nor toxic to soil microbes in a northern hardwood forest acidic soils, implying that biochar amendments can be used to sequester C without harming the soil microbial community. Gomez et al. (2014) found a significantly lower fungus/bacteria ratio in four soils amended with biochar (sand, sandy loam, clayey, and clay loam). The shift in bacteria community has been attributed to both biochar's ability to raise pH, P, K, total C, and total N (Gomez et al., 2014, Ippolito et al., 2014, Senbayram et al., 2019, Sheng and Zhu, 2018, Yao et al., 2017) and the addition of labile organic C in soil which results in a wider C/N ratios (Lehmann et al., 2011).

The abovementioned studies emphasized the potential benefits of using biochar to enhance soil bacteria population and growth. However, due to differences in feedstock type, application rates, soil type, and soil environment, the effect of biochar on bacteria communities and abundance has resulted in mixed results. In addition, the reasons on how biochar affects bacterial communities and diversity in different soils are unknown because reported findings either contradictory or inconclusive. Also, the impact of short-term biochar additions on bacteria taxonomy, however, is unknown. Therefore, if biochar is to be used as a soil amendment, a better understanding of the impact of biochar derived from various feedstocks on bacteria abundance and diversity, as well as subsequent nutrient cycling and plant responses in diverse soil ecosystems, particularly in agricultural settings, is required. Some studies used phospholipid fatty acid analysis to describe how soil microbial communities respond to biochar (Gao et al., 2017, Gao and DeLuca, 2018, Gomez et al., 2014). Changes in microbial structure as a result of biochar addition have also been investigated using the PCR-denaturing gradient gel electrophoresis method (Rutigliano et al., 2014). In addition,



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previous studies only looked at the most common microbial taxa, whereas combining DNA extraction, PCR, and high-throughput 16S rRNA gene sequencing can now detect less common taxa, providing a more complete picture of microbial communities (Xu et al., 2016).

### 2.5 Biochar use as soil amendment

Biochar use as a soil amendment can improve soil fertility and crop production through enhanced physical, chemical, and biological properties of soils, as well as improving nutrient availability while minimizing nutrient leaching, all of which improves plant productivity (Ding et al., 2016). Biochar application can help poor-resourced smallholder farmers reclaim their degraded lands and increase agricultural productivity. However, as mentioned above in section 2.4 under biochar properties, the effectiveness of biochar on soil properties and crop yield is dependent on the feedstock and pyrolysis condition used for biochar production.

#### 2.5.1 Improvement of soil chemical properties

**Soil pH-** the pH of biochars is an important characteristic for its use as a liming agent in agriculture. Because of biochar alkalinity, high pH buffering capacity, and functional group effects, the use of alkaline biochars can help to reduce soil acidity (Dai et al., 2017). Biochar's alkalinity is determined by three factors: (i) organic functional groups; (ii) carbonate content; and (iii) inorganic alkali content. During pyrolysis, mineral elements like Ca, K, Mg, Na, and Si in feedstocks form carbonates or oxides, which react with $H^+$ and monomeric Al species in acid soils to reduce exchangeable acidity and raise pH (Dai et al., 2017). The increased pH buffering capacities after biochar application were due to increased CEC. The release of cations from biochars, such as K, Ca, Mg, and Na, was a major contributor to the pH rise. The protonation of carboxyl groups on biochar surfaces, as well as the dissolution of the biochar, caused the cation release (Agegnehu et al., 2017).

Although alkaline biochar can be used as a liming material to lime acid soils, the liming potential of biochar varies depending on the soil and biochar type. For example, in a field base study, paddy straw-derived biochar (pH 10.50) increased the pH of a sandy soil (pH 5.24) by 4.5 units when compared to the control (El-Naggar et al., 2018a). A high dose of biochar (50 and 100 t/ha) with pH of 9.40 raised the pH of an Alfisol, lowering the exchangeable Al concentration in the soil (Tomczyk et al., 2020). Li et al. (2018) found that applying biochar at 10, 20, 40, and 60 t/ha had no effect on soil pH in a semi-arid region. Ippolito et al. (2016) found that adding acidic biochar (pH 5.8) to a calcareous soil reduced soil pH by 0.2 to 0.4 units while increasing the bioavailability of P, Mn, and Zn. Based on the original soil properties, such as pH, and texture, studies show that biochar application to soils can either increase or



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decrease soil pH. However, more research is needed into the production of acidic biochars, as well as their use in improving alkaline soils (Yu et al., 2019).

**Soil EC**- most biochars contain a high amount of soluble salts, their EC is generally higher than that of most agricultural soils. When applied to soil, the availability of soluble nutrient ions such as $NO_3^-$, $K^+$, and $Ca_2^+$ could be directly related to the soluble salt content and, thus, to the EC of biochar (Yu et al., 2019). Plants are harmed by excessive salts or high EC in soil due to a decrease in osmotic potential. In semi-arid soil, the EC of the soil was reported to increase with increasing biochar application rates (10-60 t/ha) and decreased tomato growth (Li et al., 2018). El-Naggar et al. (2018a) revealed that adding 30 t/ha of biochar made from paddy straw, silver grass residue, and umbrella tree residue to a sandy soil with EC of 0.07 dS/m increased the soil's EC by 385, 100, and 71 %, respectively. The soil's EC must be kept low to ensure adequate nutrient availability and plant growth.

**Soil CEC**- biochar application to agricultural soils increases CEC over time due to biochar surface oxidation and adsorption of highly oxidized organic matter onto the biochar surface, as well as its porous structure and large surface area (Tomczyk et al., 2020). El-Naggar et al. (2018a) found that applying biochar derived from crop residues at 30 t/ha increased the CEC by 3, 1, and 0.8 cmol/kg of a sandy soil which had an initial CEC of 0.5 cmol/kg. Biochar derived from wood increased the CEC by 190% in an Anthrosol (initial CEC = 2.81 cmol/kg) compared to the control treatment (Tomczyk et al., 2020). Various types of biochars produced from different feedstocks change the CEC of different soils to a different extent and the CEC affects nutrient availability and water retention of soil (Yadav et al., 2018). Only a few studies have considered the mechanisms that influence macro- and micronutrient availability in arid soils after biochar application. Soils with low CEC due to low clay and SOC would benefit in the arid region.

### 2.5.2 Biochar effect on soil nutrient retention and availability

Biochar can help improve the soil's nutrient retention capacity due to its large surface area, porosity, and presence of both nonpolar and polar surface sites (Hussain et al., 2017, Panahi et al., 2020, Tag et al., 2016, Yu et al., 2019). Also, biochar adds mineral nutrients and thus improve soil fertility because it contains organic C as well as plant nutrients such as N, P, K, Ca, Mg, S, Fe, Mn, and Si. However, the release of nutrients by biochars depends on the nature of the feedstock materials and the pyrolytic conditions. For example, the pyrolysis of high-nutrient feedstock materials at temperatures between 400 and 500 °C results in the production of nutrient-rich biochars (Hossain et al., 2020, Yu et al., 2019).



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**Major nutrients (N, P, K)-** Given its porous structure and ability to adsorb $NH_4^+$-N, biochar can help to slow the release of nitrogen from the soil. For example, the pore space of biochar facilitated water and nutrient transfer during the early stages of its application (Hussain et al., 2017). Biochar application increased $NO_3^-$-N and $NH_4^+$-N retention in soil by 33 and 53%, respectively (Gao et al. 2016). In a similar study, Hu et al. (2019) found that 59.32 % N was released after 84 days and 69 % N was released within 28 days. Liu et al. (2017) suggested that the increase in N retention after biochar application in soil is due to adsorption of $NO_3^-$-N and $NH_4^+$-N due to biochar's high CEC, reduced $NO_3^-$-N leaching due to the soil's increased ability to hold water, and increased microbial immobilisation of N in soil due to the supply of labile C. In general, biochar influences soil N availability through two mechanisms: (i) biotic factors such as fixation, mineralisation , immobilisation, denitrification, and plant uptake; and (ii) abiotic factors such as sorption, volatilization, and leaching (Nguyen et al., 2017). Apart from increased soil N, the application of biochar is associated with increased availability of soil P (Dia et al., 2017; Brantley et al., 2016). Other research (Atkinson et al., 2010, Major et al., 2010) found that adding alkaline biochar to acidic soils increased K concentration. Biochar application increases N, P, and K availability in soils, which is beneficial for plant growth. However, biochar has both negative and neutral effects on the availability of these nutrient in various soils and application rates (Agegnehu et al., 2016, Gonzaga et al., 2018, Nguyen et al., 2017, Yao et al., 2017).

**Secondary major nutrients (Ca, Mg, S)-** Biochar has a positive effect on soil nutrient retention. Biochar increased Ca and Mg availability in soil, increasing crop yield (Hussain et al., 2017), which was previously supported by Abujabhah et al. (2016), who discovered that woody biochar had a significant impact on exchangeable Ca, Mg, and Na in black clay loam, red loam, and brown sandy loam soils. In addition, the incorporation of biochar in soil increased or decreased the availability of Ca, Mg, and S. (Butnan et al., 2015, El-Naggar et al., 2018a, Lusiba et al., 2017). Biochars could be designed to meet the nutritional needs of plants grown in acid soils based on nutrient deficiency (Novak et al., 2009, Yu et al., 2019). For example, adding biochars made from rice husk or grass feedstocks to soils deficient in SI increases its concentration, while soybean-derived biochar can add N, whereas, eggshell-derived biochar can add Ca, and manure-derived biochar can add nutrient elements like Ca, Mg, K, and woody-derived biochars usually add C (Yu et al., 2019). This suggest that biochar can be chosen as a specific elemental nutrient enhancer based on these various characteristics in different feedstocks.

**Trace elements (Fe, Mn, Zn, Co, Cu, Mo, B)-** biochar alters the bioavailability of trace elements in soils by changing soil pH and balance of cations (Beesley et al., 2011) . However, these effects depend on the soil type, and the type and rate of biochar. For example, woody



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biochar improved the availability of micronutrients (B and Mo) (Hussain et al., 2017), whereas the addition of mixed hardwood-derived biochar did not influence the Cu and Zn content (Uchimiya et al., 2010). When applied in sandy soils, biochar reduced Fe and Al content, but had no effect in silt or clay soils (El-Naggar et al., 2018b). The addition of hardwood-derived biochar, on the other hand, increased Fe and Mn availability while having no effect on Zn or Cu availability (Ippolito et al., 2014). Biochar increased Mn and Na content in sand, sandy-loam, and silty-sand soils (Noyce et al., 2015). The ability of hardwood-derived biochar to enhance and decrease trace elements has also been demonstrated in studies, which is beneficial for the remediation of polluted soils. However, research on the impact of animal-derived biochar on trace elements is limited.

### 2.6 Effect of biochar on maize productivity in different soil types

The use of biochar in soils to improve soil fertility and sequester carbon has grown in popularity over the years. Biochar as a soil amendment is effective in improving soil fertility and crop productivity in different soil types (Diatta et al., 2020, Hossain et al., 2020). Biochar's impact on crop growth and yield, on the other hand, varies depending on biochar type, application rate, soil properties, and environmental factors. Xiao et al. (2016) found that when maize straw was applied in a clay loam soil, maize increased by 9 and 13% at 20 and 30 t/ha in the first year, and by 11 and 14% in the second year in a calcareous Inceptisol. In support of these findings, Zhang et al. (2017) found that wheat straw biochar applied at 20 and 40 t/ha increased maize yield in both years. Improved soil organic C, soil structure, moisture content, and additional nutrients like N, P, and K were all attributed to the increase in maize. Soil characteristics, feedstock type, pyrolysis conditions, and application rates all influenced maize response to biochar application. In addition, extensive work has been done on the effect of biochar on maize especially in other countries such as China (Xiao et al., 2016, Zhang et al., 2017); America (Cole et al., 2019, Rogovska et al., 2016); Brazil (de Sousa Lima et al., 2018, Gonzaga et al., 2018) but hardly any published research with the Africa continent (Agbede and Adekiya, 2020). Moreover, there no published research on the effects of residual biochar combined with biological nitrogen fixation by legumes on maize productivity. To make potential recommendations on biochar use, further research is required to evaluate the effect of biochar on maize and legumes like chickpea in arid and semi-arid soils in Southern Africa.

### 2.7 Use of acacia and poultry litter biochar as soil amendment

**Poultry litter as biochar feedstock-** Poultry litter is a mixture of poultry manure and bedding, such as wood shavings, sawdust, straw, or other organic materials, as well as feathers and feed spillage, used in poultry operations. Poultry litter biochar (PLB) has a high nutrient



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content, particularly N, P, K, and Ca (Tag et al., 2016), a higher CEC (Gaskin et al., 2008), a greater ability to adsorb and sequester metal ions and a higher ash content, which makes it useful as a liming agent in low pH soils (Inal et al., 2015). PLB has been studied as a soil amendment to improve soil nutrient availability and crop production (Brantley et al., 2016, da Silva Mendes et al., 2015, Joardar et al., 2020). Many studies, however, did not report on the characterization of PLB properties in terms of proximate analysis and elemental composition to determine if the biochar used was, in fact, '*biochar*' as suggested by the International Biochar Initiatives (IBI 2015). For instance, Inal et al. (2015), Joardar et al. (2020), Toluwase Oreoluwa et al. (2020) are examples of studies that did not report PLB properties such as proximate analysis and elemental composition, while studies that reported only nutrient elements includes (da Silva Mendes et al., 2015), Tian et al. (2016b), Yu et al. (2018).

The results of the few studies that reported PLB proximate analysis, elemental composition, and nutrient elements varied. For instance, Pariyar et al. (2020) recently reported that PLB pyrolyzed at 550-600 °C was highly carbonised and stable, with H/C and O/C ratios of 0.7 and 0.3, respectively, as well as higher FC (33%) and ash content (48%) and low VM (26 %). Conz et al. (2017) on the other hand, reported that PLB pyrolyzed at 550 $^{o}$C and 650 $^{o}$C had 43% VM, 49% ash content, had a low C aromaticity of 5% FC, and 33% total C, but was stable, as measured by the H/C and O/C ratios of 1.9 and 0,1, respectively. These findings were previously supported by Tag et al. (2016) who found that PLB pyrolyzed at 500 °C contained 68% VM, 23% FC, 35% total C, and 8% ash, but the biochar was highly carbonized (H/C = 0.48) and stable (O/C = 0.1). In contrast, an earlier study by Enders et al. (2012) found that PLB pyrolyzed at 500-600 °C had higher VM, which was associated with lower C, ash, N, P, Ca, K, Mg, and S content. The PLB was not effectively carbonised and stable, because the biochar had H/C and O/C values above 0.6. These studies show that the pyrolysis condition did affect PLB production, as PLB with low C content was produced at higher temperatures. The PLB produced in these studies does not qualify as "biochar," but rather as a pyrogenic carbonaceous material, according to the IBI 2015 guidelines, because the C content was less than 50%. To be certain that any produced biochar qualifies as '*biochar*', the proximate analysis and elemental composition must be determined for better recommendation on its use and application.

**Acacia wood as a biochar feedstock-** *Acacia mearnsii* is an invasive plant species found in South Africa that originated in Australia. Given the plant's widespread distribution in the country currently, using it as biochar could help to limit its spread and negative impact on local plant species. The use of biochar derived from acacia plant species referred to as to ACB is very limited in literature, also characterization of VM, FC, ash content, H/C, and O/C ratio to determine if the produced product was indeed biochar is also scarce. For example, Pituya et



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al. (2017) in Thailand pyrolyzed acacia wood (*Leucocephala glauca Benth*) at 500 $^{o}$C and found that the biochar was stable, with higher C (73%), pH (7.7), and BET-SA (359.7 $m^2$/g), but VM or ash content was not determined. On the contrary, biochar made from Acacia *mearnsii* at 450-550 $^{o}$C had higher pH, P, high C content, and exchangeable cations, despite the lack of proximate and elemental analysis (Lusiba et al., 2017, Lusiba et al., 2018). However, the properties of biochar made from *Acacia mearnsii* were not studied by (Reyes et al., 2015). On the other hand, Deng et al. (2017) reported that biochar made from crushed Acacia *seyal* charcoal contained 69 % C, 32 %VM, 24 % ash, and low levels of Ca, Mg, P, and K nutrients. Although the authors reported an increase in soil C and K, when applied at 10 Mg/ha in a Vertisol silt loam, the biochar had no significant effect on soil pH, sorghum biomass, or yield. The later research shows that proximate and elemental analysis of biochar derived from acacia wood feedstock is required to determine whether the biochar produced qualifies '' biochar'' and for use as a soil amendment as recommended by the IBI 2015 guideline.

Despite the growing interest among researchers on the use of PLB and ACB as a soil amendment to alleviate acid soils, research on the impact of PLB and ACB on improving soil nutrients and thus increasing chickpea and maize growth and yield in Southern Africa is still limited. There is a lot of evidence that woody derived biochar can improve soil properties and thus increase legume growth and yield (Hiama et al., 2019, Mia et al., 2014, Nelissen et al., 2014, Quilliam et al., 2013), but there is hardly any evidence for chickpea (Lusiba et al., 2018, Macil et al., 2017, Macil et al., 2020). Furthermore, the response of maize to biochar made from poultry litter and woody feedstocks varied greatly depending on the application rate. When PLB or woody biochar was applied at 10 to 60 t/ha, for example, maize yields increased (Brantley et al., 2016, de Sousa Lima et al., 2018, Inal et al., 2015). However, other researchers found a decrease in maize and nutrient uptake at the same application rates (Cole et al., 2019, Gonzaga et al., 2018, Taskin et al., 2019). Application rates used in previous studies, such as 50 and 100 t/ha (Chan et al., 2008b); 60 t/ha (Widowati et al., 2012); 96 t/ha (Rogovska et al., 2014); and 120 t/ha (Cole et al., 2019), may not be feasible in regions with limited feedstock availability, such as semi-arid areas, where majority of smallholder farmers reside. Consequently, studies that demonstrate the characterization of PLB and ACB properties and their impact on chickpea and maize productivity when applied at lower rates that smallholder farmers can afford are crucial.

### 2.8 Biochar effect on biological nitrogen fixation (BNF)

Biological nitrogen fixation (BNF) is the process of converting atmospheric nitrogen ($N_2$) into organic ammonia molecules ($NH_3$), through symbiotic association of rhizobia species with



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legumes. Biological nitrogen fixation is thought to contribute around 17.2 x 107 Mg of nitrogen to soils each year, with leguminous crops accounting for roughly half of the global symbiotic BNF at 21.5 x 106 Mg (Mia et al., 2014).In low-input systems, where nitrogen fertilizer inputs and availability are limited, BNF is critical. It is a sustainable source of nitrogen for agriculture, reducing the need for inorganic fertilizers (Rondon et al., 2007). Smallholder farmers may benefit from lower operating costs. Depending on soil fertility and other factors, BNF can provides 80-90% of the N available to plants in the natural ecosystem (Giller, 2001). In crop rotation or intercropping, fixed N contributed by legumes increase yields of subsequent crops (Taskin et al., 2019, Uzoh et al., 2019). This shows that BNF is a critical ecosystem phenomenon for global agriculture, and that it is essential to comprehend the potential impact of biochar application on BNF (Unkovich et al., 2008).

Studies have shown that biochar application at different rates in different soils can improve soil quality and thus improve BNF(Brewer et al., 2012, Ogawa and Okimori, 2010, Rondon et al., 2007). The effect of biochar on BNF was observed through various mechanisms, including the immobilisation of inorganic N, which is known to stimulate BNF (Rondon et al., 2007). Increased nodulation, as seen in soybean (Ogawa and Okimori, 2010, Tagoe et al., 2008) Increased P bioavailability has been linked to higher BNF in several legumes, including soybean (Tagoe et al., 2008), common bean (Rondon et al., 2007). Biochar and nodulation via adsorption of flavonoids and Nod-factors interactions (Lehmann and Joseph, 2009b) 2009). Direct and indirect addition of macro (Ca, Mg, K,) and micronutrients (B, Mo, Zn) due to a change in soil pH may be beneficial to legumes and thus enhance BNF (Brewer et al., 2012, Tagoe et al., 2008). Giller (2001) reported that biochar addition in soils with high nitrates, Ca, P and low micronutrients reduced BNF significantly. However, Lehmann et al. (2003) suggested that high application of biochar and high availability of Ca, P and micronutrients with low nitrates is ideal for maximum BNF in the soil.

The current state of knowledge on biochar and BNF is uncertain and it is still unclear how biochar application improves BNF. Despite these studies, the mechanisms underlying biochar application and BNF in soils have not been clearly identified and remain uncertain because reported findings are inconclusive. For example, In Colombia, Rondon et al. (2007) found that when Eucalyptus *deglupta* biochar pyrolyzed at 350 $^{o}$C was applied in clay loam Oxisol, BNF of common bean increased by 72% at 90 g/kg (equivalent to 100 t/ha), but the proportion of N derived from atmosphere (Ndfa) increased by 78% at 60 g/kg (equivalent to 78 t/ha). Higher BNF after biochar application was attributed to greater B and Mo availability, while higher K, Ca, and P availability, as well as higher pH and lower N availability and Al saturation, may have played a smaller role. In Japan, Tagoe et al. (2008) found that when carbonized chicken manure pyrolyzed at 500 $^{o}$C was applied to soybean, the number of nodules and nodule weight



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increased at 50 and 100 t/ha due to higher available P. In contrast, In the UK, Quilliam et al. (2013) found that increased biochar (derived from woody feedstocks) application rates from 25 to 50 t/ha reduced nodulation, but increased nitrogenase activity in a Cambisol, sandy clay loam when different tree species were pyrolyzed at 450 °C.

In Netherlands, Mia et al. (2014) found that when grass was pyrolyzed at 400 $^{o}$C and applied at 10 t/ha, it resulted in the highest nodulation and BNF (71.91 g N/pot) of red clover, but when applied at 120 t/ha, nodulations decreased and BNF was reduced to 13.87 g N/pot. Because biochar application at higher rates did not increase nodulation or stimulate higher BNF, the authors concluded that adsorption of flavonoids and Nod factors, as well as their stimulating role on nodulation, are unlikely to be the main mechanisms by which biochar affects BNF, contradicting findings by (Lehmann and Joseph, 2009b). The authors also showed that biochar application did not increase P availability or other micronutrients (B and Mo), contradicting the findings of (Rondon et al., 2007, Tagoe et al., 2008). In a recent study, Mia et al. (2018) found that red clover had a higher amount of N-fixed (89 g N/m$^2$) despite having a lower %Ndfa when eucalyptus-wood derived biochar (pyrolyzed at 550°C) was applied at 10 and 20 t/ha. However, BNF was unaffected by B, pH, P, K, Mg, and Mo, but biomass production was a significant contributor to total N-fixed in both the pot and field studies. In, Hungary, Horel et al. (2018) found that cellulose fibres and grain husk pyrolyzed at 600 $^{o}$C and applied to Luvisol at 0.5 and 2.5 % (w/w) resulted in higher BNF than woodchips biochar. Higher soil P and pH caused an increase in BNF, while the amount of nitrate had no effect on BNF.

Differences in biochar properties, soil nutrient status, biochar application rates, soil type, and legume crop response to biochar application could explain the disparity in results on these studies. Although biochar application rates of 50-100 t/ha (Horel et al., 2018, Quilliam et al., 2013, Rondon et al., 2007, Tagoe et al., 2008) has been shown to have a positive and negative effect on nodulation and BNF, this rate may not be feasible for farmers who have limited access to organic materials. This suggests that more research is essential to investigate the effect of biochar derived from different feedstocks and application rates on BNF of other legumes, such as chickpea, in South Africa, as majority of the studies were done elsewhere using crop residues feedstocks and hardly on poultry litter. Moreover, new methods to quantify BNF needs to be explored, this is because some studies used the acetylene ($C_2H_2$) reduction Assay (ARA) (Horel et al., 2018, Quilliam et al., 2013) isotope dilution method (Rondon et al., 2007), and N difference methods (Khan et al., 2020) to describe how BNF respond to biochar. However, the $^{15}$N natural abundance technique is the most accurate and effective method of quantifying BNF (Unkovich et al., 2008), and therefore it is a useful tool to investigate the mechanisms behind the effect of biochar on BNF.



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## 2.9 Chickpea and its contribution to biological nitrogen fixation

Chickpea (*Cicer arietinum L*) is a popular pulse crop that provides high-quality protein for both human and animal consumption. Chickpea is the most widely grown pulse crop in tropical, subtropical, and temperate regions of the world, with more than 50 countries participating in chickpea cultivation (FAO, 2014, FOOD and NATIONS., 2019). Chickpea's diverse domestic, industrial, and other uses, as well as its ability to grow better with low inputs under arid conditions and abrasive edaphic factors, contributes to agricultural sustainability particularly in smallholder cropping systems (Khan et al., 2020). Chickpeas, when grown in rotation or intercropped with cereals, can provide an additional source of income for farmers as well as a net input of nitrogen to the soil.

Chickpea, for example, fixed about 29 to 45 kg N/ha in Ethiopia (Meleta and Abera 2019), contributing to high soil N concentrations. Chickpea fixed more nitrogen than Faba bean in Spain (40 and 50 kg N/ha) (López-Bellido et al., 2011). Biabani et al. (2011) reported high amount of nitrogeN-fixed by chickpea in Iran which ranged from 47 to 78%. This demonstrates that chickpea has the potential to alleviate nitrogen in ecosystems with limited nitrogen availability, thereby contributing to soil fertility. Despite extensive research on chickpea growth and yield in arid and semi-arid environments (Khan et al., 2020, Lusiba et al., 2018, Macil et al., 2017, Ogola, 2015).

Studies on the symbiotic performance of chickpea are limited in South Africa, particularly in the Mpumalanga and Limpopo provinces (Macil et al., 2020). The lack of effective native chickpea rhizobia strains, as well as agronomic factors like crop management, soil acidity, and low P availability, limit maximum BNF and yield (Khan et al., 2020). Biochar use as a soil amendment can be an alternative strategy for improving soil fertility because of its potential to increase soil organic matter, nutrient availability, and water retention; in so doing improve the population of bacteria responsible for nitrogen fixation and thus increasing chickpea productivity.



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# CHAPTER 3

# PHYSICAL AND CHEMICAL PROPERTIES OF BIOCHAR DERIVED FROM POULTRY LITTER AND ACACIA FEEDSTOCKS

## Abstract

The type of feedstock used and the pyrolysis temperature have a direct impact on biochar properties, which affect the quality and performance of biochar as a soil amendment and carbon sink. The aim of this study was to compare the physical and chemical properties of two locally produced biochars made from poultry litter and acacia under similar pyrolysis temperature, but different systems and conditions. Acacia biochar (ACB) was commercially produced in a pyrolysis chamber, while poultry litter biochar (PLB) was made in a modernized kiln furnace. The biochars were pyrolyzed at a temperature of 550 $^{o}$C under limited-oxygen condition. Physical properties like moisture content, ash content, volatile matter, surface area, and particle size distribution were determined after crushing and sieving the biochars. Chemical analysis P, Ca, Mg, K, Na, S, Fe, Mn, Cu, Zn, Co, B, Mo, Na, Al, and elemental analysis of C, N, H, and S were also determined. Pyrolysis of poultry litter and acacia feedstocks at 550 $^{o}$C produced a more stable biochar product due to higher fixed C and total C of greater than 50%. Both biochars had a H/C and O/C ratio of 0.3 % and 0.1 %, respectively, making them stable for C sequestration with a potential half-life of 100-1000 years in the soil. Plant available nutrients were higher in the PLB, while P, S, and N were 48, 5, and 3 times lower in the ACB, respectively. However, PLB had higher Na content, EC, while both biochars had higher Fe, Mn, and Al concentrations than the soil toxicity levels. This suggests that PLB and ACB could have an impact on plant roots and nutrient uptake, thus, caution should be taken to ensure that trace elements in the soil do not reach critical toxicity levels. Because ACB has a low N content and a high C/N ratio, it should be supplemented with a high N fertilizer when used for soil improvement.

Keywords: biochar, feedstock, physicochemical characteristics, proximate analysis, pyrolysis



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### 3.1 Introduction

Biochar known as black carbon, is a recalcitrant, carbon rich material that when applied to soil, has the capacity to store up to 9 to 11 Gt C each year (Wang et al., 2015). A wide range of crop residues (Manolikaki and Diamadopoulos, 2019, Nguyen et al., 2016) and hardwood (Cole et al., 2019, Gonzaga et al., 2018) animal waste (Enders et al., 2012) feedstocks were converted into biochar via pyrolysis in an oxygen-depleted environment for use as a soil amendment. The benefits of converting organic wastes into biochar includes; mitigating climate change by sequestering C and reducing greenhouse gas emissions, remediating polluted environment, recycling agricultural wastes, reducing the phytotoxicity of heavy metals **,** agricultural utilization of organic materials as soil conditioner thus improving soil fertility and crop productivity (Agegnehu et al., 2016, Kätterer et al., 2019).

Biochars have different properties, including chemical composition, surface chemistry, particle and pore size distribution, and physical and chemical stabilization, due to the wide range of properties of feedstocks (Inyang et al., 2016, Jafri et al., 2018, Yuan et al., 2019). The properties of biochar are largely determined by the feedstock used and the pyrolysis conditions used, which have an impact on the quantity, quality, and performance of biochar in terms of soil improvement and carbon sequestration (Lehmann and Joseph, 2015). The elemental composition of nutrients and metals is largely controlled by the feedstock, whereas the proportion of volatiles and surface properties are largely controlled by the pyrolysis temperature (Tag et al., 2016). Biochar feedstock selection is important because it comes in a variety of compositions and chemistries, which affect soil conditioning and economic factors. Several studies (Brantley et al., 2016, Joardar et al., 2020, Uras et al., 2012) have found that biochar made from animal waste is less stable (low C) but has a higher nutrient content than biochar made from wood and plant materials, which is more stable and has a higher C content.

Poultry litter and acacia feedstocks are two agricultural by-products produced in large quantities in many warm-climate countries such as South Africa. However, information on their characterization and use as biochar feedstock is limited, if not contradictory. Many studies have been conducted to investigate the production, characterization, and application of crop residues and woodchip biochar for agronomic and environmental applications (Cole et al., 2019, Gonzaga et al., 2018, Manolikaki and Diamadopoulos, 2019, Nguyen et al., 2016). The published results on woody feedstocks can be applied to acacia feedstock, but not to poultry litter, because there were contradictions in in the characterization and use of poultry litter biochar as a soil amendment. For example, poultry litter pyrolyzed at 550˚C and 650˚C had lower FC, but higher VM and ash content (Conz et al., 2017, Enders et al., 2012). Tag et al. (2016) on the other hand, reported low VM because of high FC and total C of poultry litter



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biochar pyrolyzed at 250 and 600 ºC. Song and Guo (2012) found that pyrolysis temperature ranging from 300 to 600 ◦C decreased poultry litter total N, total C, but increased pH, ash content and OC stability, surface area. Other studies (Brantley et al., 2016, Gaskin et al., 2008, Tian et al., 2016b) reported higher chemical composition of biochar produced from poultry litter at temperature ranging from 300 to 600 ◦C.

Although differences in feedstock type and pyrolysis condition may contribute to conflicting evidence of biochar characterization and performance in the soil, a full assessment of biochar properties is required to characterize the pyrolyzed product as biochar. The International Biochar Initiative (IBI 2015) and the European Biochar Certificate (EBC 2015) established standard test methods and guidelines for biochar properties that can be used as soil amendments. The standard characterisation tests that these research bodies require to be performed on biochar samples include total elemental and nutrient contents, thermal gravimetric analysis, heavy metals and organic compound determination, specific surface area, and other basic chemical analytical methods. The C content of biochar must be greater than 50% of the dry mass, according to the IBI and EBC test methods/guidelines. Pyrolyzed organic matter with less than 50% carbon content is known as pyrogenic carbonaceous material. The molar H/C and molar O/C ratios should be less than 0.7 and 0.4, respectively. The H/C and O/C ratios show the degree of carbonisation and biochar stability in the soil. Non-pyrolytic chars or pyrolysis deficiencies indicate a H/C ratio greater than 0.7 (Břendová et al., 2017, Pariyar et al., 2020).

In addition, the pH, bulk density, water content, and specific surface area of biochar, as well as the N, P, Mg, Ca content, must be provided. Biochar's specific surface area serves as both a quality indicator and a pyrolysis method control value. Due to differences in feedstock characteristics and pyrolysis temperature, biochar properties and performance in the soil vary. The objective of this chapter was to compare the physical and chemical properties of biochar made from acacia and poultry litter feedstocks that had been pyrolyzed at a temperature of 550-650 ºC. Biochar properties and their potential use as a soil amendment were evaluated using the IBI or EBC test method/guidelines.

## 3.2 Materials and methods

### 3.2.1 Biochar used in the study

In this study, two locally produced biochars derived from poultry litter and *acacia mearnsii* feedstocks were used. The biochars were made at the same temperature of 550ºC, but with different pyrolysis systems and conditions. Lanstar Company (Pty) (Ltd) provided the acacia



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biochar, which was produced through slow pyrolysis. Poultry litter biochar was created in a kiln furnace at the University of Venda's, School of Agriculture, experimental farm.

### 3.2.2 Production of poultry litter biochar (PLB)

Fresh poultry litter was collected at the broiler production houses of the school of Agriculture experimental farm, University of Venda. The poultry litter consisted of broiler chicken manure mixed with hardwood shaving (sawdust) that was used as bedding in the poultry houses. The broilers are fed daily with starter, grower and finisher from meadow feeds which contained protein, moisture, fat, fibre, calcium, phosphorus and lysine. Prior to pyrolysis, the poultry litter was air dried under sunlight until completely dry. The kiln measuring 1.4 m (diameter) and 1.3 m (height) was manufactured by Vuthisa Company (Ltd) (Pty) and was placed in an open space. The components of the kiln were tripod and two drums all measuring 1.2 m (height) and 0.5m (diameter). The kiln had a lid with a shimmy opening for degassing. The poultry litter feedstock was placed in a tripod, which was then closed and transferred upside down at the center of the kiln furnace in between two drums filled with grass material and placed upside down to generate heat and combustion for the tripod containing the poultry litter feedstock. Firewood was placed inside the kiln and ignited. The kiln was left to burn for 15 minutes before being closed all around with soil leaving three holes open to see if the tripod had conducted heat and gassed out. The kiln was left to burn for 60 minutes under low oxygen conditions. The temperature was measured by placing a thermometer at the bottom of the kiln. To avoid spontaneous combustion, the biochar was removed from the kiln after 24 hours of reaction time.

### 3.2.3 Biochar preparation prior to analysis

The PLB and ACB were kept in polyethylene bags that were tightly sealed. The biochars were air-dried before being crushed with a mortar and pestle to reduce particle size and sieved to achieve a particle size of 2.00 mm for further analysis.

## 3.3 Data collection

### 3.3.1 Physical and chemical characterization of PLB and ACB

Proximate and elemental analyses as well as pH and EC were determined in accordance with the International Biochar Initiative Test method (IBI 2015) and European Biochar Certificate (EBC 2015).



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- **Proximate analysis and physical properties**

Proximate analysis, such as fixed C, moisture content, ash content, and volatile matter, were performed in accordance with the modified American Society for Testing and Materials (ASTM D1762-84 (2007) and (ASTM D 3172-13 (2013) as described by (Aller et al., 2017). The thermogravimetric analysis of biochar samples was used to assess their devolatilization characteristics. In summary, the moisture content was calculated as a weight loss after drying samples at 105°C. At 900°C, volatile matter was measured, and ash was measured as the residue after burning to constant weight at 750°C. After that, the fixed carbon content was calculated by subtracting the dry mass and volatile ash content. The specific surface area was determined using the $N_2$ gas on a Micromeritics ASAP2010 (Accelerated Surface Area and Porosimetry) system according to the Brunauer-Emmet-Teller (BET) specific surface area (Micromeritics Instrument Corp., USA). The biochar samples were degassed on the VacPrep 061 for 60 minutes at 90°C, followed by 16 hours at 300°C. They were then kept at 200°C on the VacPrep until they were analysed. The particle size distribution was determined using the (ASTM D 2862-82 (2016) method, which involved sieving the samples through 1.4 mm-0.6 mm sieves in a sieve shaker for 30 minutes.

- **Elemental analysis**

Elemental analysis of C, N, H and S was determined using an elemental analyser via dry combustion at 102 °C of air dried biochars using the ASTM, D1762-84 (2007) and ASTM, D 3172-13 (2013) as described by (Aller et al., 2017)**.** The oxygen (O %) content was calculated by subtracting the C, N, H, ash, and moisture contents from the total sample mass (EBC 2015). O % = 100 − (ash content, C + H+ N +S).

- **Chemical analysis**

Approximately 300 mg biochar sample was weighed and digested with 10 mL $HCl/HNO_3$ and 40 mL deionised water and heated using a microwave. The solution was analysed for P, Ca, Mg, K, Na, S, Fe, Mn, Cu, Zn, Co, B, Mo, Na, Al content through an inductively coupled plasma mass spectrometer as described by (Enders et al., 2012). The pH and EC were performed in 1: 20 w/v ratio using deionized water mixed for 90 minutes according to the method described by (Rajkovich et al., 2012). The concentration of mineral N ($NH_4^+$-N, and $NO_3^-$-N) was determined using a 2 M KCl extraction and the colorimetric method by (Bremmer and Mulvaney,1965).



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## 3.4 Results and discussion

### 3.4.1 Proximate characteristics of PLB and ACB

Biochar stability in terms of C recalcitrance is estimated using proximate analysis of moisture, volatile matter (VM), fixed carbon (FC), and ash content. The results showed higher fixed C content (73 %), ash content (16-17 %), volatile matter (3 %) and of the pyrolyzed PLB and ACB at 500 $^{o}$C. Both PLB and ACB had a fixed C content greater than 70%, with lower VM content. The labile and recalcitrant biochar fractions are determined using VM and FC (Enders et al., 2012). The high fixed C of both PLB and ACB resulted in lower VM release, indicating that the feedstocks were properly pyrolyzed at 550$^{o}$C. In addition, PLB and ACB had high total C content above 50%. The results showed that both PLB and ACB are stable and have a good potential for C sequestration, as outlined in the IBI (2015) and EBC (2015) guidelines. The fixed C of the PLB and ACB is higher than that reported in literature for biochar derived from poultry litter (Chan et al., 2008b, Tian et al., 2016a) and acacia feedstock (Deng et al., 2017). In contrast, the VM values of PLB and ACB reported in this study are lower and contradict with previous findings (Gonzaga et al., 2018). Most plant-based biochars contain elevated C content (around 70%) as compared to manure derived biochars (around 45%), however, the amount of fixed C will vary according to the temperature during pyrolysis (Ippolito et al., 2016). This implies that pyrolysis of PL and AC feedstocks at 550$^{o}$C produced more stable carbon due to the aromatic rings formed in the biochar structure in this study. The carbon structure transforms from amorphous to aromatic ring structures which are very stable (Tag et al., 2016). The slight differences in VM between ACB and PLB can be a result of the degree of breakdown of cellulose, hemicellulose and lignin in the acacia feedstock (Enders et al., 2012).

The ash content of PLB was slightly higher than that of ACB. (Enders et al., 2012, Ippolito et al., 2016) stated that manure derived biochars have relatively high concentration of inorganic elements and metals, and they tend to have higher ash contents compared to woody derived biochars. Pyrolysis of poultry litter feedstock at 550 $^{o}$C resulted in higher Ca, K and Na which ultimately contributed to higher ash content of the biochar. Meanwhile, pyrolysis of acacia feedstock at the same temperature resulted in volatilisation of Mg, K, S and P which probably resulted in lower ash content. Similarly, the ash content of the PLB in this study is lower than that reported by (Conz et al., 2017, Tian et al., 2016a)**,** who found that the ash content of PLB produced at 550$^{o}$C was greater than 50%. Although ash content values for ACB are scarce in the literature, biochar derived from woody feedstocks, produced at 550$^{o}$C had a low ash content of less than 5% (Butnan et al., 2015, Deng et al., 2017, Gonzaga et al., 2018).



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### 3.4.2 Elemental composition of PLB and ACB

The PLB contained approximately 3 times more N than the ACB, reducing its C/N ratio. However, the N content values for PLB in this study was similar with results by (Brantley et al., 2016, Yu et al., 2018) but lower than that reported in the literature (Conz et al., 2017, Rajkovich et al., 2012). The mass C/N ratio of biochars ranged from 18 to 55, with the ACB having a mass C/N ratio greater than 30 above the threshold level (EBC 2015). Generally, organic materials with a high C/N ratio above 20 results in a significant impact on net N immobilisation by microbes (Singh et al., 2010). The high C/N ratio of ACB indicates that the biochar has high potential to immobilize N and is likely to induce N deficiency after its application. Because ACB has a low N content but a high mass C/N ratio, it should be supplemented with N fertilizer or organic materials with a high N content when applied to soil.

Functional groups of biochar such as H/C and O/C ratios are important for biological degradability. The H/C and O/C ratios of the PLB and ACB were almost similar, ranging from 0.2-0.3% and 0.1%, respectively. In the literature, there are no ideal H/C or O/C ratios for biochars. However, to distinguish biochar from other thermochemically processed biomass that is not biochar, the IBI (2015) test method recommends that the H/C ratio of biochar be less than 0.7. The EBC (2015) test method, on the other hand, recommends a maximum H/C ratio threshold of 0.7 and a O/C ratio threshold of 0.4%. The low H/C (0.3%) and O/C (0.1%) atomic ratios indicated more C aromaticity, which is linked to biochar's long-term stability in soil. This means that biochar with a low H/C ratio is highly carbonised, and has a high C content, resulting in stable, condensed aromatic structures (Tag et al., 2016). Biochars have leftover structures or combustion residues with O/C ratios of less than 0.6%, which is proportional to the number and composition of substituted functional groups. In this study, PLB and ACB had a O/C ratios of 0.1%, implying that both biochars would persist for the longest period when applied to soils. Biochars with O/C ratio less than 0.2% have been shown to ensure a minimum half-life of 1000 years (Uras et al., 2012), suggesting the possibility of long-term carbon sequestration (Lehmann and Joseph, 2015). The elementary analytic results are comparable to those reported on poultry litter biochar by (Enders et al., 2012) and on woodchips biochars (Gonzaga et al., 2018).

### 3.4.3 Physical attributes of PLB and ACB

The BET surface area of PLB was significantly lower (6.20 $m^2/g$) than that of ACB (70 $m^2/g$). Biochar's surface area is known to improve soil aeration, water holding capacity and nutrient retention (Tag et al., 2016). The development of porosity during pyrolysis is influenced by the residence time and gas-flow rate, in addition to the feedstock and pyrolysis temperature. These two factors work together to determine how biogas forms during pyrolysis, which



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influences biochar surface area development. The surface area of PLB produced at 550ºC was much lower than expected. The surface area of biochar made from poultry litter under similar pyrolysis temperature had a surface area above 200 $m^2/g$ (Cely et al., 2015). The lower surface area could be due to inorganic compounds present in high amounts in the ash blocking micropores and preventing adsorbate $N^2$ gas access, resulting in relatively low surface area production (Song and Guo, 2012).

Pyrolysis conditions (temperature, pressure and heating rate) also affected the properties and reactivity of biomass chars especially the nature and content of inorganics(Carrier et al., 2012). The low BET surface areas obtained in the present study may be attributed to the presence of alkali and alkali earth metals (AAEM) mainly K, Na, Mg and Ca in the feedstock, which can decrease the surface area by blocking pores (Song and Guo, 2012). The ACB biochars produced in this study have sufficiently large specific surface areas on which reactions can take place, and a high density of chemical reaction sites where nutrients can be adsorbed. The reported BET surface area value of PLB is significantly higher than that reported by (Tag et al., 2016),and significantly lower than that reported by (Novak et al., 2009). The BET surface area value of ACB is lower than that reported by (H El-Gamal et al., 2017) but higher than that reported by (Bera et al., 2016).

In comparison to the ACB, PLB had a higher total particle size distribution than BET-SSA. The PLB had 64 % more fine particles that passed through the smallest sieve of 0.6 mm than the ACB, which had 34 % fine particles that passed through the same sieve. This suggests that PLB, rather than ACB, will play a larger role in providing habitat for microorganisms when applied to soil. Chemical sequestration is aided by both SSA and micropores, which alter chemical bioavailability and affect soil organisms (Enders et al., 2012, Uras et al., 2012). After pyrolysis, the PLB and ACB had low moisture content of less than 10%, indicating that both biochars are commercially viable, as biochar moisture levels are typically between 3–10 % (Carrier et al., 2012).

### 3.4.4 Chemical composition of PLB and ACB

The pH values of PLB and ACB were 10.3 and 9.2, respectively, indicating that they were alkaline. The high pH of the PLB in comparison to the ACB was attributed to the PLB's high alkaline metals of Ca, Mg, K and Na content, as well as the high ash content. Furthermore, the biochars were pyrolyzed at 550°C, indicating that the temperature increase was most likely a contributing factor in the biochar's pH increase, probably due to carbonate formation and the release of alkali salts from the feedstock during pyrolysis (Gonzaga et al., 2018). Moreover, (Enders et al., 2012) indicated that high pH values in biochars are attributed to the increased pyrolysis temperature of 400-600$^0$C, primarily due to the separation of alkali salts from organic



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materials. The pH range of the PLB and ACB in this study is comparable to previous findings (Enders et al., 2012, Tag et al., 2016) but significantly higher than other findings (Akça and Namli, 2015). This suggests that PLB and ACB could be used as a liming material and as a long-term solution for heavy metal stabilization in contaminated soils.

