Biochar and Poultry Litter Effects on Maize Growth, Nutrient Uptake and Selected Soil Biological Activities in Different Soil Types

By

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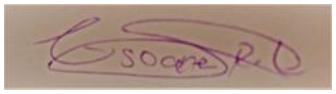
DECLARATION

I, Rethabile Linah, Ntsoane, declare that this research is my original work and has not been submitted for any degree at any other university or institution. The dissertation does not contain other persons' writing unless specifically acknowledged and referenced accordingly.

Signed (Student):  **Date** 07/ February/ 2022

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ABSTRACT

South African soils contain lesser percent organic carbon content compared to soils from many parts of the world. The loss in organic carbon content reduces soil fertility and drives an ever increasing demand for the use of soil amendments to enhance soil fertility. The study consiststed of a greenhouse pot experiment and a laboratory incubation experiment. The objective of a greenhouse study was to assess the effects of biochar and poultry litter application on maiize productivity under different soil types. Treatments consisted of different soil types and amendments of biochar and poultry litter. Soils were collected from four sites (Mutshenzheni, Rambuda, Tshiombo Irrigation and Tshiombo Madzivhandila) representing different soil types (Westleigh (We_1), Hutton (Hu), Westleigh(We_2) and Shortlands (Sd), respectively. Soils were amended with biochar and poultry litter. The amendments consisted of various mix ratios of biochar (BC) with poultry litter (PL) as treatments, which are namely, BC_0PL_0, $BC_{100}PL_0$, $BC_{75}PL_{25}$, $BC_{50}PL_{50}$, $BC_{25}PL_{75}$, and BC_0PL_{100}. Soil amendments were applied at different rates of 0- 5 t ha^{-1} PL and 0- 40 t ha^{-1} BC. Treatments were laid out in a completely randomize design (CRD) and replicated three times. Biochar and poultry litter application exerted no significant difference on soil pH of We_2, Sd, and Hu soils. However, the effect of biochar and poultry litter application was significant at soil We_1. In contrast, application of biochar and poultry litter had no effect on soil total N of soil We_1, We_2, and Hu and was significant on soil Sd. The results of this study showed that application of biochar and poultry litter treatments had no significant effect on both the maize growth and nutient uptake at early stages (Week 1 and Week 2). However, the application of biochar and poultry litter treatments had a significant difference ($p \geq 0.001$) on maize growth and nutrient uptake at a later stage (Week 3 to Week 6). Therefore, there is a potential to ameliorate fertility constraints in agricultural soils using biochar combined with poultry

litter. Though biochar possesses some essential elements required for plant growth, sole application reduces its efficiency with its effectiveness confirmed only when applied in combination with organic-based materials such as poultry litter. The laboratory incubation experiment assessed the effects of different biochar rates on soil chemical and bio-quality parameters. Each treatment consisted of a 200 g of soil (We_1, Hu, We_2 and Sd) homogenously mixed with biochar amendments (0, 10, 20, and 40, t ha^{-1}). The treatments were laid in a completely randomize design (CRD) and replicated three times. Soil sampling was done on day 0, 30, 60, 90, and 120, and samples were analyzed for soil available P and N and bio-quality parameters namely microbial biomass nitrogen, microbial biomass carbon, urease, alkaline and acid phosphatase, β glucosidase, soil organic carbon, and dehydrogenase activity. The results of the study revealed various responses of soil bio-quality parameters and selected soil chemical properties after biochar and poultry litter application. Thus, the effects of biochar rate, incubation days and soil type on soil enzymes and other bio-quality parameters elicited an understanding on microbial activity and soil enzymes mechanism . Therefore, a prolonged study (more than 120 day) is required to evaluate the effects of incubation days, biochar rate and soil type effect on soil nutrients and bio-quality parameters.

DEDICATION

I would love to dedicate my work to the man above all mankind (Modimo wa mehlolo), who gave me strength when nobody could. He taught me to love my neighbours like I love my self, he is worthy to be praised. I will never separate my work with a selfless soul, my late father ‘’Hlabirwa ‘a Gobetse ‘a Hlabirwa ‘a Phaahla ‘a Phaahla’’ and mother ‘’Mahlako’’ who taught me to believe in myself. Most importantly, my beloved sons Phomolo Bohlale Omphile and Phomelelo Babushi Onkarabile, Ntsoane, for being the only reason I kept going and never gave up.

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Table of Contents

LIST OF TABLES

List of figures

Chapter One

1. Introduction

1.1. Background information

Soil quality is an important factor that determines the nature and capacity of the ecosystem to support plants and animals (Khan and Huq, 2014). Thus, the processes carried out in soil should maintain and conserve both the nutrient and quality of the soil. Soil organic matter is a vital determinant and component of soil quality. Furthermore, numerous reports abound on the significant correlation between soil quality and soil microbes (Meliani *et al.*, 2012 and Rao *et al.*, 2019). Nonetheless, the continuous loss of organic matter in soils for most areas of the world represents a major concern (Paz-Ferreiro *et al.*, 2012). This depletion, which leads to a decline in soil fertility, is associated with a loss of both soil quality and the carrying capacity of the ecosystem to provide services. The decline in soil fertility is attributed mainly to continuous cultivation, coupled with rapid organic matter mineralization (Chivenge *et al.*, 2007). Furthermore, soil degradation has been identified as major cause of food insecurity and poverty. According to Du Preez *et al.* (2011), soil degradation in South Africa poses a serious threat to sustainable agriculture with 58% of soil containing less than 0.5% organic carbon content while only 4% contain more than 2% organic carbon. Consequently, there is a need to preserve organic carbon pools in soil. Generally, 2 to 5% of organic matter decomposes annually and the remainder constitutes the majority of carbon stored in mineral soils, which is stable on time scales of centuries to millennia (Horwath, 2005; Whitman and Lehmann, 2009). Therefore, the first role that the soil played historically as sources or sinks of carbon is associated with changes in land management (Trumbore, 2000). This distinctive characteristic illustrated by the fertility of the Terra preta soils of the Amazon that has retained charcoal over thousand years is the motive of an increasing interest in using biochar as a soil fertility enhancer

(Lehmann *et al.*, 2003). Biochar is a charcoal produced through pyrolysis in an oxygen-limited environment (Deem and Crow, 2017). Biochar's recalcitrant nature facilitates the general accrual and retention of soil organic carbon (SOC), where it is rapidly decayed. Therefore, biochar addition to soil could provide a potential sink for carbon (Duku *et al.*, 2011) and contribute to reduced global warming (Atkinson *et al.*, 2010). Biochar addition has been reported to influence the activity of soil microorganisms by enhancing available soil nutrients (dissolved organic matter, P, Ca and K), adsorption of toxic compounds and improved soil water and pH status, hence a changed soil microbial biomass (Lehmann *et al.*, 2011). In addition, biochar application to soil provides a recalcitrant food source for microbes, favourable habitat for soil microflora and alters predation rates by soil micro-fauna (Pietikäinen *et al.*, 2000; Warnock *et al.*, 2007). Khan and Huq (2014) showed that biochar has gained recognition as an effective tool for enhancing soil health by storing carbon and other nutrients in the soil. Emphasis is increasingly being turned away from a strictly chemical approach for assessing soil fertility and other important soil qualities. Instead, a biological approach is required to assess soil processes related to crop production, soil quality and overall soil sustainability (Fauci and Dick, 1994). Soil organisms provide a myriad of ecosystem services and hence, understanding its response after biochar addition to soil is critical in ensuring soil quality (Warnock *et al.*, 2007). Nevertheless, organic matter in soil changes over time due to weathering process, interactions with soil minerals and oxidation by microorganisms in soil (du Preez *et al.,* 2011). This provides the reason for using soil biological activities as indicators of soil organic carbon. Soil microbial biomass, particulate organic matter, dissolved organic carbon, dehydrogenase, β-glucosidase, acid and alkaline phosphatase are used to reflect the microbiological activities in soil (Mondini *et al.*, 2004). Soil microbial biomass C and N, are the driving force in the decomposition of organic materials and are frequently used as early indicators of agricultural ecosystem (Baaru *et al.*, 2007).

The organic matter available in soil depends mainly on the size and the activity of the microbial biomass (Piotrowska and Koper, 2010). Soil enzymatic production is also strongly connected to the soil organic matter (SOM) because reduced SOM production decreases the soil enzyme activities (Kumar *et al.,* 2013). Higher organic matter level provides enough substrate to support higher microbial biomass, hence higher enzyme production (Yuan and Yue, 2012). Enzymes are essential to all living cells since they are able to catalyse the biological and chemical processes in the soil. Soil dehydrogenase enzymes are responsible for biological oxidation of organic matter by transferring the hydrogen electrons from the organic substrate to inorganic electron acceptor (Ghaly and Mahmoud, 2006). Glucosidase enzymes are more involved in cellulose degradation while phosphatase, are intimately involved in P cycling; and both influence fertilizer use efficiency (Piotrowska and Koper, 2010). Moreover, soil microbial activities strongly affect soil function, and consequently, crop growth and yield (Piotrowska and Koper, 2010).

Direct application of poultry litter alone may cause environmental concerns such as odour and leaching of nutrients in agricultural lands (Inal *et al*., 2015). Nonetheless, poultry litter is a greater source of nutrients and improves availability of nutrients (Lentz and Ippolito, 2012). It is well known that biochar application on soil improves microbial abundance, nutrient availability and contribute to reduced global warming (Duku *et al.*, 2011; Atkinson *et al.*, 2010; Warnock *et al.*, 2007). However, Biochar efficiency with poultry litter is unclear (Inal *et al*., 2015). Chan *et al*. (2007) and Major *et al*. (2010), reported improved nutrient concentrations after application of biochar and poultry litter while Lentz and Ippolito, (2012), reported a decrease in iron after poultry litter and biochar application. In a 6-week greenhouse maize experiment showed improved maize and bean growth after biochar and poultry litter application (Inal *et al*., 2015). Therefore, biochar of poultry manure could be used effectively for agricultural purposes.

Maize (*Zea mays L*.) is a staple grain crop grown in South Africa and consumed by more than half of the population as a primary staple food (Makhaga *et al.*, 2011). Ramaru *et al.* (2000) revealed that soil fertility decline affects maize growth and yield. Furthermore, Mabapa *et al.* (2010) attributed soil fertility decline to continuous cropping without organic and inorganic fertilizer use. In addition, Gichangi *et al.* (2002) reported that fallow periods are no longer feasible with increased human population and land-use pressure. Limited rainfall, risks from erratic climate and high fertilizer cost have reduced the use of fertilizers (Odhiambo *et al.*, 2010). Therefore, there is a need for the introduction of alternative soil fertility improvement strategies to enhance and sustain crop productivity. Biochar as a soil amendment improves quality and fertility in different soil types (Blackwell *et al.*, 2009). Biochar amended soils are also characterized by high level of organic matter (OM), higher CEC, pH, base saturation and nutrients such as N, P, K, Ca and improve crop production (Kristin, 2011). There are hardly any studies done in South Africa on the interaction of biochar application with soil microbial activities. Further research is required to understand how biochar amendments in different soil types affect the activities of soil microbes, plant growth and nutrient uptake.

1.2. Problem statement

Soil fertility is generally low in the tropics, due to rapid organic matter mineralization and the presence of highly weathered secondary minerals (van Wambeke, 1992). South Africa's smallholder farming conditions are worsened by soil fertility decline, which is a principal and pervasive constraint to crop production (Lynch, 2009). However, soil fertility improvement has been successfully achieved through inorganic and organic fertilizer use. Major disadvantages inherent in the use of inorganic fertilizers include higher cost and lower accessibility to emerging farmers (Sohi *et al.*, 2010; Mabapa *et al.*, 2010). While cover crops, mulches, compost, or manure additions have also been

reported to improve nutrient use efficiency, they mineralize rapidly in soil and last for only a few growing seasons (Lehmann and Rondon, 2006). There are scant records on studies that show the interaction of biochar and poultry litter application with soil microbial activities in South African soils. Therefore, further research is required to understand how biochar amendment in different soil types affect the activities of soil microbes, plant growth and nutrient uptake.

1.3. Justification of the study

Amending soils using biochar has a potential to improve the fertility of the soil by altering the chemical and physical properties of the soil (Mbagwu and Piccolo, 1997). Thus, biochar application to soil is crucial in maintaining appropriate levels of soil organic matter and biological cycling of nutrients (Jien *et al*., 2015). Moreover, biochar application to soils has a potential of improving soil nutrient retention and water-holding capacities, and sustain carbon storage thereby reducing greenhouse gases emissions (Duku *et al*., 2011). Biochar addition to soil transforms nutrients by creating a larger microbial biomass thus increasing soil nitrification and changes the pH of the soil. Uptake of N and P by plant is stimulated by the production of mineralizing enzymes (Lehmann *et al*., 2011). On the other hand, biochar application to soil mitigates emission of other greenhouse gases because of the ability to stabilize the liable carbon in soil. Hence, improved soil fertility status has a positive response to crop production, grain yield and dry matter through soil carbon stabilisation. Consequently, improved crop yield will lead to increased food availability, which will ensure food security and lead to better nutrition and income. The findings from the study will add on knowledge that already exists about biochar and poultry litter application effects on maize production, and benefit smallholder farmers by improving their crop production through well manage soil fertility. The issue of food insecurity has been critical in many parts of the world including South Africa, and particularly at rural household level. Earlier report

has linked food insecurity closely to low crop production, low income, poverty and unemployment (van Averbeke and Khosa, 2007). However, low crop production levels still exist in South Africa compared to many other parts of the world.

1.4. Objectives

1.4.1. Overall objective

The overall objective of this study was to assess the effect of biochar and poultry litter application on maize growth, selected soil bio quality parameters and chemical properties in different soil types.