The PLB biochar had higher levels of major nutrients Ca, Mg, K, P, S and micro-nutrients Zn, Fe, Mn, and Cu as well as non-essential Na than the ACB. The high constituents of these elements in the poultry litter could have caused partial volatilization of these elements during pyrolysis, resulting in an increase in available nutrients in the PLB. In addition, the high levels of these elements in the PLB were most likely due to the poultry litter's feed supplements, and these elements were most likely secreted in large amounts in the excretion. Poultry litter as a source of nutrients is based on its high concentration of micro and macronutrients. Woody derived biochars, on the other hand, contain low amounts of macro and micro-nutrients due to low absorption of these elements from the soil during plant growth (Conz et al., 2017, Enders et al., 2012, Novak et al., 2009). The low availability of major nutrients in ACB make it less appealing as a soil amendment.

The high levels of K, S, and Mg in the PLB are consistent with the results of Gaskin et al. (2008)**,** who reported much higher K and Mg in poultry litter biochar than in the biochars made from peanut hull and pine chips. The high Ca and K content of PLB would be a problem if it is added in soils with high Ca content, as excess Ca could limit the availability of Mg and K, while excess K could bind P. This suggests that PLB application rates greater than 10 t/ha may be unsuitable unless used on very acidic soils with Ca and K deficiency. The Ca and K contents of PLB are comparable to those obtained from poultry litter activation (Singh et al., 2010). In the PLB, the content of K and Na was much higher than the content of Ca and Mg. This is because K and Na carbonates and oxides are much more water soluble than their Ca and Mg counterparts (Singh et al., 2010). The P content of ACB was 48 times lower than that of PLB, while the S content was 5% lower and the N content was 3 times lower. Biochars made from PLB had much higher P, S, and N content than biochars made from woody feedstocks (Singh et al., 2010, Uras et al., 2012). Because most biochars' P, N, and S content tends to volatilize at temperatures above 200 $^{o}$C, P and N addition would be required if ACB were to be used in acid soils deficient in these nutrients.

The high EC value of PLB in this study was due to the high amount of Na content. This means that applying PLB at high rates, such as 20 t/ha, could have a negative impact on plant growth if soluble salt levels are high, resulting in reduced water uptake by plant roots and nutrient imbalances (Carrier et al., 2012, Uras et al., 2012). When PLB with an EC of 5.6 dS/m was applied at 10 t/ha into an acid soil with an EC value of 0.11 5.6 dS/m, the EC value of the soil



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increased by 36 % than the control (Chan et al., 2008b). This suggests that the amount of biochar added to the soil should be cautiously calculated before use to avoid soil salination and nutrient imbalances. A measured EC value of more than 4 dS/m indicates salinity. The biochars' extremely high EC values, especially for manure feedstock biochars, confirm the presence of a large amount of water-soluble salts. Because of the higher relative solubility of K and Na containing salts and carbonates in water, EC values in this study were mostly affected by K and Na content of the biochar. Sodium is not an essential plant nutrient and promotes clay dispersion which has a negative impact on soil structure (Tag et al., 2016). Furthermore, Na phytotoxicity is likely to be harmful in a variety of soils and crops (Enders et al., 2012). Because ACB has low availability of soluble salts, as indicated by the low EC, application of ACB should not have a significant negative impact on soil salinity.

Fe, Cu, Mn, Zn, and Al concentrations in the PLB and Fe, Mn, and Al concentrations in the ACB were both above the toxicity level for soils. This suggests that high application rates of PLB and ACB could result in toxic levels of these trace elements, which could affect plant growth. Biochar can directly adsorb cations like $Al^{3+}$, $Fe^{3+}$, and $Ca^{2+}$, causing soil P adsorption or precipitation to be delayed especially in low buffered sandy textured soils (Singh et al., 2010). In addition, traces of iron in the biochar could be from the kiln that was used to make the biochar, but this is a presumption that needs to be confirmed. Since poultry litter contains Cu, Fe, Mn, and Zn supplements, which are added to ensure weight gain and reduce health problems in poultry. Unused trace metal supplements end up in poultry manure, and different feed formulations affect metal levels in poultry litter. Thus, when applying PLB and ACB biochar at different rates, caution should be taken to ensure that the trace elements do not reach critical toxicity levels in the soil.

The macro and micro-elements values for PLB are comparable to those reported by (Brantley et al., 2016, Enders et al., 2012, Rajkovich et al., 2012, Yu et al., 2018), while the values for ACB are lower than those reported by (Agegnehu et al., 2016, Kätterer et al., 2019). Overall, the results of this study showed that PLB and ACB had similar elemental composition and physicochemical characteristics to most biochars made from plant and poultry litter feedstocks, indicating that they could be used as soil amendments. However, because it contains relatively low levels of soluble nutrients and lacks the most important N and P nutrients, ACB biochar cannot be considered a balanced nutrient enhancer, therefore, when used as a soil amendment, it should be used in conjunction with materials with more N and P.



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### 3.5 Conclusion

PLB and ACB qualify as '*biochar*' according to the IBI and EBC test methods/guidelines and can be used as soil amendment. This is because when pyrolyzed at 550-650 °C, both biochars had fixed C and total C greater than 50% and a lower H/C ratio of 0.3, indicating the biochars' potential for soil stability. With a pH range of 9.2-10.3, both biochars were naturally alkaline, indicating liming potential. In comparison to ACB, PLB had higher available nutrient composition, making it a highly recommended biochar for use as a soil amendment to improve soil quality and crop production. When using poultry litter as a biochar feedstock, however, extreme caution should be taken because the biochar contains higher levels of micro-elements (Fe, Al, and Mn), which have been linked to the feed supplements. The high BET surface area of ACB, on the other hand, makes it an excellent biochar for C sequestration and water adsorption; however, the biochar should be applied months before planting to allow mineralisation and immobilisation of N to pass or be supplemented with high organic N materials to avoid soil N deficiency.



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**Table 3.1**: Proximate, elemental, and physicochemical analysis of poultry litter biochar (PLB) and acacia biochar (ACB) biochar according to the standard test methods/guidelines of the IBI, 2015 and EBC, 2015.

| | PLB | ACB |
|---|---|---|
| **Proximate analysis (%)** | | |
| Fixed C | 73,2 | 73 |
| Ash | 17,2 | 16,3 |
| Volatile matter | 3,2 | 3,9 |
| Moisture | 6,4 | 6,8 |
| Total | 100 | 100 |
| Total C | 56 | 56,7 |
| **Elemental analysis (%)** | | |
| N | 3,11 | 1,03 |
| H | 13,6 | 17,2 |
| S | 5 | 1 |
| O | 5,09 | 7,77 |
| C/N ratio | 18,0 | 55,0 |
| H/C | 0,2 | 0,3 |
| O/C | 0,1 | 0,1 |
| **Physical properties (%)** | | |
| > 1,4 mm | 17 | 48 |
| 1,4 -1,18 mm | 4 | 5 |
| 1,18 -0,85 mm | 4 | 4 |
| 0,85-0,6 mm | 8 | 5 |
| < 0,6 mm | 64 | 34 |
| Total PSD[a] | 80 | 48 |
| BET-SSA[b] $N_2$ gas ($m^2/g$) | 6,2 | 70 |
| BET-SSA $CO_2$ gas ($m^2/g$) | 115,1 | 908 |
| **Chemical properties (g/kg)** | | |
| Macronutrients | | |
| pH | 10,3 | 9,2 |
| EC | 4,95 | 0,49 |
| Ca | 32 | 18,4 |
| Mg | 16,2 | 2,2 |
| K | 40,1 | 5,1 |
| P | 24 | 0,5 |
| S | 5,1 | 1 |
| Na | 10,3 | 0,3 |
| Micronutrients | | |
| Fe | 4,29 | 2,04 |
| Mn | 1,16 | 1,59 |
| Cu | 1,47 | 0,43 |
| Zn | 1,05 | 0,02 |
| B | 0,06 | 0,02 |
| Mo | 0,01 | 0,01 |
| Al | 2,99 | 3,09 |

[a]PSD- particle size distribution; SSA[b]- specific surface area; IBI- International Biochar Initiative; EBC- European Biochar Certificate.



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# CHAPTER 4

## THE POTENTIAL OF BIOCHAR TO ENHANCE CONCENTRATION AND UTILIZATION OF SELECTED MACRO-AND MICRO-NUTRIENTS FOR CHICKPEA (*Cicer arietinum L.*) GROWN IN THREE CONTRASTING SOILS

### Abstract

The balance in nutrient availability and retention after addition of biochar depends on the feedstock and application rates of the biochar in different soils. A pot experiment was conducted to assess the potential effect of biochar derived from poultry litter (PLB) and acacia (ACB) feedstocks on selected rhizospheric macro and micro-nutrients, shoot and root biomass, as well as nutrient uptake by chickpea grown in three different soil types. Treatments consisted of two biochar (PLB and ACB), four application rates of 0 (control), 0.5, 1 and 2% w/w, and three soil types [Fernwood (Arenosol); Pinedene (Gleyic Acrisol); Griffin (Helvic Acrisol)]. PLB application at 2% increased rhizospheric pH, CEC, and P, K, Ca, Mg, S, Zn concentrations in Pinedene and Griffin soils. Chickpea shoot and root biomass in the Pinedene and Griffin soils were highest at 0.5 and 1% PLB application and was attributed to increase in soil N and N uptake. Application of PLB and ACB at 1 and 2% showed a significant change in rhizospheric pH and available P in Fernwood soil resulting in a reduction of Fe concentration. The higher C/N ratio of the ACB probably resulted in immobilisation of nutrients as evident from the low response in shoot and root biomass as well as shoot N uptake of chickpea in all soil types. The study confirmed that the effect of biochar on the availability of soil nutrients and plant growth is associated with the nature of the biochar feedstock, the rate of application and the soil characteristics.

**Keywords**: biochar; chickpea; macronutrients; micronutrients, poultry litter, rhizosphere



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## 4.1 Introduction

Biochar is a carbon-rich material obtained from pyrolysis of organic materials under oxygen-limited conditions. The interest in biochar as a soil amendment has been on its ability to improve and maintain soil fertility and to increase the sequestration of soil carbon. This is because biochar is relatively stable, and therefore biochar's resistant nature to decomposition plays a major role in limiting climate change (Agegnehu et al., 2017, Lehmann and Joseph, 2015). Furthermore, biochar is an alternative and relatively safer method for managing organic waste with additional advantages such as extracting usable energy from the gas and liquid fractions during pyrolysis (Cely et al., 2015). Despite the positive attributes, the application of biochar may also have adverse effects on the environment. Such adverse effects may involve N binding and immobilisation, release of some toxic heavy metals associate with biochar, increase in EC and pH causing nutrient deficiencies (Kookana et al., 2011, Singh et al., 2010, Xu et al., 2013), as well as the existence of plant germination or plant growth-related phototoxic compounds (Rogovska et al., 2012). This means that some of the unintended effects such as nutrient absorption, increased leaching of certain nutrients, and increased EC need to be considered prior to biochar application to soil (Kookana et al., 2011).

Biochars may be produced from a variety of organic materials and under different pyrolysis conditions, resulting in products of varying properties. For example, biochar derived from plant-based materials such as woody biomass or grasses have low nutrients because of their low ash content, N, P, K, but are regarded as carbon fixers because of their high recalcitrant carbon content (Agegnehu et al., 2017, Tag et al., 2016). In addition, plant-based biochars are limited to providing adequate nutrient content in the soil to meet crop nutrient requirements as compared to organic or synthetic fertilizers (Cely et al., 2015). To date, several studies have focused on the impact of biochar derived from plant materials in other countries such as China, Germany and Australia to improve soil quality (Agegnehu et al., 2017, Macdonald et al., 2014, Zhao et al., 2014). In South Africa, invasive tree species such as *Eucalyptus*, and *Acacia mearnsii* (Lusiba et al., 2017, Lusiba et al., 2018) and organic materials such as sugarcane bagasse and vineyard pruning (Sika and Hardie, 2014, Uras et al., 2012) were characterized and evaluated as potential biochar feedstock types for improving physical and chemical soil properties and crop growth in different soils. The studies indicated that biochar derived from various plant species has the potential to improve soil fertility; however, the type of feedstock and pyrolysis conditions have a greater influence on the benefit of biochar in soils. On the other hand, biochar produced from animal



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sources has a higher nutrient content but minimal studies have been conducted to evaluate its agronomic value and potential modification of soil properties. Poultry litter refers to the poultry manure and bedding material mixed together. The poultry litter is commonly used as a source of plant nutrients by smallholder farmers. There are, however, food safety and environmental concerns regarding its application in unmodified forms to agricultural land (Chan et al., 2008a). Environmental problems associated with improper application of poultry litter have focused on contamination of soil and/or surface water with N and P as well as release of toxic trace elements such as arsenic (As), copper (Cu), and zinc (Zn) (Wilkinson et al., 2003).

In addition, manures have the potential to lead to release of ammonia and methane which may intensify global warming and severe groundwater pollution and stream nutrient pollution (Ding et al., 2016). Using pyrolysis, converting poultry litter to biochar could be a safer and more effective alternative to use this resource in agriculture. Studies in Europe indicate that poultry-derived biochar acts as a nutrient releaser based on its high nutrient content, notably N and P (Chan et al., 2008b, Tag et al., 2016), enhance high soil nutrient retention due to its high CEC and liming potential (Rajkovich et al., 2012). In addition, biochar derived from poultry litter could reduce soil N leaching(Ulyett et al., 2014) compared to the use of organic materials, while P and K content from the feedstock material could be fully recovered during pyrolysis (Cely et al., 2015).

However, a loss of at least 50% N during pyrolysis of manure feedstock can be expected. Biochar application in agriculture in South Africa and elsewhere in the world is limited although there is substantial evidence that biochar can improve soil quality; increase crop yields; and mitigate climate change (Ding et al., 2016, Gul et al., 2015). However, the reported response varies according to the type of feedstock, the rate of application and the type of soil used. There are still uncertainties regarding the impact of poultry-derived biochar on nutrient absorption and use in various South African soil types. Therefore, the efficacy of biochar derived from poultry litter and acacia feedstock on nutrient availability and use in different South African soils needs to be better understood. In addition, biochar production from poultry litter and acacia, in particular the species *Acacia mearnsii,* would be beneficial in terms of waste and environmental management in South Africa.

For example, most South African poultry farms face challenges of unsafe and unplanned disposal of poultry manure, which has resulted in unwanted pollution of the environment (Ravindran et al., 2017). On the other hand, the species Acacia mearnsii has invaded millions of hectares of land in South Africa, altering the chemical and biological properties of soils resulting in a decline in native vegetation population (Souza-Alonso et al., 2017). Due to the economic and environmental



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benefits of biochar, the use of poultry litter and acacia (*Acacia mearnsii*) materials in biochar production could be a promising resource for soil fertility management in South Africa. The main objective of this study was to evaluate the potential of poultry litter and acacia-derived biochar to improve rhizospheric nutrient concentration, and nutrient utilization by chickpea grown in three South Africa soils differing in texture using a pot experiment.

## 4.2 Materials and methods

### 4.2.1 Soil classification and sampling

The three soils with contrasting texture and mineralogy were randomly collected at a depth of 0-30 cm from different smallholder farms located in Dopeni village about 38.9 km away from Thohoyandou. Prior to soil sample collection, soil profiles were excavated in the farmer's field. The soils were classified as Fernwood (Orthic A with E horizon), Pinedene (Orthic A, Apedal B, Neocutanic B), Griffin (Orthic A, Yellow Brown Apedal B, and Red Apedal B) according to (Fey, 2010) and as Arenosol, Gleyic Acrisol, and Helvic Acrisol, respectively, according to the world reference base (FAO-WRB, 2014). The Fernwood soil was collected at 22°54'51.6"S 30°12'40.4"E, while the Pinedene soils was collected at 22°54'51.6"S 30°12'40.4"E and the Griffin soil was collected at 22°54'39.4"S 30°12'32.6"E. The cropping history of these three soils includes continuous cropping with limited application of animal manure (poultry and cow dung), and NPK fertilizer 3:2:1 which is applied once per annum before planting maize. The main cropping practice is intercropping of maize with groundnuts (Fernwood and Pinedene soils), maize with sweet potato and butternut squash (Griffin soil).

### 4.2.2 Soil analysis

Soil samples collected from the field were air-dried at room temperature, crushed and sieved through a 2-mm sieve for analysis of soil texture using the hydrometer method (Bouyoucos, 1962) and chemical properties such as pH, CEC, P, total N, total C, Ca, K, S, Zn and Fe (Table 4.1). Soil pH was determined in 1:2.5 ratio of soil: water (w/v) as outlined in (Staff, 1996). Exchangeable cations ($Ca^{2+}$ $Mg^{2+}$ & $K^{+}$) and CEC were determined using the ammonium acetate extraction procedure (Peech, 1965). Available P was extracted using the Bray 1 method (Bray and Kurtz, 1945). Total N was determined using the Kjeldahl method, while ammonium nitrogen ($NH_4^{+}$-N) and nitrate nitrogen ($NO_3^{-}$-N) in the soil solution were measured using the colorimetric method (Mulvaney and Page, 1982, Bremmer and Mulvaney, 1965). Organic carbon was determined using the Walkley and Black method (Nelson and Sommers, 1983). Concentration of Zn and Fe



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content was measured by atomic absorption spectrophotometer following the Mehlich 3 extraction method(Mehlich, 1984).

### 4.2.3 Experimental setup and design

Between May and August 2017, a pot experiment was conducted in a naturally-ventilated tunnel house located at 22º58.08´S and 30º26.40´E and 595 m above sea level at the University of Venda, School of Agriculture, experimental farm, Thohoyandou, South Africa. The experiment consisted of three soil types, namely Fernwood, Pinedene and Griffin; two biochar types, poultry litter (PLB) and acacia (ACB); and four application rates, 0 (control), 0.5, 1 and 2%, (w/w) equivalent to 10, 20 and 40 t/ha, respectively. Information on how the biochars were produced and characterized, see chapter 1. The treatments were arranged in a completely randomised design and replicated four times. Pots measuring 25 cm in diameter and 25 cm in height were filled with 4 kg of air-dried sieved soil. The poultry and acacia biochar were each applied according to the specified rates of application and mixed thoroughly with the soil. Starter superphosphate fertilizer (10.5 %) was applied consistently at approximately 2 g P/pot (equivalent to 60 kg P/ha) in all pots. The pots had been watered to a field capacity of 60% before planting. Chickpea was used as a test crop. Approximately 450 g of desi chickpea cultivar seeds were inoculated with bradyrhizobium spp cicer containing 5 x $10^8$ bacterial cells per gram, soaked in a mixture of 2,25 g of bradyrhizobium powder and immediately planted. Each pot had four seeds planted and the plants were thinned out to two plants per pot after 10 days of emergence (DAE). The pots (pot + soil mixture + water) were weighed and watered every three days when necessary to target a field capacity of 60%.

## 4.3 Data collection

### 4.3.1 Postharvest plant and soil analysis

Plants in each pot were cut to crown level about 65 days after emergence. Each sample of the shoot was put into a sample paper bag. Approximately 20 g of soil from the rhizosphere was shaken, and transferred to sampling bags. Shoot samples were oven-dried to a constant weight at 65°C for 48 hours, and weighed to determine shoot biomass. The samples of the dried chickpea shoot were ground and sieved to pass a 1 mm sieve. Shoot N concentrations was determined using the 1 M HCl digestion method and the inductively couple plasma spectrophotometer. Each rhizospheric soil sample was air-dried at room temperature of 25°C for three consecutive days. The soils were sieved (2.00 mm) and weighed into air tight sample plastic bags for analysis of



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selected nutrients Ca, K, P and S as well as mineral N (extractable $NH_4^+$-N, and $NO_3^-$-N) and total N were determined using the methods for soil analysis as described in subsection 4.2.2.

### 4.3.2 Statistical analysis

The data was analysed using R software (version 3.5.2). Generalised Linear Mixed Model analysis of variance was used to test the treatment effects on rhizospheric soil; pH, CEC, P, Ca, K, Mg, S, Fe and Zn as well as shoot biomass and plant nutrient uptake (Ca, K, Mg, S, Fe and Zn). Soil type, biochar type and biochar application rates were considered as fixed factors while the variables such as pH, CEC, P, Ca, K, Mg, S, Fe and Zn were considered random factors. Tukey's honest significant difference (HSD) test was used for treatment mean separations with the threshold probability level set at $P \leq 0.05$. This was followed by plotting of the significant interactions using Sigmaplot version 14. A principal component analysis (PCA) was done to assess the variability and relationship that exist among the shoot biomass, soil macro-micro nutrients and nutrient uptake with the soil types, biochar feedstock type and application rates.

## 4.4 Results

### 4.4.1 Physico-chemical properties of soils and biochar used in the study

The Fernwood soil was a loamy-sand whereas the Pinedene and Griffin soils were sandy-clay-loam and clay-loam with pH values of 4.1, 3.8, and 5.5, respectively (Table 4.1). Acid saturation was higher in the Pinedene soil compared to that of the Fernwood and Griffin soils. Available P and K were higher in the Pinedene soil while Na, Mg, Ca, S and CEC were higher in the Griffin soil. The Pinedene soil had slightly higher concentration of Fe and Zn (Table 4.1). The SOC and TN were higher in Pinedene and Griffin soils compared to the Fernwood soil, hence, the Fernwood exhibited higher C/N ratio than the Pinedene and Griffin soils. Ammonium-N was higher in the Pinedene while nitrate-N was greater in Griffin soil (Table 4.1).



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**Table 4.1.** Soil physicochemical characteristics of three different soil types used in the study.

| Parameters | Units | Chemical Characteristics | | |
|---|---|---|---|---|
| | | **Fernwood soil (Arenosol)** | **Pinedene soil (Gleyic Acrisol)** | **Griffin soil (Helvic Acrisol)** |
| Clay | % | 10 | 22 | 38 |
| Sand | % | 84 | 57 | 44 |
| Silt | % | 6 | 21 | 18 |
| Textural class | - | Loamy sand | Sandy clay loam | Clay loam |
| pH | - | 4.1 | 3.8 | 5.5 |
| Acid Saturation | % | 6 | 14 | 0 |
| Exchangeable acid | cmol $H^+$/kg | 0.16 | 0.75 | 0 |
| P | mg/kg | 2 | 25 | 1 |
| Na | mg/kg | 17 | 38 | 54 |
| K | mg/kg | 15 | 64 | 39 |
| Ca | mg/kg | 258 | 400 | 1079 |
| Mg | mg/kg | 136 | 277 | 394 |
| CEC | cmol/kg | 2.7 | 5.3 | 9 |
| S | mg/kg | 5.31 | 13.08 | 17.11 |
| Fe | mg/kg | 45.09 | 46.07 | 3.01 |
| Zn | mg/kg | 0.56 | 2.16 | 0.26 |
| SOC | % | 1.4 | 2.5 | 1.5 |
| Total N | % | 1.06 | 2.11 | 1.53 |
| C/N ratio | g/g | 12.8 | 12.2 | 10.2 |
| $NH_4^-$-N | mg/kg | 21.23 | 27.53 | 21.43 |
| $NO_3^-$-N | mg/kg | 9.42 | 13.11 | 23.4 |



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### 4.4.2 Biochar effect on rhizospheric soil pH, CEC and macro-nutrient concentration

Application of PLB increased soil pH of the Fernwood rhizospheric soil by 1.7 units (4.1 to 5.8) and the Griffin rhizospheric soil by 1.3 units (5.5 to 6.8) and the Pinedene rhizospheric soil by 1 unit (3.8 to 4.8). On the other hand, the application of ACB increased the soil pH to 5.2 in the Fernwood, 6.6 in the Griffin soil and 4.2 in the Pinedene soil (Fig 4.1a). Application of PLB and ACB at different rates had no significant effect on rhizospheric pH of the Griffin and Pinedene soils; however, rhizospheric pH was higher at 1 and 2 % in the Fernwood soil (Fig 4.1b). Application of PLB significantly increased rhizospheric P compared to the ACB in all soil types. The Griffin soil showed the least rhizospheric P compared to Fernwood and Pinedene soils when ACB was applied (Fig 4.1c). Application of biochar from 0.5% to 2% increased rhizospheric P in the Fernwood soil. The rhizospheric soils of Griffin and Pinedene showed a higher but similar P at 1% and 2%, followed by 0.5%, compared to the control (Fig 4.2d). Application of PLB and ACB increased rhizospheric CEC from 9 cmol/kg to 13 and 11 cmol/kg in the Griffin soil, respectively. Both Fernwood and Pinedene soils showed the least CEC after ACB application (Fig 4.1e). Application of biochar at 2% resulted in higher CEC in the Griffin, while the Fernwood and Pinedene soil, showed similar CEC values at 2 % (Fig 4.1f).

Rhizospheric concentrations of K, Mg and Ca of Griffin soil were higher when PLB and ACB were applied. The Fernwood and Pinedene soil showed the lowest rhizospheric concentrations of K, Mg, and Ca when ACB biochar was added (Fig 4.2a, c & e). The concentration of K, Mg and Ca was 549, 569, 1292 mg/kg in PLB amended Griffin soil and 274, 493, 1281 mg/kg in ACB amended soil. The concentration of K in the Griffin, Fernwood and Pinedene rhizosphere increased with higher levels of biochar application (Fig 4.2b). Application of PLB and ACB at different rates had no significant effect on Mg and Ca in all soil types (Fig 4.2d & f).



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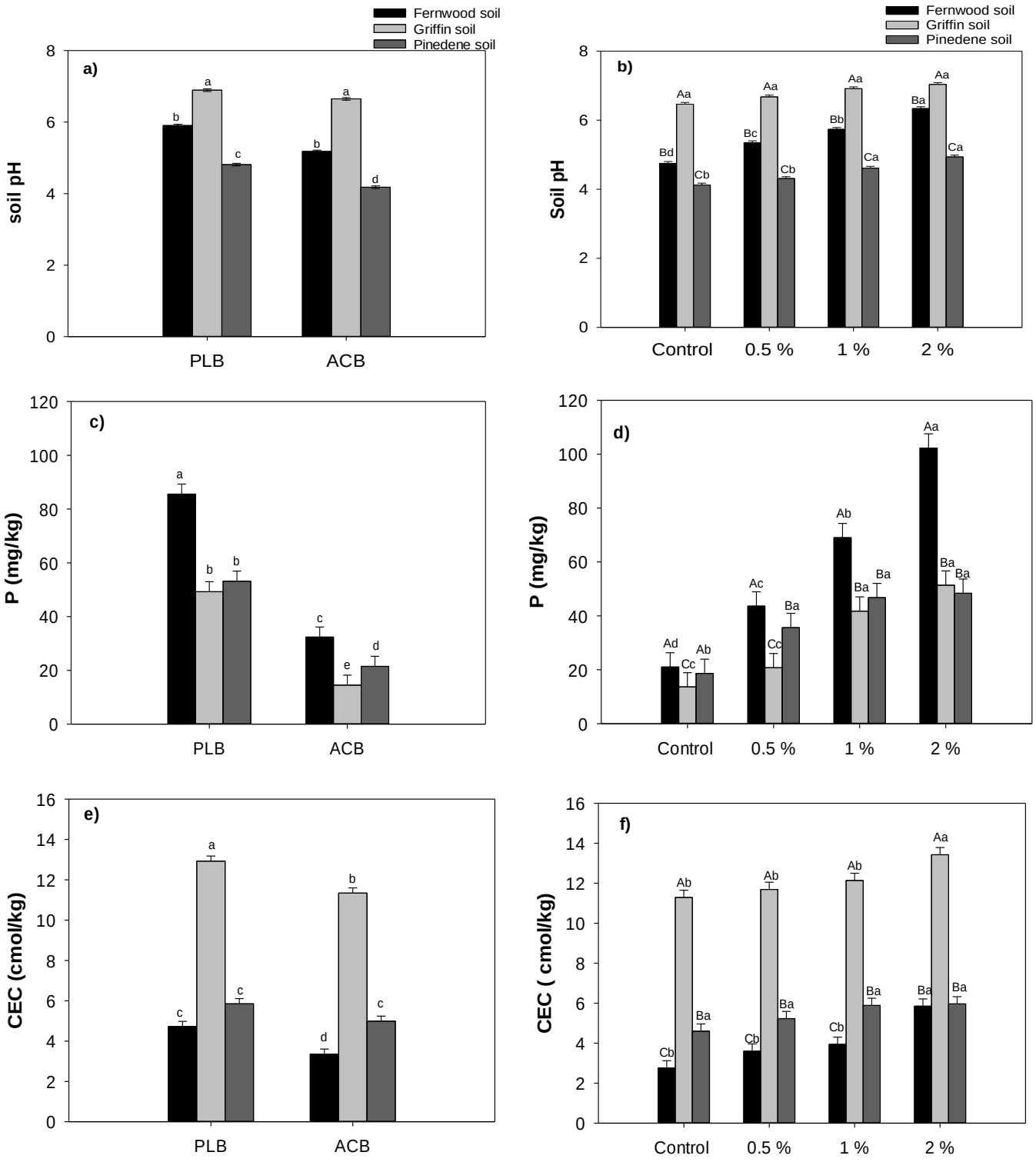


**Figure 4.1**: The interactive effect of soil type x biochar feedstock type and soil type x application rates on soil pH (a-b); P (c-d); CEC (e-f), respectively at significant probability level of $P \leq 0.05$.



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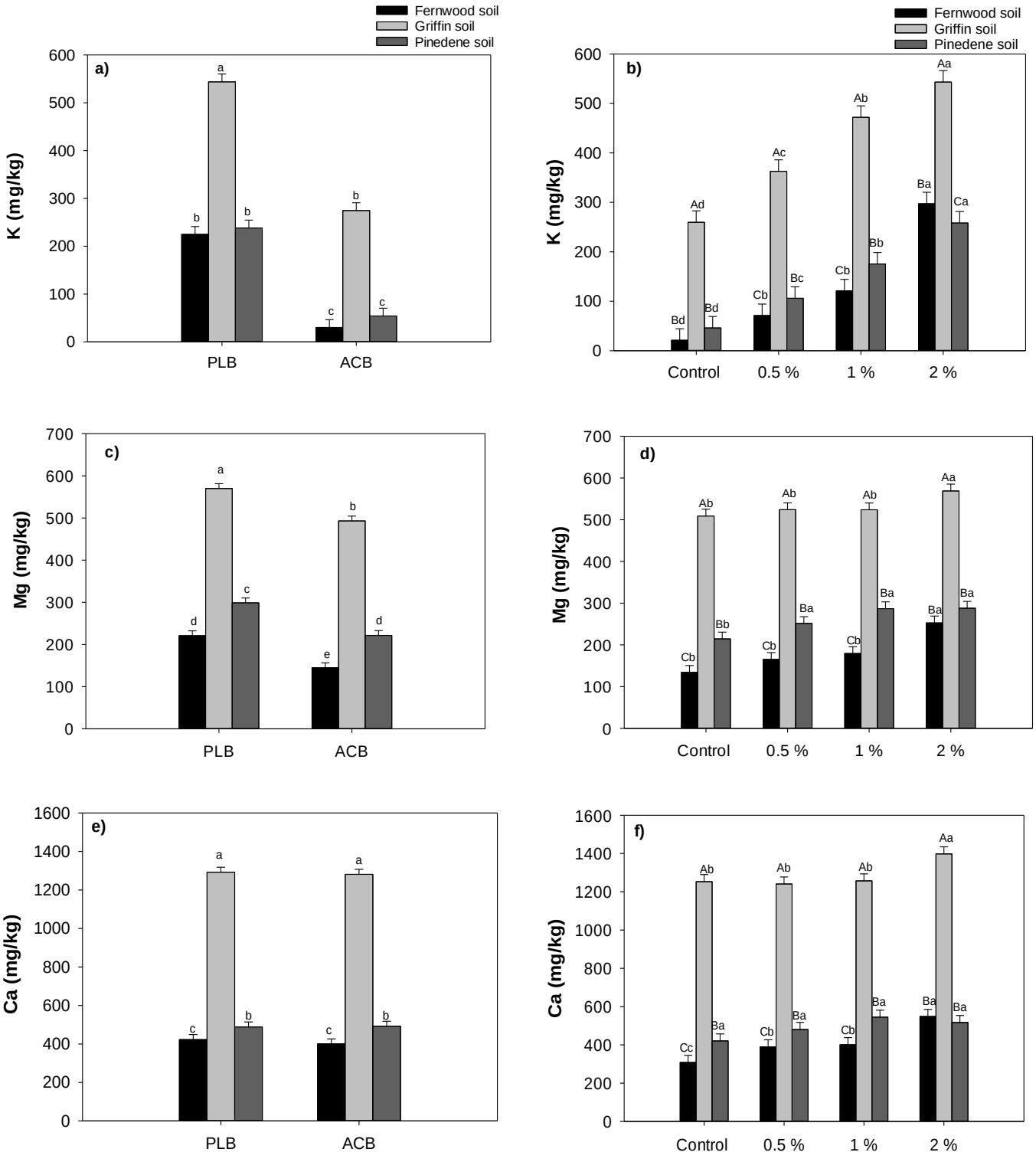


**Figure 4.2**. The interactive effect of soil type x biochar feedstock type and soil type x application rates on K (a-b); Mg (c-d); Ca (e-f), respectively at significant probability level of P ≤ 0.05.



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### 4.4.3 Biochar effect on rhizospheric micro-nutrient concentration

Griffin and Pinedene rhizospheric soils showed a higher concentration of sulfur (S) after application of PLB and ACB, with the lowest concentration in Fernwood soil (Fig 4.3a). Sulfur concentration in the Griffin soil was higher at 1 and 2 % but decreased at the same rates in the Pinedene soil (Fig 4.3b). Applying 2% biochar resulted in a higher concentration of sulfur in the rhizosphere soil of the Fernwood. Concentration of Zn was lower when ACB was applied in all soil types (Fig 4.3c). Increased application rates from 0.5 to 2% resulted in a significant increase in the concentration of rhizospheric Zn in both Fernwood and Pinedene soils. On the other hand, the rhizospheric concentration of Zn was higher at 1 and 2% and lower at 0.5% biochar application in Griffin soil (Fig 4.3d). In contrast, the rhizospheric soil of Griffin showed the lowest concentration of Fe compared to the Fernwood and Pinedene soils (Fig 4.3e). Pinedene soil showed the highest concentration of Fe at 0.5% but lower concentration at 1 % and 2%. Similarly, Fe concentration was lowest at 2% in the Fernwood soil, while the Griffin soil showed no difference in Fe concentration among biochar application rates (Fig 4.3f).



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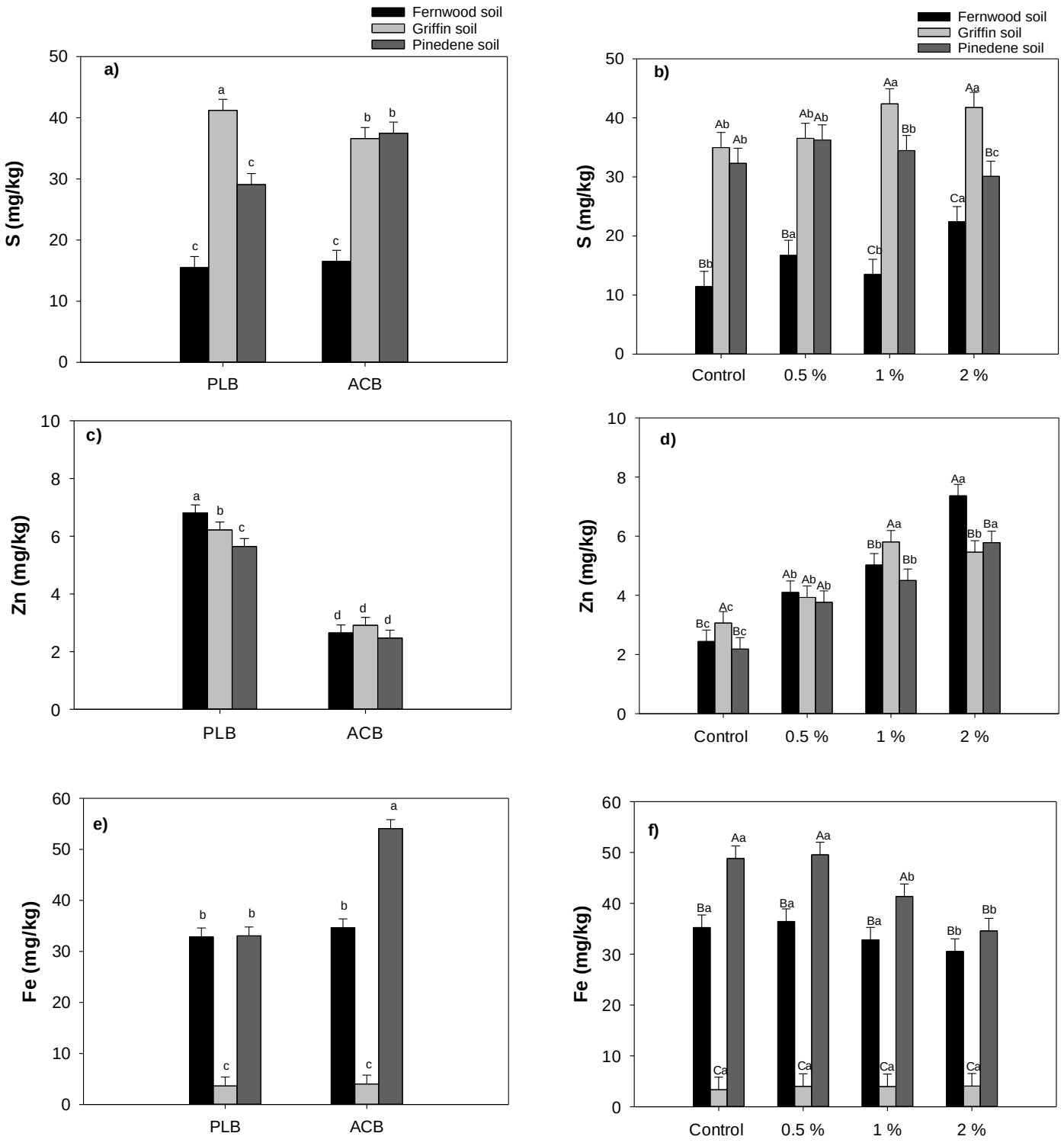


**Figure 4.3**. The interactive effect of soil type x biochar feedstock type and soil type x application rates on S (a-b); Zn (c-d); Fe (e-f), respectively at significant probability level of $P \leq 0.05$.



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### 4.4.4 Biochar effect on total C, total N, C/N ratio and soil N ($NH_4^+$-N and $NO_3^-$-N)

The soil type and biochar feedstock type had no effect on rhizospheric total C, however, application of biochar at 2% increased total C in all soil types (Fig 4.4a &b). Compared to the control, rhizospheric total C increased from 1.9, 1.7 and 1.8 % to 2.7, 2.5 and 2.4 % in the Fernwood, Griffin and Pinedene, respectively (Fig 4.4b). The Fernwood soil showed highest rhizospheric total N compared with the Griffin and Pinedene soils when PLB and ACB were applied (Fig 4.4c). Rhizospheric total N was higher at 2 % in the Fernwood soil, on the contrary different biochar application rates had no effect on total N in both the Griffin and Pinedene soils (Fig 4.4d). The rhizospheric C/N ratio of Griffin and Pinedene soils was similar either with PLB or ACB application at different rates (Fig 4.4 e & f). On the other hand, application of PLB at 2 % the Fernwood soil resulted in a lower C/N ratio (Fig 4.4 e & f).

Application of PLB and ACB resulted in higher rhizospheric $NH_4^+$-N concentration in the Pinedene soil compared to the Griffin and Fernwood soils which showed similar $NH_4^+$-N (Fig 4.5a). Application of biochar at different rates had no effect on rhizospheric $NH_4^+$-N of Fernwood and Griffin soils, however, addition of 1 and 2 % biochar resulted in lower $NH_4^+$-N in the Fernwood soil (Fig 4.5b). Similar to rhizospheric total N, the Fernwood soil had the highest $NO_3^-$-N concentration than the Griffin and Pinedene soils, especially when ACB was applied (Fig 4.5c). Rhizospheric $NO_3^-$-N was higher at 0.5 % in the Fernwood soil and lower at 1 and 2 % biochar application. Both the Griffin and Pinedene soils showed no change in $NO_3^-$-N concentration when either PLB or ACB was applied at different rates (Fig 4.5d).



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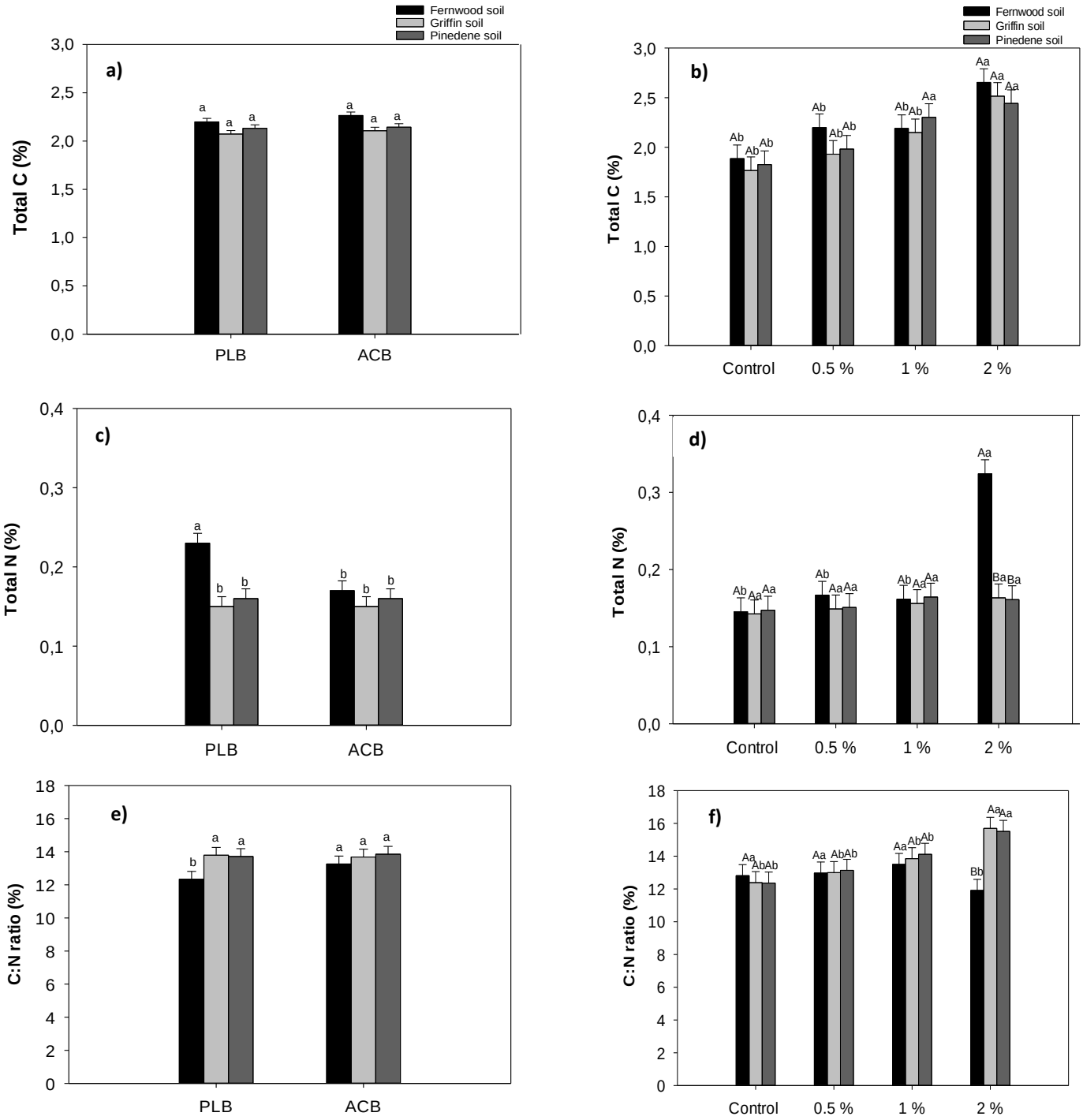


**Figure 4.4**. The interactive effect of soil type x biochar feedstock type and soil type x application rates on Total C(a-b); Total N (c-d); C/N ratio (e-f), respectively at significant probability level of P ≤ 0.05.



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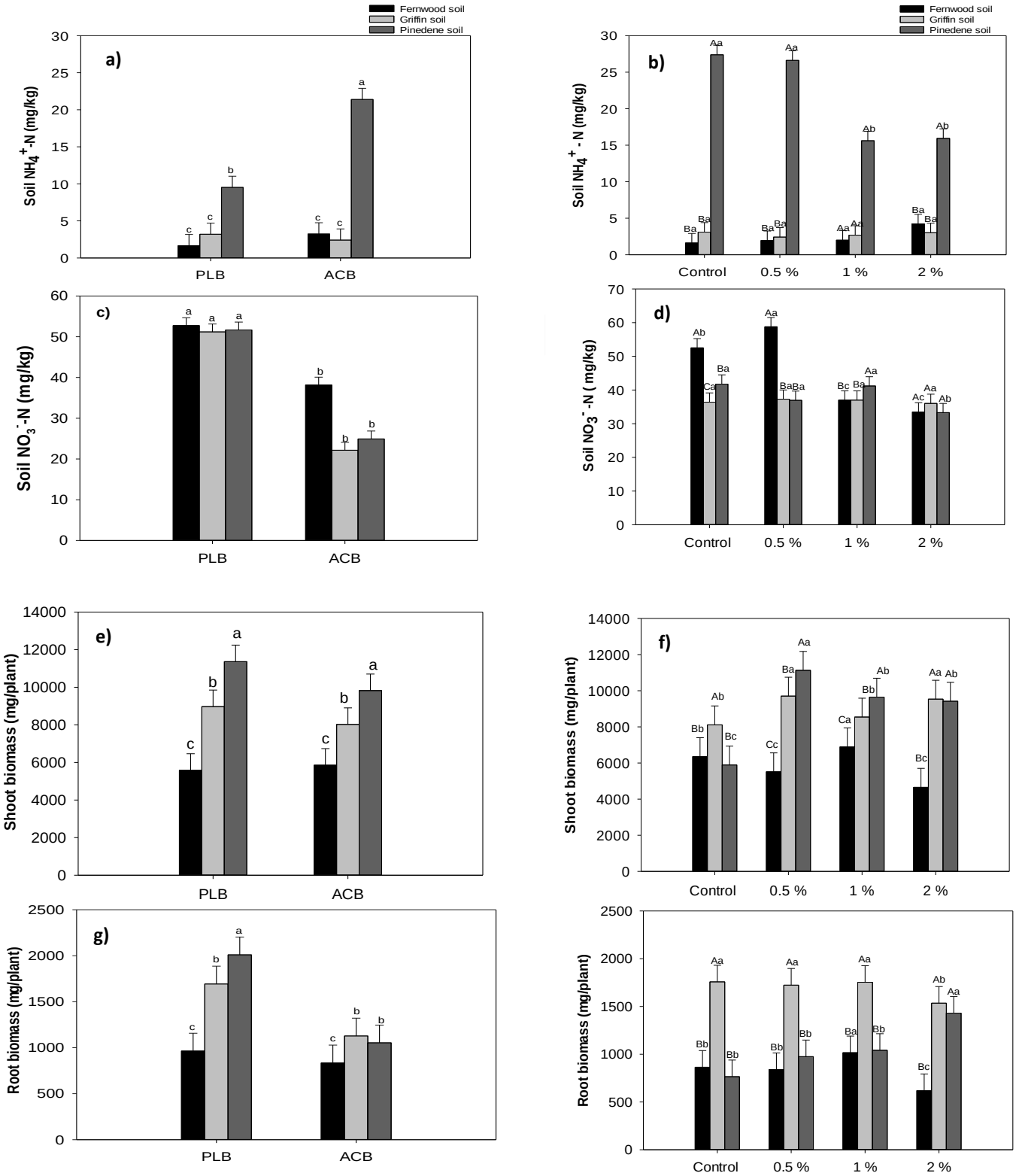


**Figure 4.5**. The interactive effect of soil type x biochar feedstock type and soil type x application rates on soil $NH_4^+$-N C(a-b); $NO_3^-$-N (c-d); Shoot biomass (e-f); Root biomass (g-h), respectively at significant probability level of $P \leq 0.05$.



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### 4.4.5 Biochar effect on shoot biomass, and nutrient uptake by chickpea

Application of PLB increased shoot and root biomass of chickpea grown in the Pinedene and Griffin soils compared to ACB. In fact, shoot biomass was 55 and 50 % higher in the Pinedene and Griffin soils, respectively, than in the Fernwood soils (Fig 4.5e & g). Compared to the control, shoot biomass was higher in all biochar application rates in both the Pinedene and Griffin soils (Fig 4.5f). The Fernwood soil exhibited the least increase in shoot and root biomass, however, when the application rate was increased to 2 %, both shoot and root biomass decreased (Fig 4.5f & h). Chickpea grown in the Fernwood soil showed the least nutrient uptake (Fig 4.6 & 4.7), while chickpea grown in the Griffin and Pinedene soils had higher uptake of N, Ca (Fig 4.7a, e & g), Fe and Zn (Fig 4.7c & e) with PLB compared to ACB application. There was no difference in P and S uptake in chickpea growth in all soil types with the application of either biochar derived from PL or AC feedstock (Fig 4.6c & 4.7a). Application of PLB resulted in higher K uptake in the Griffin soil, while chickpea grown in the Fernwood and Pinedene soil showed higher K uptake with ACB application (Fig 4.6e). Nutrient uptake differed among biochar application rates in all soil types. Compared to the control treatment, application of biochar at 0.5 to 2 % resulted in higher uptake of N, P, K (Fig 4.6b, d, f), S and Zn (Fig 4.7b & f) by chickpea in the Griffin and Pinedene soil. The opposite was observed in the Fernwood soil where chickpea uptake of N, P, K, Ca Fe and Zn decreased with 2 % biochar application (Fig 4.6 & 4.7).



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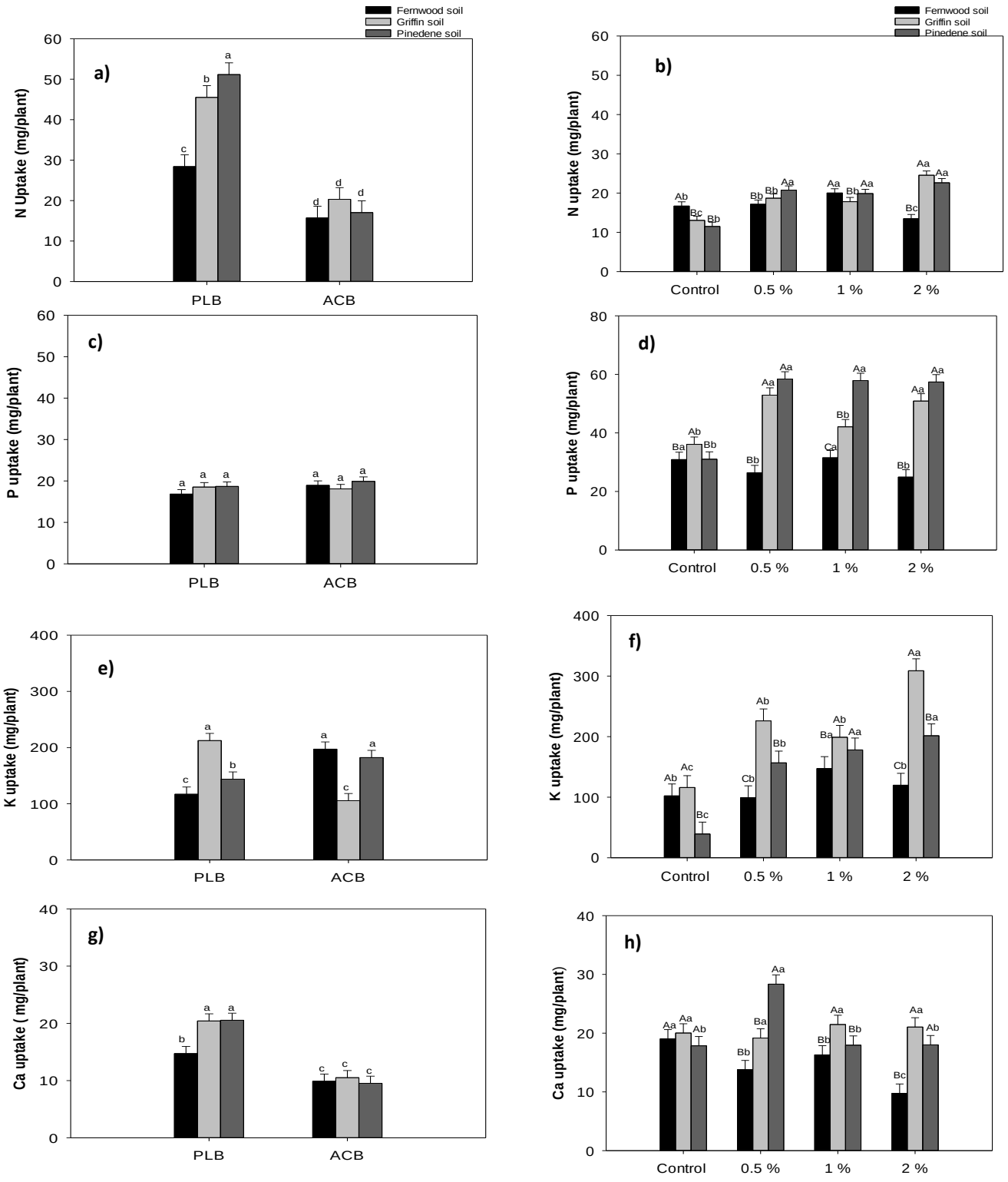


**Figure 4.6**. The interactive effect of soil type x biochar feedstock type and soil type x application rates on shoot N (a-b); P (c-d); K (e-f), Ca (g-h) uptake, respectively at significant probability level of $P \leq 0.05$.



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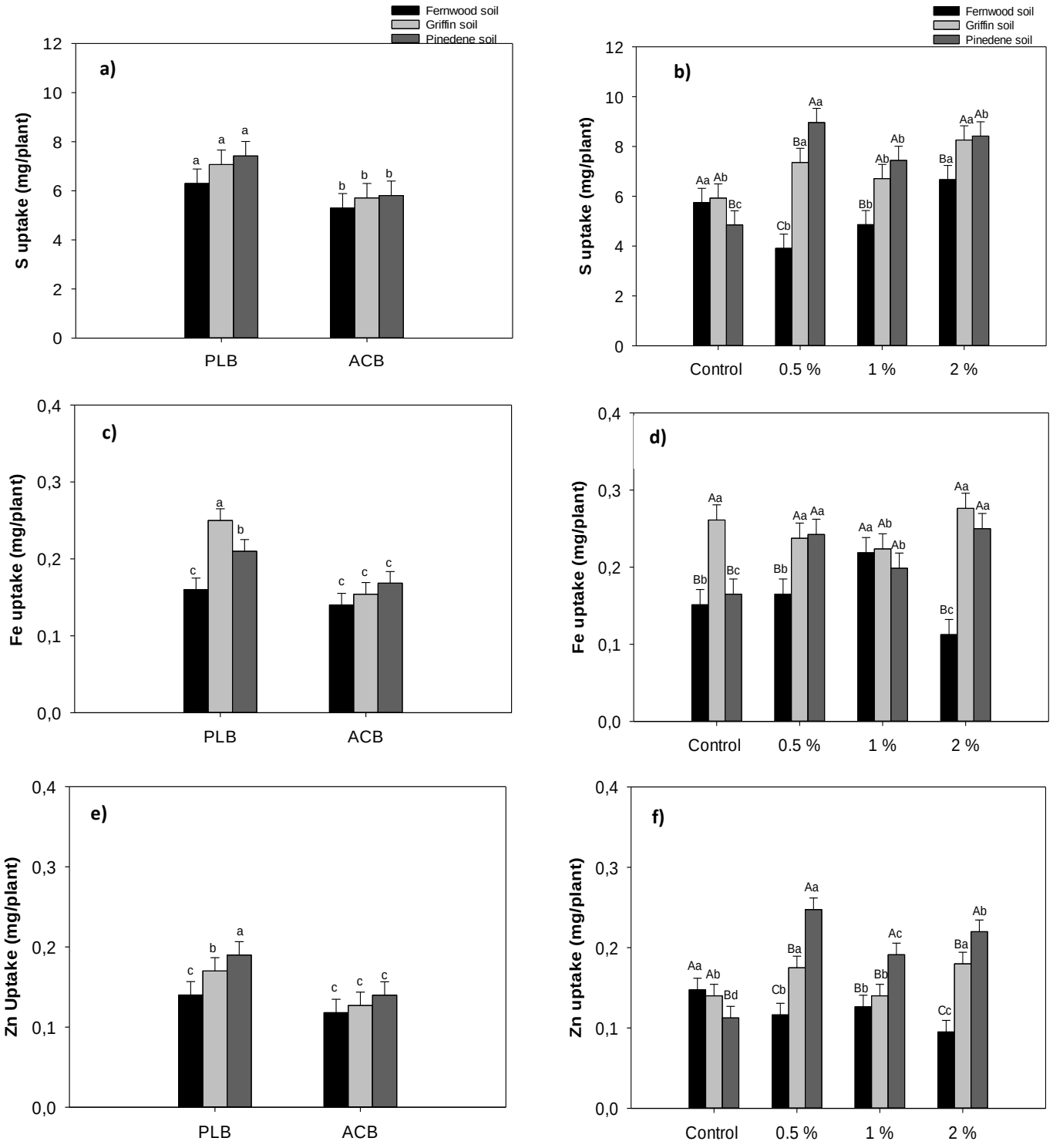


**Figure 4.7**. The interactive effect of soil type x biochar feedstock type and soil type x application rates on shoot S (a-b); Fe (c-d); Zn (e-f) uptake, respectively at significant probability level of $P \leq 0.05$.



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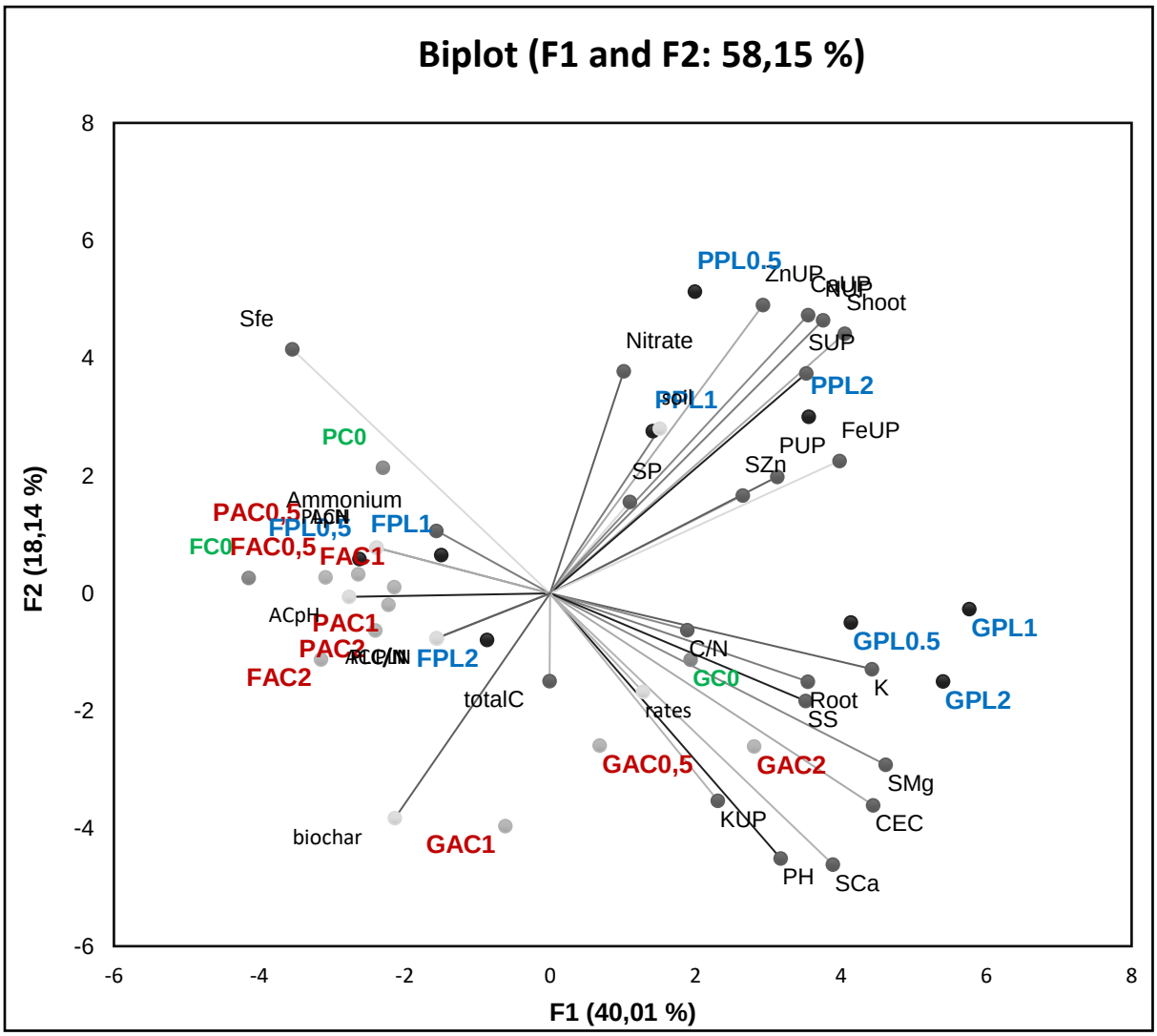


**Figure: 4.8**. PCA analysis illustrating the relationship between rhizospheric macro and micronutrients, plant growth and nutrient uptake of chickpea with biochar properties, soil type, application rates and biochar feedstock type. The soil types are donated as FP, GP, PP and FA, GA, PA representing Fernwood, Griffin and Pinedene with PLB and ACB biochar, respectively. The Fernwood, Griffin and Pinedene soils without biochar were represented as FC0, GC0 and PC0, whilst the 1, 2, 3 numbers represent the application rates at 0.5 %, 1 % and 2 % respectively.



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## 4.5 Discussion

### 4.5.1 Biochar effect on soil fertility of three different soil types

In this study, rhizospheric pH and CEC as well as macro and micro-nutrients concentration varied with soil type, biochar feedstock type and application rates. The increase in pH in the Fernwood, Griffin and Pinedene soils with PLB and ACB applications was expected. The soils in this study were acidic and biochar addition acted as a liming agent to increase the pH. Generally, biochar produced contained high concentrations of $Ca^{2+}$, $K^{+}$ and $Mg^{2+}$ in the ash content which form alkaline oxides or carbonates. Thus, biochar with high base cations when mixed with acidic soils, reacts with the $H^{+}$ and $Al^{3+}$ ions to raise the soil pH (Tag et al., 2016). In fact, PLB had more liming effect with higher cations, pH (10.3) and ash content (170 g/kg) compared to ACB with pH and ash content of 9.2 and 164 g/kg, respectively. Interestingly, the high clay content and CEC above 5 cmol/kg implied that the Griffin and Pinedene soils are highly buffered and therefore requires more lime to neutralize the acidity than sandy soils like the Fernwood which have low buffering capacity. Such soils would require application of biochar above 2% either derived from poultry litter acacia feedstock. However, the results suggest that caution should be taken when applying PLB and ACB on poorly buffered loamy sand soil such as Fernwood to avoid over liming. Over-liming may result in nutrient deficiency in poorly buffered soils and therefore adversely affecting nutrient uptake.

Applying 2% biochar was optimal to enhance CEC of Griffin soil in this study. Increased CEC due to the incorporation of biochar is likely to enhance extractable nutrients such as Ca, Mg and K (Ding et al., 2016). In fact, because of higher CEC with biochar application, Griffin soil adsorbed more macronutrients than the other two soils. Similar results have been reported (Gaskin et al., 2008, Rajkovich et al., 2012). Increased exchangeable cations and CEC in Griffin soil compared to other soils may be due to higher soil pH. Rhizospheric CEC was higher when Griffin soil pH was higher than 6.5. The availability of base cations increases as the soil pH increases and maximum availability is at pH values above 6.5 (Ding et al., 2016). This was evidently supported by the positive correlation of rhizospheric CEC, K, Mg and Ca with soil pH, particularly in the Griffin soil, as shown in the PCA analysis (Fig 4.8). The results suggest that slightly acid, clay loam soils such as the Griffin soil with high clay content will require biochar above 2% (equivalent to 40 t/ha) to lime the soil and improve CEC, nutrient retention and availability. However, soils such as Pinedene, which are very acidic, with high acid saturation (Table 4.1) are likely to require



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twice the application of 2% PLB or ACB to increase soil pH for optimum nutrient retention and availability of essential nutrients.

The low response of rhizospheric K, Mg and Ca concentration and CEC in Fernwood soil with either PLB or ACB application was probably due to low SOC, clay content and high percentage of sand particles (Table 4.1), as well as low buffering capacity of the soil. Similar findings were reported elsewhere (Novak et al., 2009, Schulz and Glaser, 2012). Soils such as Fernwood are highly leached (E horizon characteristics) and therefore likely to be infertile. However, this short-term study showed that applying 2% biochar either derived from poultry litter or acacia feedstocks has the potential to reduce nutrient leaching and improve total soil C, which means that long-term biochar application could improve soil fertility of such soils. This is because at 2% biochar application, rhizospheric K, Mg, Ca and CEC were significantly higher.

Compared to the CEC, the concentration and availability of P, Fe and Zn is pH dependent and therefore the rhizospheric P in this study was more at pH level greater than 6.0 in the Fernwood soil, particularly when PLB was applied. On the other hand, rhizospheric P was similar in both Griffin and Pinedene soils with PLB and ACB applications. The results showed that an increase in the application rates of either PLB or ACB from 1 to 2% resulted in a higher increase in rhizospheric P, particularly in the Fernwood soil, while P was significantly higher in the Pinedene and Griffin rhizospheric soils at 1 and 2%. Similarly, the concentration of rhizospheric Zn was higher at pH greater than 6.0, particularly at 2% PLB in the Fernwood and 1% in the Griffin soil. Possible mechanisms that could explain the increase in rhizospheric P with biochar application in this study are that P sorption is pH-dependent and therefore the increase in soil pH with PLB application could have led to an increase in P availability. Increased soil pH reduces the toxicity of Al and Fe, which are the dominant binding agents of P in acidic soils, and decreases the bond strength between Fe, Al and Ca and P (Singh et al., 2010).

The concentration of rhizospheric Fe decreased and P increased when the pH was above 6.0 when PLB and ACB were applied in Griffin soil and when biochar was applied at 1 and 2% in Fernwood soil and at 2% in Pinedene soil. On the contrary, Pinedene soil showed the highest concentration of Fe at pH 4.2 and a decrease in P after application of ACB. The concentration of Fe is usually dominant in acid soils and solubility increases with lower pH and decreases with higher pH because the binding capacity in the soil is related to the formation of organic complexes and is highly dependent on soil pH. The results show that soil pH and the liming potential of biochar have a greater impact on the improvement of P availability in acid soils. In addition, the Fernwood soil exhibited higher rhizospheric P than Griffin and Pinedene soils which had more



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clay content, meaning that soils with low clay content have a low binding capacity for P sorption. The results of this study are consistent with the findings of previous PLB application studies on available P (Wang et al., 2015). The low response of rhizospheric P in Griffin and Pinedene soils than the Fernwood soil, despite high pH with biochar application, was likely due to higher concentration of Ca, as Ca competes with $PO_4^{-3}$ ion in the colloidal particle exchange site. Application of ACB resulted in a lower P response in this study, which could be associated with a low P (0.50 g/kg) in the biochar, compared to PLB, which contained a higher P of 24 mg/kg. These findings on the application of biochar derived from acacia feedstock suggest that ACB may not be an alternative suitable source of P for elevating low P soil levels, such as Pinedene soil.

Interestingly, in this study, rhizospheric P did not correlate with soil pH, but Fe correlated negatively, while Zn positively correlated with soil pH. Indeed, rhizospheric P and Zn varied with biochar type and application rates, as explained in the PCA analysis where rhizospheric P and Zn positively correlated with application rates and negatively with biochar type. These findings suggest that the increase in soil pH at 2% with biochar application is likely to increase the availability of P and Zn but may reduce the concentration of Fe toxicity. Not only does the rise in soil pH with biochar application alleviate the toxicity of Fe, but also reduces Al and other heavy metals such as Zn and Cu as demonstrated by previous findings (Inyang et al., 2016). Although Al was not determined in this study, several researchers showed an increase in soil pH with biochar derived from plant feedstocks, resulting in a decrease in Al toxicity in most tropical soils (Steiner et al., 2007, Van Zwieten et al., 2010). Chen et al. (2011) reported that biochar produced from wood or corn straw can effectively adsorb copper (Cu) and zinc (Zn) in aqueous solutions. Biochar produced from animal wastes such as poultry litter have high amounts of ash and inorganic components that can bind heavy metals. Precipitation and surface complexation, have been reported to be responsible for the strong affiliations between animal-derived biochar and heavy metals (Inyang et al., 2016, Uchimiya et al., 2010).

Variations in the efficacy of biochar for immobilisation of Fe, Al and heavy metals can be attributed to rise in soil pH influencing the pH-dependent charge (Van Zwieten et al., 2010), as well as to the pyrolysis temperature and feedstock affecting the abundance of functional groups forming metal complexes (Uchimiya et al., 2010). Heavy metal immobilisation is also influenced by the ash content (rise in phosphate, and cations), surface area and porosity variations (Inyang et al., 2016). These properties rely not only on the content of the feedstock, but can be controlled by regulating the temperature of the pyrolysis and other conditions of production (Uchimiya et al., 2010). These findings suggest that biochar application particularly PLB has the essential benefit



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in alleviating the toxicity of Fe, Al and reduce heavy metals concentration such as Cu and Zn in acid soils where these elements could affect plant growth.

Total C did not vary with the type of soil and biochar feedstock used, but with biochar application rates as evidently shown in the PCA (Fig 4.8). Biochar application at 0.5 to 2% increased total C from 1.7% to 2.7%. The non-response of total C with biochar application was probably due to the short-term experiment and biochar is very stable in the soil, and decomposition is long-term. During the experiment, biochar was not significantly degraded, and improvements to SOC must be attributable to the decomposition of more labile SOM components. Adding biochar at 2% in this study indicates that PLB or ACB biochar has the potential to improve SOC in the soil, but the impact could be more apparent in the long run. Rhizospheric total N and C/N ratio varied with soil type and application rates, whereas nitrate-N and ammonium-N varied with soil type, type of biochar feedstock used and application rates. Application of 0.5 % PLB appeared to be optimal to enhance nitrate-N in the Fernwood soil. The rise in nitrate-N with biochar derived from Poultry litter feedstock compared to AC feedstock may be attributed to the biochar's low C/N ratio (Table 4.1). Both biochar's had similar total C, but PLB had higher total N than ACB (Table 4.1), meaning that as expected, more N was released from PLB due to faster mineralisation of the material by microorganisms in the soil. Because of their low C/N ratio, biochar derived from animals-based feedstocks are likely to stimulate N mineralisation more than biochar derived from plant feedstocks (Zhao et al., 2014). A high N feedstock generally results in a high N biochar that stimulates N mineralisation (Thies et al., 2015).

The C/N ratio determines net N mineralisation and soil immobilisation (Nguyen et al., 2017), and therefore, addition of ACB with high C/N ratio limited N availability in all soil types. Several studies have shown that the incorporation of organic materials with a low C/N ratio enhances faster mineralisation by microorganisms in the soil, while the addition of organic materials with a C/N ratio of more than 50.1 % leads to rapid decomposition and net immobilisation of N. Due to their high C/N ratio (N immobilisation) and high surface area (adsorption) (DeLuca et al., 2015), woody derived biochar reduce N availability. The results suggest that it is important to supplement the biochar with N fertilizer to alleviate soil N when incorporating biochar with high carbon content and low N such as the acacia biochar, since microbes usually use available soil N for their metabolism and temporary block N availability when applying such biochar in soil.

Reduction of ammonium in the Pinedene soil with biochar application especially at 1 and 2 % was not as rapid as in the Fernwood and Griffin soils. The pH change in the soil was not as drastic as the other soils, because the soil was very acid at first. The concentration of ammonium-N in very



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acidic pH less than 4 is generally greater than in neutral and alkaline soils (Nguyen et al., 2017). The results show that nitrate-N and ammonium-N are indeed pH-dependent, and soil-specific, especially when biochar is applied. In addition, both ammonium-N and nitrate-N were reduced when the biochar rates were increased to 2 %. This indicates that biochar derived from PL or AC feedstock could retain N and reduce availability of nitrate for plant growth in tropical soils due to high immobilisation as a results of enhanced soil C/N ratio and nitrate capture when biochar is applied beyond 1 %. The differences in retention and availability of nitrate and ammonium in this study with the application of PLB and ACB could be related to the method used in soil extraction of mineral N.

Several authors have recently shown that soils amended with biochar capture significant amounts of nitrate that can only be analysed by serial extractions and not by standard methods of soil analysis (Hagemann et al., 2017, Haider et al., 2016, Kammann et al., 2015). These authors indicated that the standard KCl extraction method underestimates nitrate and possibly ammonium stocks in biochar modified soils. Haider et al. (2016) reported a significant release of nitrate in aged biochar modified soils, but not so much for ammonium when repeated methods of extraction were used. Jassal et al. (2015), reported higher nitrate capture from 34 to 39 g/kg when woody or poultry litter biochar were treated with ammonium nitrate solution. Therefore, the differences in soil and root zone nitrate content following the application of PLB or ACB are likely to be due not only to ACB immobilisation, but also to ACB nitrate capture, which was not analysed in this study. These results indicate that the standard KCl method may not be an appropriate method for determining the availability of nitrate and ammonium in biochar-amended soils with a high surface area and a large particle size such as ACB, but the released nitrate could be determined using the repeated extraction method.

### 4.5.2 Biochar effect on biomass production and nutrient uptake of chickpea

Chickpea growth and nutrient uptake varied with soil type and type of biochar feedstock applied. Application of biochar derived from Poultry litter feedstock produced the highest shoot and root biomass than biochar derived from AC feedstock. Due to the higher shoot N uptake as a result of high soil N, the greatest production of biomass in the Pinedene soil was more evident than the Griffin and Fernwood soil. The results show that biochar derived from Poultry litter feedstock could increase the supply of N through mineralisation in Pinedene soil that translated into shoot biomass. In addition, the decrease in soil acidity with biochar application has created an environment that is more suited for plant growth. The alteration of soil pH has implications for the availability of nutrients such as P and S, N mineralisation and other nutrient supply, especially



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base cations such as $Ca^{2+}$, $Mg^{2+}$ and $K^{+}$. Evidence in the literature (Cely et al., 2015, Ding et al., 2016) indicates that the application of biochar directly increases the turnover of soil nutrients, specifically N, P and K, due to the biochar's inherited concentrations of nutrients in ash content, or indirectly by influencing soil properties such as soil pH, CEC, microbial population growth and activity.

Chickpea grown in highly buffered, fine textured Pinedene and Griffin soils showed a higher uptake of N, K, and Ca nutrients compared to that grown in low buffered, coarse Fernwood soil. Despite the non-significant effect of biochar on P uptake, P uptake by chickpea was higher in both the Griffin and Pinedene soils than the Fernwood soils. This means that macro-nutrients such as P, Ca, and K were supplied directly when PLB biochar was applied to both soils. These findings were supported by the significant positive correlation of root biomass with K, Ca, Mg and soil pH, whereas shoot biomass correlated positively with N, P, and Ca uptake (Fig 4.8). Biochar application rates had no significant effect on chickpea shoot and root biomass in the Fernwood; however, when 0.5 % biochar was added, shoot biomass was apparently high and decreased with increasing levels of biochar. Other crops have been widely reported for the positive response of biomass production with PLB application rates as low as 0.4 to 50 t/ha (Akça and Namli, 2015, Macdonald et al., 2014, Rajkovich et al., 2012). Nonetheless, this study contrasted previous findings with woody derived biochar on chickpea (Lusiba et al., 2018, Macil et al., 2017) and other crops (de Sousa Lima et al., 2018, Laghari et al., 2015) in different soils with low response of shoot biomass with acacia-derived biochar application in all soil types. Possible reason for the difference in this study might be due to N immobilisation as observed with low N concentration and N uptake because of high C/N ratio associated with the applications of ACB. Low N uptake with biochar application is an indication that a small fraction of the biochar (labile carbon) was easily mineralised, leading to N immobilisation. Another possible reason might be the ACB application's low ability to retain nutrients and/or less direct nutrient release such as K, Ca, Mg and P due to low CEC, which is likely to lead to the suppression of chickpea growth. The ACB had low plant-available nutrients and very low P, so its application could not have made a significant contribution to chickpea plant nutrition considering that the experiment was short term. The results suggest that biochar from acacia-derived plant species should be supplemented with organic and inorganic fertilizers to avoid nutrient deficiencies in the soil.

When PLB or ACB was applied, the Fernwood soil showed the least response in the production of chickpea shoot biomass, as well as nutrient uptake. Consequently, the low response of shoot biomass production in the Fernwood soil was unexpected because the positive effects of biochar



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as a soil amendment were mainly associated with highly weathered low nutrient sandy soils(Agegnehu et al., 2017, Jien and Wang, 2013). Furthermore, the results contradict previous findings by Major et al. (2010), which indicate that biochar derived from woody materials improved the soil nutrient status of acidic, infertile sandy soils, resulting in increased growth and crop yield. In this study, the low response of biomass and N uptake in the Fernwood soil could be attributed to soil rather than application of biochar. The soil had low total C before biochar application and lacked sufficient amounts of essential nutrients such as N, P and K for high performance of chickpea (Gaur et al., 2010). Despite the application of biochar, the Fernwood soil had the lowest concentration of nutrients such as Ca, Mg and K. Though with the application of PLB, soil P and nitrate-N increased in the soil; however, the increase did not translate into higher shoot biomass. The results suggest that to improve the performance of chickpea in high sandy textured soils such as the Fernwood, the application of biochar should be supplemented with organic fertilizers to enhance SOC and CEC, so that the soil can retain and release nutrients for plant absorption.

### 4.6 Conclusion

Application of biochar derived from poultry litter at 0.5-2% improved rhizospheric pH, CEC, nutrient concentration and nutrient uptake, resulting in higher shoot and root biomass of chickpea grown in Griffin and Pinedene soils than the Fernwood soil. The ACB high C/N ratio contributed partly to N immobilisation and therefore affected chickpea grown in Fernwood, Pinedene and Griffin soils. Application of ACB at 1% and 2%; however, is likely to increase SOC in sandy textured Fernwood soils and reduce Fe toxicity if applied in all soils beyond 2%. Although this study was short-term, the results of this study suggest that PLB has the potential to improve the availability of nutrients in low nutrient acid soils. However, soils such as Pinedene and Griffin with high buffering capacity and kaolinite clay content may require more than 2% biochar for maximum nutrient retention compared to low buffered sandy textured Fernwood soils. On the other hand, biochar derived from acacia would be a better source of carbon sequestration, so its application can improve the SOC resulting in improved soil structure. Indeed, the effect of biochar on the availability of rhizospheric nutrients and the growth of chickpea varied with the type of biochar feedstock, soil type and application rates as observed in this study. Therefore, long-term field trials focusing on the assessment of poultry litter and woody derived biochar using different soil types and application rates is necessary to undertake, to better understand the biochar's benefit as soil amendment and for making more reliable recommendations.



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# CHAPTER 5

# SHORT-TERM IMPACT OF BIOCHAR APPLICATION FROM DIFFERENT FEEDSTOCKS ON BACTERIA COMMUNITY COMPOSITION AND ABUNDANCE OF THREE CONTRASTING SOILS

## Abstract

The effect of biochar derived from both plant and animal feedstocks on microbial abundance and diversity in acidic tropical soils is not well understood. In this study, investigation was conducted to understand the shift in bacteria abundance and diversity at phylum and genus level under the influence of different factors that include biochar types, application rates, as well as soil type. Bacterial diversity and community composition varied depending on soil type, biochar type, and application rates. The most dominant bacterial phyla were Proteobacteria (30%), Actinobacteria (19%), Acidobacteria (14%), and Firmicutes (11%), while *Bacillus* (8%), *Sphingomonas* (5%), *Bryobacter* (2%), while *Microvirga, Conexibacter*, and *Bradyrhizobium* (all greater than 1%) were the most prevalent genera in the samples. Application of 1 and 2% PLB and ACB was ideal for increasing bacterial diversity and abundance in the Fernwood soil, while 1% ACB was ideal to increase the genera *Bacillus, Bryobacter, Microvirga, Conexibacter* and *Bradyrhizobium* whose species are most likely to contribute in C and N cycling in the Fernwood and Griffin soils. Addition of 2% PLB in the Griffin and Pinedene soils increased the abundance of Proteobacteria. The shift in bacterial diversity and community composition in this study was due to higher rhizospheric soil pH, P, K, total C, and total N in the Fernwood soil, while K, total C, and nitrate in the Pinedene and Griffin soils. This indicates that short-term biochar application can effectively alter soil properties and thereby change bacterial community composition, especially those involved in C and N cycling in acidic tropical soils.

Keywords, biochar; bacteria community; relative abundance; soil nutrients; 16 rRNA Gene



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## 5.1 Introduction

Community structure, abundance and activities of soil microbes such as bacteria and fungi are crucial indicators of soil quality because they drive several soil functions that are related to nutrient cycling and availability. Most bacterial species are involved in carbon and nitrogen dynamics as well as development and stability of soil through the binding of stable aggregates. A diverse microbial community structure is important for efficient nutrient transfer to crops and greater nutrient retention in soil. This is an important process with positive impacts on nutrient management through the reduction of nutrient loss from agricultural soil to the environment. Microbial abundance and activity could be enhanced through the incorporation of organic materials into soil in the form of composted materials and plant residues in both agricultural and forestry ecosystems (Xu et al., 2016). Recently, biochar technology has been recognized as a soil amendment for C sequestration due to its inherent capabilities in mitigating the release of greenhouse gases (GHG), improving soil quality in agricultural soils (Noyce et al., 2015) as well as providing an ecologically sound waste management strategy (Lehmann and Joseph, 2015). Presence of biochar influences microbial community structure, activity and population dynamics. Meanwhile, such influences could be stimulated by biochar feedstock properties, application rates and soil type (Gomez et al., 2014, Lehmann et al., 2011, Rutigliano et al., 2014).

Biochar is a solid, carbonaceous, organic material obtained by pyrolysis or thermochemical conversion of biomass under oxygen-limited conditions. Biochar is stable, resistant to decomposition, rich in nutrients, and can persist in soil for thousands of years (Lehmann et al., 2011). Historically, biochar was used as a soil amendment for at least 2000 years in the Amazon basin in South America. The “Terra Preta” soils that were regularly amended with biochar and other organic materials have higher pH, are richer in nutrients and densely populated with diverse microbial communities than unamended Oxisol, which are generally acidic and infertile (Lehmann and Joseph, 2009b, Lehmann et al., 2011). The higher productivity of Terra Preta soils than their unamended Oxisol counterparts led to world-wide interest in applying biochar to agricultural soils and is creating new markets for the biochar produced as a co-product from the thermochemical conversion of biomass via pyrolysis (Novak et al., 2009).

Biochar can vary in their physical and chemical characteristics that include C and N contents, surface area, ash content and volatile matter. These attributes would result in markedly differing reactions when applied to soils (Tomczyk et al., 2020). The effect of biochar on soil chemical and physical properties have been widely documented in literature (Gao et al., 2016, Gul et al., 2015, Kelly et al., 2015, Noyce et al., 2015, Xu et al., 2016). However, far less is known regarding the



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effects of biochar addition on microbial activity, abundance, and diversity, particularly in semi-arid soils. For example, biochar derived from various crop residues (Gao et al., 2017, Xu et al., 2016), woody feedstocks (Gomez et al., 2014) and animal manure (Muhammad et al., 2014) altered soil properties such as surface area, abundant surface functional groups, concentration of nutrients (C, N, Ca, K and P) and pH (Gul et al., 2015), such changes are capable of influencing microbial abundance, diversity and activities. In addition, the distinct physicochemical properties of biochar, which when applied provides a favourable environment for microbes, could explain the shift in microbial behaviour in the soil as a result of biochar application (Lehmann et al., 2011).

It is uncertain whether the previous findings were due to differences in the biochar used, the nature of the biodiversity assessment, differences in environments and soil types or combination of these factors. Nonetheless, the effect of short-term biochar additions on bacteria taxonomy is poorly understood. Therefore, a more comprehensive approach is needed to establish both specific and diverse impacts of biochar application on bacteria abundance and diversity. Such approach could also have implications for nutrient cycling and plant responses in agroecosystems, especially when biochar is used as a soil amendment. Moreover, it is important to assess the potential differences between abundance and diversity of bacteria with respect to the type of biochar applied, application rates and soil type. Hence, the aim of the present study was to evaluate the impact of biochar derived from poultry litter and acacia feedstocks as well as application rates on bacteria abundance and diversity in three contrasting soils from the Limpopo Province, South Africa.

## 5.2 Materials and methods

The biochar, soil and treatments used for this study was the same as in chapter 3 and 4. See section 3.2, in chapter 3 for biochar preparation and characterization, and section 4.2 in chapter 4 for description of soil and analysis, experimental design and layout.

## 5.3 Data collection

### 5.3.1 Soil sampling

Approximately 20 g of rhizospheric soil was shaken from the root and transferred to sampling bags. One part of each rhizospheric soil sample was transferred in sampling bags and stored in a freezer at 4 $^{o}$C for further analysis.



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### 5.3.2 DNA extraction

Genomic DNA was extracted from 0.5 g freeze-dried soil using the MoBio PowerSoil® DNA Isolation Kit (MoBio Laboratories, Carlsbad, CA, U.S.; Cat. No. 12888-50) according to manufacturers. The DNA concentrations from the isolated samples was quantified using the Fluorometer method using the Qubit® dsDNA HS (High Sensitivity) assay and Qubit® 2.0 Fluorometer (Thermo Fisher Scientific, Wilmington, DE, USA) according to the manufacturer's instructions. Successful extracted DNA was then confirmed using the 1% agarose Gel electrophoresis and visualized on a gel system (Gel Doc 2000TM) using Quantity One 4.2 software (Bio-Rad Laboratories, Hercules, CA).

### 5.3.3 Preparation of 16S rRNA amplicons and high throughput sequencing

The extracted DNA from each sample was subjected to polymerase chain reaction (PCR),

341F: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCCTACGGGNGGCWGCAG) and 805R:(GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAGGACTACHVGGGTATCTAATCC) primers to target the V3-V4 hypervariable region of the 16S rRNA gene (Caporaso et al., 2012). The PCR reaction for each sample contained 12.5 µl TEMPase hot start master mix, 0.5 µl 341F and 805R primers, 2 µl of DNA and 9.5 µl of water. The reaction conditions run using a thermal cycler (Bio-Rad Laboratories, Hercules, CA) which included denaturation at 94 $^{o}$C for 30s; annealing at 55 $^{o}$C for 15s and at 68 $^{o}$C for 1 min; elongation at 68 $^{o}$C for 5 min. The amplicons of the samples were sequenced using the paired-end method by Illumina Biosystems NGS platform (MiSeq, Kapa Biosystems, Wilmington, Massachusetts).

### 5.3.4 Microbial community analysis and statistical analysis

The quality of raw Illumina reads was assessed using FastQC (Babraham Bioinformatics, UK; https://www.bioinformatics.babraham.ac.uk/index.html).

The reads were trimmed using Trimmomatic version 0.38 (USADELLAB.org; http://www.usadellab.org/cms/?page=trimmomatic) to improve the quality of the reads. The software package quantitative insight into microbial ecology (QIIME2, version 2019.1.0) was used to analyse the bacterial communities in the samples (Bolyen et al., 2019). QIIME2 pipelines were used to demultiplex, denoise, dereplicate and merge sequences which were clustered into operational taxonomic units (OTUs) at 97% similarity. Taxonomic composition of the samples was determined by comparing clustered sequences to the SILVA reference database for the V3-V4 region using a pre-trained classifier (SILVA version 132_99). Calculations of alpha diversity



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(including Shannon, Simpson and Chao1 indices) were performed in QIIME2. Pearson correlation analysis was used to assess the relationships among chemical properties of soils and relative abundances of dominant communities. The relationship between bacteria community composition and the selected soil environmental variables was analysed by the principal component analysis (PCA) to determine the independent contributions of these selected variables to the variation in community composition. Sequence reads obtained in this study have been submitted to the GenBank databases under the Bio Project PRJNA703480 (Accession numbers SRX10144366 - SRX10144391).

## 5.4 Results

### 5.4.1 Shift in bacteria diversity

Bacterial abundance, diversity and richness in this study was represented by the Chao1, Shannon and Simpson indices (Table 5.1). The initial untreated Pinedene (P treatment) soil showed the lowest Chao1 and Shannon indices compared to the other two soils but the OTUs were higher than the Griffin soil. After chickpea harvesting (control treatment), the number of OTUs, Chao1 and Shannon was higher in the Fernwood soil (FC0) than in the Pinedene and Griffin soils (GC0). However, when 0.5 % and 2 % ACB (treatment GAC2, GAC0.5) and 0.5 % and 1 % PLB (treatment GPL0.5 and GPL1) were applied, the number of OTUs as well as the Chao1 and Shannon indices increased in the Griffin soil. The application of 2% PLB (GPL2) and 1 % ACB (GAC1) resulted in a decrease in the number of OTUs, Chao1 and Shannon indices in the Griffin soil. Compared to the initial untreated sample and control, the number of OTUs, Chao1 and Shannon indices in Pinedene soil was higher in all PLB and ACB treatments. In contrast, application of 0.5 and 1 % either PLB or ACB resulted in lower OTUs, Chao1 index and Shannon index in the Fernwood soil, except when 2 % was applied compared to the control and initial untreated sample. The Simpson index was similar in all treatments in the Griffin, Pinedene and Fernwood soils. These findings indicate that bacteria species in the Fernwood soil were rich and diverse due to the rhizosphere effect rather than the application of PLB and ACB. On the other hand, the diversity and richness of bacteria in the Griffin soil was more evident when PLB and ACB were applied at 0.5 and 1 % as well as at 0.5 and 2 %, respectively. Although the addition of 2 % PLB and 0.5% ACB in the Pinedene soil led to less diverse and rich bacteria species.