1.4.2. Specific objectives

The specific objectives were to determine the effects of applying biochar and poultry litter in different soil types on;

i. Soil bio-quality parameters

ii. Selected soil chemical properties (pH, EC, N, P, K, Ca, Mg, and total C)

iii. Maize dry matter (DM) yield

1.5. Hypotheses

i. Biochar and poultry litter application will affect soil bio-quality parameters

ii. Biochar and poultry litter application will affect selected soil chemical properties (pH, EC, N, P, K, Ca, Mg, and total C)

iii. Biochar and poultry litter application will affect maize dry matter (DM) production

Chapter Two

2. Literature review

2.1. Biochar

Biochar is defined as a by-product of the process of pyrolysis, which is derived from the black carbon biomass, and intended to amend soil (Lehmann and Joseph, 2009). Biochar is a solid carbon-rich material produced by heating biomass in an oxygen-limited environment. Traditional charcoal production using earthen and brick kilns, vented a large amount of volatiles causing atmospheric air pollution compared to modern pyrolyzers, which captures production of bio-fuels and syngas (Zheng *et al.*, 2010). Biochar is essentially charcoal produced for agricultural purposes. Thus, the use of biochar as a soil additive is proposed as a means to simultaneously mitigate anthropogenic climate change effects whilst improving agricultural soil fertility. Soils throughout the world contain biochar deposited by natural events such as land clearing by field fires (Krull *et al.*, 2008). Lehmann and Rondon (2006) showed that biochar is a recalcitrant organic carbon that contains soil micro- and macro-nutrients. This distinctive characteristic is illustrated by the fertility of the Terra preta soils in the Amazon, which has retained charcoal for over thousand years. In contrast to other chars, biochar comprises of mainly stable aromatic form of organic carbon, which cannot readily degrade, or be emitted to the atmosphere as carbon dioxide even under favourable environmental and biological conditions (Sohi *et al.*, 2010). Consequently, studies indicate that biochar may also decrease emission of other greenhouse gases such as nitrous oxide and methane (Zheng *et al.*, 2010).

2.2. Biochar production

Biochar is a highly stable compound created by heating biomass at high temperatures between 350˚C and 600˚C in anaerobic conditions (Whitman and Lehnmann, 2009). Biochar is one of the most abundant renewable resources available globally. Though there are varieties of feedstock, biochar in South Africa is mainly made from agricultural residues such as sugar cane bagasse, maize stock and organic wastes (Uras *et al.*, 2012). Traditionally, most agricultural residues were scarcely utilized (Duku *et al.*, 2011). Potential biochar's properties are more dependent on the biomass it originated from. Feedstock may be derived from different biomass; agricultural residues such as corn and wheat, yard waste, industrial by-products, animal manure and sewage sludge (Laird *et al.*, 2009).

Mineral ash constitutes about 1-20% by weight of lignocellulosic biomass which is composed of nitrogen (N), phosphorus (P), potassium (K), silicon (Si), calcium (Ca), cadmium (Cd), mercury (Hg) and arsenic (As), while extractives constitute 1-10% by weight (Duku *et al.*, 2011). High yield biochar is generated when a lignin rich feedstock is pyrolyzed at high temperatures (Demirbas *et al.*, 2006). Thus, high productive biomass such as grasses are highly recommended compared to biomass which are characterized by low mineral and nitrogen content.

2.3. Effect of biochar on soil microbial biomass carbon and nitrogen

Soil microbial biomass (SMB) is defined as an active component of the of ecosystem, which regulates many critical functions and properties of soil and environmental qualities. The functions and processes include nutrient cycling, decomposition of organic residues, structural stability, and indicator of soil pollution and bioremediation (Islam and Wright, 2004). Biochar effects on soil biological processes are not well understood (Lehmann *et al*., 2011). This is reflected in high variability in the response of

soil microbial biomass to biochar additions reported in the literature (Grossman *et al.*, 2010; Khodadad *et al.*, 2011; O'Neill *et al.*, 2009). There is a huge variability in physical biochar structures depending on the parent material and the conditions present at their formation (Czimczik and Masiello, 2007). Zang *et al.* (2014) showed that biochar amendments have no significant effect on soil microbial biomass and suggests a shift to a more bacteria dominant community with biochar addition.

Other studies have reported that biochar amendments reduced soil microbial biomass through toxicity effect (Dempster *et al.*, 2012). In contrast, Kolb *et al.* (2009) reported that microbial biomass and activity significantly increased with biochar application. Furthermore, Yoo and Kang (2011) reported greatly enhanced microbial biomass N with decreased C/N ratio following biochar application in soil. Increased microbial biomass N suggests microbial immobilization of N while a significant decrease in microbial C/N ratio in the biochar treated soil suggests a possible shift in microbial community structure (Haytham, 2012 and He *et al*., 2013).

Biochar application rates and soil type also affected response of soil microbial biomass (Lehmann *et al*., 2011). Dempster *et al*. (2010) showed a decline in soil microbial carbon after biochar application at rates 0, 5 and 25 t/ha and improved soil fertility of a coarse textured top-soil. In a study conducted by Chen *et al*. (2015) in the Western part of Australia, the initial microbial biomass C: N ratio of 8: 1 did not show any difference from the biochar addition at 5 t/ha, but showed a declined ratio of 5:1 with biochar addition at 25 t/ha. Chen *et al*. (2015) concluded that biochar application on soil has no significant effect on soil microbial nitrogen. Biochar addition to soil supplies carbon to upgrade microbiologically unfavourable C/N ratios in nitrogen rich environment (Odugbenro *et al*., 2019).

2.4. Effect of biochar on soil enzyme activities

Enzyme activity is defined as a biochemical technique used to reveal information about the enzyme metabolic processes (Shaw and Burns, 2006). Soil enzymatic activities are often linked to important soil parameters such as organic matter, soil physical properties and microbial activity and can be integrated to past soil biological management (Dick and Kandeler, 2005). Biochar can stimulate overall microbial activity in the short-term (Smith *et al*., 2010). As a result of possible limitations of microbial stimulation, some enzyme activities may be increased while others are reduced (Kolb *et al*., 2009). Soil enzyme assays after 7 days' incubation showed that soil with 2% biochar amendment had significantly increased activities of enzymes compared to that of a non-biochar amended soil (Swaine *et al*., 2013). Conversely, the purified enzyme assays showed that biochar application had variable effects on soil enzymatic activities (Lehmann *et al*., 2011). This suggested that the results from the first experiment conducted by Swaine *et al*. (2013) were inconsistent. Thus, a second experiment was conducted and seven days after biochar was added to three different soils, fluorescence-based assays revealed significant increased enzyme activities (Bailey *et al*., 2011).

A pot experiment conducted by Khan and Huq (2014) to study the effect of biochar on the abundance of soil bacteria showed that bacteria were not able to survive in biochar-amended soil due to nutrient deficiency, decreased sorption of enzymes as well as binding of essential enzymes. In another experiment conducted by Swaine *et al*. (2013) to determine the biochar alteration of the sorption of substrates and products in soil enzyme assays, results showed that the pine wood and barley straw biochar amended soils caused a significant reduction in substrate concentration and extractable product in soil dehydrogenase enzyme assays. Consequently, biochar effects on soil enzyme activities are not well understood and impact on assay constituents will limit the genuine biochar identification. There are variable responses relating the biochar effect on the

soil enzymatic activities. Therefore, there is a need for an extensive research, which has more conclusive data that complement the fluorescent and colorimetric assays. The data should indicate whether the biochar has caused increased or decreased enzyme activity (Ennis *et al*., 2012).

2.5. Effect of biochar on soil Nitrogen

Nitrogen is the single most limiting nutrient in primary crop production (Peake *et al*., 2014). Biochar addition to soil alters the nitrogen dynamics by reducing the environmental harmful N-fluxes (Clough and Condron, 2010). Several mechanisms are used to explain the apparent retention of N in biochar-amended soils and the reduction of N leaching (Clough *et al*., 2013). Global N cascade is as a result of consequent leaching of ammonia (NH_3) and nitrate (NO_{3-}) and enhanced emission of nitrous oxide (N_2O) due to the increasing intensification of agricultural system. Although Rondon *et al*. (2007) reported enhanced biological N fixation in biochar-amended soils, there is a need for further studies on the effect of biochar on soil biological activities (Bailey *et al*., 2011). Nevertheless, Clough *et al*. (2013) showed that biochar application on soil alters soil nitrogen (N) dynamics.

Studies are required to predict agronomic and N cycling responses since biochar implications on N immobilisation and mineralization are specific to individual soil-biochar combinations. After 10 weeks' incubation study with biochar addition at 25 t/ha, Dempster *et al*. (2010) showed that net nitrogen mineralisation decreased from an average of 11 mg N/kg dry soil to 1 mg N/kg dry soil. The results could not explain the sorption of nitrogen to biochar at the rates (0, 5, and 25 t/ha). In an apple orchard experiment, biochar addition at the rate 20 t/ha led to no significant change in Mineral-N content (Sivakumaran *et al*., 2010).

Biochar application in soil showed a reduced N_2O gas emission when incubated for 100 days (Wu *et al*., 2012). In addition, DeLuca *et al*. (2009) showed that biochar application in soil influenced nutrient transformation by increasing nitrification and N uptake by plants, respectively. Furthermore, biochar application on soil showed increased nitrogen fixing bacterium and associated mycorrhiza fungi, especially in nitrogen poor environment (Rondon *et al*., 2007). Kim *et al*. (2007) reported that un-amended Amazonian soils had 25% greater microbial diversity and more N fixing organisms compared to Amazonian dark earth soils nearby. Biochar application to the soil did not improve the inorganic nitrogen on soil due to excess nitrogen that leaches into adjacent surface water (Manning, 2012). Moreover, the excess nitrogen tends to reduce in situ soil biodiversity and causing harmful eutrophication.

2.6. Effect of biochar on soil Phosphorus

Biochar application to soil is reported to increase the availability of P in the rooting zone. The effect of biochar on P availability can be partly traced back to physical characteristics like porosity, sorption capacity, surface area, and charge density, and to biological changes (Peake *et al*., 2014). DeLuca *et al*. (2009) reported increased availability and uptake of P in acid, natural forest soils while Glaser *et al*. (2002) reported increased bioavailability of P, metal cations, and trace elements after biochar treatments in soil of the humid tropics. After three weeks' incubation study, Soinne *et al*. (2014) indicated that biochar addition did not increase the sorption of P in incubated soils as the biochar that was used had a very low P sorption affinity. Biochar solubilisation when added to soil may result in the minerals becoming available to plants.

2.7. Effect of biochar on soil Potassium

Biochar pyrolysis might volatilize other elements (e.g. nitrogen) or hold others in insoluble form (e.g. magnesium) (Angst and Sohi, 2013). However, Karim *et al*. (2017) indicated the potential of biochar- potassium as a substrate for the provision of conventional potassium (K) fertilizer since K is largely conserved and converted into K-containing soluble salts. Although soil potassium reserves are large, a small amount of exchangeable or soluble forms of K will be available during a season of intense cropping (Simonsson *et al*., 2009).

In a pot trial, Wang *et al*. (2018) investigated the effect of biochar application on soil potassium dynamics and crop uptake. The results of the study revealed an enhanced K-dissolving bacteria after biochar application on entisols and alfisols, which was associated with change in pH and water soluble K. Furthermore, biochar has been documented to affect nutrient cycling (Rahimzadeh *et al*., 2015), by enhancing activity and abundance of microbes (Grossman *et al*., 2010).

Potassium- dissolving bacteria are able to solubilise K-bearing minerals by excreting organic ions to bring K into soil solution (Sheng *et al*., 2008). Biochar is suggested as a means to enhance soil potassium and soil fertility at large. (Lehmann *et al*., 2011). Hence, a conclusion by Wang *et al*. (2018) that biochar could be a feasible soil amendment to improve available soil K, but response of crop K uptake may vary depending on soil types.

2.8. Effect of biochar on soil Ca, Mg, EC, and soil pH

Biochar decreases the possibilities of leaching and improves nutrient cycling (Steiner *et al*., 2007) by assuring improvement of degraded soil as it improves soil properties (Yamato *et al*., 2006). Biochar application has also been shown to increase soil electrical conductivity (EC) and pH (Liang *et al*., 2006). However, the effect of increased

pH and EC varied with salt contents of biochar as it differs with characteristics (Spokas, 2010). In a 10 days' incubation experiment, Shah *et al*. (2017) reported a significant increase of soil EC and pH on biochar amended soils compared to control treatment. However, the study also showed that biochar carbon percentage evolved as carbon dioxide generally declined with increasing biochar carbon levels.

The liming effect of biochar on soil pH was observed in acidic soils (Dume *et al*., 2015). However, little is known concerning the impact of biochar application on alkaline soils (Mohawesh *et al*., 2018). The effect of biochar application in soils totally depend on the methods of biochar application applied and application rate on soil (Edenborn *et al*., 2015). Biochar application to soil usually provide only modest contribution to the total soil nutrient capital because soil generally contain relatively large total nutrient pools (Chan *et al*., 2007). In addition, biochar added to soils have the potential to alter chemical properties, which in turn can influence the rate of nutrient transformation (Atkinston *et al*., 2010).

An increased soil pH when biochar is added to acid soils is associated with increased alkaline metal (Ca^{2+}, Mg^{2+} and K^{2+}) oxides in biochar and reduced soluble Al^{3+} concentration (Steiner *et al*., 2007). However, Syuhada *et al*. (2016) found that biochar rate significantly affected availability of Ca, and Mg in the soil. This suggest that biochar addition as an amendment could provide a good nutrient source (Yuan and Xu, 2010).

2.9. Maize origin, description and production

Maize (*Zea mays* L.) is the world's 3rd most important cereal crop after wheat and rice (Harris *et al*., 2007). The maize plant can grow up to 4 m tall and can be grown under diverse environments. Maize belongs to the grass family Poaceae which originated from Mesoamerica, Mexico (Paliwal, 2000). The plant has spread through various parts of the world and was introduced to Africa soon after discovery. Although some maize

types produced in Africa are derived after introductions from South America, Mexico and parts of the eastern South America (Farnham *et al*., 2003), most of South African maize trace back to varieties grown in South America. Most of maize crop in South Africa is being used as feed for animals and as staple food (Du Plessis, 2003). Pholo (2009) showed that maize is a relatively cheaper source of energy that is traditionally used to brew beer.