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**Table 5.1.** OTU Observed, and alpha diversity indexes of bacteria (Chao1, Shannon and Sampson) with PLB and ACB application in the Griffin, Pinedene and Fernwood soils**.**

| Treatments | Codes | OTU observed | Chao1 Index | Shannon Index | Simpson Index |
|---|---|---|---|---|---|
| Initial untreated sample | F | 38587 | 1510,8 | 9,6 | 1 |
| | G | 15998 | 644,5 | 8,6 | 1 |
| | P | 25099 | 399,4 | 7,1 | 1 |
| Control | GC0 | 18713 | 710,1 | 8,7 | 1 |
| | PC0 | 25421 | 611,7 | 8,6 | 1 |
| | FC0 | 103338 | 2542,5 | 10,1 | 1 |
| Fernwood soil with PLB | FPL0.5 | 32665 | 1097,2 | 9,2 | 1 |
| | FPL1 | 34656 | 1269,5 | 9,5 | 1 |
| | FPL2 | 50904 | 1550 | 9,7 | 1 |
| Fernwood soil with ACB | FAC0.5 | 32398 | 1160 | 9,3 | 1 |
| | FAC1 | 20471 | 1136,6 | 9,2 | 1 |
| | FAC2 | 40262 | 1447 | 9,6 | 1 |
| Griffin soil with PLB | GPL0.5 | 32780 | 1262,8 | 9,4 | 1 |
| | GPL1 | 33203 | 1352,7 | 9,4 | 1 |
| | GPL2 | 13591 | 472,7 | 8,1 | 1 |
| Griffin soil with ACB | GAC0.5 | 44574 | 1426,6 | 9,6 | 1 |
| | GAC1 | 7650 | 235,5 | 7,3 | 1 |
| | GAC2 | 70361 | 2410,8 | 10,3 | 1 |
| Pinedene soil with PLB | PPL0.5 | 30454 | 900,7 | 9 | 1 |
| | PPL2 | 20267 | 535,3 | 8,4 | 1 |
| Pinedene soil with ACB | PAC0.5 | 39188 | 691,3 | 8,7 | 1 |
| | PAC1 | 54564 | 1800,9 | 9,6 | 1 |
| | PAC2 | 79332 | 1649,5 | 9,6 | 1 |

PLB- Poultry litter biochar; ACB- acacia biochar; F-Fernwood, G-Griffin, P- Pinedene; C0- control, 0.5 %, 1 % & 2 %



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### 5.4.2 Shift in bacteria abundance at phylum level

A total of 1,103,992 OTUs were obtained from 23 samples. The composition of the bacteria community was calculated based on the relative abundance of OTUs at 97 % sequences similarity level. In all 23 samples, a total of 36 phyla were obtained from the OTUs generated, but 11 phyla were selected based on a total relative abundance greater than 1% for all sequence data in each sample. Proteobacteria (30%), Actinobacteria (19%), Acidobacteria (14%), Firmicutes (11%) and Chloroflexi (8%) were the five most abundant bacterial phyla in all samples (Fig 5.1). Planctomycetes, Gemmatimonadetes, Bacteroidetes, Verrucomicrobia, Patescibacteria, and Cyanobacteria were detected in lower frequencies less than 5% (Fig 5.1).

The initial untreated Griffin soil (G treatment) had more Acidobacteria (17%) and Chloroflexi (19%), while Actinobacteria (51%) were more abundant in the untreated Pinedene soil, and Proteobacteria (32%), Firmicutes (15 %) and Planctomycetes (5 %) were more abundant in the untreated Fernwood soil. (Fig 5.1). In this study, the rhizospheric effect had an impact on the abundance of bacteria at phylum levels. For example, the control (GCO-Griffin soil after chickpea harvest) had more Proteobacteria, Actinobacteria and Acidobacteria. Chloroflexi and Firmicutes were more abundant in Pinedene (PC0) soil and Fernwood (FC0) soil, respectively (Fig 5.1).

The relative abundance of Proteobacteria increased slightly from 29% (FCO treatment) to 30 % and 31 % on average in the Fernwood soil in all PLB and ACB treatments, respectively (Fig 5.1). The abundance of Actinobacteria was reduced by the application of PLB and ACB, but the relative abundance of Firmicutes was higher in all PLB and ACB treatments compared to the initial untreated sample (F) and control treatment, respectively (FCO). When ACB was applied to the Fernwood soil, the abundance of Acidobacteria and Chloroflexi changed slightly. The relative abundance of Planctomycetes, Gemmatimonadetes, Bacteroidetes, Verrucomicrobia, Patescibacteria, and Cyanobacteria were more abundant with PLB and ACB application when compared with the initial untreated samples of the studied soils (Fig 5.1).

The application of PLB and ACB in the Griffin soil increased the relative abundance of Proteobacteria, with markedly higher Proteobacteria at 2% PLB (GPL2, 42 %) and 1% (GAC1, 34 %). Addition of 0.5 and 1 % PLB resulted in a decrease in Actinobacteria, but the lowest relative abundance of Actinobacteria was at 1 and 2 % ACB (GAC1 and GAC2 treatment). Acidobacteria decreased in relative abundance with higher levels of PLB and increased with higher levels of ACB. The relative abundance of Firmicutes, on the other hand, increased by 16 % at 1% ACB



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(GAC1), whereas Chloroflexi was less abundant compared to G treatment in all PLB and ACB treatments (Fig 5.1).

The relative abundance of Proteobacteria in the Pinedene soil increased from 17% to 34% (PPL2) in PLB treatments and to 30 % (PAC0.5) in ACB treatments. The relative abundance of Actinobacteria decreased from 51 % to 17% and 11 % when PLB and ACB were applied, respectively. Acidobacteria, on the other hand, decreased with higher application levels of PLB and ACB. The relative abundance of Firmicutes decreased in all PLB and ACB treatments compared to the initial samples and control treatments (Fig 5.1).



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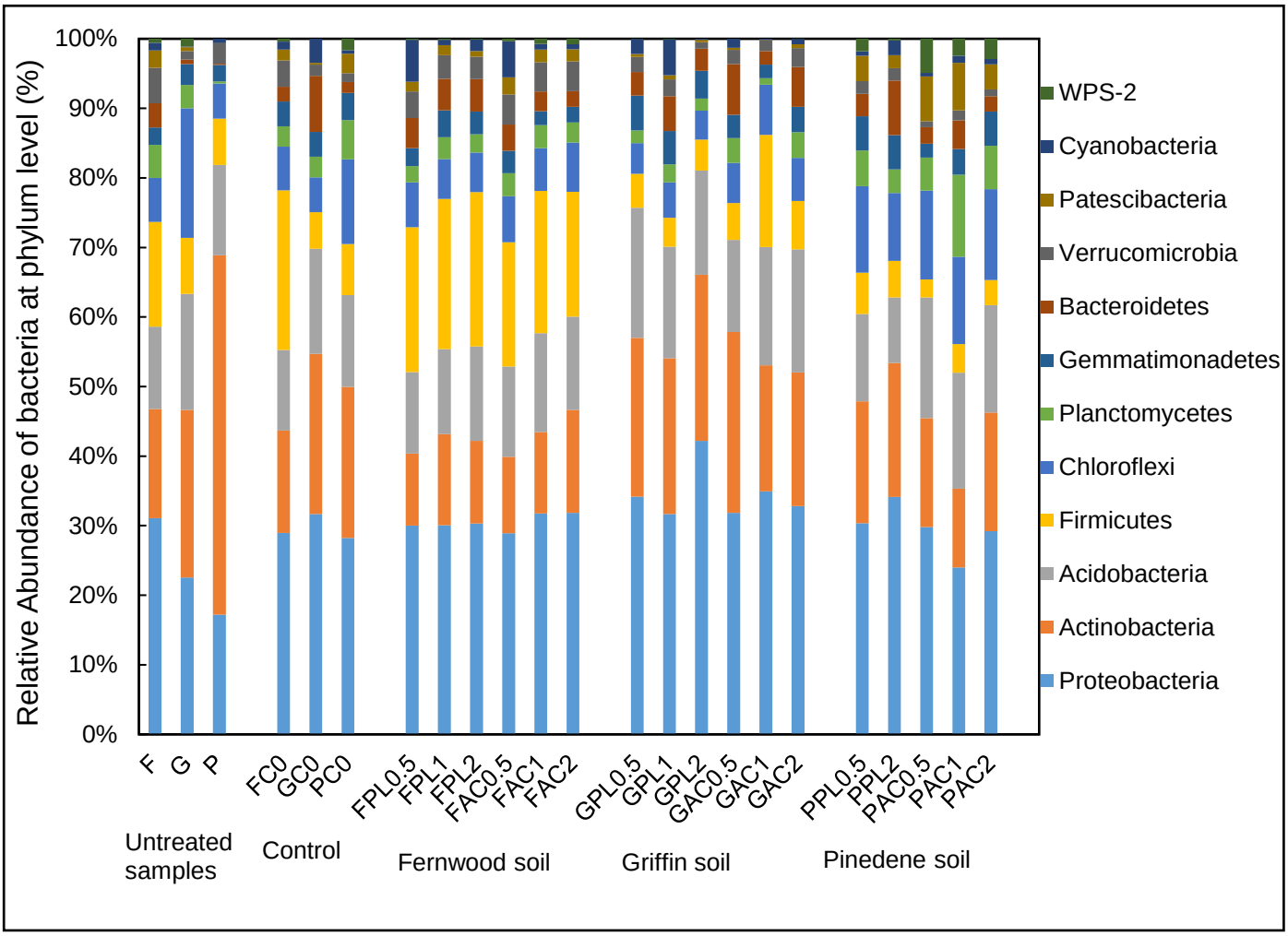


**Figure 5.1**: Relative abundance of 12 dominant bacteria taxa at phylum level of Fernwood, Griffin and Pinedene soils revealed by 16S rRNA high-throughput sequencing across 0.5%,1%, 2.0% PLB and ACB application.



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### 5.4.3 Shift in bacteria abundance at genus level

A total of 19 bacterial genera were found to be greater than 0.5 % of all samples. Only 8 genera, *Bacillus*, *Sphingomonas, Bryobacter, Microvirga, Conexibacter, Bradyrhizobium, Flavisolibacter, and Acidothermus*, were most dominant with an average relative abundance of greater than 1%, in order of highest dominant to least dominant (Fig 5.2). *Bacillus* (11%) *Sphingomonas* (7%), *and Bradyrhizobium* (1.4%) were more abundant in the untreated Fernwood soil, while *Acidothermus, Bryobacter and Conexibacter* were abundant in the untreated Griffin soil, and *Sphingomonas* was abundant in the untreated Pinedene soil (Fig 5.2). After chickpea harvesting, *Bryobacter, Sphingomonas,* and *Conexibacter* were abundant in the Pinedene soil, whereas Bacillus and *Sphingomonas* were abundant in Fernwood soil, and *Bradyrhizobium, Microvirga, and Flavisolibacter* were abundant in Griffin soil (Fig 5.2).

The relative abundance of *Bacillus* (15 %) was higher in Fernwood soil when PLB and ACB were applied at 2% and 1%, respectively (treatments FPL2 and FAC1) (Fig 5.2). The relative abundance of *Bryobacter, Bradyrhizobium*, and *Sphingomonas* in ACB treatments, on the other hand, was higher than in PLB treatments, particularly at 1% (treatment FAC1). Similar to the Fernwood soil, application of ACB at 1 % increased *Bacillus, Bryobacter*, *Microvirga, Flavisolibacter,* and *Bradyrhizobium* abundances in the Griffin soil (Fig 5.2). Sphingomonas increased from 1.94 % to 8.01 % at 2 % PLB (treatment GPL2), while *Flavisolibacter i*ncreased from 0.10 % to 2.03 % (treatment GAC0.5). The abundance of *Conexibacter* was higher at 0.5 % and 2 % ACB treatments, but only when compared to the initial untreated sample. At 0.5 % PLB (treatment PPL0.5), the Pinedene soil had the highest relative abundance of *Sphingomonas*, *Bacillus,* and *Bradyrhizobium*, while *Bryobacter* and *Acidothermus* were relatively higher at 0.5 % ACB (treatment PAC0.5) (Fig 5.2).



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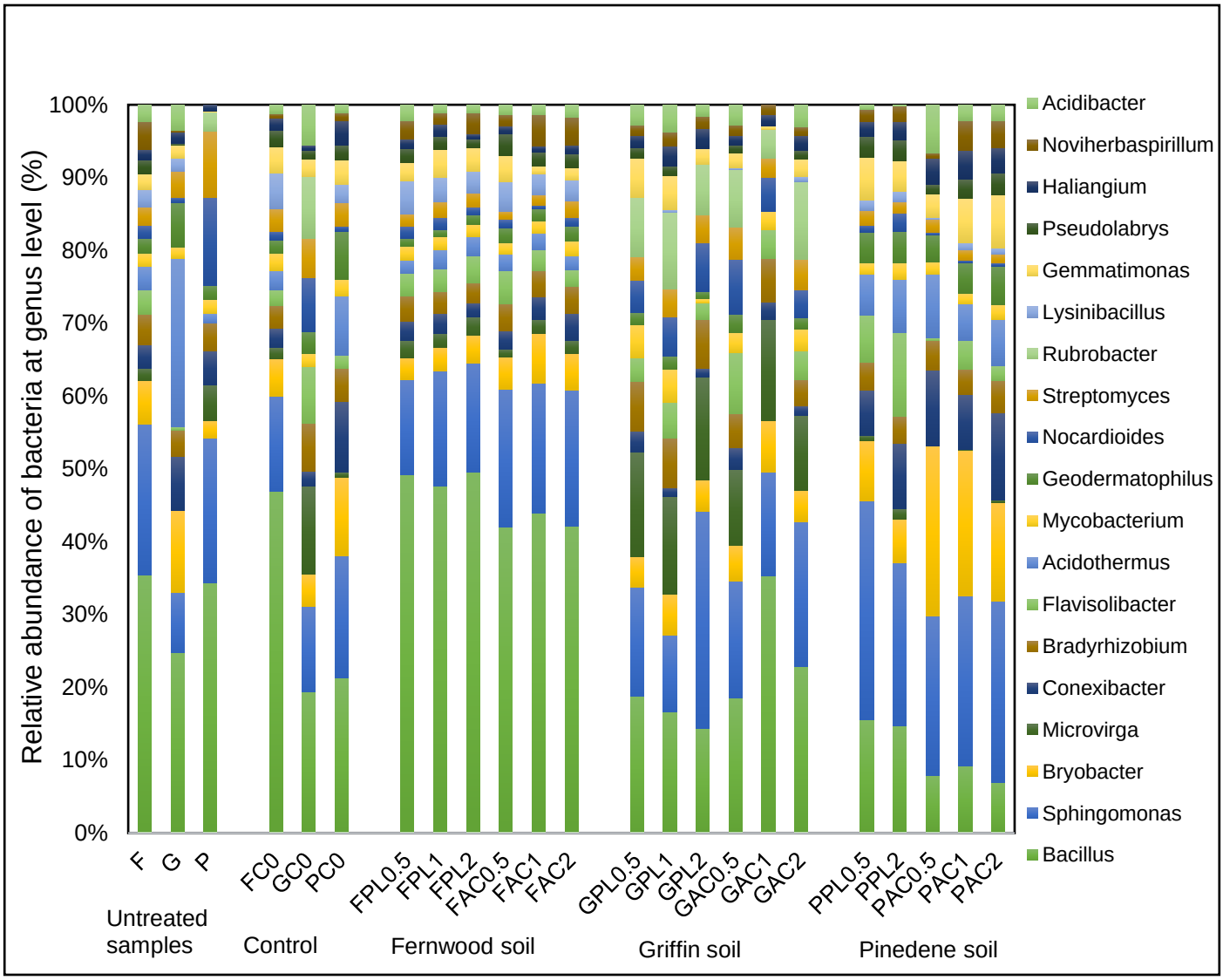


**Figure 5.2:** Relative abundance of 19 dominant bacteria taxa at genus level of Fernwood, Griffin and Pinedene soils revealed by 16S rRNA high-throughput sequencing across 0.5%,1%, 2 % PLB and ACB application.



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### 5.4.3 Principal Component Analysis

The biplot of the first two major components (PCs) showing the relationship between bacteria community at phylum level and soil properties in the Fernwood, Griffin and Pinedene soils are shown in (Fig 5.3-5.5). The first (F1) and second (F2) components explained 76.74 % of the variability in the Fernwood soil, 72.59% in the Pinedene soil and 59.98 % in Griffin soil. The Eigenvalues ranged from 8.7-6.6, 7.6-4.5, and 11.0-3.4 for F1 and F2 in the Fernwood, Griffin and Pinedene soils, respectively.

Positive loadings of Proteobacteria, Actinobacteria, Acidobacteria, Chloroflexi, Verrucomicrobia, soil pH, P and K, as well as negative loads of Gemmatimonadetes and Bacteroidetes in the Fernwood soil contributed significantly to F1 variability (Fig 5.3). While Acidobacteria, Firmicutes $NH_4^+$, total C and C/N ratio and negative loadings of Patescibacteria, Cyanobacteria, $NO_3^-$, total N contributed to F2 variability. Soil pH, P and K have a strong positive correlation with Proteobacteria Actinobacteria, Acidobacteria, Verrucomicrobia and Nitrate correlated positively with Chloroflexi and Patescibacteria; and total N with Cyanobacteria. Meanwhile, soil P, K, $NH_4^+$, total N, total C and C/N ratio are highly negatively correlated with Bacteroidetes, Patescibacteria, Actinobacteria, Firmicutes, Chloroflexi, Patescibacteria, respectively (Fig 5.3). The variation in the bacteria communities was largely explained by the application rates in the Fernwood soil as compared to the biochar feedstock type.

Positive loading of Firmicutes, Chloroflexi, soil pH, P, K, $NO_3^-$ and negative loading of Gemmatimonadetes, Cyanobacteria, C/N ratio, total C contributed to F1 variability, but Planctomycetes, Bacteroidetes, Proteobacteria, Acidobacteria contributed to F2 variability in the Griffin soil (Fig 5.4). Except for Chloroflexi and Firmicutes, which correlated positively with soil P, K and $NO_3^+$, the correlation between soil properties and phylum in the Griffin soils was not strong, while Actinobacteria and Gemmatimonadetes correlated negatively with soil pH, P, K and $NO_3^+$ (Fig 5.4). The type and application rate of biochar feedstock did not contribute to the variation in phylum-level bacterial communities in the Griffin soil, but the change in soil properties with biochar application at different rates was largely attributed to the variation. All soil properties and Acidobacteria, Chloroflexi, Planctomycetes, Patescibacteria and contributed negatively to F1 variability in the Pinedene soil (Fig 5.5), apart from $NH_4^+$, total C and C/N ratio, Proteobacteria, Actinobacteria and Verrucomicrobia. On the other hand, none of the soil properties contributed to the variation in F2, but the variation was largely due to Firmicutes, Gemmatimonadetes, Bacteroidetes and Cyanobacteria (Fig 5.5). Acidobacteria, Chloroflexi and Planctomycetes were highly positively correlated with soil pH, P, K and $NO_3^+$, whereas Proteobacteria, Actinobacteria



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and Verrucomicrobia were the opposite. Acidobacteria, Planctomycetes, Patescibacteria and were negatively associated with the total C and C/N ratio (Fig 5.5). The variation in the groups of bacteria was largely explained by the type of biochar feedstock than the application rates in the soil of Pinedene.

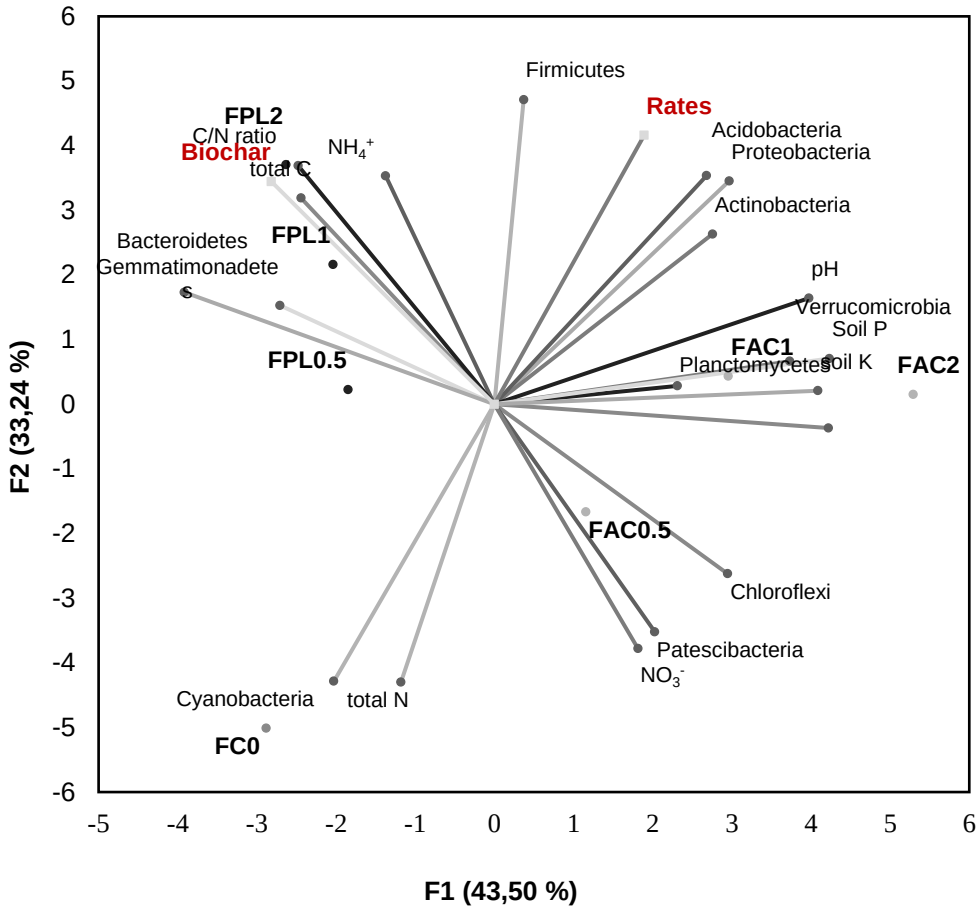


**Figure 5.3:** Principal component analysis (PCA) biplot representing the relationship between bacteria communities at phylum level and selected soil properties (pH, P, total N, total C, $NH_4^+$, $NO_3^-$, C/N ratio) in the **Fernwood soil**. The vectors represent the bacteria phyla and treatments (soil type, biochar feedstock type and application rates). The direction of the arrow indicates the steepest increase in the variable, and the length indicates the strength relative to the other variables. Bold text represents the treatments: FCO, FPL0.5, FPL1, FAC0.5, FAC1, FAC2.



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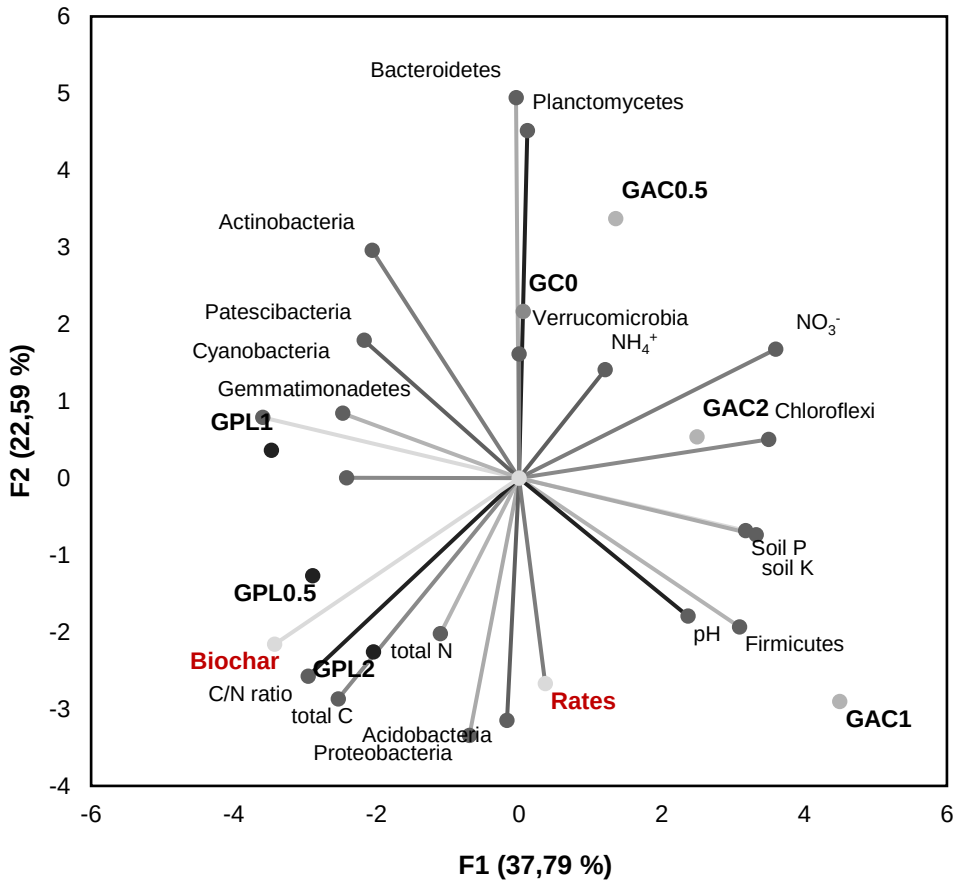


**Figure 5.4:** Principal component analysis (PCA) biplot representing the relationship between bacteria communities at phylum level and selected soil properties (pH, P, total N, total C, $NH_4^+$, $NO_3^-$, C/N ratio) in the **Griffin soil.** The vectors represent the bacteria phyla and treatments (soil type, biochar feedstock type and application rates). The direction of the arrow indicates the steepest increase in the variable, and the length indicates the strength relative to the other variables Bold text represents the treatments: GC0, GPL0.5, GPL1, GPL2, GAC0.5, GAC1, GAC2.



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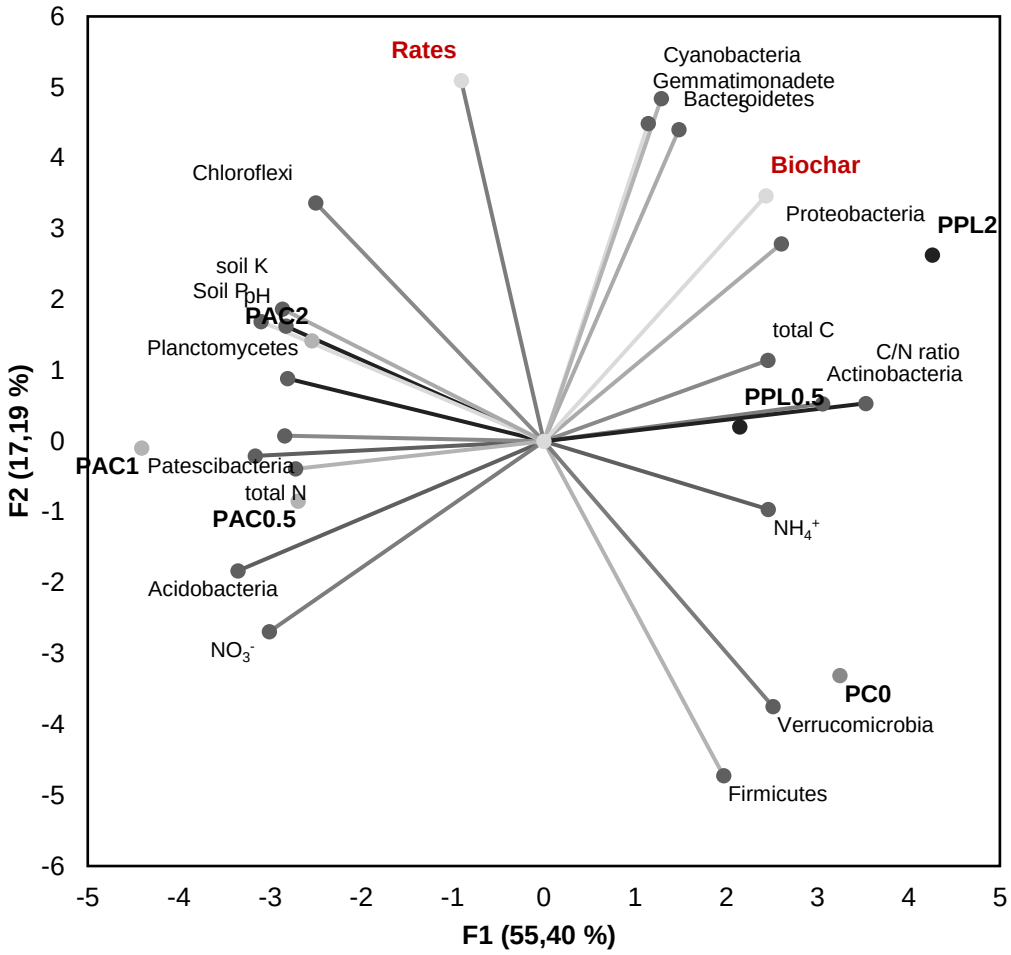


**Figure 5.5:** Principal component analysis (PCA) biplot representing the relationship between bacteria communities at phylum level and selected soil properties (pH, P, total N, total C, $NH_4^+$, $NO_3^-$, C/N ratio) in the **Pinedene soil**. The vectors represent the bacteria phyla and treatments (soil type, biochar feedstock type and application rates). The direction of the arrow indicates the steepest increase in the variable, and the length indicates the strength relative to the other variables. Bold text represents the treatments: PCO, PPL0.5 PPL2, PAC0.5, PAC1, PAC2.



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## 5.5 Discussion

### 5.5.1 Shift in bacteria diversity with biochar application

This study hypothesized that biochar derived from poultry litter and acacia feedstock would alter chemical properties of three different soil types and thus contribute to a change in the microbial community of the soils especially the increase in bacterial relative abundance and diversity. Indeed, in this study, the diversity and community composition of bacteria varied by soil type, type of biochar used and application rates. The diversity index of soil microorganisms reflects species richness and community distribution, with higher indices indicating greater soil microbial diversity and a more complex and stable soil micro-ecosystem function (Tian et al., 2016b). The Chao index reflects the community's actual number of species. In general, a higher Chao index indicates greater community richness, whereas Shannon and Simpson indexes show the species richness and evenness of the community (Fan et al., 2020). The results showed that the Chao1 and Shannon indices increased when biochar was applied in all soil types compared to non-biochar treatments. A change in bacterial communities was caused by application of 2 % PLB or ACB in the Fernwood soil, as well as 0.5 % PLB and 2 % ACB in the Griffin and Pinedene soils. This suggests that the richness of the soil bacteria community was indeed influenced by the addition of different rates of biochar applied. In similar studies, Doan et al. (2014) and Sun et al. (2016) found that diversity indices were significantly improved by biochar application. This is due to the pore structure of the biochar and its water and nutrient adsorption, which can provide microorganisms with an appropriate habitat (Lehmann et al., 2011). At the same time, microbial metabolism can be stimulated by an appropriate quantity of biochar and the species richness of the microbial community can be increased.

Bacteria communities were not evenly distributed according to the Simpson index, despite the richness of bacteria in the samples. Similarly, Fan et al. (2020) reported no change in the Simpson index at different rates despite biochar application. The results of this study indicate that bacteria in the soil were richer, but less even, which means that some species were more abundant than other species. For example, bacterial communities were richer in the untreated Fernwood soil (F treatment) and control (FCO treatment) compared to the soils of Griffin and Pinedene before biochar application. The findings of this present study are similar to those reported by (Cole et al., 2019, Fan et al., 2020) who found no effect on Shannon and Simpson index alpha-diversity indices after biochar application. This indicates that plant growth in the untreated samples and chickpea growth in the control treatment stimulated a rapid shift in communities of bacteria,



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probably due to the release of organic acids from root exudates as well as development of plant and the change in the rhizospheric soil environment (Adeleke et al., 2017). Similar results were reported by Singh et al. (2007), who indicated higher diversity of bacteria communities in the rhizosphere due to higher plant growth.

These findings on increased richness in coarse soils agrees with previous studies (Carson et al., 2010, Chau et al., 2011) suggesting that texture of the soil affects the soil nutrient status and water content, thus affecting microbial habitats and their metabolic activities. Biochar has been shown to modify soil properties, which could alter bacterial community structures. (Fan et al., 2020, Gul et al., 2015, Ippolito et al., 2014, Lehmann et al., 2011, Xu et al., 2016). Biochar can enhance aeration and water retention in soils due to its physical properties such as high porosity and large surface area, and then provide a favourable habitat for soil bacteria to escape from predators as well as enhancing their capabilities to live and grow (Lehmann et al., 2011). In addition, the chemical properties of soils can also contribute to the changes in the bacterial community structure after biochar addition (Gul et al., 2015) . Overall, these findings suggest that applying biochar to soil can increase bacterial diversity, which is important for soil stability and functionality.

### 5.5.2 Shift in bacterial abundance with biochar application

Biochar alters the microbial community composition by promoting microbial abundance and activity through changes in soil properties, adsorption, and/or nutrients (Fan et al., 2020, Quilliam et al., 2013) thus affecting microbial processes involved in nutrient cycling and organic matter decomposition (Sun et al., 2016). In the present study, the community composition of bacteria varied with soil type, type of biochar used and application rates. Proteobacteria, Actinobacteria, Acidobacteria, Firmicutes, Chloroflexi were the five most dominant bacterial phyla, while Bacillus, *Sphingomonas, Bryobacter, Microvirga, Conexibacter, Bradyrhizobium, Flavisolibacter, and Acidothermus*, were six most dominant genera at (>1%).

Proteobacteria phylum (which comprise the genera *Sphingomonas, Bradyrhizobium, Microvirga*) occupied the highest proportions (17-42 %) of the bacterial abundance in all soil samples, confirming previous findings that biochar application increased Proteobacteria (Fan et al., 2020, Xu et al., 2016). Application of PLB increased the relative abundance of *Sphingomonas* on average by 8 % in the Griffin and Pinedene soils, while in the Fernwood soil by 6 % in ACB treatments. Biochar acted as a carbon source for *Sphingomonas* in this study, as total C increased with higher levels of biochar, and *Sphingomonas* were more abundant in soils where total C was



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higher. Similar to this study, *Sphingomonas* response was higher in soils amended with biochar than in the control soil, due to higher soil organic carbon (Han et al., 2017). Species belonging to the *Sphingomonas* genera play an important role in plant abiotic stress tolerance, bioremediation, and environmental contaminant biodegradation (Asaf et al., 2020). In the Griffin soil, there was a slight shift in *Bradyrhizobium* abundance with PLB and ACB application, but the shift in the Fernwood soil soils was due to ACB application. In the Pinedene soil, however, the rhizosphere effect of chickpea increased *Bradyrhizobium* more than PLB or ACB applied at different rates, most likely due to root exudates effects. When PLB and ACB were applied, *Microvirga* were more abundant in the Griffin soil and less abundant in the Fernwood and Pinedene soils. Species from the genera *Bradyrhizobium* and *Microvirga* have a significant impact on nitrogen cycling, specifically nitrogen fixation (Han et al., 2017).

In the Fernwood soil, biochar application increased Acidobacteria, whereas in the Griffin and Pinedene soils, Acidobacteria decreased with higher levels of PLB and increased with higher levels of ACB. Acidobacteria are commonly found in low-pH soils, and their decrease in abundance after PLB application could be due to an increase in soil pH. The increase in *Bryobacter* abundance with ACB application in all soil types was related to soil pH because the species of this genera belong to the phylum Acidobacteria and are very sensitive to high soil pH. The relative abundance of Acidobacteria decreased significantly with increased biochar application rates, due to increased soil pH after biochar addition (Fan et al., 2020, Xu et al., 2016).

Firmicutes, the phylum for the genera *Bacillus*, increased in abundance when 2% PLB was applied in the Fernwood soil and 1% ACB was applied in the Griffin soil. The *Bacillus* is one of the most common bacterial genera found in soil, which thrives well in soil environments with pH above 6. The increase in Bacillus relative abundance in the Fernwood and Griffin is linked to the increase in soil pH caused by biochar application. *Bacillus* species have a variety of ecological functions that either directly or indirectly promote plant growth. This includes nitrogen fixation, phosphorus, potassium, and other nutrient solubilisation and mineralisation, phytohormone production, produce siderophores and abiotic stress tolerance. In addition, they are the most widely used microbial groups for bioremediation and biological pathogen and pest control (Saxena et al., 2020).

The variation in the abundance of Proteobacteria, Acidobacteria and Firmicutes in the Fernwood soil was largely explained by the biochar application rates and soil pH, as these phyla were increased at the highest PLB and ACB (Fig 5.3). Proteobacteria includes most bacteria that are responsible for nitrogen fixation and their growth is best at pH range of 6.5 to 7.0. On the other



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hand, Acidobacteria are responsible for biogeochemical cycling of carbon and most abundant in soils with pH less than 6, but rare or absent in soils with pH greater than 6.5 (Lehmann et al., 2011). The soil pH in the Fernwood ranged from 4.1 to 5.7-6.9 on average, while the Griffin ranged from 5.5 to 6.4-7.2 in PLB treatments and 6.6-6.9 in ACB treatments, and the Pinedene ranged from 3.8 to 4.2-5.4 in PLB treatments and 4.1-4.4 in ACB treatments. Firmicutes were more abundant in the Fernwood soil in all biochar treatments, similar to Acidobacteria, whereas Chloroflexi were more abundant in the Pinedene soils. This suggests that the application of biochar in the Fernwood soil was ideal to influence changes in soil properties, thus providing habitat and a favourable rhizospheric environment for growth and abundance of Proteobacteria, Acidobacteria and Firmicutes more than the other soils.

The decline in relative abundance of Actinobacteria in this study is in contrast with previous studies that reported higher abundance of Actinobacteria due to biochar application (Nguyen et al., 2018, Nielsen et al., 2014, Sheng and Zhu, 2018). Actinobacteria are often associated with the degradation of recalcitrant polymers and are therefore considered to be ecologically significant in soil organic matter turnover and grow best in the pH range of 5.0 to 5.5 (Khodadad et al., 2011). On average, Griffin soil had a maximum pH of 7.2 and 6.9, while Pinedene soil had a pH of 5.4 and 4.4, respectively, when 2% PLB and ACB was applied (Fig 4.1a & b). This implies that due to higher pH in the Griffin soil and less pH than their requirement in the Pinedene soil, the growth and abundance of Actinobacteria was limited. The difference of these results with previous findings (Nguyen et al., 2018, Nielsen et al., 2014, Sheng and Zhu, 2018) is more related to the soil texture as the diversity and community composition of bacteria was more in the sandy textured Fernwood soils.

Despite the negative response of the phylum Actinobacteria to biochar application, when 2% ACB was applied, the genera *Conexibacter* with species belonging to the Acidobacteria were more abundant in the Pinedene soils. This is interesting because the pH of the soil at this rate was 4.4, which is lower than the pH of Actinobacteria. *Conexibacter* is a relatively unknown genus, with only two species (*C. woesei and C. arvalis*) discovered in soil metagenomic studies. *Conexibacter* is an intriguing genus because it is thought to contribute to carbon cycling in soil environments and has the potential to contribute to nitrogen cycling by reducing nitrate. They may play a significant role in bioremediation in the future if more research is done on these genera (Hu et al., 2014).



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### 5.5.3 Biochar application changes soil chemical properties, affecting relative abundance of bacteria communities

Biochar application has been shown in recent studies to have an impact on the microbial community composition (Cole et al., 2019, Liu et al., 2017, Yao et al., 2017), and similar findings were reported in this study. The PCA analysis showed a clear association between bacteria and soil chemical properties as affected by biochar type and application rates in different soil type. The significant change in soil pH, P and K with biochar application in Fernwood soil favoured the abundance of Proteobacteria, Acidobacteria and Verrucomicrobia. The significant positive correlation between soil pH, P and K with Proteobacteria, Acidobacteria, and Verrucomicrobia has supported this shift (Fig 5.3). In a previous study, soil pH variation due to the application of rice straw biochar contributed to 56.5 % of the total bacterial change variation in Acrisol (Xu et al., 2014). Similarly, a significant positive correlation between taxa diversity and pH has also been identified due to biochar application (Jenkins et al., 2017). Other soil parameters such as nitrate, total N and total C contributed to the variation in relative abundance of Firmicutes, Chloroflexi, Patescibacteria, Bacteroidetes and Cyanobacteria (Fig 5.3).

The results are consistent with previous findings in which the application of biochar improved soil properties and thus affected the composition of the soil microbial community, primarily in the acidic and sandy soils (Han et al., 2017, Nguyen et al., 2018, Xu et al., 2016). However, the present study has shown for the first time, the response of bacterial communities at phylum level when biochar is applied to soils such as the Fernwood, Griffin and Pinedene. Contrary to the Fernwood soil, the increase in soil pH, P and K reduced the abundance of Actinobacteria and Gemmatimonadetes. However, soil P, K and nitrate resulted in greater abundance of Firmicutes and Chloroflexi in the Griffin soil. The variation in the relative abundance of bacteria in the Griffin soil was more related to the types of biochar feedstock because the effect was greater in the ACB than in soils amended with PLB (Fig 5.4). This is because the application of 1% ACB resulted in the highest abundance of Firmicutes and Chloroflexi in the Griffin soil. Pinedene soil, on the other hand, showed a higher relative abundance of Proteobacteria, which correlated positively with the C/N ratio.

Soil pH has always been shown to be a key variable in shaping the community of soil bacteria (Jenkins et al., 2017). In this study, however the largest variation in the relative abundance of bacteria community composition was explained by soil pH, P, K, total N and total C, contributing 10-12 % in the Fernwood soil, while K and total C contributed 10-13 % in the Griffin soil and



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ultimately nitrate contributed 12 % in the Pinedene soil and 14 % in the Griffin soil (Fig 5.3-5.5). This shows that the study's hypothesis was true, because the shift in the composition of the bacteria community was indeed affected by improved soil pH and other nutrients due to biochar application, but the effect was soil-specific and depended on the type of biochar and the application rate. Although biochar is regarded as a significant material to improve soil quality by enhancing soil physical-chemical properties, consequently improving microbial abundance, diversity and activity, there is still uncertainties with regards to the feedstock type (Anderson et al., 2011, Chen et al., 2015, Hu et al., 2014, Kolton et al., 2011) and application rates in various soils (Gao et al., 2017, Gomez et al., 2014, Noyce et al., 2015, Sun et al., 2016). The results of this study were essentially consistent with previous findings (Senbayram et al., 2019, Sheng and Zhu, 2018, Xu et al., 2016, Yao et al., 2017), which reported a significant shift in bacterial communities due to improved soil pH, P, K, total C and total N with biochar application. A recent study by Fan et al. (2020), showed that soil pH, total N, C/N, total C, total K, available N, and available K were all closely related to bacterial community composition and were correlated to biochar application, suggesting that biochar amendment changed bacterial community composition indirectly via changes in soil properties.

### 5.6 Conclusion

Changes in soil chemical properties due to biochar application affected the community composition and abundance of bacteria in this study and the effect depended on the type of soil, type of biochar applied and application rate. Application of PLB and ACB in the coarse sandy textured Fernwood soil at 1 and 2% resulted in greater abundance of Proteobacteria, Acidobacteria, Firmicutes. However, application of 1% ACB in the Fernwood and Griffin soils appeared to be ideal to increase the genera *Bacillus, Bryobacter, Microvirga, Conexibacter and Bradyrhizobium* whose species are most likely to contribute in C and N cycling. On the contrary, when 2% PLB was applied, both the clay loam textured, Griffin and sandy clay loam, Pinedene soil showed greater abundance of Proteobacteria, while Acidobacteria and Chloroflexi were less abundant with higher levels of biochar application. In addition to soil pH, improved soil parameters such as K, P, total C, total N and nitrate significantly contributed to the variation in the composition of bacteria community in this study. However, a major contributing factor in the decrease of Actinobacteria was a change in soil pH with biochar application. Considering the short-term nature of this experiment, further research is needed to explore the relationship between the physicochemical properties of different soils and the long-term diversity of bacteria because biochar sequestration is long-term.



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# CHAPTER 6

# BIOLOGICAL $N_2$ FIXATION, C ACCUMULATION AND WATER-USE EFFICIENCY ($\delta^{13}C$) OF CHICKPEA GROWN IN THREE DIFFERENT SOIL TYPES: RESPONSE TO ADDITION OF BIOCHAR FROM POULTRY LITTER AND ACACIA

## Abstract

The potential benefits of biochar that results in enhanced biological nitrogen fixation (BNF) in tropical soils is not fully understood. Therefore, this study assessed the efficacy of biochar derived from poultry litter (denoted as PLB) and acacia (denotated as ACB) feedstocks on BNF, C accumulation and water-use efficiency ($\delta^{13}C$) of chickpea grown in three contrasting soils of Fernwood (Arenosol), Pinedene (Gleyic Acrisol), and Griffin (Helvic Acrisol). The biochars were applied at the rate of 0.5, 1 and 2% (w/w) with a control (0%) and replicated four times. The $^{15}N$ and $^{13}C$ natural abundance technique was used to quantify BNF, C accumulation and WUE. Chickpeas grown at 2% PLB in Griffin soil produced more biomass, accumulated more N and C, and derived more N from the atmosphere, resulting in higher total N-fixed of 90 mg N/plant. Due to low biomass, total N-fixed decreased by 49% when ACB was applied, despite high nodulation and high amounts of N derived from the atmosphere. Chickpea N fixation and C accumulation were increased by the addition of 0.5% PLB and ACB in the Fernwood soil and 2% PLB and ACB in the Pinedene soil. Chickpeas used less water in all three soils when 2% ACB was applied, due to higher N accumulation rather than biomass produced. The dependence of chickpea on soil N was enhanced by the decrease in total N-fixed in the Griffin and Fernwood soils, while due to an increase in total N-fixed in the Pinedene soil. The significant increase reported for BNF, C accumulation and WUE are positively correlated with improved soil pH, availability of P, K and Mg and shoot N content when biochar was applied. Application of PLB has the potential to improve BNF and C accumulation, while ACB could enhance WUE of legumes grown in low fertile soils, but the effect could be soil specific as observed in this study.

Keywords: biochar, biomass, chickpea, nitrogen fixation, nodulation, nutrient availability



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## 6.1 Introduction

The agronomic added value of leguminous plants in cropping systems lies in their ability to fix atmospheric nitrogen through the biological nitrogen fixation process (BNF), thus reducing the use of costly nitrogen fertilizer in enhancing soil fertility (Hiama et al., 2019). Nitrogen contributed to soils through BNF is considered as a cheap, sustainable and environmentally-friendly compared to that supplied by synthetic N-fertilizers. However, the sustainability of BNF depends on factors such as the soil type, fertility of the soil, association between host plant and rhizobia, as well as the potential influence of and presence or absence of inputs such as fertilizers or biofertilizers in the establishment of the symbiotic legumes (Mpai and Maseko, 2018). Studies have shown that the dependence on $N_2$ fixation and total N-fixed is generally lower when legumes are grown in nutrient-deficient soils and largely high in fertile cropping fields (Güereña et al., 2015, Hiama et al., 2019, Mohale et al., 2014). This means that nutrient availability is proportional to nitrogen fixation in legumes.

Biological $N_2$ fixation may be improved by applying lime to reduce soil acidity (Hiama et al., 2019, Khan et al., 2020, Rondon et al., 2007) and fertilizers to reduce key nutrient deficiencies such as phosphorus and by enhancing the potential of rhizobia-legume symbiosis (Thies et al., 2015). Biochar technology has been proposed as an alternative strategy to improve soil edaphic properties (Mia et al., 2014, Rondon et al., 2007), enhance soil biological processes (Lehmann and Joseph, 2015) and crop performance in other countries including South Africa (Lusiba et al., 2017, Lusiba et al., 2018, Macil et al., 2017, Sika and Hardie, 2014). Evidence that the application of biochar influences the symbiotic performance of legumes and BNF has been provided (Hiama et al., 2019, Khan et al., 2020, Mia et al., 2018, Rondon et al., 2007). In addition, studies have shown that biochar is an excellent support material for Rhizobium inoculants (Macil et al., 2017, Quilliam et al., 2013).

The possible reasons behind the observed effect of biochar on BNF include: increased availability of macro-and micro-nutrients such as P, B and Mo in soils (Mia et al., 2014, Rondon et al., 2007, Tagoe et al., 2008). Biochar's liming potential may alter soil pH and have a positive or negative effect on BNF (Khan et al., 2020, Lehmann et al., 2011). Changes in nutrient cycling processes such as N-mineralisation or immobilisation may increase or decrease the N-fixing (Bruun et al., 2011, Güereña et al., 2015, Nelissen et al., 2012).Increased nodulation (Ogawa and Okimori, 2010, Tagoe et al., 2008) and biochar adsorptive capacity, which may enhance signaling for nodulation by adsorption of flavonoids and nod-factors (Lehmann and Joseph, 2009b, Lehmann et al., 2011). Available biochar compounds such as volatile matter (VM) may stimulate or reduce



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soil microbial activity, thereby affecting the amount of N-fixed by legumes (Lehmann and Joseph, 2009b).

Although these studies have shown that BNF is enhanced by biochar application, it is still unclear why BNF increases with biochar application. This is because the reported results (Güereña et al., 2015, Mia et al., 2014, Quilliam et al., 2013, Rondon et al., 2007) are contradictory and inconclusive. In addition, the reported results indicated a variation in BNF at different biochar application rates. For example, increasing biochar application rates from 50 t/ha to 100 t/ha increased BNF by red clover (Tagoe et al., 2008). Mia et al. (2014) reported an increase in BNF by red clover at 10 t/ha biochar application rates, but BNF was reduced at higher levels of 50 and 120 t/ha. Quilliam et al. (2013) reported a reduction in nodulation of red clover with elevated application rates of 25 and 50 t/ha, even though nitrogenase activity remained unchanged. Güereña et al. (2015) reported higher BNF by common bean at 15 t/ha biochar application.

This clearly demonstrate that the reasons/mechanism associated with the change in BNF with biochar application remains hypothetical and further studies are crucial for a better understanding of the relationship between biochar properties and BNF. Generally, the knowledge about the effect of biochar (especially from poultry litter) and the dependence of grain legumes such as chickpea on $N_2$ fixation and their N contribution under semi-arid environment is very limited and there is no published literature currently. Investigation of such a research idea could reveal whether there is a dynamic and process interdependency between the grain legumes particularly chickpea and N-fixed through atmospheric $N_2$ fixation. Moreover, it is also important to understand whether N from biochar and symbiotic chickpea contributes to the N economy of different types of soils.

Chickpea (*Cicer arietinum L*.) is the third widely grown legume in the world but it is under researched and under-utilized in African countries (FAO, 2014, Mpai and Maseko, 2018). Chickpea was introduced in South Africa a decade ago, particularly in the provinces of Limpopo and Mpumalanga. However, poor nodulation has been reported, partly due to a lack of, or insufficient populations of effective native rhizobia in the soil, despite the use of biochar derived from woody feedstock (Lusibisa, 2015, Macil et al., 2020, Ogola, 2015). Therefore, there is an urgent need to better understand how biochar produced from different feedstocks affects BNF of legumes to draw more reliable conclusions about its use. Moreover, the majority of relevant studies used biochar derived from plant-based feedstocks, with no studies using animal litter as a feedstock. Furthermore, data on the effects of biochar derived from various feedstocks such as poultry litter on BNF in tropical soils is still lacking. It is worth to understand that biochar



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characteristics produced from a variety of feedstocks and production conditions vary widely depending on the soil type (Enders et al., 2012, Kloss et al., 2012, Singh et al., 2010) . The efficacy of biochar to improve nitrogen fixation, C accumulation and WUE by chickpea is scarcely studied, despite its positive effect on soil management and crop production.

The inclusion of legumes such as chickpea in the current cropping systems not only provides protein nutrition, but could also improve soil fertility and reduce farmers' dependence on commercial inorganic fertilizers through its nitrogen fixing ability, as the crop obtains 80% of its N requirement through BNF (Biabani et al., 2011). Although, chickpea is generally regarded as being drought tolerant, possible strategies to improve nitrogen fixation by ensuring adequate mineral nutrition are essential for the crop. Similar to BNF, carbon nutrition in plants is affected by soil nutrient status and water deficit as these factors can alter $CO_2$ fixation and overall plant growth. However, in dry environment where water is limiting, plant species with high water-use efficiency are preferred to those with low water-use efficiency (Mohale et al., 2014).

The objective of this study was therefore, to assess the potential impact of biochar derived from poultry litter and acacia on biological $N_2$ fixation, C accumulation and WUE of chickpea grown in three contrasting soils differing in texture and fertility. We hypothesized that (i) biochar derived from poultry litter and acacia feedstocks would improve BNF, C accumulation and WUE by chickpea due a change in rhizospheric properties of the contrasting soils and thus improve biomass production. (ii) Depending on the type of biochar feedstock used and application rates, N fixation, C accumulation and WUE by chickpea will vary amongst the contrasting soils. Furthermore, (iii) due to a change in rhizospheric soil properties caused by biochar application at different rates, there is a linear relationship between the produced biomass and the total N-fixed, C accumulation and WUE of chickpea, but this could depend on the type of soil, biochar feedstock and application rates.

### 6.2 Materials and methods

The biochar, soil and treatments used for this study was the same as in chapter 3 and 4. See section 3.2, in chapter 3 for biochar preparation and characterization, and section 4.2 in chapter 4 for description of soil and analysis.

#### 6.2.1 Experimental setup and design

The experiment consisted of three types of soils namely Fernwood, Pinedene and Griffin; two types of biochars, poultry litter (PLB) and acacia (ACB) as well as four application rates which



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were 0% (control), 0.5%, 1% and 2% w/w). The treatments were arranged in a completely randomized design with four replicates. Pots with a diameter of 25 cm and height of 25 cm were filled with 4 kg of air-dried sieved soil. The poultry and acacia biochar were each applied according to the stated application rates and thoroughly mixed with the soil. Starter superphosphate fertilizer (10.5% P) was applied uniformly in all pots at approximately 2 g P/pot (equivalent to 60 kg P/ha). Prior to planting, the pots were watered to 60% field capacity. Chickpea was used as a test crop. Approximately 450 g seeds of desi cultivar were inoculated with bradyrhizobium *spp cicer* containing 5 x $10^8$ bacterial cells per gram and soaked in a mixture of 2.25 g bradyrhizobium powder and planted immediately. Four seeds were planted into each pot. Ten days after emergence (DAE), the plants were thinned to two plants per pot. Every three days, the pots (pot + soil mixture + water) were weighed and watered when necessary to targeted 60 % field capacity. Non-$N_2$-fixing crop amaranthus (*Amaranthus retroflexus)* was used as a reference crops to estimate the percentage of N derived from the atmosphere (%Ndfa) by chickpea. The amaranthus was planted using the same soil and biochar treatments.

### 6.3 Data collection

#### 6.3.1 Postharvest plant and soil analysis

At flowering stage, approximately 65 days after emergence, chickpea and reference crops amaranthus were harvested and each separated into shoot and root. Approximately 20 g of rhizosphere soil was shaken from the root and transferred to sampling bags. The roots were placed in paper sample bags and transferred to the laboratory where the chickpea roots were washed with tap water. Thereafter, nodules were detached from the roots, counted and oven-dried at 60$^o$C for 48 hours for nodulation assessment. Shoot and root samples of chickpea and amaranthus were oven-dried at 65 °C for 48 hours to a constant weight and then weighed to determine shoot and root dry weights. The dried chickpea and amaranthus shoot samples were immediately ground to fine powder. The samples were stored in a tight zip lock plastic bag and taken for analysis of $^{15}N/^{14}N$ and $^{13}C/^{12}C$ isotopes as well as shoot N concentration using the 1 M HCl digestion method and the inductively couple plasma spectrophotometer.

#### 6.3.2 Analysis of shoot $^{15}N/^{14}N$ isotope

The natural abundance$^{15}N$ analysis allows the identification of the source of nitrogen present in legumes, and the proportion that it derived from the atmosphere (through BNF), respectively, from the soil N pool (Unkovich et al., 2008). Finely ground plant materials were analysed for isotope



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$^{15}N$ and $^{13}C$ composition at the stable light isotope unit, University of Cape Town. Approximately 1.2 mg samples of both chickpea and amaranthus was weighed into tin capsules, and analysed for %N and $^{15}N/^{14}N$ ratio using a Carlo Erba NA1500 elemental analyser (Fisons Instruments SpA, Strada, Rivoltana, Italy) coupled to a Finnigan MAT252 mass spectrometer via Conflo II open-split device. The $^{15}N$ natural abundance is expressed as δ (delta) notation expressed as the deviation of the $^{15}N$ natural abundance of the sample from atmospheric (atm) $N_2$ (0.36637 atom % $^{15}N$). The isotopic composition ($\delta^{15}N$) was calculated as described by Unkovich et al. (2008) using equation 1. This allowed calculation of the %N derived from the atmosphere (%Ndfa; i.e. the portion of N obtained through BNF).

$$\delta^{15}N(‰) = \frac{\left[^{15}N/^{14}N\right]_{sample} - \left[^{15}N/^{14}N\right]_{standard}}{\left[^{15}N/^{14}N\right]_{standard}} \times 1000 \quad (1)$$

where $^{15}N/^{14}N_{sample}$ is the abundance ratio of $^{15}N$ and $^{14}N$ of the chickpea plant or reference crop amaranthus; while $^{15}N/^{14}N_{standard}$ is the abundance ratio of $^{15}N$ and $^{14}N$ in the atmosphere.

**Percent N derived from the atmosphere (%Ndfa)**

The percentage of N derived from symbiotic nitrogen fixation from atmospheric $N_2$ was estimated using the equation (Shearer and Kohl, 1988, Unkovich et al., 2008):

$$\%\,Ndfa = \frac{\delta^{15}N_{ref} - \delta^{15}N_{leg}}{\delta^{15}N_{ref} - B} \times 100 \quad (2)$$

where, $\delta^{15}N_{ref}$-is the mean value of the $^{15}N$ natural abundance of non-fixing reference plants (amaranthus) dependent on soil N; $\delta^{15}N_{leg}$,-is the $^{15}N$ natural abundance value for the $N_2$-fixing chickpea crop; and B-the $^{15}N$ natural abundance of chickpea plants depending solely on $N_2$ fixation for their N nutrition. The B value used in this study was -2.00 (Unkovich et al., 2008).

**Shoot nitrogen content-** was determined as the product of shoot N concentration (% N) and shoot dry weight (mg/plant) of chickpea plant as described by (Pausch et al., 1996)

Shoot N (mg N/plant) = (% N x shoots dry weight) /100 (3)

**Total N-fixed-** was calculated as described by (Maskey et al., 2001)

N-fixed (mg N/plant) = %Ndfa x shoot N (4)

**Soil N uptake**- was calculated by computing the difference between shoot N and N-fixed.



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Soil N uptake (mg N/plant) = Shoot N – total N-fixed 5

### 6.3.3 Analysis of shoot $^{13}C/^{12}C$ isotope

Studies have shown that $\delta^{13}C$ can be used as an indicator of water-use efficiency especially in $C_3$ plants (Farquhar et al., 1989). Low $^{13}C$ discrimination (less negative $\delta^{13}C$ values) implies greater water-use efficiency during photosynthesis, while high $^{13}C$ discrimination (more negative $\delta^{13}C$ values) implies low water-use efficiency. This forms the basis for using shoot $\delta^{13}C$ as a tool for measuring water-use efficiency in chickpea as a $C_3$ plant. Shoot samples of chickpea of approximately 3 mg were weighed into a tin capsule and run on a mass spectrometer, as described for $^{15}N/^{14}N$ isotopic ratio. The ratio of $^{13}C/^{12}C$ in each sample was used to calculate the $^{13}C$ natural abundance or $\delta^{13}C$ (‰) as described by (Farquhar et al., 1989).

$$\delta^{13}C = \left[ \frac{\left(^{13}C/^{12}C\right)_{sample}}{\left(^{13}C/^{12}C\right)_{standard}} - 1 \right] \times 1000 \quad 6$$

where $^{13}C/^{12}C_{sample}$ is the isotopic ratio of the chickpea sample, and $^{13}C/^{12}C_{standard}$ (0.0112372), is the isotopic ratio of PDV, a universally accepted standard from Vienna Pee-Dee Belemnite (Craig, 1957). The carbon concentration (%C) per plat was obtained directly from mass spectrometric analysis. The shoot C content/plant was calculated as the product of C concentration (% C) and shoot dry matter weight (mg/plant).

Shoot C (mg C/plant) = (% C x shoots dry weight) /100 7

### 6.3.4 Statistical analysis

The data was analysed using R software (version 3.5.2). Generalised Linear Mixed Model analysis of variance was used to test the treatment effects and their interactions on shoot, N content, $\delta^{15}N$, Ndfa, N-fixed, C content, and $\delta^{13}C$ variables. Soil type, biochar feedstock type and application rates were considered as fixed factors while the variables were considered as random factors. Tukey's honest significant difference (HSD) test was used for treatment mean separations with the threshold probability level set at $P \leq 0.05$. The Pearson's correlation analysis was performed using Minitab version 19 to assess the correlation between soil and chickpea biomass, BNF attributes and C accumulation of chickpea. Principal component analysis (using XLSTART software) was done to assess the variability and relationship that exist among the biomass, BNF



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attributes and C accumulation of chickpea with the biochar treatments and soil types. This was followed by plotting the graphs and significant linear regression of N-fixed with shoot dry weight, C content and N content using Sigmaplot 14.

## 6.4 Results

### 6.4.1 Effect of biochar on nodulation and BNF attributes of chickpea

Chickpea grown in the Fernwood soil had higher nodulation irrespective of PLB or ACB application, with a higher number of nodules and nodule dry weight at 0.5 and 2% (treatments FPL0.5, FAC1 and FAC2), respectively. In treatments GAC1 and GAC2, chickpea produced more nodules and had a higher nodule dry weight. In the Pinedene soil, however, applying PLB or ACB at different rates had no effect on the number of nodules or the dry weight of nodules (Table 6.1). Chickpea shoot $\delta^{15}N$ values varied when PLB and ACB was applied in the Fernwood soil, with chickpea grown in ACB treatments having the lowest values. In the Griffin and Pinedene soils, however, chickpea shoot $\delta^{15}N$ did not differ depending on whether PLB or ACB was applied. Application of PLB and ACB had a significant effect on %Ndfa of chickpea grown in the Fernwood and Griffin soils. In the Fernwood soil, %Ndfa of chickpea ranged from 3-21% in PLB treatments and 22-30 % in ACB treatments, whereas in the Griffin soil, %Ndfa by chickpea ranged from 31-42% and 26-30 % in PLB and ACB treatments, respectively (Table 6.1). On the contrary, PLB and ACB application had no effect on the %Ndfa of chickpea grown in Pinedene soil. In all the soil types, total N-fixed and soil N uptake by chickpea varied in all PLB and ACB treatments.

Chickpea N fixation decreased from 14 to 1 mg N/plant as PLB levels increased, whereas soil N uptake increased from 64 to 81 mg N/plant in the Fernwood soil, while treatment FPL0.5 had higher total N-fixed than the other treatments. The total N-fixed in ACB treatments ranged from 18 to 37 mg N/plant, with treatment FAC0.5 having the most N-fixed. In contrast, chickpea grown in the Griffin soil fixed more N, resulting in lower soil N uptake when PLB was applied, and the opposite was possible when ACB was applied. Chickpea grown in treatment GPL2 fixed the most N (90 mg N/plant), whereas chickpea grown in treatment GAC2 only fixed 17 mg N/plant. When 1% of either PLB (treatment GPL1) or ACB (treatment GAC1) was applied, soil N uptake decreased from 52 to 40 mg N/plant and increased from 52 to 137 mg N/plant, respectively. Conversely, the total N-fixed and soil N uptake of chickpea grown in Pinedene soil was higher in PLB treatments than ACB treatments. However, the total N-fixed and soil N uptake followed a similar pattern, treatments with the most N-fixed having higher soil N uptake. In PLB treatments,



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the average N-fixed was 53 mg N/plant and soil N uptake was 299 mg N/plant, while in ACB treatments, N-fixed and soil N uptake were 43 and 156 mg N/plant, respectively (Table 6.1). Similarly, chickpea shoot N concentration and uptake were higher in treatments with high N-fixed, especially at 2% PLB or ACB (treatment PPL2 and PAC2).

**Table 6.1:** Effect of PLB and ACB biochar at different rates on nodulation, N uptake, and total N-fixed of chickpea grown in the Fernwood, Griffin and Pinedene soils.

| Treatments | Nodule no/plant | Nodule Dwt (mg/plant) | $\delta^{15}N$ (‰) | Ndfa (%) | Total N-fixed (mg N/plant) | Soil N uptake (mg N/plant) | N concentration (%) | N content (mg/plant) |
|---|---|---|---|---|---|---|---|---|
| **Fernwood soil** | | | | | | | | |
| FC0 | 28c | 93b | 7.5b | 18.3b | 14.0b | 64.4 | 1.2 | 78.4 |
| FPL0.5 | 55a | 130a | 7.5ab | 21.3b | 15.3b | 53.4 | 1.2 | 87.9 |
| FPL1 | 1e | 2.5b | 8.8a | 6.7c | 6.0c | 78.2 | 1.3 | 68.7 |
| FPL2 | 3e | 7.5b | 9.6a | 2.8c | 0.5c | 81.9 | 1.7 | 78.7 |
| FAC0.5 | 3e | 5b | 2.6c | 30.9a | 36.7a | 48.4 | 1.4 | 70.1 |
| FAC1 | 13d | 15b | 6.8ab | 25.7ab | 25.3ab | 71.5 | 1.3 | 92.1 |
| FAC2 | 60a | 170a | 7.1b | 22.3ab | 17.6c | 50.3 | 1.5 | 67.0 |
| **Griffin soil** | | | | | | | | |
| GC0 | 21b | 100b | 6.0 | 36.3b | 29.5c | 52.3b | 1.1 | 88.5 |
| GPL0.5 | 13b | 60b | 6.0 | 32.4c | 70.8b | 53.1b | 1.3 | 168.4 |
| GPL1 | 19b | 80b | 6.8 | 31.6c | 71.5b | 40.1b | 1.5 | 184.8 |
| GPL2 | 10b | 40b | 6.4 | 42.2a | 89.6a | 50.3b | 1.7 | 183.4 |
| GAC0.5 | 13b | 61b | 6.0 | 30.8c | 22.9bc | 116.1a | 1.4 | 78.1 |
| GAC1 | 55a | 230a | 6.0 | 26.4c | 21.3c | 137.4a | 1.2 | 57.4 |
| GAC2 | 29ab | 100b | 4.8 | 30.6c | 17.9b | 126a | 1.1 | 86.8 |
| **Pinedene soil** | | | | | | | | |
| PC0 | 3 | 5 | 7.0 | 23.6 | 39.1b | 126.4b | 2.9c | 158.9b |
| PPL0.5 | 5 | 5 | 7.8 | 16.5 | 52.3ab | 324.5a | 2.7c | 395.5a |
| PPL1 | 1 | 2 | 7.2 | 22.6 | 47.4ab | 259.1a | 2.8c | 330.6a |
| PPL2 | 1 | 2 | 7.2 | 19.9 | 57.4a | 313.5a | 3.0c | 403.5a |
| PAC0.5 | 1 | 2 | 7.4 | 20.0 | 37.8c | 143.3b | 2.9c | 181.2b |
| PAC1 | 1 | 2 | 7.5 | 20.9 | 41.8b | 174.3b | 3.4b | 216.1b |
| PAC2 | 2 | 5 | 7.0 | 23.4 | 47.5ab | 154.1b | 3.7a | 201.7b |

Values with dissimilar letters in a column the means are significant different at $P < 0.05$
PLB- Poultry litter biochar; ACB- Acacia biochar; Dwt- Dry weight



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### 6.4.2 Effect of biochar application on $\delta^{13}C$, C content and C/N ratio of chickpea

The effect of biochar on water-use efficiency ($\delta^{13}C$) and C accumulation (shoot C content and C/N ratio) of chickpea in different soil types is presented in (Table 6.2). When PLB and ACB were applied at different rates in the Fernwood, Griffin, and Pinedene soils, the values of shoot $\delta^{13}C$, C accumulation, and shoot C/N ratio varied (Table 6.2). In all soil types, shoot $\delta^{13}C$ values were lower and more negative in PLB treatments, but higher and less negative in ACB treatments. Higher levels of PLB resulted in higher shoot $\delta^{13}C$ in Fernwood soils, but at 2% ACB shoot $\delta^{13}C$ increased to -28.7 ‰ (treatment FAC2) when compared to the control (treatment FC0). Similarly, in the Griffin and Pinedene soils, chickpea shoot $\delta^{13}C$ values decreased with increased PLB levels and increased with increased ACB levels, with the highest shoot $\delta^{13}C$ value at in treatments GAC2 (-28.3 ‰) and PAC2 (-27.8 ‰), respectively. Chickpeas grown with PBL and ACB at different rates had no change in shoot C concentration as well as shoot C/N ratio in the Fernwood soil. When PLB and ACB were applied at different rates in the Griffin and Pinedene soils, however, shoot C content and C/N ratio of chickpea were significant. Shoot C content of chickpeas grown in the Griffin and Pinedene soils was higher at 0.5% PLB (GPL0.5 and PPL0.5) and ACB (GAC 0.5 and PAC0.5) treatments, but decreased in all PLB and ACB treatments, with higher biochar application in both soils. Similarly, in PLB treatments in the Fernwood soil and ACB treatments in the Pinedene soil, the shoot C/N ratio of chickpea were lower than the control treatments, but shoot C/N values decreased with higher levels of biochar application. On the other hand, chickpea shoot C/N ratios were higher in ACB treatments in the Griffin soil and in PLB treatments in the Pinedene soil than the control treatment, but C/N values decreased with higher ACB application rates in both soils (Table 6.2).



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**Table 6.2:** Effect of PLB and ACB on shoot C concentration, C content, C/N ratio, and Water-use efficiency (WUE) of chickpea in the Fernwood, Griffin and Pinedene soils.

| Treatment | $\delta^{13}C$ | C concentration (%) | C content (mg C/plant) | C/N ratio (g/g) |
|---|---|---|---|---|
| **Fernwood soil** | | | | |
| FC0 | -29,9ab | 41,1 | 1774 | 43,3 |
| FPL0.5 | -29,7ab | 40,9 | 2711 | 36,9 |
| FPL1 | -30,0ab | 40,3 | 2744 | 39,9 |
| FPL2 | -30,1c | 39,7 | 2075 | 29,79 |
| FAC0.5 | -29,2b | 41,1 | 2921 | 34,2 |
| FAC1 | -29,4ab | 40,5 | 3017 | 30,9 |
| FAC2 | -28,7a | 40,7 | 2777 | 32,3 |
| **Griffin soil** | | | | |
| GC0 | -29,6ab | 41,1 | 2404bc | 26,9b |
| GPL0.5 | -29,5ab | 42,3 | 6656a | 16,7c |
| GPL1 | -29,3ab | 40,6 | 4430ab | 14,6c |
| GPL2 | -29,6ab | 41,5 | 3751ab | 13,6c |
| GAC0.5 | -30,4c | 40,7 | 2886bc | 38,3ab |
| GAC1 | -29,0b | 40,7 | 2299bc | 31,7b |
| GAC2 | -28,3ab | 40,2 | 1530c | 25,5c |
| **Pinedene soil** | | | | |
| PC0 | -29,1b | 40,8 | 2448c | 29,4b |
| PPL0.5 | -29,9ab | 40,4 | 5136ab | 46,1a |
| PPL1 | -30,4c | 39,6 | 4977ab | 41,42ab |
| PPL2 | -30,4c | 39,5 | 4489ab | 36,4ab |
| PAC0.5 | -28,6ab | 41,4 | 3244bc | 18,6c |
| PAC1 | -28,3ab | 42,7 | 2458c | 17,8c |
| PAC2 | -27,8a | 42,4 | 1635c | 17c |

Values with dissimilar letters in a column the means are significant different at P<0.05
PLB- Poultry litter biochar; ACB- Acacia biochar; C/N ratio by weight



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### 6.4.3 Pearson's Correlation Analysis

Total N-fixed by chickpea positively correlated with shoot dry weight, N content, and soil N uptake, with Parsons' coefficients (r) of 0.79, 0.92, and 0.86, respectively (Table 6.3). Figure 6.1 shows that shoot dry weight contributed to higher N-fixed, with increased shoot biomass and N content resulting in higher N-fixed irrespective of PLB or ACB application (Fig 6.1). On the other hand, PLB application increased shoot dry weight, total N-fixed, and N content, resulting in higher C accumulation in chickpea shoots (Fig 6.2). This was demonstrated by the significant correlation between shoot dry weight with N and C content (Table 6.3). Interestingly, The %Ndfa negatively correlated with shoot $\delta^{15}N$ (r = -0.80) and not with total N-fixed (Table 6.3). In this study, the change in soil properties caused by biochar application directly and indirectly attributed to the increase in total N-fixed. The total N-fixed was significantly correlated with the change in soil P and soil $NO_3^-$, with r values of 0.77 and 0.82, respectively, while Parsons' coefficients (r) for N content were 0.87 and 0.72, respectively. Similarly, shoot dry weight and %Ndfa, correlated significantly with soil $NO_3^-$, while soil N uptake had a strong relationship with soil P and $NO_3^-$ (Table 6.3).



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**Table 6.3**: Pearson's correlation (r) among plant growth, BNF and WUE attributes of chickpea grown.

| Variables | Shoot Dwt mg/plant | % N concentration | N content mg/plant | C content mg/plant | % Ndfa | $\delta^{15}N$ ‰ | Soil N uptake | $\delta^{13}C$ ‰ | C/N ratio g/g |
|---|---|---|---|---|---|---|---|---|---|
| N-fixed | **0.794***** | 0.600*** | **0.920***** | 0.5139* | 0.4187*** | | **0.864***** | | -0.506*** |
| Ndfa | | 0.2090* | 0.3397*** | | | **-0.806***** | | | |
| $\delta^{15}N$ | | | | | **-0.806***** | | | | |
| Nodules No | | -0,3811** | -0,3213** | | | | | | |
| $\delta^{13}C$ | 0.2011* | -0.4253* | 0.696* | | | | | | |
| N content | **0.621***** | 0.5435*** | | | | -0.3252** | | | |
| C content | **0.9921***** | | 0.5204** | | | | | | |
| %C | | | 0.2648*** | | | | 0.490* | | |
| C/N ratio | | -0.871 | -0.5251*** | | | | | 0.5235** | -0.4574* |
| PNUE | | | -0.3835*** | | | 0.6133** | | 0.3067* | 0.2357* |
| Soil N uptake | 0.533* | **0.756***** | **0.985**** | | | | | | **-0.709**** |
| **Variables** | **soil pH** | **soil P** | **Soil $NO_3^-$** | **Soil K** | **Soil B** | **Soil Mo** | | | |
| N-fixed | **0.901***** | **0.775***** | **0.822***** | 0.2041* | -0.697* | | | | |
| N content | | **0.877***** | **0.720***** | 0.246* | | | | | |
| shoot Dwt | | 0.504* | **0.777**** | 0.635 | -0.482* | | | | |
| Ndfa | 0.2678* | -0.5555** | 0.646* | 0.3672*** | 0.2062* | -0.2425* | | | |
| $\delta^{15}N$ | -0.2625* | 0.5117** | | -0.3598*** | -0,1956* | 0,2391* | | | |
| Soil N uptake | | **0.879***** | 0.646* | | | | | | |

Values with asterisk symbol represents significant correlation at *** P<0.001; **P<0.01; * P<0.05;
Bold values represent high correlation, Dwt- Dry weight, C/N ratio by weight



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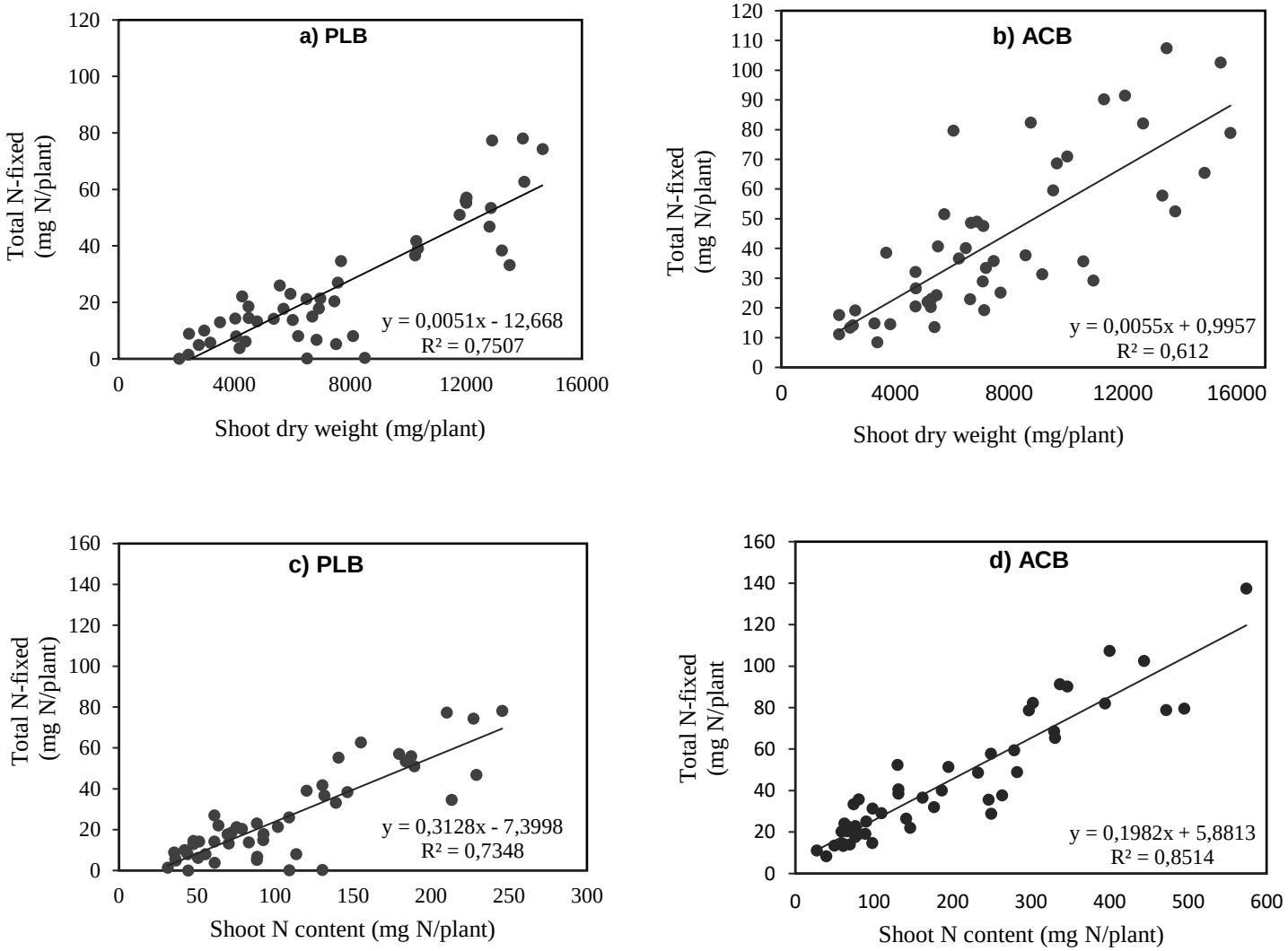


**Figure 6.1:** Relationship between the total N-fixed with shoot dry weight (a &b) and N content (c & d) of chickpea as influenced by PLB and ACB application.



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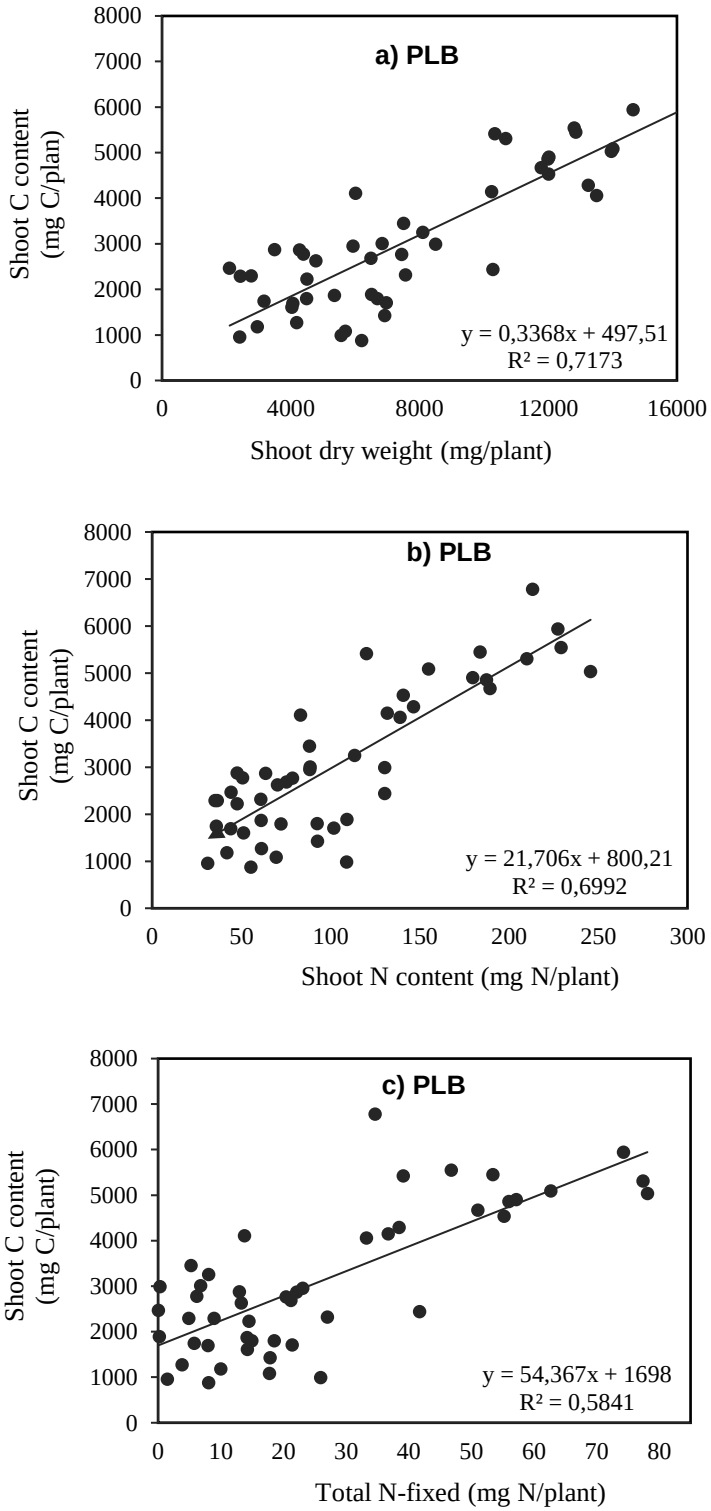


**Figure 6.2**: Relationship between shoot C content and shoot dry weight (a); total N-fixed (b) and shoot N content (c) of chickpea as influenced by PLB and ACB application.



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### 6.4.4 Principal Component Analysis

The biplot of the first two major components (PCs) showing the relationship between between growth, BNF attributes and WUE parameters of chickpea with selected soil properties in the Fernwood, Griffin and Pinedene soils are shown in (Fig 6.3-6.5). The first (F1) and second (F2) components explained 72.07 % of the variability in the Fernwood soil, 71.95 in the Pinedene soil and 70.91% in the Griffin soil with the F1 having the most variability. The Eigenvalues ranged from 11.8-9.07, 14.9-5.9 and 14.9-5.7 for F1 and F2 in the Fernwood, Pinedene and Griffin soils, respectively.

in the Fernwood soil (Fig 6.3), positive loadings of %N, soil N uptake, $\delta^{15}N$, $\delta^{13}C$, soil pH, P, K, Ca, Mg, B and negative loadings of %Ndfa, total N-fixed and %C significantly contributed to the variability in F1, with %Ndfa, total N-fixed, soil pH, P, K, Mg, and B having greater variability above 8 % than the other variables. In the second component (F2), %N, soil $NO_3^-$, and $NH_4^+$ contributed positively to the variability in F2, whereas C content and shoot C/N ratio contributed negatively, with C content, soil $NO_3^-$, and $NH_4^+$ having 9% variability. Total N-fixed had a positive correlation with %Ndfa (r = 0.96), whereas $\delta^{15}N$, soil N uptake, soil pH, P, K, Mg, B had a strong negative correlation with %Ndfa and total N-fixed (r = 0.80 on average).

In the Griffin soil (Fig 6.4), positive loadings of shoot biomass, %N, N content, total N-fixed, soil N uptake, C/N ratio, $\delta^{15}N$, $\delta^{13}C$, soil P, K, Mg and negative loadings of %Ndfa and %C significantly contributed to the variability in F1, with N content, total N-fixed, soil N uptake, C content, soil P, K, and Mg having variability above 5%. Soil pH, Ca, and B all contributed positively to F2 variability, with soil Ca and B having greater variability of 13 %, respectively. Total N-fixed and soil N uptake were strongly correlated positively with shoot biomass, %N, C content, C content, and soil $NO_3^-$, P, K, Mg ($r > 0.75$ on average), whereas %N and $\delta^{15}N$ were negatively correlated with %Ndfa.

In the Pinedene soil, shoot biomass, N content, total N-fixed, soil N uptake, C content, $\delta^{13}C$, soil $NO_3^-$, pH, P, K, Mg negatively contributed, to the variation in F1, but % N, C/N ratio, and soil $NH_4^+$ positively contributed, with N content, total N-fixed, soil N uptake, $\delta^{13}C$, soil pH, P, Mg having the largest variation of greater than 5%. Soil Ca was responsible for 9.8 % of variation in F2. Total N-fixed and soil N uptake had strong positive correlation ($r > 0.85$ on average) with shoot biomass, N content, C content, $\delta^{13}C$, soil $NO_3^-$, P, K, Mg, whereas C/N ratio and $NH_4^+$ had a strong negative correlation ($r > -0.80$ on average), while %Ndfa strongly correlated negatively with $\delta^{15}N$ and C content ($r > -0.75$ on average).

The type of biochar used, rather than the application rates, was largely responsible for the variation in total N-fixed in this study (Fig 6.3-6.5). This is because the proportion of N derived from the atmosphere (%Ndfa) and total N-fixed of chickpea varied with the type of biochar in



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the Fernwood soil (r > 70), whereas the type of biochar contributed negatively to the variation in total N-fixed and soil N uptake in the Griffin and Pinedene soils (r > -70), implying that either PLB or ACB had the largest variation. On the other hand, chickpea C accumulation and WUE ($\delta^{13}C$) varied greatly depending on the type of biochar and application rates. In the Fernwood soil (Fig. 6.3), for example, biochar application rates negatively correlated (r > -0.89) with percent C and C/N, but biochar type was positively correlated (r = 0.81) with % C and negatively correlated (r > - 0.80) with C/N and $\delta^{13}C$ in the Griffin soil (Fig. 6.4), whereas content and $\delta^{13}C$ were negatively correlated (r > -70) with biochar type (Fig 6.5).

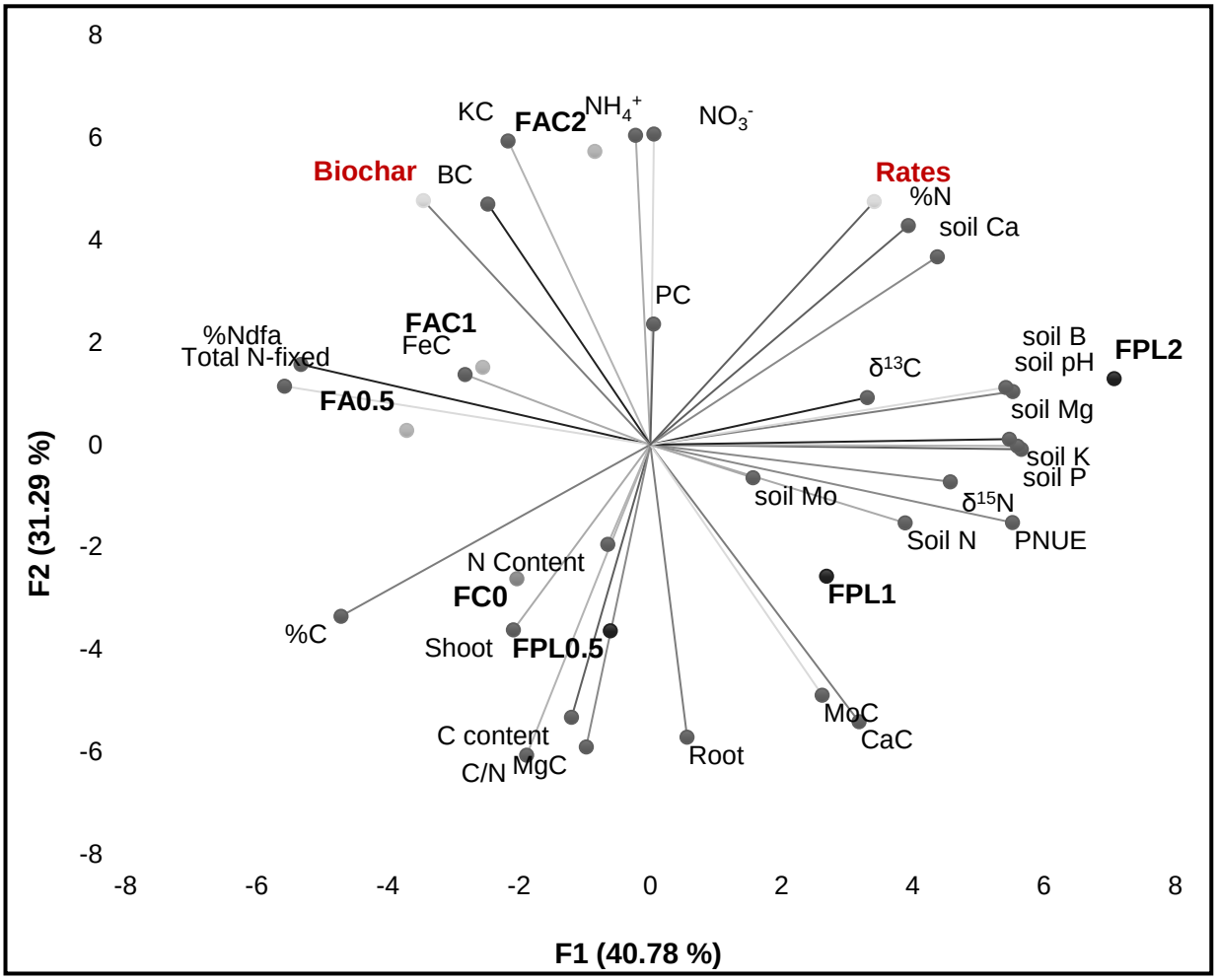


**Figure 6.3**: PCA-biplot illustrating the relationship between growth, BNF attributes and WUE parameters of chickpea with selected soil properties of **Fernwood soil**. The vectors represent the growth, BNF and WUE variable and treatments (soil type, biochar feedstock type and application rates). The direction of the arrow indicates the steepest increase in the variable, and the length indicates the strength relative to the other variables. Bold text represents the treatments: FCO, FPL0.5, FPL1, FAC0.5, FAC1, FAC2.