South Africa produces 48% of white maize and the remaining 52% is yellow maize. White maize is primarily used for human consumption while the yellow maize is mostly used for animal feed production (DAFF, 2014). In addition, worldwide consumption of maize is more than 116 million tons, with Africa consuming 30% and South America 21%. The southern parts of Africa use 85% of maize production as food compared to other parts of the world, which use most of the maize as feed for animals. Although economic review of the South African agriculture 2019/ 2020 has reported a 21.7% increase in field-crop production, the contribution of agriculture to GDP has decreased by 9.8% which was estimated at R81 337 million in 2019 (DALRRD, 2020). In addition, BFAP BASELINE Agricultural Outlook 2018-2027, reported a 31% year on year decline in white maize production in South Africa (BFAP, 2018). Therefore, there is a need to advance the increased production and yield of maize in South Africa.

2.10. Effect of biochar on growth and yield of maize

Several studies have demonstrated the significant effect of biochar addition to soil on improved crop production (Chan and Xu, 2009; Laird *et al.*, 2010; Lehmann *et al.*, 2011; Rondon *et al.*, 2007). Biochar application to soil enhanced the overall soil quality by altering the soil physical, chemical, and biological properties and subsequently increasing crop productivity. Maize yields were much higher in plots amended with biochar and fertiliser than the non-fertilised and non-biochar amended control on the terra preta soils (Steiner *et al.,* 2007). Furthermore, the study showed that it is strictly

incorrect to say that the effect of the treatments had increased maize yield since the yields for all treatments displayed post-clearance decline and control yields were ultimately very low. A field experiment with biochar application under a maize-soybean rotation showed that, availability of nutrients such as Ca and Mg were greater with biochar, and crop tissue analysis showed that Ca and Mg were limiting in this system. Increased soil pH and decreased exchangeable acidity trends were observed with biochar application. In another experiment, biochar application to soil showed an overall increase in crop yield and nutrient uptake from 77–320% with greater available Ca and Mg (Major *et al.*, 2010**)**. The use of a low dosage biochar tested on minimally tilled soil had significantly increased crop yield in Zambia's sandy acidic soils (Cornelissen *et al.*, 2013). However, field trials carried out in a red sandy clay loam ultisol east of Lusaka, central Zambia, showed a moderate but non-significant effects on maize yield (Cornelissen *et al.*, 2013). Therefore, the effect of biochar on chemical, biological and physical properties are soil type dependent.

2.11. Effect of biochar and organic fertilizers on soil and crops

Application of biochar with organic fertiliser is an efficient way of increasing soil organic matter (SOM) (Fischer and Glaser, 2012). Application of an organic fertilizer combined with biochar is more beneficial to the soil compared to applying biochar and organic fertilizer individually. Kuzyakov *et al.* (2009) reported increased biochar decomposition during co-composting biochar with an organic material. In addition, compost-biochar amendments are likely to benefit the soil by biological activation of biochar, higher long-term carbon sequestration and enhanced nutrient use efficiency compared to individual compost and biochar application. Moreover, in a greenhouse pot trial carried out for 4 months, Karami *et al.* (2011) reported a significant increase in maize crop biomass yield after an application of green waste combined with biochar. In contrast, Schulz *et al.* (2014) showed that biochar integration with compost was outweighed by compost alone. Therefore, single biochar application yielded different results integrated one.

2.12. Effect of biochar and poultry litter on soil and crops

Poultry litter produced as a by-product on intensive production facilities may pose environmental concerns as it decomposes quickly and release greenhouse gasses (Chan *et al.*, 2007). While biochar usage can be a good strategy for recycling wastes and promote plant growth (Atkinson *et al.*, 2010). Musumuvhi *et al.* (2018) indicated that co-composting biochar with poultry litter improves performance of maize, and soil physical and chemical properties. Biochar co-composting is also beneficial for providing a habitat for microbes, promote aeration, and increase pH, N, P, K, Ca, and Mg concentrations (Li *et al.*, 2014). Moreover, co-composting biochar with poultry litter significantly increased nutrient availability (pH, N, P, K, Ca, and Mg) and their use efficiency in both pot trials and field trials (Maru *et al*., 2015; Olasekan *et al.*, 2019).

Chapter Three

3. Experiment 1 (Greenhouse study)

An 8-week controlled greenhouse experiment was carried out to assess maize nutrient uptake and dry matter production in different soil types, following combined application of biochar with poultry litter, and sole application of both poultry litter and biochar

3.1. Materials and methods

3.1.1. Soil, biochar and poultry litter characterization

3.1.1.1. Soil characterization

Soil samples used in the greenhouse experiment were collected randomly using an auger, at a depth of 20 cm at four sites (Mutshenzheni, Rambuda, Tshiombo Irrigation and Tshiombo Madzivhandila) representing different soil types (Westleigh$_1$, Hutton, Westleigh$_2$ and Shortlands), respectively (Soil Classification Working Group, 1991). The top layers that is normally rich in organic debris was removed in each collected soil. Westleigh soil form originate from Orthic topsoil of a soft plinthic subsoil which is usually gleyed, shallow, and imperfectly drained. On the other hand, Hutton soil form originates from an Orthic topsoil of a red apedal subsoil, often deep, structureless soils, with wide variety of texture and a base status. Shortlands soil form originates from an Orthic topsoil of a red structured subsoil, which is often deep, structured, and formed from a basic parent material. Soils were collected on fallowed farms with a history of limited use of pesticides and inorganic fertilizer. In the past five years, maize and vegetables were the main crops grown on these farms. Samples from each site were thoroughly mixed, air-dried and passed through a 2-mm sieve. Afterwards, selected chemical and physical properties were determined. Soil pH was measured in both 1 N KCl and H_2O (1:2.5, soil: solution ratio) using pH meter (White, 1997). Electrical conductivity (EC) was measured in water using conductivity meter with the soil solution ratio 1:2:5

(Okalebo *et al.*, 2002). Soil texture was determined following the hydrometer method as described by Bouyoucos (1962). Organic carbon content was determined using the Walkely and Black (1934) method. Available P and total N, were determined using the Bray 1 method (Bray and Kurtz, 1945) and Kjeldahl procedure (Bremner, 1960), respectively. Ammonium acetate extraction procedure was used to determine cation exchange capacity (CEC) and exchangeable cations as described by Peech (1965).

3.1.1.2. Physico-chemical properties of soils before planting

Soils test results were interpreted using Labserve's and Wingerdbemesting's test and interpretation (Conradie,1994). Table 2 shows that the soil Westleigh$_1$ (We$_1$), Hutton (Hu), Westleigh$_2$ (We$_2$), and Shortlands (Sd) had pH values of 7.02, 7.09, 7.06, and 7.06, respectively (Appendix 1). We$_1$ had a low total N content compared to Hu, while We$_2$ and Sd soils had the highest total N content compared to the other two soils (Table 2, Appendix 2). All soils (We$_1$, Sd, Hu and We$_2$) had higher K content (Table 2). All the soils had high Mg content, optimum Na content and extremely low exchangeable acidity with higher Ca contents (Table 2). We$_2$ soil had considerably low available P content, followed by Sd, while the Hu and We$_1$ soil had optimum available P. All the four soils (We$_1$, We$_2$, Hu, and Sd) had high amounts of soil micronutrient (Zn, Cu, Mn, and Fe) content, except for We$_2$ soil which had a very low Zn content (1.0 mg kg^{-1}) (Appendix 2). In terms of soil textural classification, We$_1$ is loamy, both Hu and We$_2$ are sandy clay loam, while Sd is clayey (Table 2). The soils had very low CEC status with high organic matter content in all soils except We$_2$ soil with very organic matter content (Table 2).

3.1.1.3. Biochar and poultry litter characterization

The biochar used in the experiment was generated by slow pyrolysis (400-450 °C) of pine wood biomass. Biochar used was obtained from Lanstar Energy Company, a commercial supplier based in Johannesburg. Biochar used was analyzed for selected chemical properties shown in Table 1, using methods of analysis described for soil

analysis in section 3.1.1.1. On the other hand, poultry litter used in the greenhouse experiment was collected from the University of Venda, School of Agriculture experimental farm. Poultry litter consists of feces, feathers, spilled feed, straws and saw dust. The collected poultry litter was allowed to decompose for three weeks, dried and passed through a 2-mm sieve. Thereafter, the poultry litter was analyzed for selected chemical properties using the analysis procedure described for soil analysis in section 3.1.1.1.

3.1.1.4. Chemical composition of Pine wood biochar (BC) and poultry litter (PL)

Both biochar and poultry litter had high nutrient content (N, Ca, Mg, K, and Na) as shown in Table 2. Biochar had a strongly alkaline pH of 8.90, higher than that of poultry litter, which had a neutral pH of 7.03. PL had higher organic matter percentage (OM %), higher than that of BC by 20.22 % (Table 2). Biochar had lower CEC status of 1.32 $cmol_c\ kg^{-1}$, while poultry litter had higher CEC status of 17.74 $cmol_c\ kg^{-1}$ (Table 2). Table 2 also shows that BC had low percent moisture, less volatile matter, and ash content.

3.1.2. Greenhouse experimental set-up

The treatments were set up in 25 cm diameter plastic pots of 29 cm in height. The plastic pots were placed on a steel table, filled with 5000 g of soil containing biochar and poultry litter treatments described in Table 1. Biochar and poultry litter treatments were applied 2 weeks before planting to allow thorough mix up and reaction with the soil. Treatment combinations comprised of biochar and poultry litter as follows: BC_0PL_0, $BC_{100}PL_0$, $BC_{75}PL_{25}$, $BC_{50}PL_{50}$, $BC_{25}PL_{75}$ and BC_0PL_{100} with biochar and poultry litter maximum rates at 40 t ha^{-1} and 5 t ha^{-1}, respectively as shown in Table 1 below. The treatments were laid out in a completely randomize design (CRD) and replicated three times. The pots were watered and brought to 60% field capacity (FC) then left to equilibrate for 24 hours prior to planting. A total number of 72 pots were used in the experiment, with 24 pots used per replicate. Two maize (*Zea mays* L., cultivar:

PANNAR 5R- 575R) seeds were sown per pot but later thinned to one plant per pot at 4 weeks after seedling emergence. Weeding and other management practices were undertaken when necessary. Each pot was uniformly supplied with 1.5 l of water per week depending on the prevailing root zone water saturation. The pots were randomly rotated weekly to different positions within a replicate for the duration of the trial in order to minimize the effects of variation in greenhouse climatic condition (light levels, temperature and humidity). Maize plants were left to grow up to 8 weeks.

Table 1. Treatment description of biochar, and poultry litter addition to four soils

Treatment	Description	BC (t ha^{-1})	PL (t ha^{-1})	BC: PL (%)
BC_0PL_0	Non-amended soil (no biochar and no poultry litter)	0	0	0%:0%
$BC_{100}PL_0$	Soil amended with biochar, without poultry litter	40	0	100%:0%
$BC_{75}PL_{25}$	Soil amended with higher biochar and lower poultry litter	30	1.25	75%:25%
$BC_{50}PL_{50}$	Soil amended with equal % of both biochar and poultry litter	20	2.5	50%:50%
$BC_{25}PL_{75}$	Soil amended with lower biochar and higher poultry litter	10	3.75	25%:75%
BC_0PL_{100}	Soil amended with poultry litter, without biochar	0	5	0%:100%

BC= Biochar treatment; PL= Poultry litter treatment; BC:PL= Ratio of biochar to poultry litter (in percentages)

Table 2. Selected chemical and physical properties of biochar (BC), poultry litter (PL), and soils (We_1, Hu, We_2, and Sd) used in the experiment

Parameter/ Analyte	Units	We_1	Hu	We_2	Sd	BC	PL
pH (H_2O)	cmol kg^{-1}	7.02	7.09	7.06	7.06	8.90	7.30
Total Nitrogen (N)	mg kg^{-1}	18.00	39.00	42.00	61.00	36.53	60.67
Calcium (Ca)	mg kg^{-1}	779.00	880.00	539.00	910.00	2795.58	4479.86
Magnesium (Mg)	mg kg^{-1}	182.00	217.00	119.00	203.00	207.61	1217.57
Potassium (K)	mg kg^{-1}	172.00	92.00	67.00	189.00	1871.43	2497.03
Sodium (Na)	mg kg^{-1}	10.00	11.00	9.00	9.00	145.85	202.79
"S" Value	me%	5.90	6.50	3.90	6.80	21.29	63.01
Ca Ratio		66.00	68.00	69.00	67.00	-	-
Mg Ratio		26.00	28.00	25.00	25.00	-	-
K Ratio		8.00	4.00	4.00	7.00	-	-
Na Ratio		0.70	0.80	0.90	0.60	-	-
Phosphorus (P)	mg kg^{-1}	51.00	36.00	3.00	19.00	-	-
Zinc (Zn)	mg kg^{-1}	3.50	2.20	1.00	2.80	-	-
Copper (Cu)	mg kg^{-1}	6.90	9.90	2.90	10.80	-	-
Manganese (Mn)	mg kg^{-1}	163.00	118.00	65.00	157.00	-	-
Iron (Fe)	mg kg^{-1}	52.00	39.00	38.00	59.00	-	-
Exch. Acidity	cmol kg^{-1}	0.00	0.00	0.00	0.00	-	-
Organic Matter	%	0.77	0.80	0.56	0.96	3.18	23.40
CEC	$cmol_c$ kg^{-1}	5.90	6.50	3.90	6.77	1.32	17.74
Clay (<0.002 mm)	%	18.00	32.00	26.00	57.00	-	-
Silt (0.002-0.05 mm)	%	35.00	12.00	8.00	27.00	-	-
Sand (0.05- 2 mm)	%	47.00	56.00	66.00	16.00	-	-
Textural class		Loam	Sandy Clay Loam	Sandy Clay Loam	Clay	-	-
Soil Form		Westleigh	Hutton	Westleigh	Shortlands	-	-
Moisture	% mass (AR)	-	-	-	-	<10%	-
Volatiles	% mass (Dry)	-	-	-	-	<20%	-
Ash	% mass (Dry)	-	-	-	-	<5%	-

We_1 = Westleigh at Mutshenzheni**;** Hu = Hutton at Rambuda ; We_2 = Westleigh at Tshiombo irrigation; Sd = Shortlands at Tshiombo Madzivhandila soil; AR= As Received; Dry= Moisture free; - = No analysis for parameter/ analyte.

Figure 3. 1. Image shows 30 days biochar incubation termination

Figure 3. 2. Picture of incubation jars containing soil ameliorated with biochar at 30 days of incubation

Figure 3. 3. An image shows packed and labelled soil samples of different treatments ready for analysis

3.1.3. Data collection

3.1.3.1. Plant sampling and agronomic data

Crop data was collected from each pot of one plant stand. Maize growth and phenological data was collected by measuring the following selected variables: date of emergence, plant height, stem diameter and plant biomass. The date of emergence for each plant was recorded as soon as emergence was recognized. Plant height and stem diameter were obtained on weekly basis using the measuring tape and digital caliper, respectively.

Figure 3.4. Determination of stem diameter, below and above ground biomass, and plant height, using digital caliper, weigh balance, and the measuring tape, respectively

3.1.3.2. Plant harvesting and handling

Harvesting of maize is defined as a time when you reap what you sow through picking and gathering. Manual harvesting of all maize plants was by hand from each experimental pot; and harvested maize placed in labeled brown bags, sealed, handled with care and then taken to the laboratory. The dry weight of above and below ground biomass was determined at harvest by oven drying at 70˚C for two days until constant mass is achieved.

3.1.3.3. Post-harvest soil analysis

Soil samples for all biochar and poultry litter treatments set up in section 3.1.2. were collected at harvest, air dried, passed through a 2-mm sieve, and analyzed for soil chemical and physical properties following the procedures described in sub-section 3.1.1.1. to provide a clear picture of soil's nutritional state by evaluating nutrient supply and crop uptake.

3.1.4. Statistical analysis

Using the Complete Randomize Design (CRD) which is a type of experimental design where the experimental units are assigned to different treatments of controlled factors of the experiment (Completely Randomized Design, 2008). Analysis of variance was conducted using the general linear model (GLM) procedure of SAS software version 9.4 package (SAS, Institute, 2013). The effect of biochar and poultry litter treatments on maize productivity in different soil types, were analysed using the ANOVA. Where significant differences between the treatments is observed, means were separated using Duncan's Multiple Range Test (DMRT) at $P \leq 0.05$.

3.2. Results

3.2.1. Effects of biochar and poultry litter treatments on selected soil chemical properties

3.2.1.1. Soil pH and total nitrogen

Biochar and poultry litter treatments had no significant effects on soil pH in We_1, Sd, and Hu soils (Table 3). However, biochar and poultry litter treatments had significant effect on We_1 soil. A significant increase in soil pH was observed in all biochar and poultry litter treatments of We_1 soil when compared with control BC_0PL_0 (Table 3). Application of 40 t ha^{-1} of biochar with 0 t ha^{-1} of poultry litter significantly increased soil pH by 6.22 %, compared to non-amended soil. In contrast, biochar and poultry litter treatments had no significant effect on total N content of soils We_1, We_2, and Hu (Table

3), but the effects were significant in Sd soil (Table 3). Soil Sd showed a significant N increase at treatment $BC_{25}PL_{75}$ and BC_0PL_{100}, compared to BC_0PL_0, $BC_{100}PL_0$, $BC_{75}PL_{25}$, and $BC_{50}PL_{50}$ (Table 3).

3.2.1.2. Soil Ca, Mg, K & available P

Biochar and poultry litter treatments had no significant effect on magnesium content in all soil types (We_2, We_1, Sd, and Hu), but it had a significant effect on the Ca content of We_1, We_2 and Hu soils (Table 3). A significant increase of 9 % was observed in all biochar and poultry litter amended Hu soil, compared to non-amended Hu soil. Soil We_1 had at least 8 % significant increase in soil Ca, when different rates of biochar with poultry litter were applied, compared to non-amended soil. Application of 40 t ha^{-1} of biochar with 0 t ha^{-1} of poultry litter in We_2 soil, significantly ($p< 0.05$) increased soil Ca by 103 mg kg^{-1}, compared to non-amended soil (Table 3). However, Soil Ca in Shortlands was not significantly affected by all biochar and poultry litter treatments. Biochar and poultry litter treatments had significant ($p< 0.001$) effect on K content in $Westleigh_1$ and Shortlands soils, but no significant effects on $Westleigh_2$ and Hutton (Table 3). More than 20 % significant increase of soil K was observed after We_1 soil was amended with all biochar and poultry litter treatments ($BC_{100}PL_0$, $BC_{75}PL_{25}$, $BC_{50}PL_{50}$, $BC_{25}PL_{75}$, BC_0PL_{100}), compared to non-amended soil (BC_0PL_0). Soil K in Sd soil also increased after biochar and poultry litter application at different rates, compared to the non-amended soil. However, significantly greater soil K increase in Sd soil with poultry litter application as biochar at the rate 40, 30, 20 t ha^{-1} BC with 0, 1.25, 2.5 t ha^{-1} PL, followed by 10, 0 t ha^{-1} BC with 3.75, 5 t ha^{-1} PL when compared to non-amended soil at rate 0 t ha^{-1} BC with 0 t ha^{-1} PL. On the other hand, Table 3 shows that biochar and poultry litter treatments significantly ($p< 0.001$) improved the availability of P in We_1 soil only. A significant ($p< 0.001$) available P increase in We_1 soil was observed after biochar and poultry litter treatments $BC_{50}PL_{50}$, $BC_{25}PL_{75}$, and BC_0PL_{100}, compared to BC_0PL_0,

$BC_{100}PL_0$, and $BC_{75}PL_{25}$.

3.2.1.3.Trace elements, sodium and percentage organic matter

Biochar and poultry litter treatments had no significant effects on all the trace elements analysed (Cu, Fe, Mn, and Zn) and the percentage organic matter in all soil types (Table 3). However, biochar and poultry litter treatments had significant ($p<0.01$) effect on Na content in We_1 soil (Table 3). Soil Na in We_1 soil increased when a rate of 20 t ha^{-1} biochar was applied with 2.5 t ha^{-1} poultry litter ($BC_{50}PL_{50}$) compared to control and all other biochar and poultry litter treatments.

Table 3. The effect of biochar (BC) and poultry litter (PL) treatments on selected soil chemical properties

Soil	Treatments	pH	N	Ca	Mg	K	P	Cu	Fe	Mn	Na	Zn	OM
	(t ha^{-1})	cmol kg^{-1}	mg kg^{-1}										%
Westleigh$_2$	BC_0PL_0	7.073	24.33	708b	154.0	75.3	6.7	5.90	52.7	41.3	13.00	1.500	0.7
	$BC_{100}PL_0$	7.183	24.33	811a	151.3	103.0	7.0	5.27	43.3	36.0	14.33	1.467	0.7
	$BC_{75}PL_{25}$	7.197	26.00	789a	147.7	97	7.3	5.07	44.0	37.7	13.67	1.467	0.7
	$BC_{50}PL_{50}$	7.213	25.67	870a	170.7	112.7	9.7	5.47	43.3	44.7	13.33	1.433	0.7
	$BC_{25}PL_{75}$	7.133	27.67	852a	166.3	97.3	11.3	5.50	43.7	39.7	13.67	1.533	0.8
	BC_0PL_{100}	7.123	26.67	808a	161.3	95.3	11.7	4.48	44.7	38.3	12.67	1.467	0.7
	F test	ns	ns	p<0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns
Westleigh$_1$	BC_0PL_0	6.593b	23.33	798b	184.3	110.7b	54.7b	6.23	52.3	77.3	12.67b	3.867	0.6
	$BC_{100}PL_0$	6.940a	26.33	947a	192.7	171.0a	59.0b	6.13	44.3	72.3	15.33b	4.033	0.8
	$BC_{75}PL_{25}$	6.963a	26.33	921a	190.3	149.0a	63.3b	5.90	47.3	79.3	15.67b	3.900	0.9
	$BC_{50}PL_{50}$	7.030a	26.33	977a	209.7	179.3a	81.7a	6.40	55.0	74.0	18.33a	4.367	0.8
	$BC_{25}PL_{75}$	7.030a	26.33	920a	194.7	147.0a	74.0a	5.93	48.3	66.3	16.67b	4.033	0.7
	BC_0PL_{100}	7.000a	28.67	867a	188.7	139.3a	84.7a	5.90	48.7	84.3	14.00b	4.067	0.7
	F test	p<0.05	ns	p<0.05	ns	p<0.001	p<0.001	ns	ns	ns	p<0.001	ns	ns
Shortlands	BC_0PL_0	7.143	23.00b	788	173.3	86.7ab	9.3	8.43	52.0	55.0	13.00	2.133	0.7
	$BC_{100}PL_0$	7.200	22.67b	894	171.0	146.3a	10.3	7.77	52.0	43.3	15.33	2.100	0.9
	$BC_{75}PL_{25}$	7.203	22.67b	938	187.3	148.3a	14.0	8.57	51.3	50.7	15.67	2.200	0.8
	$BC_{50}PL_{50}$	7.203	24.67b	877	176.7	119.7a	13.0	7.90	51.3	47.3	13.67	2.100	0.8
	$BC_{25}PL_{75}$	7.097	28.67a	913	189.7	113.7b	16.7	8.53	55.7	48.3	14.67	2.367	0.8
	BC_0PL_{100}	7.047	29.67a	840	179.3	115.3b	18	8.13	53.3	55.7	14.33	2.167	0.8
	F test	ns	p<0.05	ns	ns	p<0.001	ns	ns	ns	ns	ns	ns	ns
Hutton	BC_0PL_0	6.997	21.00	817b	202.3	50.3	27.3	9.03	42.3	41.7	16.00	2.000	0.7
	$BC_{100}PL_0$	7.123	22.00	966a	216.0	96.0	35.0	9.20	42.3	43.7	18.33	2.067	0.7
	$BC_{75}PL_{25}$	7.113	19.00	907a	203.3	95.0	34.0	8.67	41.7	38.3	17.33	2.100	0.7
	$BC_{50}PL_{50}$	7.150	19.33	955a	213.3	88.3	41.3	8.80	39.7	46.3	16.67	2.267	0.7
	$BC_{25}PL_{75}$	7.173	21.00	940a	217.7	77.7	44.0	9.23	40.0	47.3	16.00	2.367	0.7
	BC_0PL_{100}	7.090	20.33	899a	214.7	60.3	50.7	9.43	39.0	50.7	16.00	2.467	0.7
	F test	ns	ns	p<0.05	ns	ns	ns	ns	ns	ns	ns	ns	ns
Overall CV%		3.3	3.3	5.2	5.4	3.3	10.0	5.9	2.0	6.9	7.4	3.9	6.5

Means with same letters in each column are not significantly different at p≤ 0.05 for each soil type, ns= non-significant; CV= coefficient of variation, means separated using DMRT

3.2.1.4. Cation exchange capacity (CEC) and exchangeable acidity

Exchangeable acidity of all soil types were not significantly affected by all biochar and poultry litter treatments (Table 4). Table 4 also shows that biochar and poultry litter treatments had no significant effect on CEC in all soil types except Hu soil, which was significant at $p<0.05$. A significant increase of 0.58 $cmol_c\ kg^{-1}$ CEC was observed in Hu soil after all biochar and poultry litter treatment application, compared to the controlled non-amended soil.

Table 4. Effect of biochar and poultry litter application on soil Cation excha-
-nge capacity (CEC) and exchangeable acidity

Treatment	CEC	Exchangeable Acidity
	$cmol_c\ kg^{-1}$	$cmol\ kg^{-1}$
Westleigh$_2$ Soil		
BC_0PL_0	5.10	0.02
$BC_{100}PL_0$	5.63	0.00
$BC_{75}PL_{25}$	5.48	0.00
$BC_{50}PL_{50}$	6.12	0.00
$BC_{25}PL_{75}$	5.95	0.00
BC_0PL_{100}	5.71	0.00
Westleigh$_1$ Soil		
BC_0PL_0	5.94	0.07
$BC_{100}PL_0$	6.87	0.03
$BC_{75}PL_{25}$	6.66	0.02
$BC_{50}PL_{50}$	7.18	0.02
$BC_{25}PL_{75}$	6.71	0.04
BC_0PL_{100}	6.36	0.03
Shortlands Soil		
BC_0PL_0	5.68	0.02
$BC_{100}PL_0$	6.36	0.03
$BC_{75}PL_{25}$	6.73	0.03
$BC_{50}PL_{50}$	6.25	0.03
$BC_{25}PL_{75}$	6.53	0.03
BC_0PL_{100}	6.10	0.04
Hutton Soil		
BC_0PL_0	5.99b	0.02
$BC_{100}PL_0$	6.98a	0.03
$BC_{75}PL_{25}$	6.57a	0.02
$BC_{50}PL_{50}$	6.85a	0.00
$BC_{25}PL_{75}$	6.78a	0.00
BC_0PL_{100}	6.59a	0.00
P (F- test)		
Westleigh$_2$ Soil	ns	ns
Westleigh$_1$ Soil	ns	ns
Shortlands Soil	ns	ns
Hutton Soil	p<0.05	ns
CV %	4.9	4.3

Means with same letters in each column are not significantly different at p≤ 0.05 for each soil type, ns= non-significant; CV= coefficient of variation, means separated using DMRT.

3.2.2. Effects of biochar and poultry litter treatments on selected physiological growth parameters of maize

3.2.2.1.Stem diameter

In week 3, biochar and poultry litter treatments had significant effects on maize stem diameter of We_2, and We_1 soils at $p< 0.001$ level of significance, but had no significant effects on stem diameter in Hu and Sd soils (Table 5). A larger stem diameter of greater than 0.35 cm was observed in We_2 soil at week 3, after biochar and poultry litter treatment application $BC_{75}PL_{25}$ and $BC_{25}PL_{75}$ compared to the rest of biochar and poultry litter treatment. Soil We_1 was also affected significantly after biochar and poultry litter treatment application during week 3. A significant stem diameter of 10.80 cm was recorded after application of biochar and poultry litter treatment ($BC_{75}PL_{25}$) compared to the other biochar and poultry litter treatment. On the other hand, the treatments had no significant effects on stem diameter in Hu soils from week 1, 2, 3, 4 and 6, except for week 5 (Table 5).