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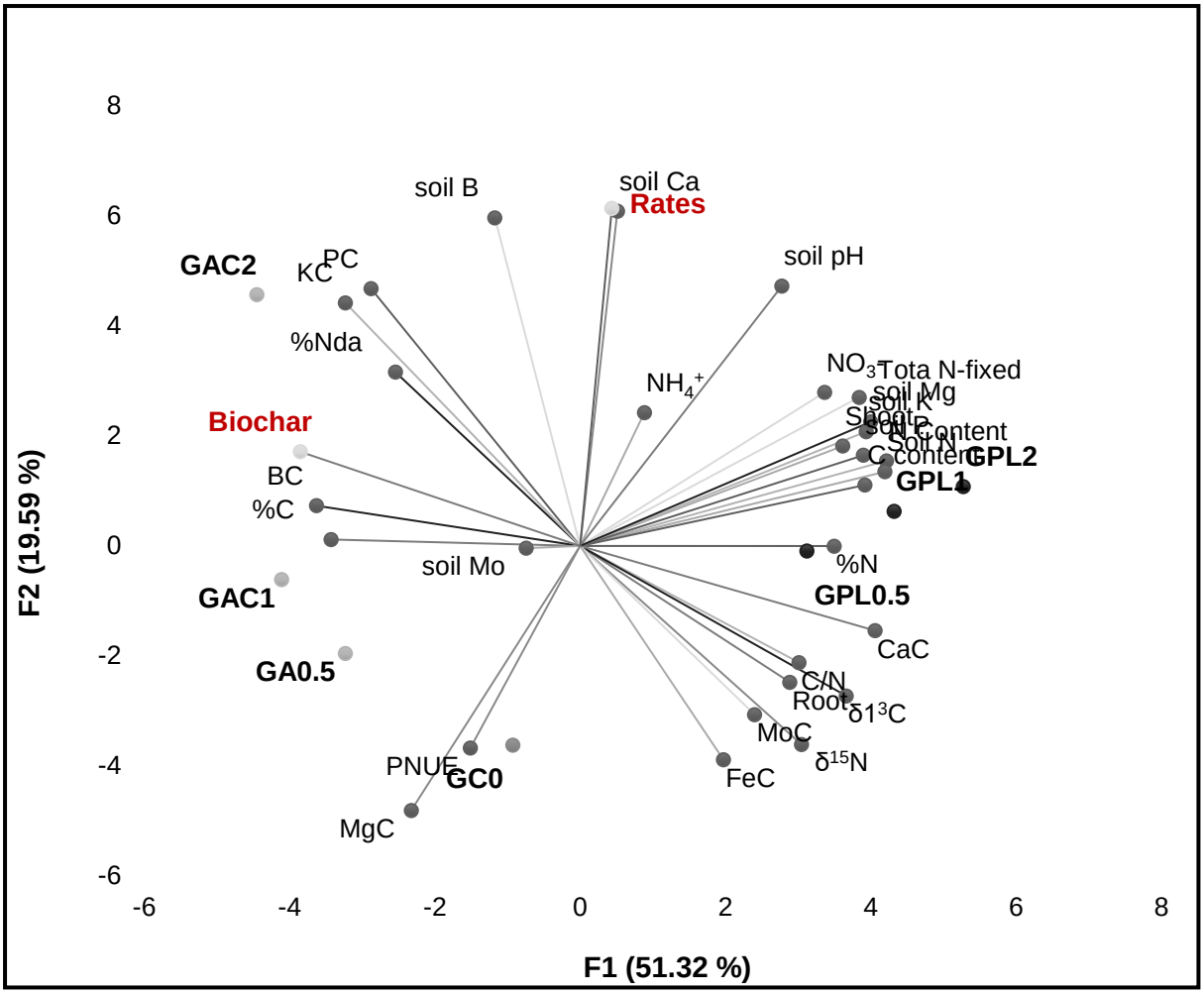


**Figure 6.4**: PCA-biplot of illustrating the relationship between growth, BNF attributes and WUE parameters of chickpea with selected soil properties of **Griffin soil.** The vectors represent the growth, BNF and WUE variable and treatments (soil type, biochar feedstock type and application rates). The direction of the arrow indicates the steepest increase in the variable, and the length indicates the strength relative to the other variables. Bold text represents the treatments: GC0, GPL0.5, GPL1, GPL2, GAC0.5, GAC1, GAC2.



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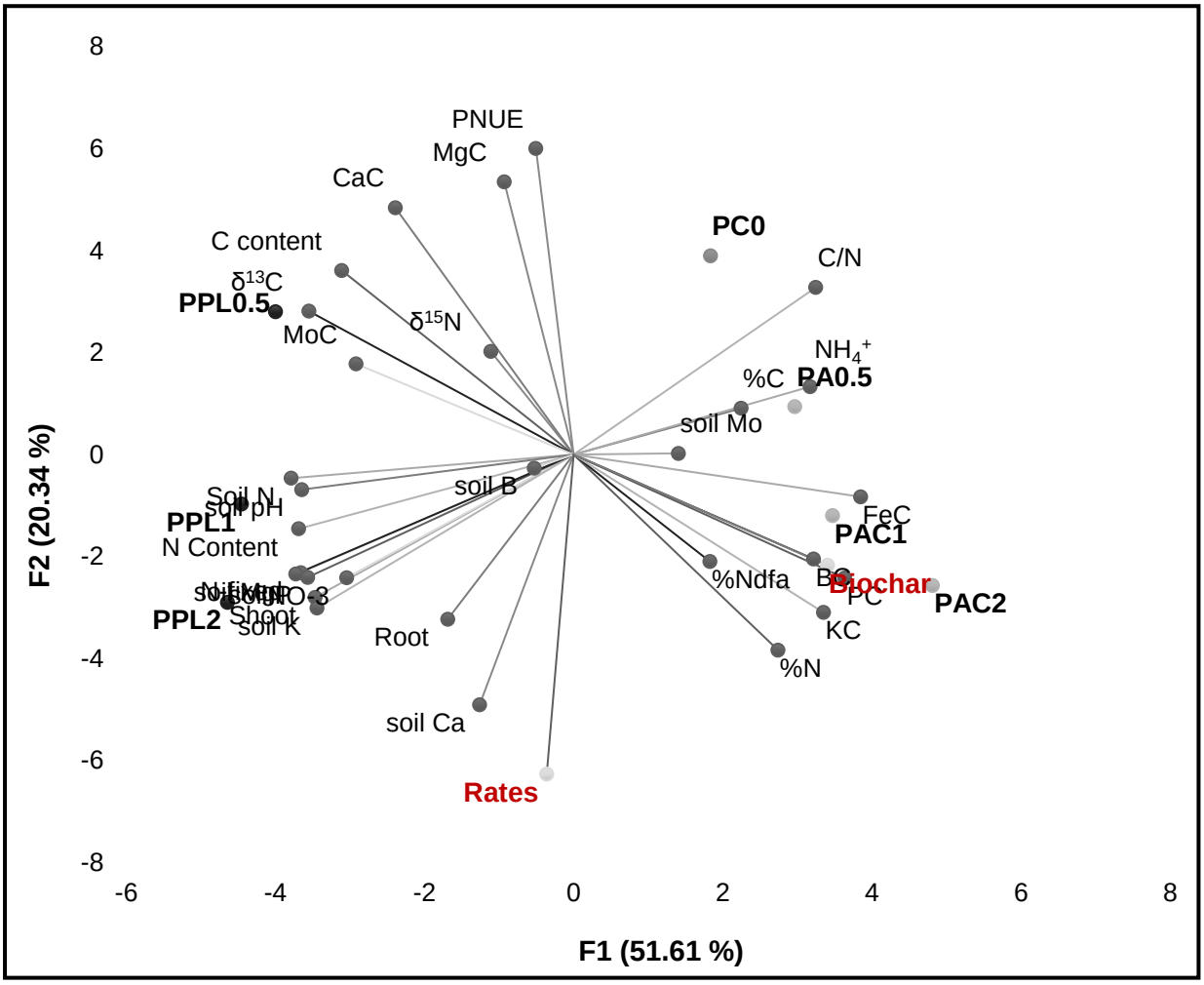


**Figure 6.5**: PCA-biplot of illustrating the relationship between growth, BNF attributes and WUE parameters of chickpea with selected soil properties of **Pinedene soil.** The vectors represent the growth, BNF and WUE variable and treatments (soil type, biochar feedstock type and application rates). The direction of the arrow indicates the steepest increase in the variable, and the length indicates the strength relative to the other variables. Bold text represents the treatments: PCO, PPL0.5 PPL2, PAC0.5, PAC1, PAC2.



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## 6.5 Discussion

### 6.5.1 Effect biochar on biological N fixation of chickpea in three contrasting soils

In this study, chickpea $N_2$ fixation varied across the three soil types, depending on the type of biochar used and the rate at which the biochar was applied. Chickpea grown in the Fernwood soils derived more N from the atmosphere at 0.5% PLB (21%) and at 0.5 ACB (30%), resulting in higher total N-fixed (15 mg N/plant and 37 mg N/plant, respectively). On the other hand, chickpea grown in PLB treatments in the Griffin soil derived more N from the atmosphere (35% on average), resulting in higher total N-fixed (77 mg N/plant on average), however, as ACB application rates increased, so did %Ndfa, resulting in a 30 % decrease in total N-fixed. In the Pinedene soil, despite the lower %Ndfa (20% on average) in PLB and ACB treatments than the control, chickpea grown had higher total N-fixed (57 and 48 mg N/plant) at 2% PLB and ACB respectively.

Similarly, Mia et al. (2018), found that applying 20 t/ha of eucalyptus-wood biochar to a sandy clay loam soil reduced %Ndfa by 73%, but total N-fixed of red clover was compensated at this rate by increased biomass production. The presence of biochar derived from plant materials has been shown to increase %Ndfa, resulting in higher total N-fixed (Güereña et al., 2015, Mia et al., 2014, Quilliam et al., 2013, Rondon et al., 2007) but the effect of PLB biochar on BNF has not been documented. The total N-fixed values of chickpea reported in this study are consistent with previous findings using biochar (Khan et al., 2020) and without biochar (López-Bellido et al., 2011, Meleta and Abera, 2019). The low N-fixed values reported when ACB was applied in the Griffin soil contradicts those reported by previous findings on woody derived biochar (Horel et al., 2018, Mia et al., 2018, Rondon et al., 2007).

The findings of this study have shown for the first time that the use of biochar derived from poultry litter, in a clay textured soil such as the Griffin and Pinedene which are highly buffered, with high C content, and high nutrient retention capacity (CEC) could improve biological nitrogen fixation by chickpea. The greater increase in total N-fixed by chickpea grown in the Griffin and Pinedene soils than the Fernwood soil when PLB was applied was linked to the fertility of the soils. The Griffin and Pinedene soils had higher macronutrients and micronutrient concentration than the Fernwood soil which contained the lowest concentrations of nutrients after biochar application (see chapter 4; Fig 4.1-4.5). The effect of biochar acting as a liming agent, supplying and enhancing nutrients to soils has been reported elsewhere (Ding et al., 2016, Wang et al., 2015), thus contributing to enhanced legume crop growth and higher BNF (Hiama et al., 2019, Khan et al., 2020, Rondon et al., 2007). Total N-fixed in the Griffin and Pinedene soils was also driven by increased biomass production of chickpea grown with PLB



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application, resulting in higher N-fixed. This was demonstrated by the strong and positive relationship between total N-fixed and shoot dry weight (r= 0.79) as shown in (Table 6.3). Chickpea grown in the Griffin soil with 0.5 to 2% PLB (equivalent to 10-40 t/ha) exhibited the highest shoot dry weight and total N-fixed. The addition of 2% PLB and ACB increased chickpea shoot dry weight and markedly enhanced N-fixed in the Pinedene soil.

The results are consistent with the findings of Mia et al. (2014) who reported higher N-fixed by red clover due to higher biomass when 10 t/ha of biochar derived from plant species was applied. Higher production of chickpea shoot biomass in the presence of PLB in the Griffin and Pinedene soils was shown in chapter 4 (Fig 4.5), as well as the application of biochar derived from poultry litter to other crops (Macdonald et al., 2014, Rajkovich et al., 2012). Other than %Ndfa and biomass production, the N concentration in legumes affects total N-fixed (Mia et al., 2018). Total N-fixed of chickpea increased in magnitude where shoot biomass and N content were high, especially when 2% PLB and ACB was applied in the Griffin and Pinedene soils. The Pearson correlation showed that shoot N concentration significantly correlated with total N-fixed (r = 0.60), whereas shoot N content strongly correlated with total N-fixed (r = 0.92). On the other hand, the PCA analysis revealed that the variation in total N-fixed in the Griffin soil was largely due to N concentration and N content, whereas only N content was found in the Pinedene soil (Fig 6.4 & 6.5). However, the higher N-fixed at 0.5 PLB and ACB in the Fernwood soil is largely attributed to the increase in %Ndfa (Fig 6.3).

Soil mineral N has a significant effect on the efficiency of N fixation by legumes and determines whether legumes would rely on soil N or obtain N through fixation (Mia et al., 2018). Chickpea grown in the Pinedene, Griffin and Fernwood soils depended more on soil N than $N_2$ fixation, where total N-fixed was lower. The dependence of chickpea on soil N varied with PLB and ACB application in all soil types. For example, chickpea grown in the Griffin soil relied heavily on soil N when ACB was applied at 0.5-2% because total N-fixed decreased as biochar levels increased. Chickpea in the Fernwood soil relied heavily on soil N when 1 and 2% PLB and 1% ACB were applied. On the other hand, chickpea grown in both PLB and ACB treatments in the Pinedene soil dependent on both total N-fixed and soil N. In the Griffin and Pinedene soils, soil N uptake strongly and positively correlated with total N-fixed (r > 0.80), whereas in the Fernwood soil, soil N uptake negatively correlated with %Ndfa and total fixed N-fixed (Fig 6.3-6.5). This is because, as %Ndfa and total N-fixed increased in the Griffin and Fernwood soils, soil N uptake decreased, whereas in the Pinedene soil, soil N uptake fluctuated to the point where it was extremely high where total N-fixed and %Ndfa were higher (see Fig C.3 Appendix).



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The relatively lower dependence of chickpea on BNF for its N nutrition as demonstrated in this study (3% in the Fernwood soil; 17% in the Pinedene soil and 26 % in the Griffin soil) has been observed elsewhere. Makhura et al. (2015) showed low dependence of chickpea on atmospheric $N_2$ fixation when grown in South Africa even with rhizobia-inoculation, especially during the first year of the study (a two-year field study). The observed low %Ndfa in this study could be due to possible ineffectiveness of the commercial rhizobia strain which can be attributed to the competitiveness of native rhizobia bacteria (Chibeba et al., 2017). Furthermore, nodulation by chickpea was reduced when PLB rates were increased from 1-2% in the Fernwood soil (93%) and Pinedene soil (33%), and from 0.5-2% in the Griffin soil (33%), while application of 0.5% ACB reduced nodulation across all soil types. This is intriguing because the Fernwood soil had the lowest nutrient availability compared to the other soils (chapter 3, Fig 4.3-4.5), but in treatments where nodulation was low, %Ndfa and total N-fixed was also low, and the crop relied heavily on soil N, whereas high nodulation by chickpea resulted in higher %Ndfa and total N-fixed, and the crop relied less on soil N in 0.5% PLB treatments. When compared to the control, despite high nodulation, %Ndfa was slightly higher in ACB treatments (1 & 2%), but total N-fixed was lower and the crop relied more on soil N. Although chickpea nodulation was lower in PLB treatments in the Griffin soil, the nodules were more effective in fixing nitrogen because %Ndfa and total N-fixed was higher and the crop relied less on soil N, whereas in ACB treatments, where nodulation was higher, %Ndfa was also high but total N-fixed was low and the crop relied heavily on soil N.

### 6.5.2 Biochar changes soil properties thus enhance BNF

Improved BNF of legumes in different soils has been attributed to increased soil pH, high concentrations, and availability of P, K, Ca, Mg, B, and Mo nutrients due to biochar application (Güereña et al., 2015, Hiama et al., 2019, Mia et al., 2014, Quilliam et al., 2013, Rondon et al., 2007). Changes in rhizospheric soil pH and nutrient availability caused by biochar application at various rates were hypothesized to improve chickpea growth and nodulation, resulting in higher total N-fixed, and thus improved C accumulation and WUE. However, this effect will vary depending on the application of PLB or ACB in the Fernwood, Griffin and Pinedene soils. Indeed, higher total N-fixed by chickpea in this study was attributed to increased soil pH with PLB and ACB application, supporting the findings by (Güereña et al., 2015, Khan et al., 2020, Ogawa and Okimori, 2010). Chickpeas grown at pH levels ranging from 6.0 to 7.0 had higher total N-fixed (see Table B.5, Appendix). Because $N_2$ fixing bacteria thrive in the pH range of 6.5 to 7.0, it's possible that PLB application created a favourable environment for bacteria at this pH range. For example, the phylum Proteobacteria and Firmicutes, as well as the genera *Bradyrhizobium* and *Bacillus*, were most abundant in treatments where pH level was above 6.0 (chapter 5, Fig 5.1 and Table B.5 Appendix). This



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suggests that biochar, particularly biochar derived from poultry litter, has the potential to lime acid soils, improving rhizospheric conditions for soil organisms like bacteria to thrive and function, as well as contributing to improved soil quality.

Increased nodulation of various crops as a result of increased in soil pH, available P, B, and Mo content (Ogawa and Okimori, 2010, Rondon et al., 2007, Tagoe et al., 2010) as well as interaction between biochar and signaling for nodulation through adsorption of flavonoids and Nod factors is another reason for improved BNF, as previously reported (Lehmann and Joseph, 2009b). In this study, nodulation was higher in the PLB and ACB treatments (0.5 %) where soil pH and P were high (Table B.5 appendix), resulting in higher %Ndfa and total N-fixed in the Fernwood soil. This suggest that application of poultry litter-derived biochar and acacia-derived biochar as low as 10 t/ha is likely to stimulate chickpea nodulation in low fertile soil such as the Fernwood soil. However, in the Griffin and Pinedene soils, where rhizospheric soil pH was high, nodulation was higher at 20 t/ha ACB and 10 t/ha PLB treatments, respectively, supporting the findings of (Mia et al., 2014). Although %Ndfa was lower, total N-fixed was only higher in chickpea grown in the Pinedene soil and not in the Griffin soil. This suggest that greater symbiosis between the rhizobia and the host plant was influenced by biochar application in this study, but the nodules in the Griffin soil were not effective to stimulate BNF when ACB was applied. Moreover, nodulation by chickpea was reduced at higher rates of PLB application (20 and 40 t/ha) in the studied soils.

Increased nodulation and BNF were also reported at 10 t/ha (Mia et al., 2014), and as high as 95 t/ha biochar application (Khan et al., 2020). However, Quilliam et al. (2013) found that elevated biochar applied at 25 and 50 t/ha reduced red clover nodulation. Mia et al. (2018) found that 50 t/ha biochar application reduced nodulation of red clover, resulting in a lower BNF. As a result, this study agrees with Mia et al. (2014) that flavonoids and Nod factors adsorption, as well as their stimulating role on nodulation, are unlikely to be the main mechanisms by which biochar affects BNF, because higher biochar application rates should lead to an increase in nodulation, resulting in higher BNF, but this was not observed in their study or in this study, contradicting findings by (Lehmann and Joseph, 2009b). However, to be conclusive on the optimum biochar application rate for nodulation in chickpea, more research is needed on various biochar feedstocks as biochar derived from plants and animal feedstocks differ in their properties and benefits in soils as observed in this study.

Moreover, increased soil pH with biochar application resulted in higher nutrient retention and availability, which led to greater biomass production in the Griffin and Pinedene soils. Treatments with higher available P, K, and Mg contributed significantly to the increase in total N-fixed by chickpea in the Griffin and Pinedene soils. Soil pH, P, K and Mg significantly and



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positively correlated with total N-fixed (r > 0.80) in the Pinedene and Griffin soils (Table 6.3). In contrast, enhanced available P, K and Mg by biochar application in the Fernwood soil did not results in an increase in total N-fixed as observed by the negative correlation (Fig 6.3), suggesting that soil pH was the only most attributing factor to the increased total N-fixed in the Fernwood soil. These findings are consistent with previous reports (Horel et al., 2018) however, they contradict results report by (Mia et al., 2018, Rondon et al., 2007). Increased BNF in several legumes, including soybean (Tagoe et al., 2008), common bean (Rondon et al., 2007) has been linked to increased available P with biochar application (Brewer et al., 2012). Biochar has been shown in other studies to increase the concentration and availability of B and Mo, thereby stimulating %Ndfa and BNF (Mia et al., 2014, Rondon et al., 2007). However, in this study, soil B concentration negatively correlated with %Ndfa and total N-fixed, while shoot B concentration correlated positively with total N-fixed in the Fernwood soil (Fig 6.3), indicating that higher soil B concentration due to biochar application is likely to inhibit total N fixation by chickpea. Furthermore, when PLB or ACB was applied at different rates in the Fernwood, Griffin, and Pinedene soils, the concentration of B and Mo was not significantly different (Table B.1 Appendix).

The concentration of nitrogen in the soil is a major factor determining whether legumes would fix atmospheric $N_2$ and utilize the fixed N for growth or rely on soil N. The %Ndfa and total N-fixed of various legumes grown in different soils has been shown to be affected by soil N status. Despite the negative relationship between total N-fixed and %Ndfa in the Griffin and Pinedene soils, rhizospheric nitrate concentration correlated significantly with total N-fixed in this study (Table 6.3). Although chickpea grown in the Griffin soil derived less nitrogen from the atmosphere, the crop had higher total N-fixed in treatments where soil nitrate was high, but the crop largely dependent on N-fixed for growth. Reduced soil nitrate when ACB was applied resulted in higher %Ndfa, but total N-fixed was reduced and the crop relied on soil N, indicating that soil nitrate was not a factor in improving or reducing BNF in the Griffin soil, but other factors such as low P availability and low biomass reduced total N-fixed when ACB was applied. Application of PLB and ACB in the Fernwood soil at 0.5% resulted in higher soil nitrate, higher %Ndfa and total N-fixed than the control. However, at higher rates of PLB nitrate was lower than the control, but resulted in less nodule formation, lower %Ndfa and total N-fixed, whereas in ACB treatments nitrate was reduced or immobilized at higher rates, and resulted in greater formation of nodules, lower %Ndfa and total N-fixed. This suggests that high soil nitrate availability caused by biochar application did inhibit chickpea nodulation and thus affected nitrogen fixation by the crop in the sandy textured soil, supporting the findings by (Bruun et al., 2011, Nelissen et al., 2012, Rondon et al., 2007) that immobilisation of inorganic N enhance BNF.



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PLB application reduced nodulation in both the Pinedene and Griffin soils due to higher levels of soil nitrates as biochar levels increased; however, %Ndfa and total N-fixed by chickpea were unaffected. When 1 and 2% ACB were applied, soil nitrate was immobilized and reduced, resulting in higher nodulation and low %Ndfa, but total N-fixed by chickpea was higher in the Pinedene soil and lower in the Griffin soil. This means that soil nitrate was not a significant factor in determining whether chickpea grown in the Pinedene and Griffin soils will have a higher or lower total N fixation, but soil pH and biomass production attributed to higher total N-fixed by chickpea in both soils. Mia et al. (2018) also noted a reduction in %Ndfa due to greater soil nitrate when eucalyptus wood biochar was applied at 20 t/ha in a sandy clay loam soil, but BNF was not affected. Previous findings have shown that low soil N availability stimulate high BNF in legumes when other soil factors are not limiting (Mapope and Dakora, 2016, Rondon et al., 2007). However, this was not the case in this study, because total N-fixed by chickpea was not affected by the concentration of nitrate-N or ammonium-N after biochar application in the Griffin and Pinedene soils.

Overall, the results of this study showed that biochar application increased shoot biomass, N concentration, N content, and changes in soil properties such as soil pH, K, Mg, P, and shoot concentrations of Fe, B, and Mo which attributed to the low or high total N-fixed by chickpea, irrespective of the soil type. This is because, despite low soil nitrate, high soil pH (6.0), P, K, Mg, and low shoot Fe concentrations, total N-fixed was reduced due to low nodulation and biomass production in higher PLB treatments in the Fernwood soil. Meanwhile, when ACB was applied at higher rates, total N-fixed was reduced due to low biomass production, resulting in less N derived from the atmosphere due to high shoot Fe and B concentrations, despite high nodulation and soil K, Mg levels and pH (Fig 6.3). In the Griffin soil, total N-fixed was reduced due to low biomass production, despite high pH (7.0), high nodulation, high nitrogen derived from the atmosphere, as a result of low nitrate and high soil K and Mg content and high shoot B concentration in ACB treatments (Fig 6.4).

When PLB was applied to the Pinedene soil, despite high nitrate and low nodulation, total N-fixed was higher due to high biomass production, high rhizospheric soil concentrations of Mg, K, P, and high shoot concentrations of Mo, as well as low shoot concentrations of Fe and B. Despite low rhizospheric soil P, low nodulation, low shoot Mo concentration, and high shoot Fe and B concentrations, total N-fixed was high when ACB was applied due to high biomass production, high soil K and Mg. Moreover, N-fixed by chickpea differed with the type of biochar feedstock and application rates in all soil types. This is because application of biochar derived from poultry litter feedstock as low as 10 t/ha and as high as 40 t/ha has the potential to improve BNF by chickpea in slightly acid, fertile soils such as the Griffin and acidic soils such as the Pinedene soil. However, application of poultry litter and acacia at 10 t/ha could possibly



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enhance BNF in low fertile sandy loam soils such as the Fernwood soil. Very acid soils, such as the Pinedene soils, will, on the other hand, require higher application rates of PLB or ACB at 2 t/ha to raise the pH of the soil, improve nutrients, and thus improve BNF.

### 6.5.3 Effect of biochar application on C accumulation and WUE of chickpea

In this study, shoot C concentration was invariable to the application of PLB and ACB at different application rates. Although, the lack of differences in shoot %C is intriguing, it is not surprising because this trend has been shown in different plant genotypes and treatments (Pule-Meulenberg et al., 2011, Yahaya et al., 2019). Despite being similar, on average, shoot %C values of chickpea ranged from 39 to 43 % with the application of PLB or ACB in the three types of soils (Table Fig 3c &d). These values are interesting because normally, the expected shoot C concentration in legumes should be about 30% as estimated by (Sprent et al., 1996). However, studies by (Mapope and Dakora, 2016, Maseko and Dakora, 2016, Mohale et al., 2014) reported greater shoot %C in various legumes. According to Post et al. (2007), shoot %C values of legumes that are above 30% and/or 35%, could be an indication of high lipid distribution within the plant organ. However, in this study, lipid distribution in shoot of the selected chickpea cultivar was not determined.

Both N and C content are products of the shoot dry weight and the shoot N or C concentration. The results of this study confirmed the relationship between C content and shoot dry weight (Fig 6.2). In the Fernwood soil, chickpea shoot dry weight was higher at 0.5% and least at 2% in PLB and ACB amended soils, respectively. Application of 0.5-2% PLB in the Griffin soil resulted in higher shoot dry weight, therefore greater C content. Meanwhile, 1% ACB resulted in the lowest growth and C accumulation. Application of 1 and 2% PLB in the Pinedene soil, chickpea had the largest shoot dry weight and the second-highest C content while the addition of 2% ACB decreased growth and C accumulation. C accumulation by chickpea grown in the Fernwood and Griffin soil, was largely associated with shoot dry weight and where growth was greater, it contributed to a larger N content and C assimilation. The direct relationship between shoot dry weight and C content was confirmed by a positive and significant linear relationship (Fig 6.2a). Mapope and Dakora (2016) reported similar findings, in which shoot C content positively correlated with soybean shoot biomass. Therefore, the results of this study confirmed that C content is associated with the biomass which is mainly derived from photosynthetically fixed carbon. In addition to biomass, lower soil nitrate and ammonium increased chickpea C accumulation in the Fernwood soil (Fig 6.3), whereas higher N-fixed and soil N uptake, as well as higher soil P, K, and Mg, increased chickpea C accumulation in the Griffin soil (Fig 6.4). The linear relationship between C content and N content, as well as total N-fixed, demonstrates the increase in C accumulation with high shoot N accumulation



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(Fig 6.2 b & c). In the Pinedene soil, C accumulation of chickpea decreased as the crop accumulated more N, P, B, and Fe, whereas an increase in soil pH, Ca, and Mo uptake resulted in higher C accumulation (Fig 6.5).

The legume C/N ratio is considered not only to be a measure of photosynthetic N-use efficiency (PNUE) but also as a good indicator of plant N status (Maseko and Dakora, 2016). In general, residues of legumes especially aboveground plant organs typically undergo faster mineralisation when their C/N ratio ranges between 9.4 and 22.7 g/g (Hobbie, 1992). The implication of this is that, when such residues are incorporated into the soil, they satisfy the nutrient demand of microbes and result in early net mineralisation of nutrients. By contrast, the opposite is largely true with residues of legumes with a C/N ratio above 23 which result in net immobilisation of N (Nguyen and Marschner, 2016). In this study, the shoot C/N ratio of chickpea varied largely with each type of soil. In the Fernwood soil, chickpea exhibited a C/N ratio that ranged from 29 to 43 g/g, while in the Griffin and the Pinedene soils the values were 14 to 38 g/g, and 17 to 46, respectively.

The C/N ratio values of chickpea grown in the Griffin soil are similar to findings reported by (Mapope and Dakora, 2016, Mohale et al., 2014); however, C/N ratio values obtained in chickpea grown in the Fernwood and Pinedene soils contradict those stated by (Hobbie, 1992, Mapope and Dakora, 2016). It is most likely that chickpea shoot biomass produced in the Griffin soil would undergo faster decomposition than shoot biomass produced in the Fernwood and Pinedene soils. This means that, if shoot biomass of chickpea were to be incorporated into the soil to improve soil fertility, the preferred biomass will be that from the Griffin soil. Studies have shown that the shoot C/N ratio of plants grown in relatively fertile soil is increased (Mohale et al., 2014). The Griffin soil was relatively more fertile than the other soils (see chapter 4; Table 4.1). The shoot C/N ratio was indeed affected by biochar feedstock type and application rates in this study, because when compared to the control, the C/N ratio decreased with high levels of PLB and ACB in the Griffin and Pinedene soils, except in the Fernwood soil where C/N ratio was lower at 2% PLB and 1 % ACB amended pots.

This is because C/N ratio decreased as N content increased in the Fernwood, Griffin and Pinedene soils as a result of higher accumulation of N and greater biomass. This was demonstrated by the negative relationship between shoot C/N ratio and N content (Table 6.3), while shoot C/N ratio correlated negatively with shoot biomass, N content, and total N-fixed in the Pinedene soil (Fig 6.5), whereas with nitrate and ammonium in the Fernwood soil (Fig 6.3). Shoot $\delta^{13}C$ of chickpea varied significantly between soil types, biochar feedstock and application rates. In the Fernwood soil, shoot $\delta^{13}C$ of chickpea ranged from -29.70 to -30.0‰ while in the Griffin and Pinedene soils mean values ranged from -28.30 to -30.40‰ as well as



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-27.80 to -30.40‰, respectively. When comparing the type of biochar, shoot $\delta^{13}C$ values on average ranged from -29.9‰, -29.5‰, -30.2‰ in PLB treatments to -29.1‰, -29.2‰, and –28.2‰ in ACB treatments in the Fernwood, Griffin and Pinedene soils, respectively. Interestingly, the most water-use by chickpea was observed in soils amended with ACB across the soil types. This is because chickpea grown in the Pinedene soil (-27.8) had the highest $\delta^{13}C$ at 2% ACB, followed by Griffin soil (-28.3‰) and Fernwood soil (-28.7‰).

This study agrees that the type of biochar influence the discrimination of $^{13}C$ differently. In general, like the C/N ratio, shoot $\delta^{13}C$ is affected by the fertility status of the soil (Mohale et al., 2014). It is intriguing that chickpea grown in the acidic, fertile Pinedene soil was more water-use efficient but did not translate to higher biomass especially when ACB was applied than that grown in the slightly acid Fernwood and Griffin soils. Despite lower shoot biomass, chickpea used less water in the Pinedene soil due to increased soil pH and P, as well as higher N uptake in the soil and C accumulation in the shoots (Fig 6.5). Furthermore, Chickpea grown in the Griffin and Pinedene soils, where both total N-fixed and N content were higher, had a higher WUE at 2% ACB than chickpea grown in the Fernwood soil, where N content was higher. Chickpea in the Griffin soil produced more biomass, accumulated more N, and had a lower shoot C/N ratio, all of which contributed to higher WUE (Fig 6.4). Mohale et al. (2014) reported a significant positive correlation between shoot $\delta^{13}C$ with total N-fixed, N content, shoot dry weight, and C content of Bambara groundnut. However, in this study, shoot $\delta^{13}C$ did not correlate with total N-fixed, but correlated positively with shoot dry weight, C content and N content (Table 6.3). This suggest that there is a close link between WUE and BNF as well as photosynthetic activity by legumes, therefore improved WUE supports N and C assimilation in legumes.

### 6.6 Conclusion

Biological nitrogen fixation, C accumulation, and water-use efficiency measured by the natural abundance of $^{15}N$ and $^{13}C$ differed with biochar feedstock type and application rates in three contrasting soils in this study. Biochar derived from poultry litter (PLB) resulted in a higher total N-fixed by chickpea in both Griffin and Pinedene soils. The application of 0.5% PLB or ACB in acid-sandy textured soils such as Fernwood could be optimal to improve BNF and C accumulation of chickpea. Meanwhile, soils with a high clay content and buffering capacity such as the Griffin and Pinedene soils would require a higher application of up to 2% irrespective of the biochar feedstock. The main reasons for improved BNF and C accumulation in this study were related to greater biomass production and high accumulation of N in shoots due to improved soil pH, nutrient composition of the soils (higher P, K and Mg). On the other hand, chickpea used less water when ACB was applied at 2% in the studies soils. High WUE



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of chickpea was stimulated by higher production of biomass, N content and N-fixed. The results of this study show potential for increasing N inputs through BNF with biochar application in acid sandy and clay textured soils. The results also indicate that there is a close relationship between BNF with photosynthesis and the use of water in legumes as influenced by biochar application. More research is needed to confirm the potential effect of poultry litter and acacia biochar on BNF and WUE by chickpea in various soils using the $^{15}N$ and $^{13}C$ natural abundance technique to be conclusive on the effect of biochar on BNF and WUE by chickpea. This is because, as shown in this study, biochar derived from plant and animal feedstocks differ in their properties and benefits in soils differing in texture and fertility status.



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# CHAPTER 7

## RESIDUAL BIOCHAR AND N CONTRIBUTED BY CHICKPEA INFLUENCES GROWTH AND N UPTAKE OF SUCCEEDING MAIZE IN SOILS VARYING IN TEXTURE

### Abstract

There is hardly any evidence in literature that reported the impact of residual biochar derived from poultry litter and acacia on maize growth and nutrient uptake in varying soil types. In addition, the benefit of excess N from residual biochar in conjunction with nitrogen-fixed by chickpea on growth and N uptake of maize has not been studied. Therefore, the aim of this study was to assess the potential effect of residual poultry litter and acacia biochar and the contribution of nitrogen-fixed by chickpea to maize growth and nutrient uptake. The treatments consisted of three soil types [Fernwood (Arenosol); Pinedene (Gleyic Acrisol); Griffin (Helvic Acrisol)]; two biochar types [PLB and ACB] and four application rates of [0 (control), 0.5, 1 and 2% w/w]. Residual PLB increased N uptake of maize grown after maize, with average N uptake of 137, 98, and 92 mg/kg in the Pinedene, Fernwood, and Griffin soils, respectively. The average N uptake by maize grown where total N-fixed by chickpea was high was 700 and 349 mg/kg in residual PLB treatments and 603 and 282 mg/kg in ACB treatments in the Pinedene and Griffin soils, respectively. Maize root was 385 and 230 % and shoot dry weight was 262 and 212 % higher in all PLB treatments than ACB treatments in Griffin and Pinedene soils where total N-fixed was higher. In Fernwood soils maize, largely dependent on other nutrients than excess N through mineralisation of PLB and ACB and total N-fixed. Direct supply of nutrients and mineralisation of residual PLB resulted in higher uptake of Ca, K, P, Mg, Zn by maize than residual ACB. The impact of residual biochar is greater in chickpea-maize rotations due to excess N from biochar mineralisation and N fixation by chickpea. However, caution should be exercised when using ACB biochar, as it may increase Fe toxicity at higher levels and reduce N and P availability due to immobilisation.

Keywords: chickpea, maize, nutrient uptake, residual biochar, soil type



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## 7.1 Introduction

The demand for maize (*Zea mays*) to maintain food security is increasing in most African countries and is expected to double by 2025 (Smith et al., 2016) Maize is a major staple food crop in Africa, especially in Southern Africa, and is the most important component of smallholder cropping systems in the continent. However, maize yields in most smallholder farming systems are insufficient to meet rising demand, and maize production is frequently constrained by abiotic factors such as drought and low rainfall, as seen in Southern Africa during the 2018 drought (Lengwati et al., 2020). Furthermore, while commercial farmers in South Africa can achieve maize yields of 4,210 to 6,470 kg/ha, maize grain yields on smallholder fields are still less than 2,000 kg/ha due to inherently soil nutrient depletion, severe soil degradation, intensive cereal monoculture, inappropriate land use, and anthropogenic climate change (Lengwati et al., 2020).In addition, inadequate N nutrient supply due to insufficient fertilizer inputs continues to be a major factor limiting maize production (Manolikaki and Diamadopoulos, 2019) as, as smallholder farmers in Africa apply only about 8.8 kg of NPK fertilizer per hectare on average (Lengwati et al., 2020).

Although synthetic nitrogen fertilizers like ammonium nitrate and urea have improved maize yield for centuries, they are out of reach for resource-poor farmers due to their high cost, which is often linked to global energy prices. Furthermore, their use has become unsustainable, environmentally harmful, and unlikely to meet food demand (Ali et al., 2018, Vanlauwe et al., 2011). As such nitrate leaching and volatilization results in decreased crop productivity, eutrophication, excess nitrate in groundwater, and increased nitrous oxide ($N_2O$) emissions (Vanlauwe et al., 2011). Nitrous oxide accounts for 8% of global greenhouse gas emissions (GHG), with agricultural sources accounting for roughly 60% of total anthropogenic $N_2O$ emissions. Increased N fertilizer inputs were primarily the main cause of the increase in atmospheric $N_2O$ concentration for decades (Brassard et al., 2016). Manure, compost, and crop residues, on the other hand, are unsustainable in tropical climates due to their rapid decomposition, and require repeated application to build up soil fertility. Carbon emissions from these organic materials also contribute to GHG emissions (Ding et al., 2016).

Current agricultural systems must become more productive and resilient in order to meet the growing food demand while working within the constraints of limited resources and a changing climate (Adhikari et al., 2018). Improving soil fertility and productivity while reducing negative environmental consequences is one of the most difficult challenges in the management of agricultural production systems. Innovative technologies, such as the application of biochar, that recycle nutrients within agricultural systems while minimizing environmental impacts, are critical to addressing these complex issues. Biochar application to soils improves soil organic



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matter, moisture, and pH, which stimulates N mineralisation and nitrification, resulting in improved soil mineral N ($NH_4^+$-N and $NO_3^-$-N) and uptake (de Sousa Lima et al., 2018, DeLuca et al., 2015, Nguyen et al., 2017). Biochar helps to increase inorganic N for plant assimilation by increasing N retention in soil through decreased leaching (Xu et al., 2016). Soil mineral N availability and uptake are influenced by biochar C/N ratio, pyrolysis temperature, and surface area. Several studies have found significant effects of residual biochar on maize growth and yield, depending on the type of biochar, application rates, soil texture, and experiment duration (Butnan et al., 2015, Nguyen et al., 2017, Rogovska et al., 2014).

The use of biochar as a soil amendment to improve maize productivity has been controversial and highly variable, with varying results, due to the complexity of interactions between biochar, soils, and crops (Brantley et al., 2016, Cole et al., 2019, Gonzaga et al., 2018, Taskin et al., 2019). Nevertheless, several studies (Kätterer et al., 2019, Smith et al., 2016, Taskin et al., 2019, Uzoh et al., 2019) have shown that integrating $N_2$-fixing legumes in maize-based cropping systems (in rotation or intercropping) improves maize production by providing additional nitrogen. For smallholder farmers with limited access to fertilizer inputs, legumes are frequently considered key components of integrated soil fertility management strategies and agroecological resilience (Smith et al., 2016). Despite overwhelming evidence that incorporating legume residues to the soil during crop rotations improves soil properties and microorganism activity (Uzoh et al., 2019); smallholder farmers are frequently forced to prioritize food production and crop sales over potential soil fertility benefits because some legumes that are used to improve soil fertility do not produce edible grain (Smith et al., 2016, Uzoh et al., 2019). As a result, smallholder farmers in Africa who practice mixed farming commonly use cowpea, groundnut, and Bamabara groundnut, pigeon pea, and common bean in rotation and/or intercropping with maize to improve soil fertility through residual N supply from fixation, while using grain for food and residual leaves to feed their livestock.

According to the literature search conducted and existing knowledge, the use of chickpea (*Cicer arietinum* L.) in rotation and/or intercropping with maize is uncommon in practice and in the literature, especially in Southern Africa. Incorporating chickpea, a nitrogen-fixing grain legume crop, will not only add value to improve soil fertility to improve maize productivity, but will also provide smallholder farmers with cash income from grain production and dietary fiber for their livestock. Furthermore, smallholder farmers will also benefit from the introduction of low-cost and effective approaches such as biochar plus the incorporation of chickpea into their cropping systems, which can supply N for higher maize yields through biological nitrogen fixation. The objective of this present study was therefore to determine the potential impact of residual poultry litter biochar (PLB) and acacia biochar (ACB) at different rates and the contribution of N-fixed by chickpea to the growth, N accumulation, and nutrient uptake of maize



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in three soil types varying in texture. The study hypothesized that: i) maize grown in residual PLB and ACB after chickpea will have higher biomass, tissue N accumulation and nutrient uptake than sole maize grown after maize; ii) maize grown in soils with high N content fixed by chickpea will have higher biomass and N accumulation; iii) growth and nutrient uptake of maize will increase with an increase in the biochar application rate and this will vary with the type of soil.

### 7.2 Materials and methods

The biochar, soil and treatments used for this study was the same as in chapter 3 and 4. See section 3.2, in chapter 3 for biochar preparation and characterization, and section 4.2 in chapter 4 for description of soil and analysis.

#### 7.2.1 Experimental setup and design

The experimental design consisted of three factors: three soils differing in texture, pH, nutrient composition and management practices which were classified as Fernwood (denoted as F), Griffin (denoted as G), and Pinedene (denoted as P). Two biochars were derived from poultry litter feedstock (denoted as PLB) and acacia feedstock (denoted as ACB). The biochars were applied at three rates of 0.5, 1 and 2% (w/w) equivalent to 10, 20 and 40 t/ha including a control (0 %). Pots with a diameter of 25 cm and height of 25 cm were filled with 4 kg of air-dried sieved soil. The pots were arranged in completely randomized design and replicated four times.

#### 7.2.2 Maize experiment

Maize grown after maize (denoted as first maize) was planted in pots which had maize that was used as reference crop. Maize grown after chickpea (donated as second maize) was planted immediately after chickpea harvesting. Both the first and second maize were planted in the same soil and biochar treatments. Two seeds were sown and thinned to one plant at 15 days after emergence. The maize crop was grown for 72 days (10 weeks) and harvested before tasselling stage. The pots were maintained at 60% field capacity by constantly weighing the pots every 3 days and watered when necessary. The pots were not supplemented with no fertilizer prior to the second maize planting. The assumption of this experiment was that maize planted after maize (used as reference crop) will depend on N supplied through biochar mineralisation and soil N, while maize planted after chickpea will rely on the residual N supplied through biochar mineralisation plus excess N that was fixed by chickpea.



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## 7.3 Data collection

### 7.3.1 Maize growth

Maize growth was determined by measuring shoot and root dry weight after harvest at 72 days after planting. Shoot and root dry weight were obtained by drying the plant's samples at $65^0$C for 48 hours. The dried shoot of maize was ground and sieved to pass through a 1mm sieve for further analysis. Maize tissue concentration of N, P, K, Mg, Ca, Zn, and Fe were measured by inductively coupled plasma spectrometry (Mehlich, 1984). Nutrient uptake by maize crop was calculated by multiplying the shoot dry weight with tissue concentration of N, P, K, Mg, Ca, Zn, and Fe. Soil N uptake was calculated as the difference between N uptake and total N-fixed. Soil samples collected after harvest in each pot were air-dried at room temperature of 25°C for three consecutive days.

### 7.3.2 Statistical analysis

The data was analysed using R software (version 3.5.2). Generalised Linear Mixed Model, analysis of variance was used to test the treatment effects and their interactions on shoot dry weight, N content, N-fixed of chickpea, as well as shoot dry weight, root, nutrient concentration and uptake of N, P, K, Mg, Ca, Zn, and Fe by maize crop. Soil type, biochar feedstock type and biochar application rate were considered as fixed factors while the parameters were considered as random factors. Tukey's honest significant difference (HSD) test was used for treatment mean separations with the threshold probability level set at $p \leq 0.05$.

## 7.4 Results

### 7.4.1 Dry matter, N concentration and N uptake of maize

Shoot and root dry weight of the first maize (maize after maize) was not significant following residual PLB and ACB at different rates in the Fernwood, Griffin and Pinedene soils (Table 7.1). However, maize grown in the Griffin and Pinedene soils had higher N uptake in all residual PLB and ACB treatments compared to the control treatments (C0). On average, N uptake by maize grown in residual PLB treatments was 98 mg N/plant in the Fernwood soil, 92 mg N/plant in the Griffin soil and 137 mg N/plant in the Pinedene soil, whereas in residual ACB treatments, N uptake was 70 mg N/plant, 75 mg/plant and 97 mg N/plant in the Fernwood, Griffin and Pinedene soils, respectively (Table 7.1). In contrast, residual PLB and ACB had a significant effect on shoot and root dry weight of the second maize (maize after chickpea) in all soil types (Table 7.1). Treatment PL2 in the Fernwood, Pinedene and Griffin soils showed the highest shoot and root dry weight compared to the control (C0). Maize shoot dry weight



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increased by 24% in the Fernwood soil; 13% in the Griffin soil and 30% in the Pinedene soils. Similarly, maize grown in residual ACB of 2% had higher shoot and root dry weight in all soil types. Maize shoot in the Griffin soil increased by 5%, followed by 7% in the Fernwood, and by 6% in the Pinedene soil. On average, root dry weight of maize grown in the Fernwood, Griffin and Pinedene soils was 14032, 12960 and 13306 mg/plant in PLB treatments, and 10818, 12514, and 9608 mg/plant in ACB treatments, respectively (Table 7.1). Residual PLB and ACB treatments in all soil types affected N concentration and N uptake of the second maize (Table 7.1). Compared to the control treatment, N concentration and N uptake of maize increased with PLB and ACB application in all soil type. Maize grown in treatments FPL0.5-FPL1, FAC0.5-FAC2, in the Fernwood soil and GPL1-GPL2 and GAC1-GAC2 in the Griffin soil and PPL1-PPL2, and PAC0.5-PAC2 in the Pinedene soil had most N concentrations and N uptake than the control (C0) treatment. The average N uptake by maize grown in residual PLB treatments was 317, 349, 700 mg/kg and in ACB treatments was 299, 282, 603 mg/kg in the Fernwood, Griffin and Pinedene soils, respectively.