In week 5, biochar and poultry litter treatments significantly affected the stem diameter in We_1 and Hu soils at $p < 0.001$ level of significance (Table 5). A significantly affected stem diameter was observed in week 5 on We_1 soil at $p< 0.001$ level of significance, where a larger stem diameter of 14.23 cm followed by 14.05 cm was recorded after biochar and poultry litter treatment application rate $BC_{25}PL_{75}$ and $BC_{75}PL_{25}$, respectively as compared to the rest of biochar and poultry litter treatments. However, plant grown on Hu soil showed a significant increase in stem diameter after all treatment application compared to control (BC_0PL_0) at $p < 0.001$ level of significant, except for $BC_{75}PL_{25}$ which did not show any significant effect on stem diameter. In week 6, the stem diameter from all soils were affected significantly after biochar and poultry litter treatment application at $p < 0.001$ level of significance, except for Hu soil.

A significant increased stem diameter of 15.65 cm and 13.59 cm was recorded for both We_1 and Sd, respectively after biochar and poultry litter treatment application $BC_{25}PL_{75}$ as compared to other treatments (Table 5). However, We_1 soil only showed a significant increased stem diameter of 13.49 cm after biochar and poultry litter was applied at rate BC_0PL_{100}, when compared to the rest of biochar and poultry litter treatments.

Table 5. Effect of biochar and poultry litter treatments on weekly stem diameter (cm)

Soil	Treatments	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Westleigh$_2$	BC_0PL_0	4.53	5.26	6.82b	8.00	9.54	10.18ab
	$BC_{100}PL_0$	3.47	5.82	6.67b	8.00	8.64	9.68ab
	$BC_{75}PL_{25}$	4.61	7.18	7.44a	10.00	10.24	10.69ab
	$BC_{50}PL_{50}$	3.48	25.09	6.60b	8.00	9.87	10.50ab
	$BC_{25}PL_{75}$	5.02	6.88	7.92a	10.00	11.07	11.72b
	BC_0PL_{100}	4.14	6.04	6.92b	9.00	11.66	13.49a
Westleigh$_1$	BC_0PL_0	3.97	7.90	9.31b	11.00	11.92b	13.10b
	$BC_{100}PL_0$	5.48	8.42	9.43b	11.00	11.58b	12.79b
	$BC_{75}PL_{25}$	4.82	10.57	10.80a	14.00	14.05a	14.14b
	$BC_{50}PL_{50}$	4.09	7.68	9.17b	455.00	12.23b	13.22b
	$BC_{25}PL_{75}$	4.33	8.31	9.95b	11.00	14.23a	15.65a
	BC_0PL_{100}	4.48	9.40	9.53b	12.00	12.57b	13.72b
Shortlands	BC_0PL_0	4.45	6.54	8.30	10.00	10.71	11.48b
	$BC_{100}PL_0$	5.11	6.95	8.71	9.00	11.01	11.96b
	$BC_{75}PL_{25}$	3.89	6.89	7.88	9.00	11.48	11.75b
	$BC_{50}PL_{50}$	5.33	7.79	9.59	11.00	12.87	12.88b
	$BC_{25}PL_{75}$	6.28	8.02	10.01	11.00	12.30	13.59a
	BC_0PL_{100}	4.38	6.79	8.93	10.00	11.45	12.63b
Hutton	BC_0PL_0	4.71	7.47	9.13	10.00	11.42b	12.11
	$BC_{100}PL_0$	4.45	8.22	8.83	11.00	12.75a	13.24
	$BC_{75}PL_{25}$	3.55	6.45	8.29	9.00	11.28b	12.75
	$BC_{50}PL_{50}$	4.78	7.42	9.51	11.00	13.33a	13.74
	$BC_{25}PL_{75}$	4.77	9.46	10.39	363.00	13.30a	13.73
	BC_0PL_{100}	5.42	8.46	9.88	12.00	13.34a	13.65
	P (F- test)						
	Westleigh$_2$	ns	ns	p<0.001	ns	ns	p<0.001
	Westleigh$_1$	ns	ns	p<0.001	ns	p<0.001	p<0.001
	Shortlands	ns	ns	ns	ns	ns	p<0.001
	Hutton	ns	ns	ns	ns	p<0.001	ns
	CV%	1.4	17.3	3	67.6	1.1	1.0

Means with same letters in each column are not significantly different at p≤ 0.05 for each soil type, ns= non-significant; CV= coefficient of variation, means separated using DMRT.

3.2.2.2. Plant height

Biochar and poultry litter treatments had no significant effects on plant height in all soil types during week 1, week 2 and week 3, except for Sd soil at week 3 where the highest plant height of 98.30 cm was recorded (Table 6). At week 4, 5 and 6, biochar and poultry litter treatments had no significant effect on plant height in all soil types, except for soil We_2. However, We_2 soil showed a significant ($p< 0.001$) increase in plant height after biochar and poultry litter treatment application $BC_{100}PL_0$, $BC_{75}PL_{25}$, and $BC_{50}PL_{50}$, when compared to the control treatment (BC_0PL_0).

Table 6. Effect of biochar and poultry litter treatments on weekly plant height (cm)

Soil type	Treatment	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Westleigh$_2$	BC_0PL_0	38.67	62.0	67.7	73.17ab	86.00ab	91.33ab
	$BC_{100}PL_0$	38.00	65.8	74.7	85.17b	94.67b	101.33b
	$BC_{75}PL_{25}$	42.00	75.3	83.3	92.33b	105.17a	106.83b
	$BC_{50}PL_{50}$	32.50	62.3	76.8	89.50b	103.00a	104.50b
	$BC_{25}PL_{75}$	46.00	77.5	91.8	104.00a	117.67a	119.83a
	BC_0PL_{100}	32.67	65.3	80.8	97.33b	115.33a	118.00a
Westleigh$_1$	BC_0PL_0	32.67	66.2	93.5	105.00	116.0	120.33
	$BC_{100}PL_0$	46.33	82.5	94.7	104.00	118.67	124.33
	$BC_{75}PL_{25}$	53.67	88.7	96.8	105.17	118.00	119.67
	$BC_{50}PL_{50}$	37.67	71.7	90.7	102.67	118.67	124.67
	$BC_{25}PL_{75}$	39.30	69.3	97.0	106.50	113.33	117.33
	BC_0PL_{100}	38.80	74.2	89.3	101.83	118.67	127.17
Shortlands	BC_0PL_0	38.83	80.7	91.2a	103.67	111.33	113.00
	$BC_{100}PL_0$	45.50	78.5	90.2a	96.50	106.33	110.33
	$BC_{75}PL_{25}$	42.17	79.0	71.8b	98.67	109.33	109.67
	$BC_{50}PL_{50}$	46.58	85.0	94.3a	104.67	114.00	115.67
	$BC_{25}PL_{75}$	47.00	88.3	98.3a	108.67	115.00	119.67
	BC_0PL_{100}	32.13	74.0	90.8a	97.17	111.67	115.67
Hutton	BC_0PL_0	41.33	71.3	94.3	102.33	113.00	115.00
	$BC_{100}PL_0$	37.80	77.5	87.3	99.83	111.33	112.67
	$BC_{75}PL_{25}$	37.67	65.8	95.7	100.67	109.67	112.67
	$BC_{50}PL_{50}$	32.47	61.0	92.7	104.50	121.33	123.00
	$BC_{25}PL_{75}$	36.17	72.0	100.0	108.00	118.67	119.67
	BC_0PL_{100}	42.87	74.0	93.5	105.67	118.33	120.33
P (F- test)							
Westleigh$_2$		ns	ns	ns	p<0.001	p<0.001	p<0.001
Westleigh$_1$		ns	ns	ns	ns	ns	ns
Shortlands		ns	ns	p<0.05	ns	ns	ns
Hutton		ns	ns	ns	ns	ns	ns
CV%		9.6	5.9	1.9	2.5	2.7	2.7

Means with same letters in each column are not significantly different at p≤ 0.05 for each soil type, ns= non-significant; CV= coefficient of variation, means separated using DMRT

3.2.2.3. Above and below ground biomass

The biochar and poultry litter treatments had significant effects on both the below and above ground biomass in all soil types (Table 7). In We_1 soil, biochar and poultry treatments significantly ($p < 0.001$) improved the above ground biomass from 8.97 g to the highest above ground biomass of 14.41 g, a significant increase by at least 5.44 g above ground biomass (Table 7). Biochar and poultry treatments also significantly ($p< 0.001$) improved the above ground biomass in Hu, We_2, and Sd soils, where the highest above ground biomass of 11.73, 9.35, and 9.96 g, respectively, were recorded at rate $BC_{25}PL_{75}$ (Table 7). On the other hand, biochar and poultry litter treatments significantly ($p < 0.001$) affected the below ground biomass in all soils. In Hu soil, the highest value of 2.48 g for the below ground biomass was recorded (Table 7). In We_1 soil, the biochar and poultry litter treatments significantly ($p< 0.001$) affected the below ground biomass with the highest recorded below ground biomass with a value of 2.09 g for treatment BC_0PL_{100}, which is greater by 77.99 % when compared to control and the rest of biochar and poultry litter treatments (Table 7).

Table 7. Effect of biochar and poultry litter treatments on above and below ground biomass

Soil	Treatments	Above ground biomass (g)	Below ground biomass (g)
Westleigh$_2$	BC_0PL_0	6.34b	1.74b
	$BC_{100}PL_0$	4.92b	0.52a
	$BC_{75}PL_{25}$	7.06a	1.33b
	$BC_{50}PL_{50}$	5.21b	0.58a
	$BC_{25}PL_{75}$	9.96a	1.38b
	BC_0PL_{100}	8.42a	1.23b
Westleigh$_1$	BC_0PL_0	8.97c	1.17a
	$BC_{100}PL_0$	10.28ab	1.43a
	$BC_{75}PL_{25}$	14.41a	1.42a
	$BC_{50}PL_{50}$	9.88ab	1.18a
	$BC_{25}PL_{75}$	9.83ab	1.63a
	BC_0PL_{100}	12.46b	2.09b
Shortlands	BC_0PL_0	5.70b	0.23a
	$BC_{100}PL_0$	7.10b	1.13b
	$BC_{75}PL_{25}$	6.95b	0.83b
	$BC_{50}PL_{50}$	8.98a	0.97b
	$BC_{25}PL_{75}$	9.35a	1.31b
	BC_0PL_{100}	7.23b	0.66b
Hutton	BC_0PL_0	7.65ab	1.3b
	$BC_{100}PL_0$	10.91b	1.65b
	$BC_{75}PL_{25}$	7.88c	0.84a
	$BC_{50}PL_{50}$	10.21ab	2.48b
	$BC_{25}PL_{75}$	11.73a	1.67b
	BC_0PL_{100}	11.62a	2.06b
P (F- test)			
Westleigh$_2$		p<0.001	p<0.001
Westleigh$_1$		p<0.001	p<0.001
Shortlands		p<0.001	p<0.001
Hutton		p<0.001	p<0.001
CV%		4.5	6.3

Means with same letters in each column are not significantly different at p≤ 0.05 for each soil type, ns= non-significant; CV= coefficient of variation, means separated using DMRT.

3.3. Discussion

3.3.1. Effect of biochar and poultry litter treatments on selected soil chemical properties

3.3.1.1. Soil pH and total nitrogen

Biochar had the highest pH of 8.90 compared to poultry litter and all unamended soils (We_1, Hu, We_2, and Sd) used in the experiment prior to planting (Table 2), that is why biochar is suggested a potential use as a liming material (Faridullah *et al.,* 2009). Biochar and poultry litter treatments had no significant effect on pH in, We_2, Sd, and Hu soils, which may be due to high initial soil pH (7.06, 7.06, and 7.09, respectively) compared to that of We_1 (7.02) (Table 3). The liming or alkaline effect of biochar is beneficial for acidic soils than those having high pH greater than 8. In this case, a high pH effect of biochar might be due to the displacement of exchangeable acidity and high buffering capacity of biochar, thereby retarding a further liming effect (Abbasi and Anwar, 2015; Jean and Wang, 2013). However, the significant soil pH increment of We_1 soil may be due to lower clay percentage of 18, compared to Hu, We_2, and Sd soils with clay percentage of 32, 26, and 57, respectively (Table 2 and Table 3). According to Xu *et al*. (2013), the insignificant increment pH in soils with high clay contents may be due high buffering capacity of clay soils compared with sandy soils. On the other hand, compared to the control, Sd soil showed a significant increase in soil N content of 23.00 to 28.67 and 29.67mg kg^{-1} after application rate $BC_{25}PL_{75}$ and $BC_{100}PL_0$ of biochar and poultry litter treatment, respectively (Table 3). The increase in total soil N content of soil Sd may be due to higher initial total N content of 61 mg kg^{-1} compared to We_1, Hu, We_2 soils, with initial total N content of 18, 39 and 42, respectively. This is consistent with the findings of Hailegnaw *et al.* (2019) who confirmed the dependence of biochar treatments effectiveness on original soil properties.

3.3.1.2. Soil Mg, Ca, K & available P

Nutrient availability is greatly affected by soil pH and it has been determined that most plant nutrients are optimally available at soil pH between 6.5-7.5 compatible to plant root growth (Miller, 2016). All soil had neutral pH levels above 6.5 but less than 7.5, which assumes vast nutrient availability for plant use (Table 2). Moreover, all soils had desirable Ca: Mg ratio, which ranges from 2.5:1 to 2.7:1 and high Mg^{2+} content (Table 2). However, the insignificant effects observed after biochar and poultry litter treatment application on soil Mg of all soil types (Table 3) had showed by Wu *et al*. (2011) who reported significant Mg^{2+} content increment in soils with low original exchangeable Mg^{2+} content. This suggests that exchangeable Mg^{2+} was released from biochar and poultry litter when applied in soils with relatively low available Mg content (Wu *et al*., 2011).

The biochar and poultry litter treatments had no significant effects on Ca content in Sd soil. However, the treatments significantly improved Ca content in, We_2, We_1, and Hu, soils that had 66, 47 and 47% sand, respectively. The improvement of Ca content in, We_2, We_1, and Hu, soils showed effectiveness of cation exchange capacity and exchangeable bases in sandy soils (Sika, 2012). Studies have shown that among other nutrients, Ca is most likely to be influenced by soil texture (Bonomelli *et al*., 2019; Clark *et al*., 2003). Zhao *et al*. (2015) reported that clay textured soil had reduced Ca content, due to poor soil aeration, soil impedance, root growth and metabolism. High soil impedance may cause cessation of root growth, increased ethylene production, accumulation of osmotic solutes in roots apices, and reduction in root length that will later affect the water uptake together with Ca content.

The treatments had no significant effects on available P in all soil types except for We_1 soil which was significant at $p<0.001$. Soil available P increased in We_1 soil which may have been due to higher P content of 51 mg kg^{-1} prior to planting as compared to all other soil types Hu, We_2, and Sd with original P nutrient of 36, 3, 19, mg kg^{-1}, respectively. According to Phares *et al*. (2017), the upsurge in P content is probably due to the increase in solubilizing fungal biomass in soils with P abundance. On the other hand, the treatments significantly increased the K content in both soil We_1 and Sd owing to the higher original soil K content of 172 mg kg^{-1} and 189 mg kg^{-1}, respectively, prior to planting. Relatively improved soil K content observed is in agreement with the findings of Romheld and Kirk, (2010) who attributed a higher K content to the presence of clay soil that facilitated K fixation.

3.6.1.3. Trace elements (Cu, Fe, Mn, and Zn), Na, and organic matter

Trace elements occur naturally in soils and some (Beryllium, Helium, Neon etc.) are not essential for plant growth. However, trace elements may be potentially toxic at elevated levels (Hooda, 2010). In this study, the biochar and poultry litter treatments had no significant effect on all selected trace elements and the Na level in all four soils. The non-significance in trace elements (Cu, Fe, Mn, and Zn) content and Na, is in agreement with an earlier study by Riedel *et al*. (2015) who reported a decreased mobility of trace elements which was partly caused by an enhanced retention of metal-binding and dissolved organic matter in biochar amended soils. Similar to the trace elements, the biochar and poultry litter treatments had no significant effects on soil organic matter of all four soil types. This could be due to sorption of labile soil organic matter on to biochar particles, thus decreasing its mineralization (Bot and Benites, 2005). Moreover, according to Cowie and Singh (2015), biochar may transiently supress soil organic matter mineralization through a short-term inhibitory effect on microbial activity of biochar-associated volatile organic compound.

3.3.1.4 Exchangeable acidity and cation exchange capacity

Exchangeable acidity measures the amount of acid cations, aluminium and hydrogen, occupied on the CEC (Logan *et al*., 2008). Cation exchange capacity on the other hand measures the ability of soil to hold positively charged ions. Cation exchange capacity is a very important soil property as it can influence nutrient availability, soil reactions and other ameliorants (Hazlenton and Murphy, 2007). Nevertheless, the biochar and poultry litter treatments had no effects on exchangeable acidity in all soils (Table 4). This may be due to higher biochar and poultry litter CEC, which has a capacity of binding Al and Fe with the soil exchange sites (Mensah and Frimpong, 2018).

The non-significant difference of exchangeable acidity might also be due to the reduction of exchangeable H^+ + Al^{3+} content by biochar forming Al complex by oxidised organic functional groups such as carboxylic and phenolic (Vithanage *et al*., 2017). The biochar and poultry litter treatments significantly improved CEC in Hu soil, which is in conformity with earlier work reported by Ndor *et al*. (2015) that biochar used as a soil amendment can boost soil fertility and improve soil quality by reducing soil acidity, improving cation exchange capacity (CEC) and retaining nutrients in soil.

3.3.2 Effect of biochar and poultry litter treatments on selected physiological growth parameters of maize

3.3.2.1 Stem diameter, plant height, above and below ground biomass

The biochar and poultry litter treatments had no significant effects on both the stem girth and plant height at week 1 and 2 (Table 5 & Table 6), in all soil types due mainly to concurrent slow release of nutrients and greater cation exchange capacity that

allows for the retention of cations (Sohi *et al.*, 2010). However, the biochar and poultry litter treatments had a significant effect on stem diameter from week 3 to 6, in various soil types (Table 5 & Table 6). This finding is in agreement with Lehmann *et al*. (2011) and Anderson *et al*. (2011) who reported that biochar and organic amendments could lead to a change in microbial abundance and activities in soils, which could promote the release of plant growth hormones and improve bioavailability of nutrients.

Biochar and poultry litter treatments significantly affected plant height. The highest plant height of 98.3 cm was recorded at week 3 in Sd soil (Table 6 and Figure 4.1). The increased plant height may be due to the highest total N content in Sd soil (61.0 mg kg^{-1}) before planting. This is in agreement with the work of Khan *et al*. (2008) who observed an increase in plant height and attributed it to a positive effect of N on vigorous vegetative growth. Moreover, the biochar and poultry litter treatments had no significant effects on plant height at week 1 and 2 (Table 6). We_2 soil had the highest sand content of 66 %, compared to other soil types. This results are in agreement with Novak *et al*. (2009) where similar improvement in plant height occurred after addition of different biochar rates in sandy soils, owing it to mixing of soil with less dense material (Celik *et al*., 2004).

A more detailed analysis of soil pores in relation to biochar and poultry litter rate showed an increased volume of larger soil pores compared to the smaller ones (Glab *et al*., 2016). Contrary to the results obtained with all selected physiological growth parameters, the treatments significantly improved the below and above-ground biomass in all soils (Table 7). Each soil type recorded the highest above ground biomass of 9.96, 14.41, 9.35, and 11.73 g, in We_2, We_1, Sd, and Hu soils, respectively, with the highest below ground biomass of 1.74, 1.63, 1.31, and 2.48 in

We_2, We_1, Sd, and Hu soils, respectively (Table 7). The improved below and above ground biomass after biochar and poultry litter application may relate to soil quality improvement (Demir *et al*., 2010), nutrient release into soil solution, increased chemicals/ beneficial organisms, balanced plant nutrition and availability of micro elements (Gunes *et al*., 2014).

3.4. Conclusion

The results of this study showed that the biochar and poultry litter treatments improved selected soil chemical properties (pH, Ca, Mg, K and available P) and maize DM yield in different soil types. Biochar and poultry litter treatments increased soil pH through the displacement of exchangeable acidity. The Increment in total nitrogen, phosphorus and magnesium were dependent on the initial soil chemical properties prior planting. However, biochar and poultry litter treatment effects on soil potassium might be dependent on clay percentage and K fixation. Soil calcium mainly depends on exchangeable base and sand percentage. Biochar and poultry litter treatments enhanced retention of metal-binding and dissolved organic matter, which reduced trace elements (Cu, Fe, Mn, and Zn) availability and Na was due to high element-soil association or interaction that causes retention. In addition, the biochar and poultry litter treatments decreased mineralization of organic matter through sorption of labile organic matter in biochar particles. Biochar and poultry litter treatment reduced exchangeable H^+ + Al^{3+} content forming Al complex by oxidised organic functional groups that did not affect the exchangeable acidity but improved CEC due to reduced soil acidity. On the other hand, the biochar and poultry litter treatments had no effects on stem diameter and plant height due to concurrent slow release of nutrients and greater cation exchange capacity, which allows retention of cations. The treatments impact on soil quality yielded an increased below and above-ground biomass of maize crop through nutrient release into soil solution, balanced plant nutrition and availability

of micro elements. Therefore, the treatments revealed a potential to ameliorate agricultural soils using different biochar and poultry litter rates. Though biochar possesses some essential elements required for plant growth, it is not effective when applied alone. Consequently, the effectiveness of the treatment combinations confirmed a potential to improve soil characteristics and crop growth, maize in particular.

Chapter Four

4. Experiment 2: Incubation experiment

Laboratory incubation studies are commonly used methods to estimate how soil organic matter decompose in a controlled environment. A controlled laboratory incubation experiment was carried out to determine effects of biochar application at different rates on N, P and bio-quality parameters of different soil types over a period of 120 days.

4.1. Materials and methods

4.1.1. Soil and biochar characterization

4.1.1.1. Soil characterization

Soil samples used in the controlled laboratory incubation experiment are the same as those used in the experiment described in subsection 3.1.1.1.

4.1.1.2. Biochar characterization

Biochar properties are the same as described in subsection 3.1.1.2.

4.1.2. Set-up of the laboratory incubation experiment

The experiment consisted of four soil types (We_1, We_2, Hu, and Sd) and four biochar application rates (0, 10, 20, and 40 t ha^{-1}), in a factorial arrangement as shown in Table 8 below. Each treatment consisted of 200 g of soil homogenously mixed with biochar as amendments at the stated rates in 500 ml canning jars. Deionized water was added to each jar to bring moisture to approximately 60% water holding capacity of the mixture. Amended soils were sealed and incubated in the dark at 25ºC, and opened every seven days to exchange air and readjust moisture levels. The treatments were laid in a completely randomize design (CRD) and replicated three times. Soil samples collection from each treatment was at 30, 60, 90 and 120 days of incubation. Analyses of collected samples for P, N and bio-quality parameters using

procedures were similar to those procedures described in subsection 3.1.1.1., 4.1.3., 4.1.4., 4.1.5., 4.1.6., and 4.1.7.

4.1.3. Soil microbial biomass Carbon and Nitrogen (MBC & MBN)

Soil active microbial biomass carbon and nitrogen were measured by fumigation extraction method (modified from Vance *et al*., 1987) where a non-fumigated 10 g of soil was extracted immediately shaking with 40 ml of 0.5M K_2SO_4 solution, filtered through a Whatman No.1 filter paper and stored in a freezer. Another 10 g of soil was fumigated with chloroform for 24 hours and then 40 ml of 0.5M K_2SO_4 was added and placed in a shaker for 30 minutes. The mixture was filtered similarly to the non-fumigated soil. Organic carbon extracted was determined using the acid digestion, $K_2Cr_2O_7$ and ferrous ammonium sulphate back titration. Microbial biomass carbon was determined by the difference between the carbon in fumigated and non-fumigated sample using the formula below (Beck *et al*., 1997).

Microbial biomass carbon (MBC) = (OCF- OCNF) / kEC…… (1)

Where: OCF= organic carbon extracted from fumigated

OCNF= organic carbon extracted from non-fumigated

kEC= soil specific constant, which is often estimated as 0.45

For microbial biomass nitrogen, 20 ml of the extract was digested using the Kjeldahl digestion method. Where a 20 ml sulfuric acid (98 %) together with 2 Kjeldahl 5 g tablets of missouric catalyst were added in the extract. The mixture was heated at 300-380 ºC until a white fume was seen. After 180 minutes, the vapor was bubbled through a sodium hydroxide solution. When the extract was completely transparent, the sample was allowed to cool at room temperature. Then 100 ml water was added and the glass content was transferred to the distillation unit. During distillation, 50 ml sodium hydroxide 50 % solution was added to neutralize pH and convert NH_4^+ into

NH_3. Stream of water vapor was bubbled in the sample to entrain the NH_3 formed. A condensed NH_3 was captured in 50 ml boric acid solution. When NH_3 reacted with boric acid, the solution turned red violet to green (pH 4.4 – 5.8). Around 150 ml condensate was captured in boric acid solution and titrated with 0.25 mol/l of HCl until the solution turned violet. With the volume and concentration HCl, number of mol nitrogen was determined. The difference in nitrogen between the fumigated and the non-fumigated is a liable N pool which is proportional to microbial biomass nitrogen that was calculated using the formula below. (Brookes *et al*., 1985).

Microbial biomass nitrogen (MBN) = (ONF- ONNF) / kEC…… (2)

Where: OCF= organic nitrogen extracted from fumigated

OCNF= organic nitrogen extracted from non-fumigated

kEN= soil specific constant, which is often estimated as 0.45

4.1.4. Soil organic carbon (SOC)

Soil organic carbon was determined by the sulphuric acid ($H_2SO_4^-$) and aqueous potassium dichromate ($K_2Cr_2O_7$) method described by Nelson and Sommers (1975). The method involves the digestion of 0.5 g of dry soil sub-sample in 5 ml of $K_2Cr_2O_7$ and 7.5 ml of concentrated $H_2SO_4^-$. The digestion tubes containing the soil was placed on pre-heated block at 145-155 ºC for 30 minutes. The digests were quantitatively transferred into a 100 ml conical flask and 0.3 ml of indicator was added to the solution then stirred using a magnetic stirrer. Thereafter, the digests were titrated with ferrous ammonium sulphate solution until a greenish to brown end point was reached. The titre value was recorded, and using the formula below soil organic carbon was calculated.

$$\% \text{ Soil organic carbon (\%)} \quad \frac{\mathbf{T} \times 0.2 \times 0.3}{\text{Sample weight}} = \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (3)$$

Where: **T** = titration volume

4.1.5. Alkaline and Acid phosphatase (PA) and β-glucosidase (GA)

Alkaline and acid phosphatase and *β*-glucosidase activities were determined following the methods reported by Tabatabai and Bremner (1971) and Eivazi and Tabatabai (1988), respectively. The method was based on the use of 0.5 g of soil, and 2 ml of modified universal buffer (MUB) containing the following substrate: alkaline phosphatase activity assay which were performed at pH 11 using *p*-nitrophenyl phosphatase (PNPP) as substrate, while acid phosphatase activity assay was performed with the same substrate at pH 5.5. Then 0.5 ml of $CaCl_2$ 0.5 M and 2 ml of NaOH 0.5 M was added in both the phosphatase; β-glucosidase activity was measured using the spectrophotometric assays where 1 g of air-dried soil was incubated for 1 hour with p-nitrophenyl-β-glucoside at 37˚C (Sigma Chemical Co., St. Louis, MO, USA) in modified universal buffer (pH 6.0). Enzymatic reactions were stopped by cooling in ice for 15 minutes. Thereafter, 2 ml of Tris (hydroxymethyl) aminomethane-sodium hydroxide (THAM-NaOH) 0.1 M pH 12 was added. The three enzymatic assays formed *p*-nitrophenol (PNP) product from reaction. The products were then determined calorimetrically at a wavelength of 398 nm.