### 7.4.2 Contribution of N-fixed by chickpea to maize growth and N nutrition

Maize growth and nitrogen nutrition were affected by total N-fixed by chickpea with biochar application, as well as residual PLB and ACB (Table 7.1). Maize grown in the Fernwood soil with residual PLB of 0.5% (FPL0.5) and ACB of 0.5-2% (FAC0.5, FAC1, FAC2) where total N-fixed by chickpea was higher than the control, maize had slightly higher root and shoot dry weight, despite higher N concentration, and total N uptake. On the other hand, maize grown where total N-fixed was lower in treatment FPL1 (6 mg N/plant) and FPL2 (1 mg N/plant) had the highest maize shoot and root dry weight, as well as the highest N uptake in the Fernwood soil. In the Griffin soil, maize grown in residual PLB treatments (GPL1 and GPL2) with greater total N-fixed (72 and 90 mg N/plant), respectively had greater shoot and root dry weight as well as N uptake and low soil N uptake. Maize grown in treatment GAC0.5 and GAC2 with low total N-fixed had higher root and shoot dry weight, and total N uptake. In the Pinedene soil, residual PLB and ACB treatments with higher total N-fixed of 57 mg N/plant (PPL1) and 48 mg N/plant (PAC2), respectively, had greater shoot and root dry weight and total N uptake. Soil N uptake by maize varied at different rates of residual PLB and ACB, with higher soil N uptake by maize in residual ACB treatments than PLB treatments in the Fernwood and Griffin soils. Furthermore, soil N uptake by maize was higher in all residual ACB treatments where total N-fixed by chickpea was greater in the Fernwood soil. In the Griffin soil, higher total N by chickpea resulted in lower soil N uptake by maize in residual PLB treatments, but soil N uptake was higher in ACB treatments with higher total N-fixed than the control. Soil N uptake by maize was higher in the Pinedene soil in all residual PLB and ACB treatments, despite high total N-fixed by chickpea.



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**Table 7.1:** Response of maize growth, N concentration and N uptake prior harvesting and after harvesting of chickpea to residual PLB and ACB at different rates in the Fernwood, Griffin and Pinedene soils.

| | Maize prior chickpea harvesting | | | | maize after chickpea harvesting | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot dry weight | Root dry weight | N concentration | N uptake | Shoot dry weight | Root dry weight | N concentration | N uptake | Soil N uptake | Total N-fixed |
| Treatments | mg/plant | | % | mg N/plant | mg/plant | | % | | Mg N/plant | |
| **Fernwood soil** | | | | | | | | | | |
| FC0 | 7953 | 8050 | 0,52 | 41,4d | 20498b | 11168b | 1,26 | 242,3b | 7,94d | 14,03b |
| FPL0.5 | 14543 | 9753 | 0,49 | 72,1c | 22658ab | 14210a | 1,39 | 301,2b | 13,89c | 15,27b |
| FPL1 | 25743 | 9610 | 0,52 | 133,1a | 25060a | 12978ab | 1,37 | 337,1a | 4,62e | 5,99c |
| FPL2 | 18200 | 11867 | 0,50 | 89,4bc | 25620a | 14908a | 1,27 | 313,4a | 0,76e | 0,51c |
| FAC0.5 | 13237 | 12087 | 0,50 | 67,2c | 20165b | 10210b | 1,42 | 293,3b | 29,75b | 36,7a |
| FAC 1 | 14623 | 11367 | 0,51 | 76,5c | 20545b | 10630b | 1,32 | 283,4b | 39,7b | 25,3ab |
| FAC2 | 14457 | 10760 | 0,45 | 66c | 21923b | 11613b | 1,45 | 319,5a | 45,6a | 17,6c |
| **Griffin soil** | | | | | | | | | | |
| GC0 | 7747 | 11770 | 0,50 | 36,6d | 22135b | 11720b | 1,23 | 272,3b | 17,6b | 29,5c |
| GPL0.5 | 18337 | 11293 | 0,45 | 82,5bc | 21973b | 11885b | 1,40 | 311,3a | 20,4b | 70,8b |
| GPL1 | 19380 | 12467 | 0,48 | 95,7b | 23373b | 11930b | 1,60 | 381,9a | 18,9b | 71,5b |
| GPL2 | 19567 | 13507 | 0,50 | 97,8b | 25040a | 15065a | 1,72 | 354,0a | 15,1c | 89,6a |
| GAC0.5 | 16743 | 19087 | 0,59 | 99,2b | 22613b | 13578ab | 1,27 | 239,9b | 69,7a | 22,9c |
| GAC1 | 11593 | 13430 | 0,55 | 60,7c | 22043b | 11493b | 1,30 | 312,6ab | 70,0a | 21,3c |
| GAC2 | 14460 | 17227 | 0,47 | 63,9c | 23208ab | 12470ab | 1,50 | 292,7b | 88,4a | 17,9c |
| **Pinedene soil** | | | | | | | | | | |
| PC0 | 9913 | 9477 | 0,61 | 60,3c | 18358bc | 8233b | 2,41c | 493,1c | 23,4c | 39,1b |
| PPL0.5 | 18663 | 9907 | 0,89 | 145,8a | 21350b | 12210ab | 2,87b | 628,1b | 33,3ab | 52,3ab |
| PPL1 | 18480 | 11100 | 0,99 | 128a | 21165b | 13518a | 2,98a | 714,6a | 30,7ab | 47,4ab |
| PPL2 | 23370 | 12700 | 0,54 | 163,3a | 23918a | 14190a | 2,94b | 757,3a | 43,8a | 57,4a |
| PAC0.5 | 13630 | 15097 | 0,54 | 73,4bc | 18315bc | 8722b | 2,93b | 618,6b | 34,9b | 37,8b |
| PAC1 | 18573 | 10700 | 0,52 | 95,9b | 17575c | 9433b | 2,87b | 504,6c | 39b | 41,8b |
| PAC2 | 22103 | 16500 | 0,56 | 119,6a | 19378c | 10668ab | 3,51a | 686,8b | 44b | 47,5ab |

Values with dissimilar letters in a column the means are significant different at P < 0.05; PLB- Poultry litter biochar; ACB- acacia biochar



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### 7.4.3 Nutrient concentration and uptake of maize

Concentration of P in maize shoots was not significant in all treatments in the Griffin and Pinedene soils, but P concentration was higher in treatment PL2 in the Fernwood soil. Maize grown in residual PLB had higher P uptake in all soil types than that grown in ACB (Fig 7.1a). The uptake of P by maize was greater in treatment PL1 (113 mg/kg) and PL2 (127 mg/kg) in the Fernwood soils, in PL2 (90 mg/kg) in the Pinedene soil and lower for treatment AC0.5 and AC1 in both the Griffin and Pinedene soils (Fig 7.2a).

Residual PLB and ACB at different rates had no significant effect on Mg concentration, but maize grown in residual PLB had higher concentration of Mg than that grown in residual ACB in all types of soils (Fig 7.1b). On the other hand, Mg uptake by maize was higher in treatment PL1 and PL2 in the Fernwood, Griffin and Pinedene soils and lower in treatment PL0.5 in the Pinedene soil and AC0.5 in the Griffin compared to control treatment (C0) (Fig 7.2b). On magnitude, Mg uptake by maize grown in residual PLB treatments was greater in the Fernwood soil (113 mg/kg), followed by Pinedene soil (109 mg/kg), and lastly in the Griffin soil (109 mg/kg).

There was no difference in the concentration of Ca in maize shoots between Griffin and Pinedene soils in all treatments, but higher concentration of Ca in maize shoots was observed in PL1 and PL2 treatments. The concentration of Ca in maize shoot decreased with higher levels of residual PLB in the Fernwood soil (Fig 7.1c). Similarly, Ca uptake by maize was lower in all residual PLB treatments in the Fernwood soils and the reverse was true in the Griffin soil. The uptake of Ca by maize decreased from 177 to 103 mg/kg in the Fernwood soil, while increased from 161 to 208 mg/kg in the Griffin soil maize. Maize grown in the Pinedene soil had higher Ca uptake in the AC0.5 (229 mg/kg) treatment (Fig 7.2c).

Treatments PL0,5, PL1 and PL2 showed a higher concentration of K in maize grown in the Fernwood soil; however, the concentration of K in maize grown in the Griffin and Pinedene soils was high in treatment PL2 (Fig 7.1d). Similarly, the uptake of K by maize grown in the Fernwood and Pinedene soils was higher in all residual PLB treatments, while the Griffin soil showed similar K uptake in all treatments (Fig 7.2d). Compared to the control (C0 treatment), K uptake by maize in the Fernwood soil increased from 441 to 860 (PL2) and in the Pinedene soil from 365 to 920 (PL2). The concentration and uptake of K was lower in all residual ACB treatments in all soil types with the Pinedene soil exhibiting the least K uptake by maize (Fig 7.1d & 7.2d).

Maize grown in the Fernwood, Griffin and Pinedene soils showed no difference in S concentration with residual PLB and ACB in this study. Compared to the control (treatment



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C0), maize grown in treatment AC2 had higher S concentration in all soil types, while S concentration decreased in treatment PL0,5 and PL1 in the Griffin and Pinedene soils (Fig 7.3a). Maize grown in treatment AC1 and AC2 in the Fernwood soil (133 and 146 mg/kg) and Griffin soil (112 and 137 mg/kg) had higher S uptake, while that in the Pinedene soil had the least S Uptake in all PLB and ACB treatments (Fig 7.4a).

Maize grown in residual ACB treatments had higher Fe and Zn concentration (Fig 7.3b & c) as well as Fe and Zn uptake (Fig 7.4b & c) than that grown in PLB treatments. Treatment AC0.5 showed the highest Fe concentration of maize grown in the Griffin and Pinedene soils (Fig 7.3b), whereas Fe uptake was higher in treatment AC2 (938 mg/kg) in the Pinedene soil (Fig 7.4b). On the other hand, maize grown in the Fernwood soil had greater Zn concentration and uptake than the other two soils (Fig 7.3c & 7.4c). Interestingly, the concentration of Fe and Zn of maize grown in treatment PL2 (Fig 7.3b & c) was higher, but the uptake of Fe and Zn in the same treatment soil was extremely low in the Pinedene soil (Fig 7.4b & c).



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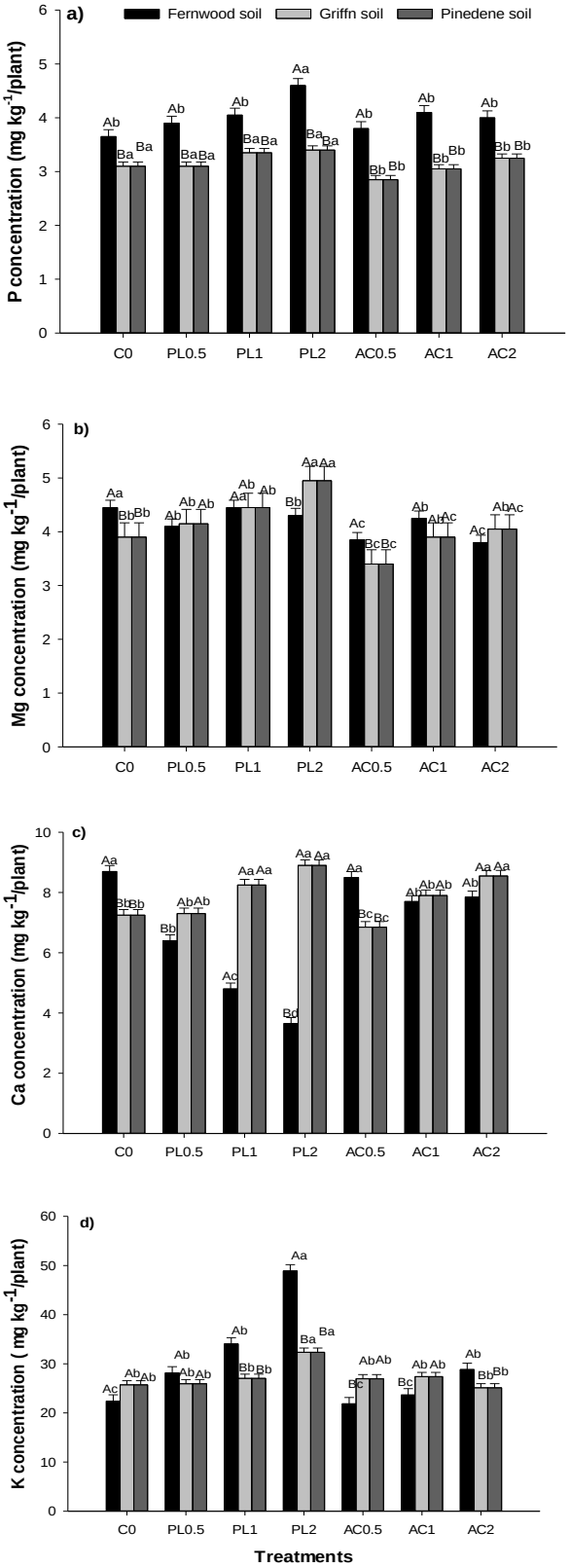


**Figure 7.1:** Effect of residual PLB and ACB on maize shoot nutrient concentration; P (a), Mg (b), Ca (c), K (d). The different capital letters indicate significant differences among soil types under each treatment, while different lowercase letters indicate significant differences among soil types at p< 0.05.



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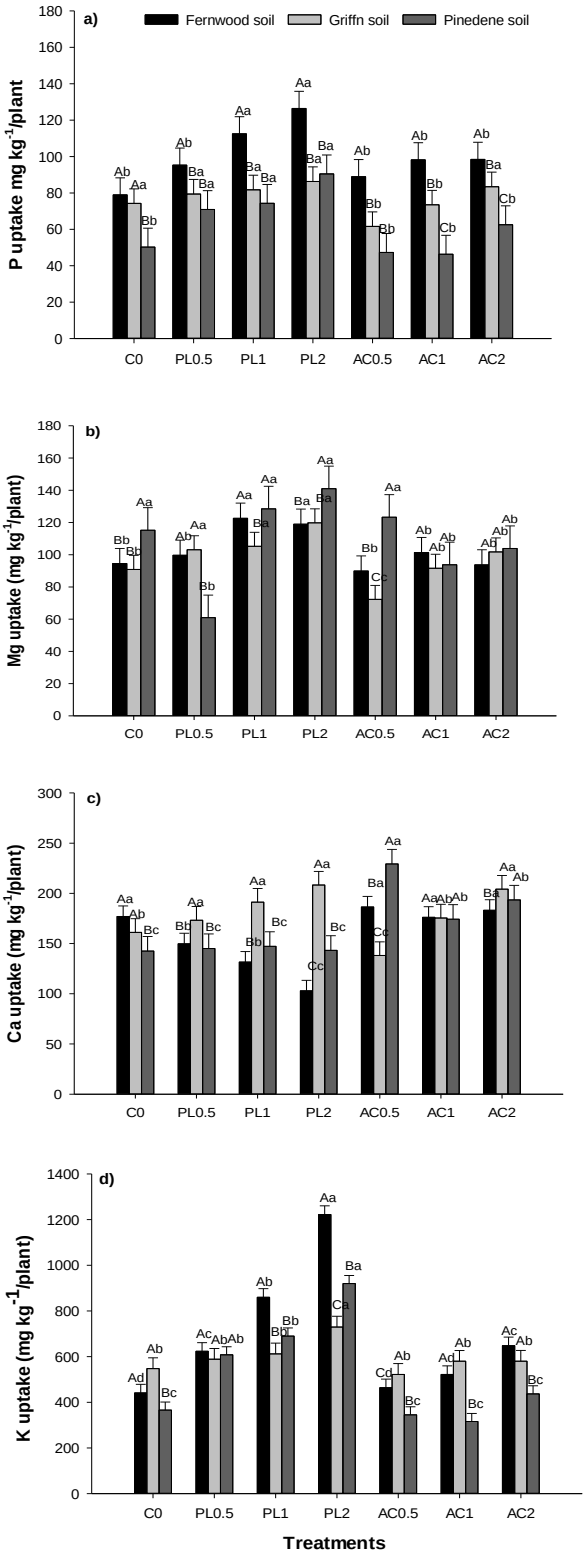


**Figure 7.2:** Effect of residual PLB and ACB on maize nutrient uptake; P (a), Mg (b), Ca (c), K (d). The different capital letters indicate significant differences among soil types under each treatment, while different lowercase letters indicate significant differences among soil types at $p< 0.05$.



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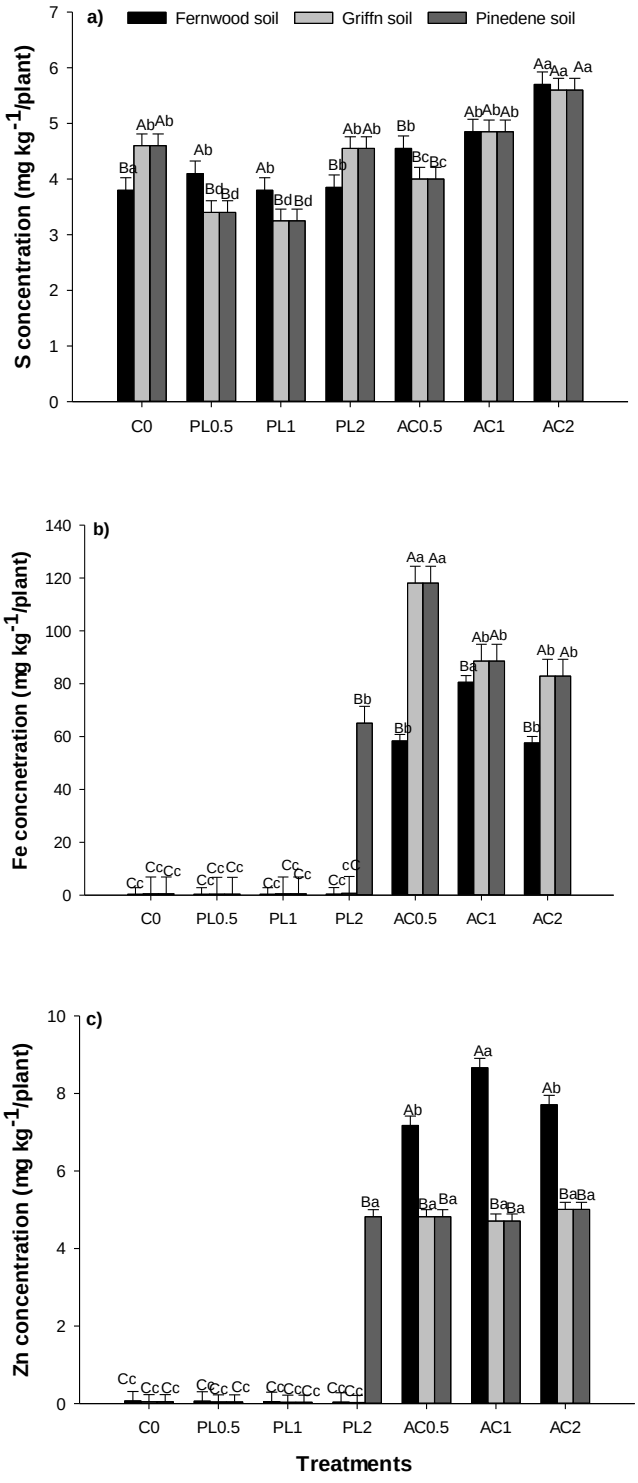


**Figure 7.3:** Effect of residual PLB and ACB on maize shoot nutrient concentration; S(a), Fe (b), Zn (c). The different capital letters indicate significant differences among soil types under each treatment, while different lowercase letters indicate significant differences among soil types at p< 0.05.



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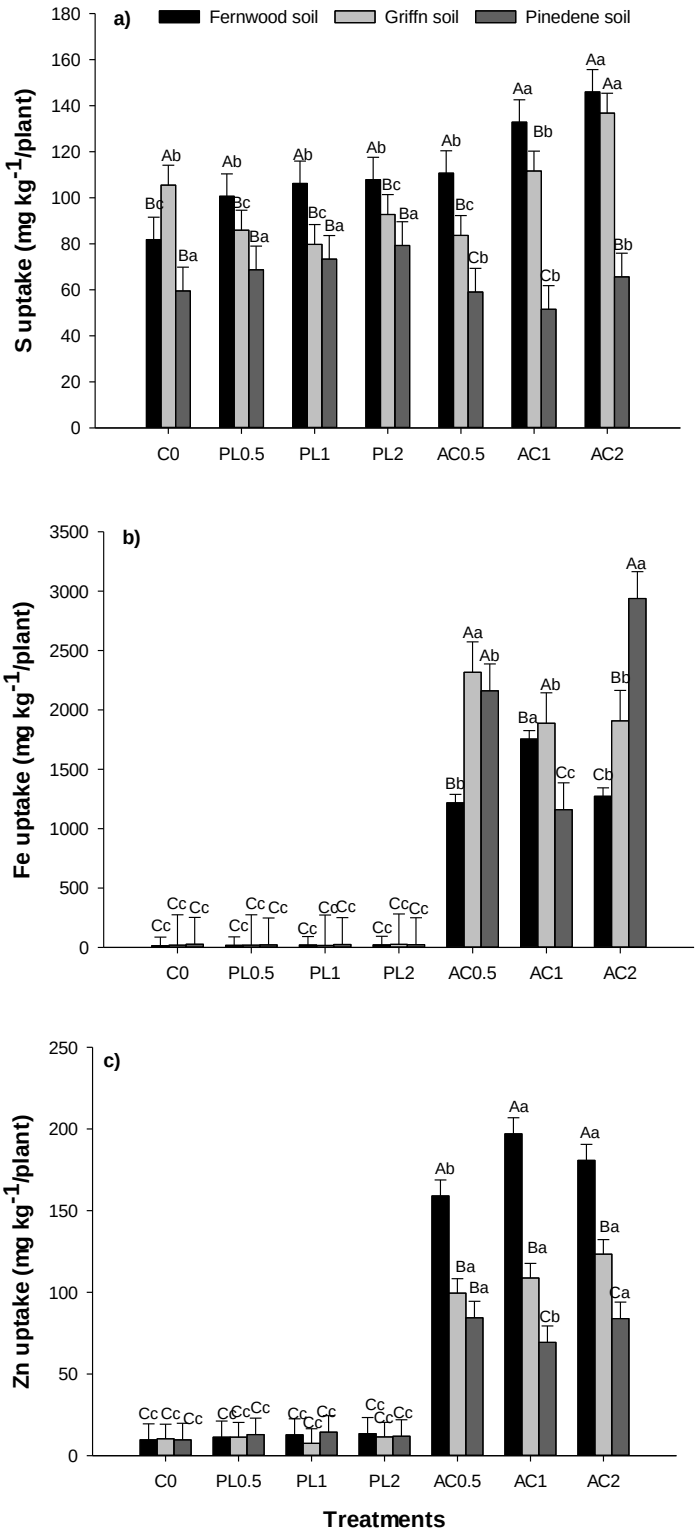


**Figure 7.4**: Effect of residual PLB and ACB on maize nutrient uptake; S(a), Fe (b), Zn (c).

The different capital letters indicate significant differences among soil types under each treatment, while different lowercase letters indicate significant differences among soil types at $p< 0.05$.



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## 7.5 Discussion

### 7.5.1 Residual effect of PLB and ACB on maize growth and N uptake

Several studies reported the increase in maize growth and nutrient uptake with biochar application alone (Cole et al., 2019), biochar + N fertilizer (Brantley et al., 2016) and biochar + compost (Manolikaki and Diamadopoulos, 2019). According to the literature search conducted and existing knowledge, there is no published study in literature that evaluated the impact of residual PLB or ACB on maize growth and nutrient uptake in varying soil types. In addition, available studies have only assessed the impact of different biochars derived from poultry or hardwood feedstocks on maize only (Rogovska et al., 2014, Xiao et al., 2016, Zhang et al., 2017) with soybean (Taskin et al., 2019), beans (Inal et al., 2015), or groundnuts (Martinsen et al., 2014). This study therefore reported for the first time the residual effect of biochar and contribution of N-fixed by chickpea to the growth of maize in different soil types.

The uptake of N by maize differed in response to residual PLB and ACB at different rates in the three contrasting soil types, varying in texture and pH. Despite the non-significant effect of residual PLB and ACB on shoot and root dry weight or N concentration of maize grown after maize (first maize), N uptake was greater in residual PLB and ACB treatments in the Griffin Pinedene, Fernwood soils than the control. Furthermore, application rates and soil type in this study contributed to the variation in maize biomass production and N uptake. In the Pinedene soil, for example, residual PLB and ACB at 2% appeared to be ideal to increase maize biomass and N uptake, whereas the Griffin soil required 2% PLB and 0.5 % ACB. Maize grown in the Fernwood soil with 1% residual PLB and ACB had higher shoot dry weight and total N uptake, but maize grown with 2% residual PLB and 0.5 % residual ACB had higher root dry weight.

The increase in maize biomass and N uptake as observed in this study with residual PLB and ACB is comparable to previous findings (Gunes et al., 2014, Manolikaki and Diamadopoulos, 2019). Similarly, in a short-term experiment lasting less than 115 days, maize growth and N uptake increased when poultry manure biochar was applied at a rate of about 10 t/ha (Inal et al., 2015, Rajkovich et al., 2012). In contrast, in a short-term experiment of less than 95 days, (Taskin et al., 2019) found no significant effect of biochar applied at a rate greater than 10 t/ha on maize growth and total N uptake. These findings suggest that biochar influences maize growth and nutrition; however, maize response will vary depending on the type of biochar used and the rate at which it is applied. Furthermore, the findings of this study showed that residual PLB can be used effectively in maize monocropping systems, as PLB treatments had higher maize biomass and N uptake than ACB treatments. Higher biomass production and N uptake were attributed to additional nutrients from the PLB, as well as faster mineralisation, when



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compared to the ACB. After harvesting maize that was used a as a reference crop, ACB-added soils had less available nutrients than PLB-added soils, as a result, maize grown in residual PLB was more likely to have the highest biomass and total N uptake.

### 7.5.2 Residual biochar and contribution of N-fixed by chickpea to maize growth and N Uptake

The increase in maize growth and nutrient uptake in the Fernwood, Griffin, and Pinedene soils was attributed to residual PLB and ACB as well as the total N-fixed by chickpea at different biochar rates. The highest root and shoot dry weight was found in maize grown after chickpea (second maize) in residual PLB and ACB at 2% in the studied soils. The average root and shoot dry weight of maize was significantly higher by 385%, 277%, 232% and 262%, 258%, 218% in all PLB treatments relative to the control in the Pinedene (sandy clay loam), Fernwood (sandy loam) and Griffin (clay loam) soils, respectively. When ACB was applied, maize root dry weight increased by 250%, 220%,191% and shoot dry weight increased by 201%, 207%, 206%, in the Pinedene, Griffin and Fernwood soil. The findings are consistent with previous studies, which found that applying biochar at a rate of 1% or 2% to a sandy loam soil, clay loam soil, loamy sand and silty clay loam soil increased maize root and shoot dry weight from 98 to 600 % (Inal et al., 2015, Manolikaki and Diamadopoulos, 2019).

The application of biochar may alter root growth and traits such as root length, volume, surface area, diameter and thus affect plant performance (Xiang et al., 2017). Although root traits were not measured in this study, changes in the rhizospheric environment due to the application of PLB and ACB may have improved root development, resulting in higher nutrient and water absorption and therefore lead to increased maize growth. Brantley et al. (2016) suggested that PLB could improve the accessibility of plant nutrients by promoting root growth and structural change. These findings suggest that the persistence of residual PLB in all soil types potentially increased maize growth mainly due to improved soil's environmental characteristics such as nutrient status, pH and cation exchange capacity (CEC) (Fig 4.1-4.5**).**

Maize grown in the Griffin soil in residual PLB treatments had higher biomass and N uptake where total N-fixed by chickpea was greater. This is because the dependence of maize on soil N was very low as the application of PLB increased so did total N resulted in less soil N uptake by the crop. However, ACB application at 0.5 to 2% reduced total N-fixed, thus maize depended largely on soil N as biomass and N uptake increased with lower total N-fixed but higher soil N uptake. On the other hand, maize grown in residual PLB and ACB treatments in the Pinedene soil depended largely on the total N-fixed by chickpea and soil N, because biomass and N uptake were greater at 2% where total N-fixed and soil N uptake were higher.



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Conversely, maize grown in PLB treatments in the Fernwood soil did not depend on total N-fixed by chickpea or on soil N, because higher biomass and N uptake were higher in treatments which had low total N-fixed and soil N uptake. This means that other nutrients such as available P from residual PLB attributed to the higher biomass and N uptake of maize in the Fernwood soil than the other two soils since soil P concentration was higher at 1 and 2 % PLB in the Fernwood soil than the other two soils (Fig 4.1). In contrast, maize grown in residual ACB treatments dependent largely on soil N as greater biomass and N uptake of maize was observed in soils with low N-fixed by chickpea.

The results suggest that chickpea can be used effectively in legume-maize rotation when PLB is applied at 0.5 to 2% in soils such as Griffin and Pinedene. This is because both soils had higher pH, macro and micro nutrient concentrations after PLB application than the Fernwood soil, which had the lowest nutrient concentrations after biochar application (Fig 4.1-4.3). The Griffin and Pinedene soils were moderately fertile than the Fernwood soil due to attributes like high buffering capacity, soil organic carbon, clay content, and CEC which allowed more nutrients to be retained. Plant growth and the addition of biochar have been linked to improved soil quality, nutrient release into soil solution, an increase in chemicals and/or beneficial organisms, and balanced plant nutrition (Gunes et al., 2014).

Residual PLB was effective in improving the growth and N uptake of maize than residual ACB, probably due to the difference in biochar properties such as low C/N ratio and high N in PLB compared to ACB. Thies et al. (2015) have shown that faster mineralisation of low C/N ratio biochars from animal feedstocks is more likely to stimulate N mineralisation than biochars derived from plant feedstocks. On the other hand, (DeLuca et al., 2015, Nguyen et al., 2017) found that woody derived biochar with a high C/N ratio reduces N availability due to high immobilisation. In addition, Rajkovich et al. (2012) observed a lower N concentration and N uptake of maize in biochar-modified fertile Alfisol because of N immobilisation due to the application of biochar with low N and high C/N ratio. Similar results have also been noted earlier (Gaskin et al., 2010, Nelissen et al., 2014, Rondon et al., 2007).

The European biochar initiatives guidelines (EBI, 2015) have shown that organic materials with a large C/N ratio above the threshold of 30 (w/w) have a significant effect on net N immobilisation when used as soil modification as such, affecting plant growth. Over a short period in a greenhouse experiments, nutrient-rich poultry biochar (Inal et al., 2015) or plants like switchgrass, corn stalks, or rice husks (Rajkovich et al., 2012) generally improved maize growth and N uptake than biochar made from hardwoods like pine or eucalyptus (Gaskin et al., 2010, Gonzaga et al., 2018, Rogovska et al., 2014). Since maize requires high N for its development and grain yield, it is therefore suggested from the results of this study that ACB



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biochar with a high C/N ratio of more than 30 g/g should be supplemented with N fertilizer or high N organic material for increased maize growth and yield especially in low buffered, acid soils such as the Fernwood. Moreover, given that N dynamics are highly complex, future studies need to address soil N interactions in biochar-treated soils.

### 7.5.3 Effect of residual PLB and ACB on nutrient uptake of maize

The higher N, P, Mg Ca, and K uptake by maize grown in all residual PLB treatments was due to the significant increase in rhizospheric nutrient concentration with PLB application in the Griffin and Pinedene soils (Fig 4.1-4.5). Although Fernwood soil showed the least increase in rhizospheric nutrient concentration, maize grown in residual PLB treatments especially at 1 and 2% had higher P, Mg, and K uptake, while Ca uptake decreased in all residual PLB treatments. The direct supply of residual PLB nutrients contributed to a large increase in the uptake of nutrients by maize. These findings suggest that the PLB served as a source of available plant nutrients and maintained levels above sufficiency after the initial application. The results are consistent with previous studies which reported higher N, P, K, Ca uptakes of maize with the addition of a poultry litter biochar (Brantley et al., 2016, Tag et al., 2016).The low response of Fe and Zn to PLB application in all soil types, on the other hand, suggests that biochar derived from poultry litter could be an alternative source for reducing element toxicity that affects plant growth by raising the pH of acid soils. Only a few studies have assessed the effect of poultry litter biochar amendments on Fe availability in various soils. For example, Inal et al. (2015) found that pelleted PLB application in a clay loam soil, increased maize growth, despite reduced Ca, Mg, and Fe concentrations in maize shoots. The concentration of Fe in maize shoots was lower at higher PLB application rates (Taskin et al., 2019). Poultry litter biochar can be used as a low-cost sorbent to remove inorganic and organic pollutants from the environment because of its large surface area, increased ion exchange capacity and different chemical composition. According to Xu et al. (2014), biochar can bind $Fe^{+3}$, $Al^{+3}$, and $Ca^{+2}$ ions by increasing the pH of the soils thus decrease Fe and increase P availability in the soil.

The decrease in Ca uptake in maize grown in the Fernwood soil is due to an increase in rhizospheric P and K concentrations at 2% PLB application (Fig 4.2), thereby a high concentration of P or K competes with Ca solubility and restricts Ca uptake (Manolikaki and Diamadopoulos, 2019)**.** Butnan et al. (2015) reported higher K content in maize tissue at 4% biochar application in a loamy soil, attributing their results to the competitive effect of K with Ca and Mg. Inal et al. (2015) suggested that reduced soil exchangeable Ca concentration, following the highest rate of biochar applied, could also cause reduced absorption of Ca by



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plants. Similarly, Gunes et al. (2014) found lower Ca concentrations after applying biochar or P-enriched biochar to lettuce plants.

Residual ACB did not have a significant effect on N, Mg, Ca, or K uptake, but resulted in a lower P uptake at 1% for maize grown in both Griffin and Pinedene soils, with no change in the Fernwood soil. The nature of the feedstock and final biochar product after pyrolysis, which had lower N and base cations than PLB, explains ACB's low response to N, Mg, Ca, and K uptake (see chapter 3, Table 3.1). The results are comparable to other findings, for example, 96 t/ha hardwood biochar applications did not affect plant tissue concentrations of N, K, S, Mg, or Zn, however resulted in lower concentrations of P, Ca, and Fe in Midwest Mollisol for 2 consecutive years (Rogovska et al., 2014).

In addition, Gaskin et al. (2010) found no differences in maize tissue concentrations of N, P, K, or Ca due to application of pine chip biochar at 11 and 22 t/ha in a loamy sand, Ultisols for two growing seasons. Gonzaga et al. (2018) observed a reduction in maize biomass, N and P nutrition with addition of pinewood chip biochar at 10, 20 and 60 t/ha in a sandy soil. However, the authors noted that soils treated with coconut husk biochar with high plant nutrients resulted in a 90% increase in maize biomass as well as tissue N and P concentrations. Plant-based biochar with low plant available nutrients clearly results in lower nutrient supply, which may affect maize production in cropping systems regardless of soil type.

Interestingly, maize grown in residual ACB had higher Fe uptake at 1 and 2 % in the Fernwood soil, resulting in less P uptake of maize despite a high rhizospheric concentration of P in the soil (Fig 4.3). In addition, higher Fe uptake of maize grown in Griffin and Pinedene soils with ACB resulted in lower P uptake of maize grown in both soils. The results show that there is a negative linear relationship between soil availability of Fe or P with crop development and P nutrition regardless of soil type. Furthermore, these results suggested that the application of ACB at 0.5 to 2% is likely to increase the concentration of Fe, which will then increase the binding and adsorption of P in acid soils, thus affecting plant growth. This is noteworthy because the soils had low Fe concentrations and during growth P deficiency in maize was observed during the experiment. This clearly demonstrates that more work on P and Fe nutrition with biochar application is required. However, these findings are in contrast with those obtained by Cole et al. (2019) who reported a linear decrease in the concentration of Fe when sugar maple hardwood biochar was applied at 2, 4, 6, and 8% in Winooski silt loam.

Overall, residual PLB at 0.5 to 2% had a positive effect on maize growth and nutrient uptake in the present study, whereas ACB had a negative or no effect on nutrient uptake, depending on the soil type, application rate, and maize response. The impact of biochar application rates on maize growth and nutrient uptake has been controversial and highly variable. For example,



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biochar derived from poultry litter and woody feedstocks at 10 t/ha to more than 60 t/ha increased (Brantley et al., 2016, de Sousa Lima et al., 2018, Inal et al., 2015, Rajkovich et al., 2012) decreased (Cole et al., 2019, Gaskin et al., 2010, Gonzaga et al., 2018, Taskin et al., 2019) maize biomass and nutrient uptake in various soil types. Further research is needed to determine the impact of different biochar application rates from different feedstocks on soil productivity and maize growth and yield. Farmers will be able to choose the best biochar type as biochar derived from various materials or pyrolysis conditions differ significantly in structure, nutrient content, pH, and stability. As this study shows, the properties of biochar, as well as its application rate and total amount applied can affect the soil environment, thus affecting plant development.

### 7.6 Conclusion

This study showed that residual PLB has the potential to increase maize growth and N uptake of maize than ACB in maize-maize monocropping systems, but the effect is greater in chickpea-maize rotation, because of the high nutrient supply and excess N through mineralisation of the biochar and N fixation by chickpea. Clay textured soils such as the Pinedene and Griffin, which are moderately fertile, will require the application of 40 t/ha PLB, whereas 20t/ha PLB will be ideal for low fertile sandy textured soils such as the Fernwood. Residual PLB at 10 to 40 t/ha had a positive effect on maize growth and nutrient uptake in the present study, whereas ACB had a negative or no effect on nutrient uptake, irrespective of the soil type, application rate and maize response. Further studies are therefore needed to determine the appropriate biochar application rates of various biochar feedstocks which are feasible for smallholder farmers for optimum maize production.



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# CHAPTER 8

# GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATION

## 8.1 General discussion

The following sections provide a summary of the study's findings as well as recommendations for future research.

### 8.1.1 Characterization of poultry litter biochar (PLB) and acacia biochar (ACB)

The physical and chemical characteristics of the biochars varied, but they all met the IBI and EBC criteria for use as a soil amendment. Biochar derived from poultry litter and acacia feedstocks pyrolyzed at 550 to 600 °C had total C greater than 50%, H/C and O/C ratio of less than 0.6 and 0.4, respectively, suggesting that when applied in the soil, both biochars could be stable and persist in the soil for about 1000 years, ensuring long-term C sequestration. PLB exhibited higher fine particles than ACB suggesting that when applied to the soil, PLB could provide a favourable habitat for microorganisms. Furthermore, both biochars had a low moisture content of less than 10% after pyrolysis, indicating that poultry litter and acacia feedstocks are commercially viable as biochar feedstocks.

Biochar produced from poultry litter feedstock retained more nitrogen and had low C/N ratio than biochar produced from acacia feedstock which had low N with high C/N ratio. This indicates that ACB biochar has the potential to immobilise N and is likely to cause N deficiency after application. Both biochars were alkaline with pH greater than 9.0 and could be used as a liming material and as a long-term solution for heavy metal stabilization in contaminated soils. On the other hand, PLB had more macro-and micronutrients, as well as a higher Na content than ACB. If added to soils with high Ca content, PLB's high Ca and K content could be a problem, as excess Ca could limit Mg and K availability, while excess K could bind P.

The high Na content in PLB resulted in a high EC value, which could have a negative impact on plant growth if soluble salt levels are high, resulting in less water and nutrient uptake by plant roots when PLB is applied at higher rates. To avoid soil salinization and nutrient imbalances, the amount of biochar added to the soil should be carefully calculated before use. Both biochars had higher levels of Fe, Mn, and Al than the toxicity levels for soils, implying that when applied at higher rates, both biochars could result in toxic levels of these trace elements, affecting plant growth. Caution should therefore be taken when using these biochars.



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Overall, the PLB biochar is highly recommended for use as a soil amendment to improve nutrients and soil quality, but caution should be taken when applied at higher rates. Meanwhile, the ACB biochar is an excellent biochar for C sequestration and water adsorption, but it should be applied months before planting or supplemented with high organic N and P materials when used as a soil amendment to improve nutrient availability, thus to avoid soil nutrient deficiency as it may temporarily block available N.

### 8.1.2 Pot experiment: PLB and ACB affects rhizospheric nutrient concentration and utilization for chickpea growth in different soil types

The findings suggest that increasing soil pH with biochar application is likely to increase nutrient availability in acid soils while lowering Fe toxicity concentrations. However, when using PLB or ACB on a poorly buffered loamy sand soil like the Fernwood, care should be taken to avoid over liming, which can cause nutrient deficiency and negatively affect nutrient uptake. Due to the short-term nature of the experiment, application of PLB and ACB at different rates had no effect on total C in the studied soils. Adding biochar at 2% in all soil types, on the other hand, indicates that PLB or ACB biochar has the potential to improve soil organic carbon, though the impact may be more noticeable in the long term. PLB contained more N, so its application at 0.5% in the Fernwood and Griffin soils and at 1% in Pinedene soils resulted in higher mineralisation by microbes, which increased soil nitrate, but at 2%, nitrate was reduced. When ACB was applied at 0.5-2% in all soil types, nitrate was immobilized due to its high C/N ratio.

Chickpea grown in highly buffered, fine-textured Pinedene and Griffin soils produced more root and shoot biomass than chickpea grown in sandy-textured Fernwood soil when PLB was applied at 0.5-2%, due to greater N, P, K, and Ca uptake. Despite high rhizospheric nutrient concentrations and retention in the Fernwood, Griffin and Pinedene soils at 2% ACB, chickpea shoot and root biomass was reduced due to low N uptake. After biochar application, the Fernwood soil had the lowest nutrient concentration; however, when PLB and ACB were applied at 0.5% rhizospheric P and nitrate increased, resulting in slightly higher chickpea biomass than the control. PLB and ACB applied at 0.5 or 1% (10 or 20 t/ha) could retain N and improve N availability for plant growth regardless of the soil type, as per the findings of this study. However, to improve chickpea performance in sandy textured soils like the Fernwood, ACB should be combined with organic materials that enhances soil aggregation, SOC and CEC, allowing the soil to retain and release nutrients for plant uptake.



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### 8.1.3 Molecular characterization of bacteria in response to biochar application

Bacterial diversity and community composition varied depending on soil type, biochar type, and application rates. In all soil types, biochar application increased the Chao1 and Shannon indices. The diversity of bacteria communities was higher at 2% PLB and ACB in the Fernwood soil, as well as 0.5% PLB and 2% ACB in the Griffin and Pinedene soils. In all soil types, however, biochar application had no effect on the Simpson index. This means that bacteria communities were abundant in all soil types, but not evenly distributed, with some bacteria species being more abundant than others. For example, after biochar application, the phylum Proteobacteria, which includes the genera *Sphingomonas, Bradyrhizobium,* and *Microvirga*, had the highest proportions of bacterial abundance in all soil types. This suggests that 0.5-2% PLB and ACB application has the potential to enhance nitrogen-fixing bacteria and *Sphingomonas* species, which are important for soil management in bioremediation and biodegradation to control environmental contaminants.

The phyla Actinobacteria, which includes the genera *Conexibacter*, and Acidobacteria, which includes the genera Bryobacter, were both affected by the increase in soil pH caused by biochar application, irrespective of the soil type. In this study, the relative abundance of Actinobacteria decreased as a result of biochar application, but the genera *Conexibacter* were more abundant at 2% ACB in the Pinedene soil. This is intriguing because the soil pH at this rate was 4.4, which is lower than Actinobacteria's pH. More research is needed on species from the *Conexibacter* genus, as they may be useful in bioremediation. On the other hand, PLB and ACB application increased phylum Acidobacteria in the Fernwood soil, but Acidobacteria increased with higher levels of ACB and decreased with PLB application in the Griffin and Pinedene soils. Furthermore, higher levels of ACB increased the relative abundance of *Bryobacter* in all soil types. Actinobacteria and Acidobacteria thrive in acid soils with pH less than 6, so this variation was related to the pH of the soils. Increased soil pH to 6.3 and 5.4 in the Fernwood soil favoured Acidobacteria but not Actinobacteria abundance, whereas pH of 7.0 and 5.0 in the Griffin and Pinedene soils hindered both Actinobacteria and Acidobacteria abundance.

With the genera *Bacillus,* Firmicutes is the fourth most dominant phylum. Firmicutes and *Bacillus* were found to be more abundant when 2% PLB was applied to the Fernwood soil, and 1% PLB was applied to the Griffin soil. The abundance of Firmicutes and *Bacillus* in the Pinedene soil did not change. Firmicutes species thrive in soils with a pH greater than 6, so PLB and ACB application in the Fernwood and Griffin soils favoured their abundance over the Pinedene soil. Soil pH, P, K, total N, and total C in the Fernwood soil, K and total C in the Griffin soil, and finally nitrate in the Pinedene and Griffin soils, explained the most variation in



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the relative abundance of bacteria community composition. The findings suggest application of PLB and ACB at 1% and 2%, respectively in the sandy-textured Fernwood soil could be ideal to increase bacteria species belonging to the phylum Proteobacteria, Acidobacteria, and Firmicutes, which are important for C and N cycling and for use in bioremediation of contaminated soils. However, high clay-textured Griffin and Pinedene soils will require the application of PLB at 2% to cause a shift in bacterial abundance.