4.1.6. Soil dehydrogenase activity (DHA)

Soil dehydrogenase activity (DHA) activity was measured by spectrophotometry using the hydrolytic reaction of formazan formation, where 2, 3, 5 triphenyltetrazolium chloride (TTC) solution was added to 2 g soil. The soil was shaken and incubated in the dark at 25˚C for 24 hours. The products of hydrolyses reaction were extracted using 10 ml methanol, centrifuged at 4500 rpm for 10 minutes and then sample absorbance was determined at 485nm (Casida *et al.*, 1964).

4.1.7. Available phosphorus (P) and nitrogen(N)

Available P was obtained by mixing 6.67 g of soil with 50 ml Bray-1 extracting solution. The mixture was shaken on a reciprocal for 60 seconds, filtered through No. 42 Whatman filter paper and the extract analyzed for available P content at 854 nm using spectrophotometer (Bray and Kurtz, 1945). Extractable N was determined using spectrophotometer by colorimetric technique that provides measurements of nitrate (NO_3^-), and ammonium (NH_4^+) from a single soil extract. A 100 ml of 2 M KCl solution was added to 10 g soil sample and the mixture was shaken for an hour then filtered through Whatman No. 42 filter paper. The concentrations of ammonium and nitrate in sample extracts were measured at the absorbance 655 nm and 419 nm, respectively (Bremner and Keeney, 1965; Freney and Wetsleaar, 1967).

4.1.8. Statistical analysis

Using the CRD model, analysis of variance was conducted using the Statistix software version 10.0 package (Statistix Institute, 2013). The effect of incubation days, biochar application rate, and soil type on selected soil nutrients and bio-quality parameters, were analyzed. The significant differences between the treatments was observed, the mean separation was done using LSD at 5% level of significance.

4.2. Results

4.2.1. Physiochemical properties of soils before planting

The result of physiochemical properties of soils before planting were described in subsection 4.1.1.

4.2.2. Chemical composition of pine wood biochar (BC)

The results of a chemical composition of pine wood biochar (BC) were described in subsection 4.2.

4.2.3. Effect of biochar application rates on N, P, and bio-quality parameters

4.2.3.1.The effect of incubation period, biochar rate, and soil type on N, P and bio-quality parameters

Incubation period, biochar rate and soil type had significant effect ($p \leq 0.05$) on N, P and all selected bio- quality parameters except for AP which was not affected significantly by soil type (Table 8).

Table 8. The effect of incubation period, biochar rate, and soil type on enzyme activities, N, P, and bio-quality parameters

Treatments	DHA(INF mg^{-1} kg^{-1} $2h^{-1}$)	U(NH_4-N mg^{-1} kg^{-1} $2h^{-1}$)	AP(P-nitrophenol mg^{-1} kg^{-1} h^{-1})	PAGA(P-nitropher mg^{-1} kg^{-1} h^{-1})	P (mg kg^{-1})	N (mg kg^{-1})	SOC (%)	MBC (%)	MBN (%)
Incubation period									
30	27.58	13.88a	3048.10	896.65	23.61a	29.08a	1.28a	13.71a	10.32
60	21.31	11.38b	632.00	573.72	27.47b	29.47b	1.50b	14.10b	4.86
90	21.00	9.04c	652.40	538.54	30.75c	36.60c	1.98c	15.76bc	6.63
120	28.20	9.83c	641.40	565.68	33.64d	40.20c	2.68c	25.40c	10.92
F- value (0.05)	ns	*	ns	ns	*	*	*	*	ns
LSD (≤0.05)	_	0.88	_	_	0.92	2.59	0.39	2.00	_
Biochar rate									
0	24.80	11.42a	447.20	417.20	27.83a	31.27a	1.52a	15.62a	5.05
10	22.60	10.42a	728.10	742.30	28.69b	39.68c	1.73ab	16.68ab	5.74
20	24.43	11.46b	3082.80	720.95	28.83bc	33.43c	2bc	17.55b	13.74
40	26.26	11.46b	715.80	694.15	30.12c	30.97c	2.19c	19.11b	8.19
F- value (0.05)	ns	*	ns	ns	*	*	*	*	ns
LSD (≤0.05)	_	0.88	_	_	0.92	2.59	0.39	2.00	_
Soil type									
Sd	44.75a	17.18a	1275	1408.80a	25.87a	18.33a	2.01a	16.50a	8.87a
Hu	22.89b	5.50b	1160.80	818.60b	34.54b	37.93b	1.69b	14.50b	1.35ab
We_2	18.77bc	11.64c	102	145.90c	27.07b	10.62c	1.02b	22.35b	4.81ab
We_1	11.69c	9.81d	2436.10	201.40c	28c	68.47d	2.72c	15.61b	17.69b
F- value (0.05)	*	*	ns	*	*	*	*	*	*
LSD (≤0.05)	11.13	0.88	_	385.30	0.92	2.59	0.39	2.00	13.32
CV	86.62	15.23	652.74	114.18	6.09	14.65	40.31	22.18	310.42

Means with same letters in each column are not significantly different at p≤ 0.05 for each soil type, biochar rate and incubation period, ns= non-significant; CV= coefficient of variation, 30, 60, 90, 120= Incubation period (days), 0, 10, 20, 40= Biochar rate (t ha^{-1}), Sd, Hu, We_2, We_1= Soil type

4.2.3.1.1. The effect of incubation period on N, P and bio- quality parameters

Urease activity measured at 30 days of incubation was high by 13.88 units compared to 11.38 at 60 days of incubation, decreased 9.04 units at 90 days of incubation, but picked-up marginal increase at 120 days of incubation (Table 8). On contrary, soil available P increased with increase in incubation days, while available N increase was observed at 90 and 120 days of incubation. Soil organic carbon and microbial biomass carbon increased with increasing incubation days until peak at 90 incubation days (Table 8). On the other hand, available N increased and peaked with biochar application rate of 10 t ha^{-1} and then decreased at biochar application rates of 20 and 40 t ha^{-1}(Table 8).

4.2.3.1.2. The effect of biochar rate on N, P and bio- quality parameters

Soil P and bio-quality parameters (SOC, and MBC) increased consistently at a very low rate from the lower biochar application rate (0 and 10 t ha^{-1}) to 20 and 40 t ha^{-1} application rate (Table 8). Urease activity decrease was observed at 10 t ha^{-1} and 40 t ha^{-1} biochar application rates with an increase observed at 20 t ha^{-1} biochar application rate (Table 8).

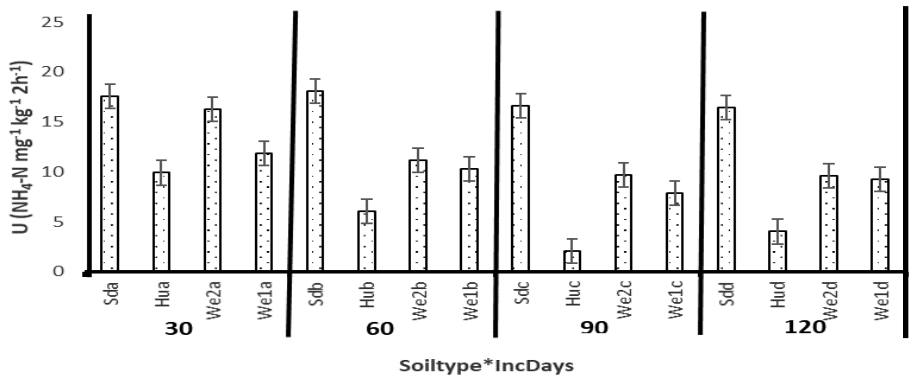
4.2.3.1.3. The effect of soil type on N, P and bio- quality parameters

Westleighe$_1$ soil had higher available N, SOC, and MBN, while We$_2$, and Sd soils had lower SOC, MBN, and available N, respectively (Table 8). Available P was high in We$_2$ soil and lower in Sd soil. In contrast, Sd soil had the highest U and DHA enzyme activities. The lowest U activity was measured in We$_2$ soil, while the lowest DHA activity was recorded in We$_2$ soil. The MBC was high in soil We$_2$ and lower in soil Hu. PAGA had the highest value in Sd soil and the lowest in We$_2$ soil (Table 8).

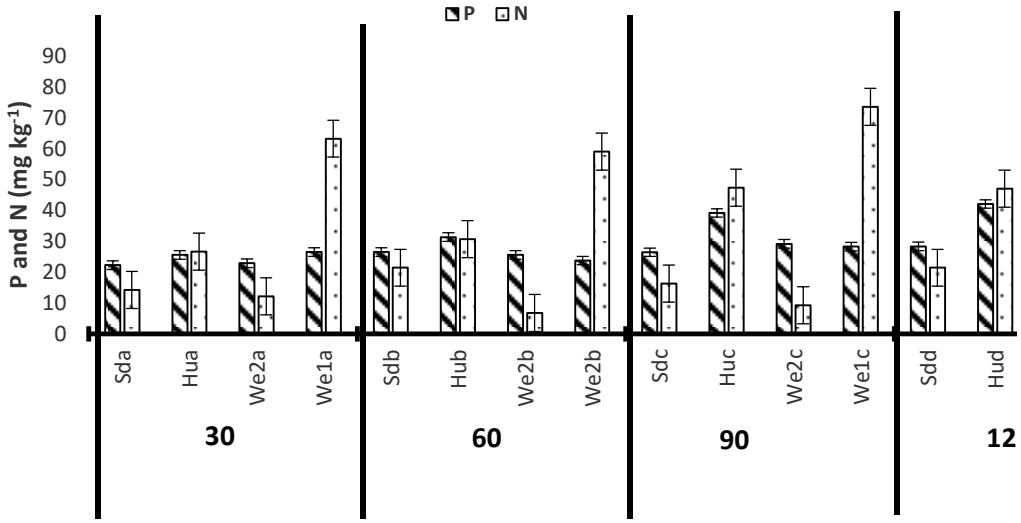
4.2.3.2. The effect of treatment interaction (incubation days x soil type) on selected soil chemical properties and bio-quality parameters.

The results from ANOVA showed that the interaction of incubation days and soil type had no significant effect ($p \leq 0.05$) on measured soil DHA, AP, PAGA, SOC, and MBN (Table 9). However, the interaction of soil type with incubation days exerted significant effect on U, P, N and MBC (Figure 6.1). Figure 6.1 shows the interaction of soil type with incubation days, where U activity was optimum when Sd soil was incubated for 60 days and lowest when Hu soil was incubated for 90 days. Available N was optimum when We_1 soil was incubated for 90 days and lower when Sd soil was incubated for 30 days. Soil type x incubation period interaction Hu_d had the highest available P compared to all soil type x incubation period interaction. While, Sd_a soil type x incubation period interaction had the lowest available P compared to all other soil type x incubation period interaction. On the other hand, $We2_d$ soil type x interaction had the highest MBC which dropped at We1a soil type x incubation period interaction (Figure 6.1).

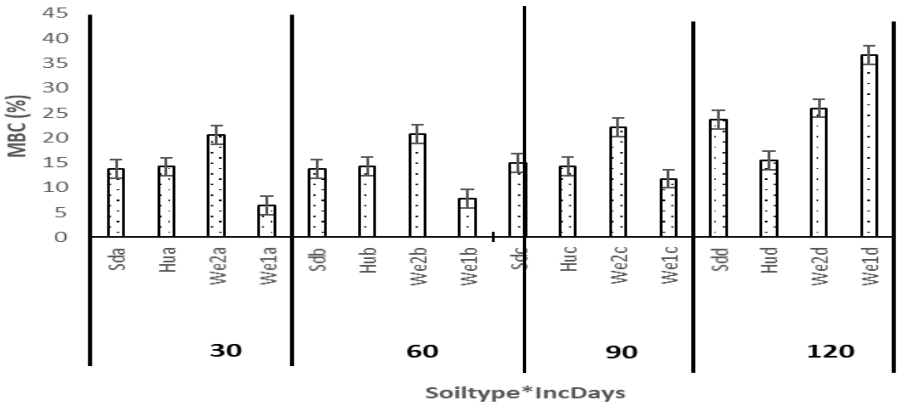
Table 9. ANOVA table for 120 days incubation of different soils with biochar application in various rate										
source	**DF**	**DHA**	**U**	**AP**	**PA+GA**	**P**	**N**	**SOC**	**MBC**	**MBN**
IncDays	3	0.191^{ns}	0.000***	0.377^{ns}	0.0612^{ns}	0.000***	0.000***	0.000***	0.000***	0.599^{ns}
BiocRate	3	0.870^{ns}	0.006**	0.350^{ns}	0.1131^{ns}	0.000***	0.000***	0.000***	0.000***	0.332^{ns}
Soiltype	3	0.000***	0.000***	0.582^{ns}	0.000***	0.000***	0.000***	0.000***	0.000***	0.014***
IncDays*Bcrates	9	0.159^{ns}	0.000***	0.439^{ns}	0.2819^{ns}	0.000***	0.000***	0.852^{ns}	0.006**	0.382^{ns}
IncDays*Soiltype	9	0.498^{ns}	0.000***	0.451^{ns}	0.0640^{ns}	0.000***	0.000***	0.098^{ns}	0.000***	0.775^{ns}
Bcrates*Soiltype	9	0.122^{ns}	0.000***	0.440^{ns}	0.0019***	0.000***	0.000***	0.937^{ns}	0.8163^{ns}	0.402^{ns}
IncDays*Bcrates*Soiltype	27	0.124^{ns}	0.000***	0.483^{ns}	0.2842^{ns}	0.000***	0.000***	1.000^{ns}	0.9576^{ns}	0.492^{ns}



(a)



(b)



(c)

Figure 6. 1. Error bars for the effect of interaction of soil type (We_1, We_2, Hu, and Sd) with incubation days (a - 0, b - 30, c - 90, and d - 120 days) on (a) U, (b) P and N, and (c) MBC

4.2.3.3.The Interaction effect of incubation days with biochar rate, on N, P and bio-quality parameters

Soil P, N and bio-quality parameters (U, SOC, MBC, and MBN), were affected significantly by the interaction of incubation days' x biochar rate (Figure 6.2.). Other bio-quality parameters were not affected by the interaction of incubation days' x biochar rate (Appendix 1). At 30 days of incubation, the interaction of incubation day x biochar rate revealed that U activity at a1 was the lowest and highest at a4. While U activity at incubation day 60 revealed the increase from 9.982 (interaction 60 incubation period with 0 t h^{-1}) to 13.999 at interaction b3, and thereafter U activity dropped to 8.754 at interaction b4 (Figure 6.2 a). At incubation day 90, the interaction of incubation day with biochar rate revealed that U activity at c1 was the highest and lowest at c4. On the other hand, U activity at incubation day 120 showed a decrease from interaction d1 of 12.279 to interaction d2 of 6.742, and thereafter U activity started picking up and increased at interaction d3 and d4, respectively (Figure 6.2 a). Figure 6.2 b and 6.2 c, shows that available P, SOC, MBC, and MBN uniformly increased from incubation x biochar rate interaction a1 to d4. Available N varied much with the rest of the parameters in that, it started with a lower incubation day x biochar rate interaction of 26.542 at a1, then increased to 40.708 at interaction a2, and finally dropped to 28.333 and 20.75, at incubation day x biochar rate interaction of a3 and a4, respectively (Figure 6.2. (b)). Figure 6.2. also shows the rise of available N at interaction b1, increasing at interaction b2 and then dropped at b3 and b4. Similar patterns continued at interaction c1 – c4, and d1- d4, respectively.