### 8.1.4 BNF, C accumulation and WUE of chickpea in three contrasting soils, response to biochar application

The proportion of N derived from the atmosphere, total N-fixed, C accumulation and WUE by chickpea varied with the type of biochar applied at different rates in the study soils as demonstrated by the ANOVA (Table B.2, appendix). PLB application at 2% in Griffin and Pinedene soils reduced nodulation, increased %Ndfa, and total N-fixed in chickpea; however, as the rates were increased, chickpea accumulated less C and used more water. The increase in soil pH, P, K, and Mg caused by PLB application, particularly at 2%, resulted in increased shoot biomass and N accumulation, which was attributed to higher total N-fixed by chickpea in the Griffin and Pinedene soils. In addition, high Mo uptake and low Fe and B uptake by chickpea in Pinedene soils increased total N-fixed when PLB was applied. Furthermore, at 2% PLB, Proteobacteria, which are responsible for $N_2$ fixation, were more abundant in both soils. The reduction in chickpea nodulation caused by PLB application was due to higher soil nitrate, and thus the crop was dependent on both soil N and total N-fixed, particularly chickpea grown in the Pinedene soil. The decrease in total N-fixed by chickpea in ACB treatments in the Griffin soil, despite high soil pH, K, and Mg, high nodule formation, and %Ndfa, was due to low nitrate concentration and available P, which resulted in less biomass produced and thus affecting total N-fixed. On the contrary, despite low soil pH (5.0) and available P, low Mo uptake, and high Fe and B uptake, application of ACB at 0.5-2% in the Pinedene soil reduced nodulation and %Ndfa, but total N-fixed was higher at 1 and 2% compared to the control treatment.

Apart from soil pH, available nutrient, and biomass production; high N accumulation in chickpea shoots increased total N-fixed and C accumulation in Griffin and Pinedene soils, as there was a linear relationship between N content and total N-fixed, as well as N content and C content and total N-fixed and C content. Furthermore, at 0.5% PLB, where N content and total N-fixed were higher in both soils, chickpea accumulated more carbon but used more water than the control and other treatments. In contrast, chickpea grown in ACB treatment, particularly at higher rates in Griffin and Pinedene soils, produced less biomass and accumulated less C, but more N and used less water. However, at 0.5% ACB and PLB, the



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crop appeared to use less water than the control while producing more biomass and accumulating more C and N. Overall, the results show that high kaolinite clay soils like Griffin and Pinedene will require 40t/ha of either PLB or ACB to raise the pH, improve nutrient availability, and thus increase BNF by chickpea. When PLB is applied, chickpea may use more water to produce more biomass and accumulate N and C, but when ACB is applied, the opposite is true. Chickpea nodulation is greatly affected by high soil nitrate, particularly when PLB is applied in soils such as Griffin and Pinedene, but it has no effect on total N-fixed; however, ACB may reduce nitrate and increase nodulation while decreasing total N-fixed due to reduced biomass.

Higher rates of PLB reduced nodulation, %Ndfa, and total N-fixed of chickpea in the Fernwood soil, so the crop relied heavily on soil N, accumulated less C, and used more water, whereas higher rates of ACB resulted in higher nodulation and soil N uptake, but low %Ndfa and total N-fixed, so the crop accumulated more C and used less water. Application of 0.5% PLB and ACB in the Fernwood soil appeared to be ideal for increasing %Ndfa and total N-fixed of chickpea due to higher soil pH. The increase in chickpea shoot biomass at 0.5% PLB and ACB in the Fernwood soil was attributed to higher nitrate, which resulted in an increase in the amount of N derived from the atmosphere and total N-fixed. The decrease in %Ndfa and total N-fixed of chickpea at higher rates (1 and 2%) of PLB and ACB application in the Fanwood soil, despite higher P, K, Mg and higher nodulation in ACB treatments was related to the lower biomass production, reduced nitrate and low nodulation in PLB treatments. In contrast, chickpea grown in ACB treatments accumulated more C than the PLB treatments and the control, particularly at 1%, where N uptake was higher.

Moreover, chickpea used less water ($\delta^{13}C$ = -28.7) at 2% ACB, when 0.5 % PLB and ACB was applied. This suggests that applying 10 t/ha of poultry litter-derived biochar and acacia-derived biochar will likely raise soil pH, increase nutrients, including nitrate, and stimulate $N_2$ fixing bacteria, improve the amount of N derived from the atmosphere, and thus increase total N-fixed in low fertile soils like the Fernwood soil. Furthermore, because of increased biomass production and N accumulation, chickpea is likely to accumulate more C while using less water.

### 8.1.5 Residual biochar and N-fixed by chickpea affects maize growth and N uptake

The residual PLB and ACB had no effect on the shoot and root biomass of the first maize (maize grown after maize), but N uptake by maize varied with soil type, application rate, and biochar type. For example, maize grown in the Fernwood soil at 1% PLB and ACB, and at 2% PLB and ACB in the Pinedene soils, produced more biomass and accumulated more N,



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whereas maize grown in the Griffin soil produced only slightly more biomass and accumulated slightly more N at 2% PLB and 0.5% ACB. These findings indicate that sandy textured soils, such as the Fernwood soil, are likely to improve maize growth and N uptake when biochar derived from poultry litter or acacia is applied at a rate of 10t/ha; however, clay textured soils, such as the Pinedene and Griffin soils, will require a rate of 20t/ha poultry litter biochar to improve soil nutrients and thus improve maize growth and N uptake. Furthermore, the results of this study demonstrated that residual PLB can be used effectively in maize monocropping systems, as maize grown in PLB treatments produced more biomass and N uptake than maize grown in ACB treatments.

Residual PLB and ACB had a significant effect on maize shoot and root biomass and nutrient uptake, but this varied depending on the type of soil and biochar applied at different rates. Total N-fixed by chickpea, for example, did not account for maize growth and N uptake in the Fernwood soil, because maize produced more biomass and accumulated more N in residual PLB and ACB treatments with lower total N-fixed, particularly at 2% application rate. Maize growth in the Fernwood soil was aided by increased uptake of P, K, and Mg as well as other nutrients in residual PLB treatments because the crop did not rely on soil N or total N-fixed by chickpea; however, maize growth in ACB treatments was largely dependent on soil N because available P decreased with higher Fe uptake.

On the contrary, where total N-fixed was higher, maize grown in Griffin and Pinedene soils at with 2% residual PLB produced more biomass and accumulated more N. Also, maize produced more biomass and accumulated more N in residual ACB treatments of 2% where total N-fixed was higher in Pinedene soils, whereas, maize biomass was higher despite low total N-fixed in residual ACB treatments of 2% in the Griffin soil. Maize growth in the Pinedene and Griffin soils was attributed to higher uptake of N, P, Mg, Ca, and K in PLB treatments. Though P uptake was reduced due to high Fe uptake, as well as the non-significant effect of residual ACB on N, Mg, Ca, and K uptake, higher maize biomass in the Griffin soil was attributed to soil N, and higher maize biomass in the Pinedene soil was attributed to both total N-fixed by chickpea and soil N in residual ACB treatments. These findings show that when applied to acidic sandy and clay textured soils, PLB is persistent and can directly release or enhance nutrient availability even at a higher rate of 40t/ha. Furthermore, PLB has the potential to improve maize growth; however, maize productivity will be higher when rotated with chickpea, particularly in clay textured soils. ACB application, on the other hand, can improve maize growth when applied at 2%, but if soil N is limited, Fe concentration can inhibit maize growth.



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## 8.2 General conclusion

The main goal of this study was to investigate whether locally made biochar from poultry litter and acacia feedstocks could improve soil nutrient availability, improve the abundance of bacteria involved in C and N cycling, and thus enhance chickpea nodulation and nitrogen fixation in three different acid soils varying in texture and nutrient status. In addition to identify possible factors that influence the improvement of biological nitrogen fixation when biochar is applied, as well as to assess whether N-fixed by chickpea combined with residual N from biochar mineralisation can improve maize growth and nutrient uptake in the future.

The study showed that after pyrolysis, biochar made from poultry litter retained more nutrients than biochar made from acacia feedstock. As a result, when poultry litter biochar was applied at 0.5-2% (10-40 t/ha), the biochar improved rhizospheric pH, CEC, nutrient concentration (N, P, K, and Ca), resulting in higher biomass produced and nutrient uptake of chickpea grown in clay-textured, highly buffered and slightly acid, Griffin and very acidic Pinedene soils compared to low buffered, sandy-textured, acidic Fernwood soil. The findings of this study demonstrated that increasing soil pH with biochar application is likely to increase nutrient availability in acid soils while lowering Fe toxicity concentrations. Poultry litter and acacia biochar could retain N and improve N availability for plant growth regardless of the soil type when applied at rates of 10t/ha or 20t/ha, but at higher rates of 40 t/ha, N may be reduced and immobilized, thus affecting plant growth. However, when using poultry litter or acacia biochar on a poorly buffered loamy sand soil like the Fernwood, care should be taken to avoid over liming, which can cause nutrient deficiency and negatively affect nutrient uptake. Moreover, to improve chickpea performance in sandy textured soils like the Fernwood, biochar should be applied regularly or combined with organic materials to improve soil organic carbon thus improve soil aggregation, and CEC, allowing the soil to retain and release nutrients for plant uptake.

The sequence data from the metabarcoding analysis showed that applying poultry litter and acacia biochar at rates of 10 t/ha and 40 t/ha in the Fernwood soil was ideal for increasing the relative abundance of bacteria communities from the phylum Proteobacteria, Acidobacteria, and Firmicutes, as well as the genera *Sphingomonas, Bacillus, Bryobacter, Microvirga, Conexibacter,* and *Bradyrhizobium*. These microorganisms are important for C and N cycling as well as for their use in bioremediation. In high clay, textured soils like the Griffin and Pinedene, however, only the application of 40 t/ha of poultry litter biochar can cause a change in bacteria abundance.



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The pot experiment study showed that biological nitrogen fixation, C accumulation and water-use efficiency of chickpea as well as growth of maize in residual biochar and N-fixed vary with soil types, type of biochar applied, and application rates. For instance, the results revealed that poultry litter and acacia biochar applied at 10 t/ha in sandy textured soils like the Fernwood, 10-40 t/ha in soils like the sandy-clay-loam-textured Pinedene, and only 10-40 t/ha poultry litter biochar in soils like the clay-loam-textured Griffin could be ideal for improving BNF and C accumulation of chickpea. Chickpea could use more water to produce more biomass and accumulate N and C at higher rates of poultry litter biochar, whereas chickpea could use less water but accumulate less biomass, N, and C at higher rates of acacia biochar due to reduced nitrate and P. The main reasons for the improved BNF in this study are related to increased soil pH and rhizospheric nutrient availability (N, P, K, Mg, and Mo), which resulted in higher root growth, which stimulated higher abundance of Proteobacteria (probably due to increased root exudates), greater biomass production, and high N accumulation. Despite high nodulation as a result of low nitrate concentration, higher rates of acacia biochar could limit BNF by chickpea due to low biomass produced as a result of high Fe concentration, especially in soils like the Fernwood and Griffin.

The findings of this study also demonstrated that residual poultry litter biochar can be used effectively in maize monocropping systems, as maize grown in residual poultry litter biochar treatments produced more biomass and accumulated more N and other nutrients than maize grown in residual acacia biochar treatments, especially at 10 t/ha in the Fernwood soil and 20 t/ha in the Griffin and Pinedene soils. Moreover, improved BNF by chickpea combined with residual poultry litter biochar increased growth and nutrient uptake of maize grown in the clay textured soils of Griffin and Pinedene. Furthermore, poultry litter biochar has the potential to enhance maize growth; however, maize productivity will be higher when rotated with chickpea, especially in clay-textured soils. Acacia biochar can improve maize growth when applied at 40 t/ha, but if soil N is limited, Fe concentration can inhibit maize growth.

Overall, the findings of this study showed that biochar made from poultry litter can be used as a soil amendment to increase nutrient availability and the abundance of bacteria involved in N and C cycling in acidic, low-nutrient soils like the Fernwood, Griffin, and Pinedene soils. Furthermore, incorporating chickpea into smallholder farmers' current maize cropping systems and applying biochar made from poultry litter can help reduce the cost of nitrogen inputs by adding residual nitrogen through BNF, thus improving soil quality and maize production. • Heavy metals (Al, Se, Cr, As, Ni, Pb, Cd, Sr, Ba, and Si), polycyclic aromatic hydrocarbons (PAHs), and morphological properties (bulk density, porosity, and SEM images-to show the surface structure of the biochar material) of biochar derived from poultry litter and acacia were



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not measured in this study; therefore, future studies should evaluate those properties, according to the IBI and EBC guidelines.

### 8.3 Recommendation for future studies

- Heavy metals (Al, Se, Cr, As, Ni, Pb, Cd, Sr, Ba, and Si), polycyclic aromatic hydrocarbons (PAHs), and morphological characteristics (bulk density, porosity, and SEM images-to show the surface structure of the biochar material) of biochar derived from poultry litter and acacia were not measured in this study; therefore, future studies should evaluate those properties, according to the IBI and EBC guidelines.
- This study used the standard KCl method to measure nitrate and ammonium concentrations in poultry litter and acacia biochar amended soils, future studies could use other analytical methods, such as the serial extraction method, to compare or detect any potential equipment-related error when measuring nitrate and ammonium concentrations in biochar amended soils.
- The increase in nitrate levels as a result of poultry litter biochar application reduced nodulation but had no effect on total N-fixed. More research is needed to understand how poultry litter biochar as well as application rates affect nitrate concentration and chickpea nodulation in sandy and clay-textured soils in the field.
- In this study, shoots were only used to estimate BNF and soil N uptake. Future studies can quantify BNF by assessing N content in roots, shoots, pods, or even grain of chickpea in biochar amended soils to compare with the findings of this study.
- The findings of this study can be used as a groundwork for long-term field studies to determine the efficacy of poultry litter and acacia biochar in improving soil organic carbon, nutrient availability, and soil biological properties, as well as biological nitrogen fixation of chickpea and other legumes in various soils.
- The application rate of 40 t/ha in clay-textured soils may not be practical for farmers with limited access to organic feedstocks; consequently, a survey for farmers should be conducted to determine the application rate that may be evaluated for them.



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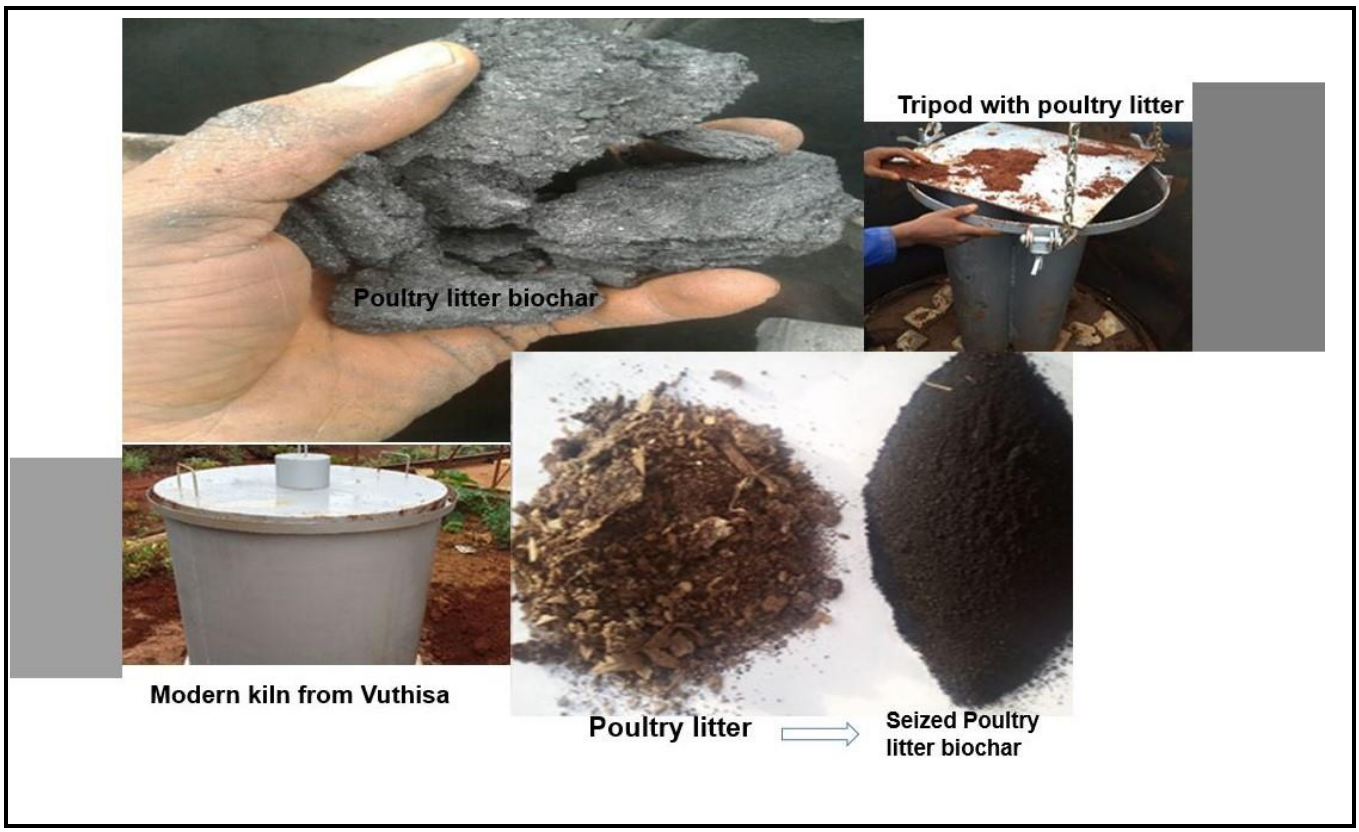
# APPENDIX A




**Picture A.1:** Pyrolysis of poultry litter biochar using modern kiln from Vuthisa Pty, Ltd.



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## APPENDIX B

**Table B.1:** ANOVA table representing the significance level of the treatments; soil type, biochar feedstock type and application rates and their interaction on selected soil macro and micro-nutrients at probability level P<0.05.

| Parameters | Soil type (ST) | Biochar feedstock type (BFT) | Application rates (APR) | ST*BTF | ST*APR | BFT*APR | ST*BFT*APR |
|---|---|---|---|---|---|---|---|
| pH | 0,001 | 0,001 | 0,001 | 0,001 | 0,001 | 0,001 | 0,003 |
| CEC | 0,001 | 0,001 | 0,001 | 0,053 | 0,001 | 0,001 | 0,022 |
| P | 0,001 | 0,001 | 0,001 | 0,664 | 0,076 | 0,001 | 0,845 |
| K | 0,001 | 0,001 | 0,001 | 0,01 | 0,001 | 0,001 | 0,795 |
| Ca | 0,001 | 0,636 | 0,001 | 0,673 | 0,01 | 0,461 | 0,148 |
| Mg | 0,001 | 0,001 | 0,001 | 0,01 | 0,001 | 0,001 | 0,052 |
| S | 0,001 | 0,551 | 0,071 | 0,01 | 0,01 | 0,017 | 0,198 |
| Total N | 0,01 | 0,666 | 0,001 | 0,251 | 0,003 | 0,053 | 0,078 |
| $NH_4^-$-N | 0,001 | 0,001 | 0,01 | 0,437 | 0,499 | 0,569 | 0,986 |
| $NO_3^-$-N | 0,001 | 0,001 | 0,001 | 0,001 | 0,975 | 0,006 | 0,001 |
| Total C | 0,755 | 0,702 | 0,001 | 0,978 | 0,928 | 0,728 | 0,872 |
| C/N ratio | 0,01 | 0,303 | 0,001 | 0,238 | 0,001 | 0,267 | 0,166 |
| Fe | 0,001 | 0,001 | 0,001 | 0,001 | 0,331 | 0,365 | 0,479 |
| Zn | 0,01 | 0,001 | 0,001 | 0,766 | 0,016 | 0,001 | 0,582 |
| Mo | 0,399 | 0,221 | 0,656 | 0,615 | 0,639 | 0,781 | 0,132 |
| B | 0,151 | 0,624 | 0,053 | 0,689 | 0,439 | 0,685 | 0,886 |
| Cu | 0,001 | 0,001 | 0,01 | 0,059 | 0,718 | 0,737 | 0,841 |
| Co | 0,001 | 0,981 | 0,611 | 0,045 | 0,001 | 0,75 | 0,097 |
| Mn | 0,001 | 0,357 | 0,126 | 0,001 | 0,001 | 0,004 | 0,009 |

Soil type- Fernwood, Griffin and Pinedene soils; biochar feedstock type- poultry litter and acacia biochar; Application rates- 0.5, 1, 2 % & control (0 %)



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**Table B.2:** ANOVA table representing the significance level of the treatments; soil type, biochar feedstock type and application rates and their interaction on chickpea and maize parameters at probability level P<0.05.

| | Soil type (ST) | Biochar feedstock type (BFT) | Application rates (APR) | ST*BTF | ST*APR | BFT*APR | ST*BFT*APR |
|---|---|---|---|---|---|---|---|
| **Chickpea parameters** | | | | | | | |
| Shoot biomass | 0,002 | 0,001 | 0,349 | 0,002 | 0,034 | 0,908 | 0,677 |
| Root biomass | 0,001 | 0,014 | 0,519 | 0,634 | 0,038 | 0,015 | 0,273 |
| Number of Nodules | 0,005 | 0,034 | 0,163 | 0,001 | 0,054 | 0,161 | 0,051 |
| Nodule dry Weight | 0,053 | 0,031 | 0,224 | 0,047 | 0,001 | 0,084 | 0,052 |
| N uptake | 0,001 | 0,001 | 0,01 | 0,001 | 0,321 | 0,576 | 0,051 |
| P uptake | 0,001 | 0,001 | 0,001 | 0,001 | 0,01 | 0,119 | 0,018 |
| K uptake | 0,048 | 0,001 | 0,007 | 0,142 | 0,819 | 0,296 | 0,33 |
| Ca uptake | 0,031 | 0,001 | 0,935 | 0,002 | 0,431 | 0,028 | 0,492 |
| Mg uptake | 0,001 | 0,001 | 0,166 | 0,05 | 0,463 | 0,204 | 0,548 |
| S uptake | 0,062 | 0,01 | 0,001 | 0,001 | 0,001 | 0,01 | 0,001 |
| Fe uptake | 0,012 | 0,01 | 0,307 | 0,003 | 0,387 | 0,693 | 0,074 |
| Zn uptake | 0,001 | 0,001 | 0,001 | 0,001 | 0,001 | 0,58 | 0,01 |
| B uptake | 0,001 | 0,001 | 0,001 | 0,001 | 0,846 | 0,239 | 0,009 |
| Mo uptake | 0,001 | 0,001 | 0,001 | 0,001 | 0,009 | 0,315 | 0,001 |
| Shoot % N | 0,001 | 0,288 | 0,001 | 0,001 | 0,065 | 0,369 | 0,272 |
| Shoot N content | 0,001 | 0,001 | 0,001 | 0,001 | 0,834 | 0,631 | 0,059 |
| δ15N (‰) | 0,001 | 0,001 | 0,05 | 0,001 | 0,001 | 0,791 | 0,001 |
| % Ndfa | 0,001 | 0,395 | 0,108 | 0,01 | 0,687 | 0,01 | 0,001 |
| Total N-fixed | 0,001 | 0,001 | 0,001 | 0,001 | 0,915 | 0,737 | 0,052 |
| Soil N uptake | 0,001 | 0,001 | 0,001 | 0,001 | 0,810 | 0,976 | 0,041 |
| PNUE | 0,001 | 0,001 | 0,001 | 0,001 | 0,010 | 0,05 | 0,216 |
| δ13C (‰) | 0,072 | 0,001 | 0,001 | 0,001 | 0,05 | 0,001 | 0,051 |
| Shoot C (%) | 0,103 | 0,001 | 0,045 | 0,001 | 0,105 | 0,11 | 0,261 |
| shoot C | 0,01 | 0,001 | 0,157 | 0,001 | 0,050 | 0,746 | 0,882 |
| shoot C/N | 0,001 | 0,01 | 0,001 | 0,001 | 0,050 | 0,604 | 0,607 |
| **Second maize parameters** | | | | | | | |
| Shoot dry weight | 0,001 | 0,001 | 0,001 | 0,069 | 0,057 | 0,038 | 0,659 |
| Root dry weight | 0,053 | 0,001 | 0,001 | 0,001 | 0,051 | 0,158 | 0,751 |
| N uptake | 0,001 | 0,003 | 0,001 | 0,502 | 0,050 | 0,054 | 0,224 |
| P uptake | 0,001 | 0,001 | 0,001 | 0,005 | 0,021 | 0,499 | 0,556 |
| K uptake | 0,001 | 0,001 | 0,001 | 0,001 | 0,001 | 0,001 | 0,856 |
| Ca uptake | 0,003 | 0,001 | 0,274 | 0,001 | 0,001 | 0,983 | 0,001 |
| Mg uptake | 0,002 | 0,236 | 0,004 | 0,038 | 0,023 | 0,001 | 0,001 |
| S uptake | 0,001 | 0,001 | 0,001 | 0,001 | 0,177 | 0,016 | 0,035 |
| Fe uptake | 0,004 | 0,001 | 0,004 | 0,005 | 0,001 | 0,005 | 0,004 |
| Zn uptake | 0,001 | 0,001 | 0,005 | 0,368 | 0,103 | 0,875 | 0,983 |

Soil type- Fernwood, Griffin and Pinedene soils; biochar feedstock type- poultry litter and acacia biochar; Application rates- 0.5, 1, 2 % & control (0 %)



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**Table B.3**: Pearson's correlation analysis of chickpea biomass and nutrient uptake with soil nutrient availability and some biochar properties.

| Variables | Shoot biomass | Root biomass | N uptake | P uptake | K uptake | Ca uptake | S uptake | Fe uptake | Zn uptake |
|---|---|---|---|---|---|---|---|---|---|
| PH | 0,083 | 0,516 | 0,080 | 0,087 | 0,284 | 0,125 | 0,077 | 0,190 | -0,143 |
| P | 0,108 | 0,085 | 0,199 | 0,046 | -0,317 | 0,304 | 0,103 | 0,001 | -0,001 |
| K | 0,490 | **0,654** | 0,467 | 0,344 | 0,250 | 0,540 | 0,405 | 0,508 | 0,228 |
| Ca | 0,284 | 0,586 | 0,209 | 0,279 | 0,635 | 0,167 | 0,245 | 0,412 | 0,090 |
| Mg | 0,493 | **0,686** | 0,428 | 0,348 | 0,500 | 0,418 | 0,394 | 0,542 | 0,261 |
| Ammonium | -0,122 | -0,185 | -0,170 | -0,015 | 0,175 | -0,189 | -0,091 | -0,063 | -0,024 |
| Nitrate | 0,369 | 0,145 | 0,395 | -0,009 | -0,544 | 0,634 | 0,061 | 0,220 | 0,117 |
| Total C | -0,216 | -0,171 | -0,120 | 0,125 | 0,229 | -0,305 | 0,110 | -0,174 | -0,049 |
| C/N | 0,256 | 0,275 | 0,234 | 0,545 | 0,599 | 0,029 | 0,338 | 0,402 | 0,329 |
| S | 0,434 | 0,440 | 0,332 | 0,405 | **0,641** | 0,291 | 0,384 | 0,500 | 0,316 |
| Fe | -0,242 | -0,597 | -0,227 | -0,093 | -0,358 | -0,198 | -0,215 | -0,336 | -0,030 |
| Zn | 0,375 | 0,353 | 0,436 | 0,231 | -0,189 | 0,519 | 0,321 | 0,255 | 0,205 |
| N uptake | **0,950** | 0,367 | 0,000 | 0,572 | 0,064 | 0,811 | **0,877** | **0,640** | **0,886** |
| P uptake | **0,644** | 0,232 | 0,572 | 0,000 | **0,625** | 0,420 | 0,548 | **0,672** | **0,615** |
| K uptake | 0,153 | 0,222 | 0,064 | **0,625** | 0,000 | -0,160 | 0,216 | 0,385 | 0,145 |
| Ca uptake | **0,893** | 0,411 | **0,811** | 0,420 | -0,160 | 0,000 | **0,677** | **0,756** | **0,664** |
| S uptake | **0,856** | 0,237 | **0,877** | 0,548 | 0,216 | **0,677** | 0,000 | **0,628** | **0,868** |
| Fe uptake | **0,810** | 0,513 | **0,640** | **0,672** | 0,385 | **0,756** | **0,628** | 0,000 | **0,605** |
| Zn uptake | **0,871** | 0,130 | **0,886** | **0,615** | 0,145 | **0,664** | **0,868** | **0,605** | 0,000 |
| Soil type | 0,519 | 0,161 | 0,503 | 0,320 | 0,228 | 0,319 | 0,490 | 0,372 | **0,611** |
| BFT | -0,547 | -0,344 | -0,559 | -0,049 | 0,512 | **-0,790** | -0,332 | -0,363 | -0,254 |
| pH-PLB | -0,374 | -0,408 | -0,345 | -0,006 | -0,204 | -0,287 | -0,513 | -0,283 | -0,264 |
| pH- ACB | -0,472 | -0,524 | -0,417 | -0,195 | -0,124 | -0,447 | -0,257 | -0,365 | -0,316 |
| N- ACB | -0,374 | -0,408 | -0,345 | -0,006 | -0,204 | -0,287 | -0,513 | -0,283 | -0,264 |

Bold text represents Pearson's significant correlation between the measured variable, BFT- biochar feedstock type, ACB- acacia biochar, PLB-poultry biochar



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**Table B.4:** Mean shoot $\delta^{15}N$ values of non-legume amaranthus as a reference plant used in calculating %Ndfa in each soil types and biochar application rate. Biochar is denoted as poultry litter biochar (PLB) and acacia biochar (ACB).

| | Control | PLB | | | ACB | | |
|---|---|---|---|---|---|---|---|
| Application rates | 0 % | 0.5 % | 1 % | 2 % | 0.5 % | 1 % | 2 % |
| Fernwood | 9.67b | 9.66b | 9.67b | 9.81b | 9.71b | 9.62a | 9.80a |
| Griffin | 9.69b | 9.64b | 9.86a | 10.08a | 9.91a | 9.79a | 9.79a |
| Pinedene | 9.86a | 9.75a | 9.71b | 9.89b | 9.82b | 9.84a | 9.82a |



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**Table B.5**: Relationship between BNF attributes and selected soil properties as well as abundance of N fixing bacteria in the Fernwood, Griffin and Pinedene soils

| Treatments | Shoot | Nodule No | %Ndfa | Total N-fixed | Soil N uptake | soil pH | soil $NO_3^-$ | soil P | soil K | soil Mg | Proteobacteria (PH= 6,5-7,0) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Fernwood soil** | | | | | | | | | | | |
| FC0 | 5030 | 28 | 18 | 14 | 64 | 5 | 1 | 18 | 15 | 134 | 29 |
| FPL0.5 | 6658 | 55 | 21 | 15 | 53 | 6 | 62 | 58 | 123 | 186 | 30 |
| FPL1 | 5778 | 1 | 7 | 6 | 82 | 6 | 47 | 105 | 214 | 207 | 30 |
| FPL2 | 4880 | 3 | 3 | 1 | 78 | 7 | 51 | 162 | 549 | 357 | 30 |
| FAC0.5 | 7133 | 3 | 30 | 37 | 48 | 5 | 55 | 30 | 19 | 145 | 29 |
| FAC1 | 5253 | 13 | 25 | 25 | 72 | 5 | 26 | 33 | 29 | 152 | 31 |
| FAC2 | 4432 | 60 | 22 | 18 | 50 | 6 | 15 | 43 | 46 | 149 | 31 |
| **Griffin Soil** | | | | | | | | | | | |
| GC0 | 8115 | 21 | 31 | 39 | 52 | 6 | 36 | 14 | 257 | 490 | 31 |
| GPL0.5 | 12560 | 13 | 32 | 71 | 53 | 7 | 53 | 39 | 450 | 557 | 34 |
| GPL1 | 10588 | 19 | 32 | 72 | 40 | 7 | 51 | 69 | 687 | 604 | 31 |
| GPL2 | 12790 | 10 | 42 | 90 | 50 | 7 | 50 | 86 | 782 | 629 | 42 |
| GAC0.5 | 6848 | 13 | 31 | 22 | 116 | 7 | 22 | 23 | 275 | 491 | 31 |
| GAC1 | 4303 | 55 | 26 | 21 | 137 | 7 | 23 | 15 | 257 | 444 | 34 |
| GAC2 | 8485 | 29 | 30 | 17 | 126 | 7 | 22 | 17 | 304 | 509 | 32 |
| **Pinedene soil** | | | | | | | | | | | |
| PC0 | 5893 | 3 | 24 | 29 | 62 | 4 | 50 | 85 | 38 | 214 | 28 |
| PPL0.5 | 15880 | 5 | 17 | 51 | 325 | 6 | 47 | 40 | 169 | 301 | 30 |
| PPL2 | 13343 | 1 | 23 | 57 | 314 | 5 | 41 | 80 | 451 | 344 | 34 |
| PAC0.5 | 6385 | 1 | 20 | 38 | 143 | 4 | 27 | 31 | 43 | 202 | 30 |
| PAC1 | 6885 | 1 | 20 | 42 | 174 | 4 | 24 | 20 | 55 | 237 | 24 |
| PAC2 | 5500 | 2 | 23 | 48 | 154 | 4 | 25 | 16 | 65 | 232 | 29 |



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# APPENDIX C

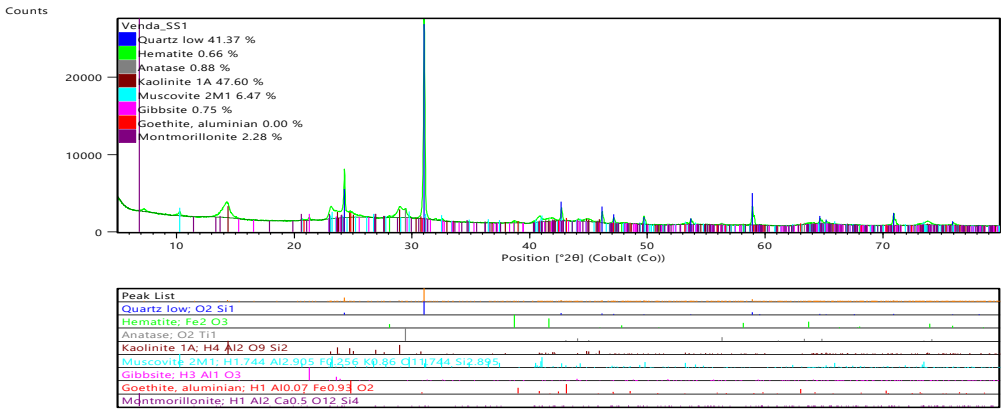


**Figure a: Fernwood soil**

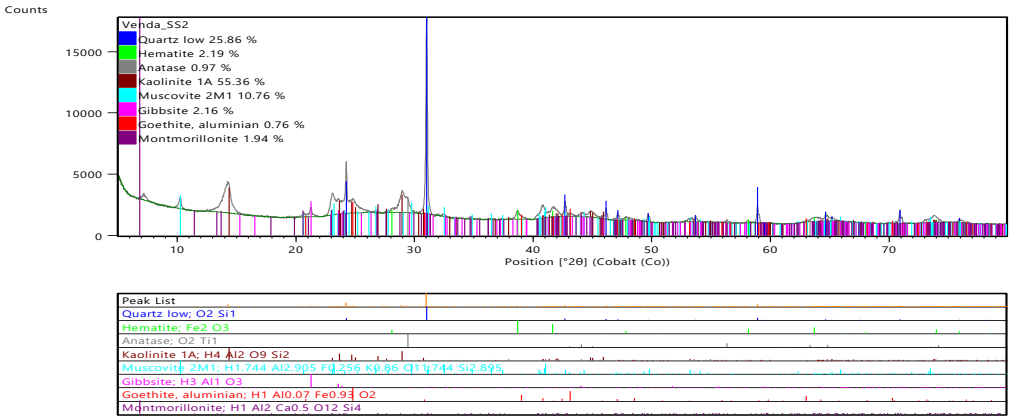


**Figure b: Pinedene soil**

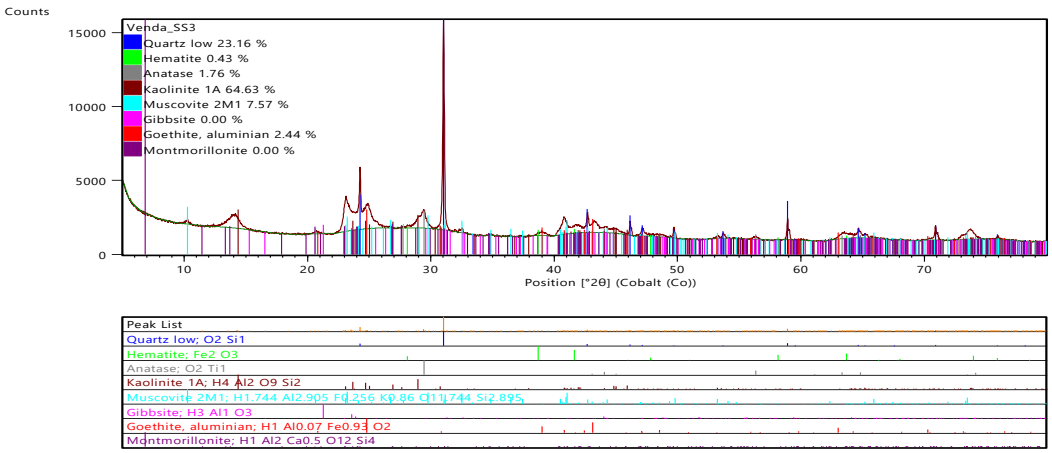


**Figure c: Griffin soil**

**Figure C.1**. Clay mineral composition of the Fernwood, Pinedene and Griffin soils, measured using the X-ray diffraction (XRD), the phases were identified using the X'Pert Highscore plus software.



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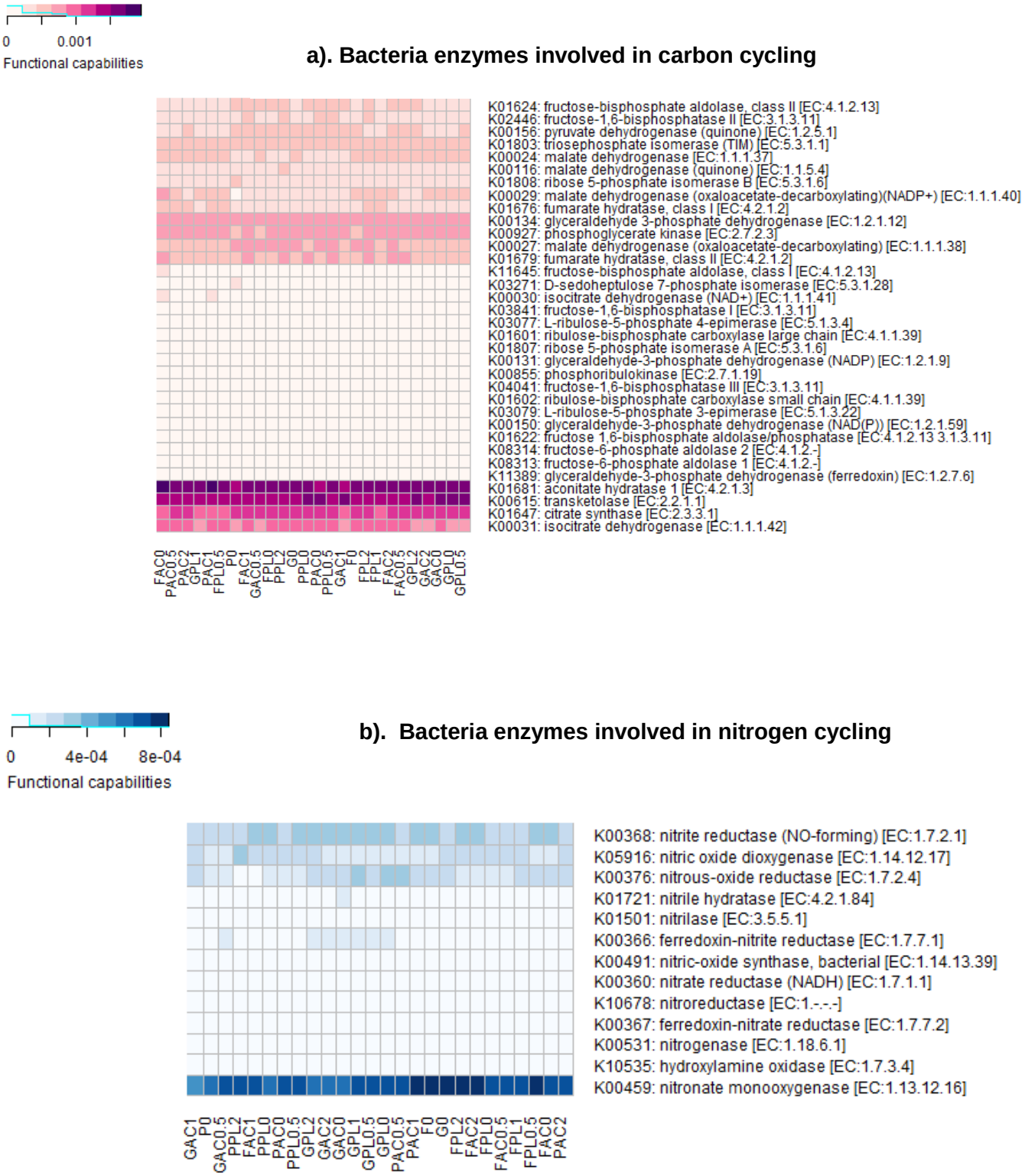


**Figure C.2:** Heatmap illustrating the functional capabilities of bacteria enzymes involved in a) carbon and b) nitrogen cycling in the Fernwood, Griffin and Pinedene soils, influenced by PLB and ACB application at 0, 0.5, 1 & 2 %.



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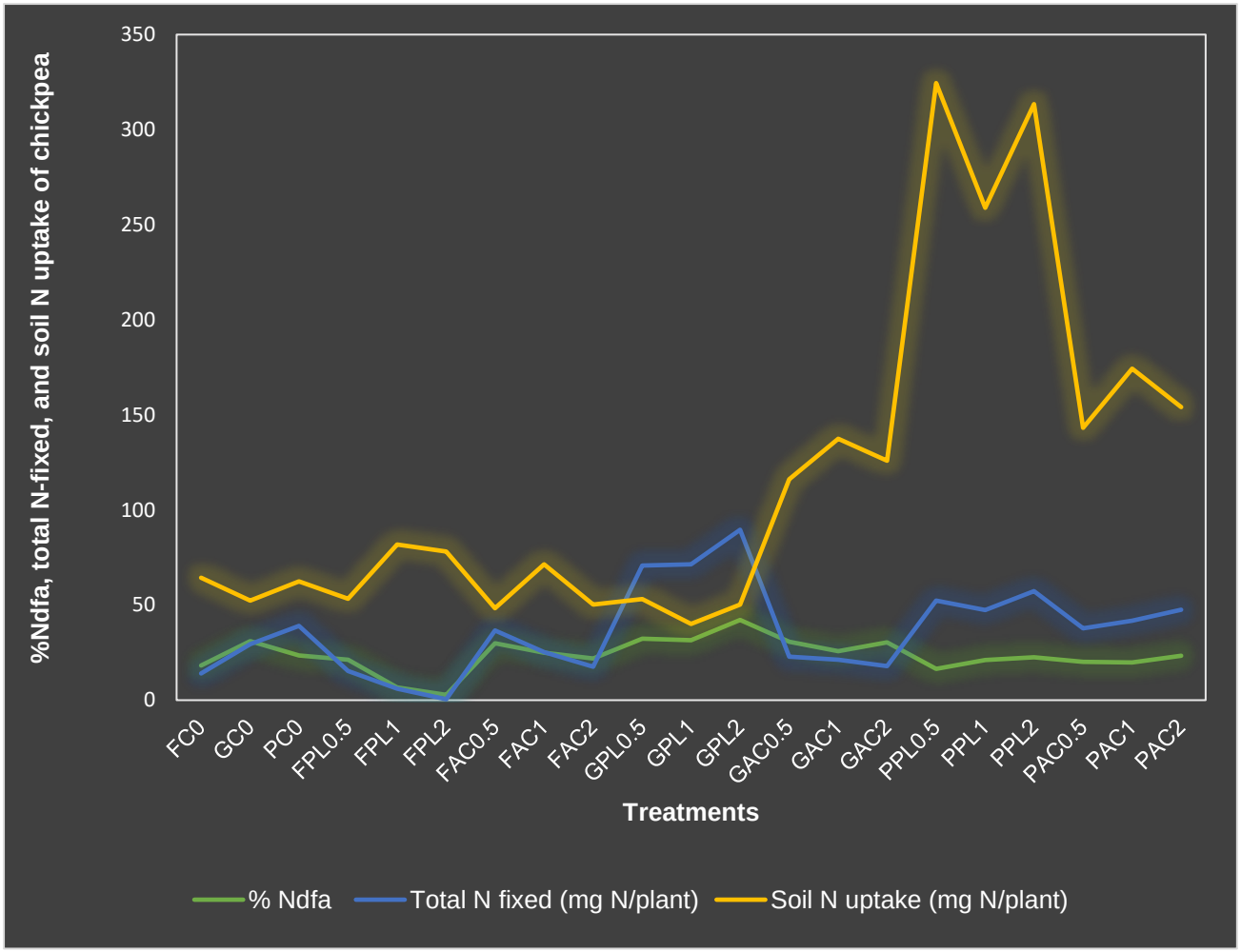


**Figure C.3:** The response of nitrogen derived from the atmosphere (%Ndfa), total N-fixed and soil N uptake of chickpea grown in the Fernwood soil, Griffin soil and Pinedene soils with PLB and ACB application at different rates of 0.5, 1 and 2 % and a control (0 %).



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