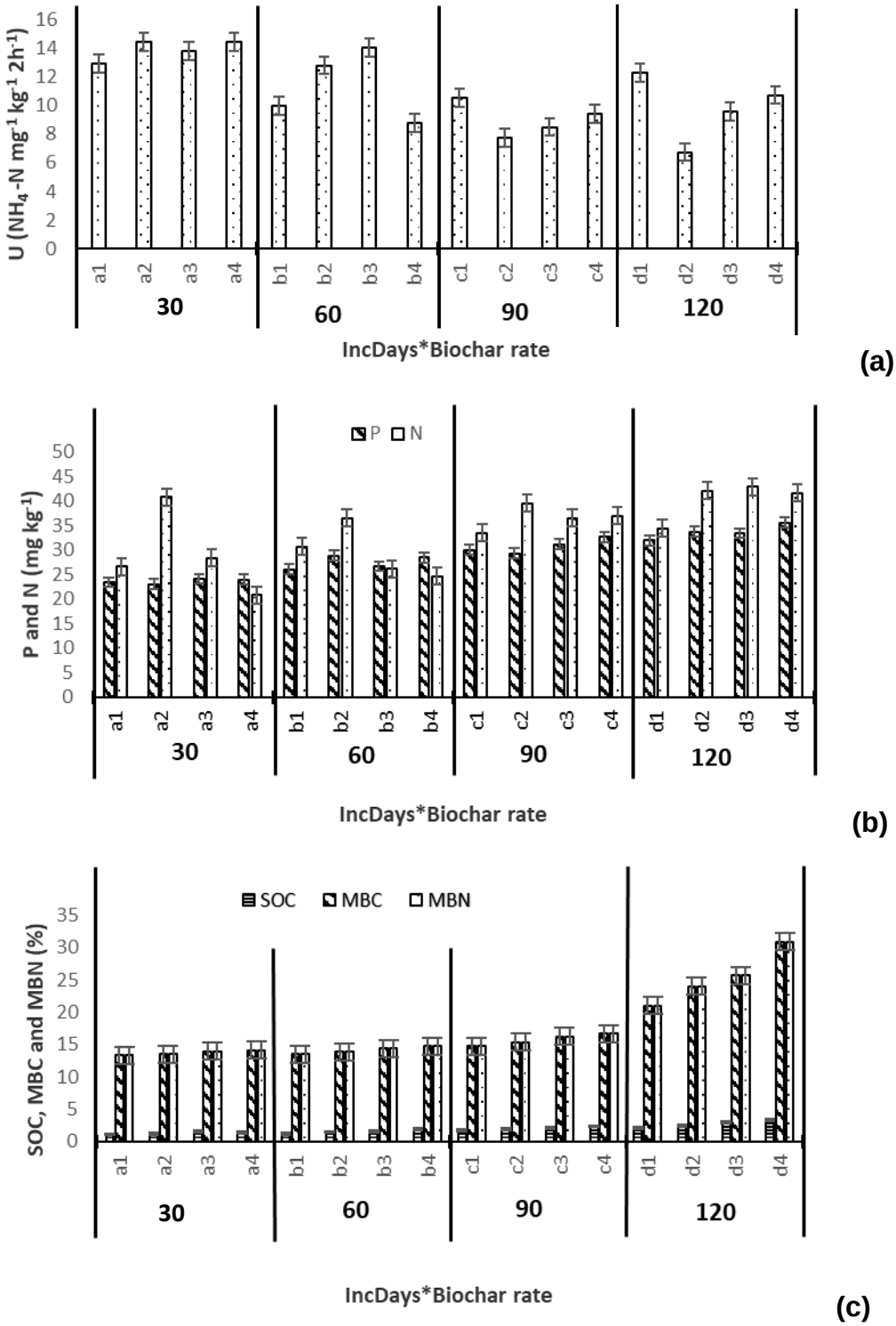
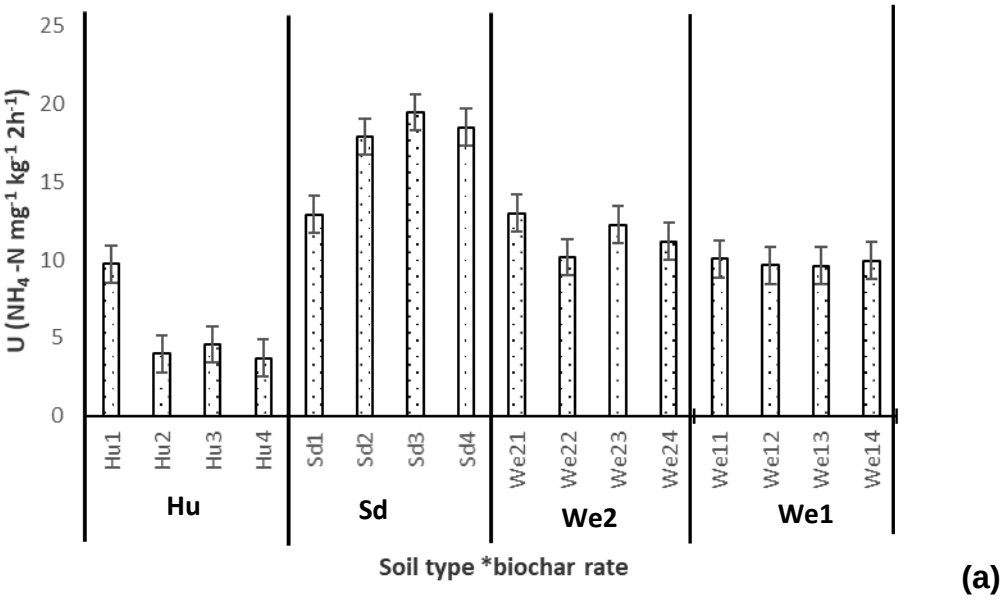


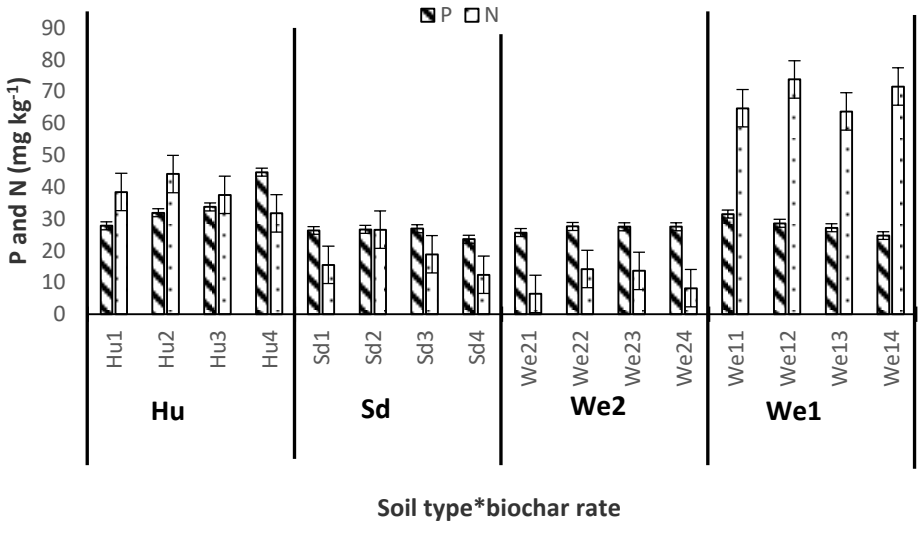
Figure 6. 2. Error bars for the effect of Interaction of incubation days (a - 0, b - 30, c - 90, and d - 120 days) with biochar rate (1 - 0, 2 - 10, 3 - 20, and 4 - 40 t ha^{-1}), on (a) Urease activity, (b) P and N, and (c) SOC, MBC, and MBN.

4.2.3.4. Soil type x biochar rate interaction effect on N, P and bio-quality parameters

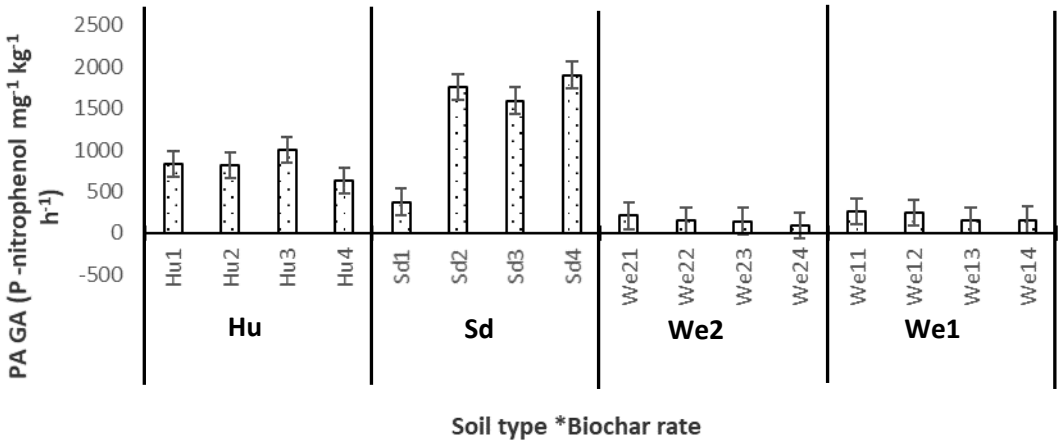
Figure 6.3 shows that the interaction of soil type x biochar rate had significant ($p < 0.001$) effect on U, PAGA, P and N. Appendix 1 shows that the interaction effect of soil type x biochar rate had no significant effects on other bio-quality parameters (DHA, AP, SOC, MBC, and MBN). Nitrogen availability was higher in soil type interaction with biochar in We_22 and We_24 soils and lower on soil type interaction with biochar in Sd1 and We_21 soils (Figure 6.3 b). Phosphorus availability was higher in soil type x biochar interaction in We_24 and We_23 soils. It was lower on soil type x biochar interaction Sd4 and We_24 soils (Figure 6.3b). Figure 6.3 a shows higher U activity on soil type interaction with biochar in Sd3 and Sd4 soils and lower on soil type * biochar in We_24 and We_22. On the other hand, PAGA activity was higher after soil type biochar interaction Sd4 and lower on soil type biochar interaction We_24 (Figure 6.2 c).



(a)



(b)



(c)

Figure 6. 3. Error bars for the ffect of Interaction of soil type (We_1, We_2, Hu, and Sd) with biochar rate (1 - 0, 2 - 10, 3 - 20, and 4 - 40 t ha^{-1}), on (a) Urease activity and (b) Available soil P and N.

4.2.3.5. The effect of interaction of incubation days, soil type and biochar rate on selected chemical properties and bio- quality parameters

Incubation days' x soil type x biochar rate interaction had significant effect on U, P, and N only and the rest of bio-quality parameters were not affected (Table 9).

4.3. Discussion

4.3.1.The effect of incubation days, biochar rate, and soil type on N, P and bio-quality parameters.

The results of this study revealed that the effect of incubation days and biochar rate on Urease activity and agrees with the pot experiment of five organic amendments (pig manure, cow dung, chicken manure, rapeseed meal and biochar) study conducted by Yang *et al*. (2018), which revealed a decrease in U activity with increased incubation days due to hydrolysis of C-N bond of some amides and urea. Urease activity increased after biochar application at the rate of 20 t ha^{-1} (Table 8) and was probably due to increased organic matter forming soil enzyme clay/complex to protect the enzyme within (Demisie and Zhang, 2015). Increased MBC and SOC with incubation days was also observed by Zhu *et al.* (2017) who concluded that it might be due to decomposition of organic materials which led to a release of nutrients to provide energy for microorganisms (Wardle *et al*., 2008). The observed increased MBC and SOC may also be due to the limited (120 days) incubation days. Jindo *et al*. (2014) reported that a decrease in MBC and SOC was only observed after 150 days of incubation with biochar. Biochar addition and days of incubation increased N and P availability when compared to control soil (Atkinson *et al*., 2010). An increased nutrient availability may be as a result of an increase in alkaline phosphatase activity and P/N levels in biochar treatments (Xiao *et al*., 2016). Though there were no significant differences observed on the effect of incubation days on

acid phosphatase activity, a general decrease in AP activity as compared to the control was observed. A decrease of acid phosphatase activity would mean an increased alkaline phosphatase activity that might be a result of an increased inorganic P in soil (Lemanowicz *et al*., 2019)

4.3.2.The effect of Interaction of incubation days with soil type, on N, P and bio-quality parameters

Table 3 Shows a significant interaction between incubation days with soil type on available P. An increased incubation period x soil type significantly increased available P. An increase in available P in relation to soil type and incubation period is due to the effect of soil pH on available P as shown in Table 8. Phosphorus availability was maintained for longer period in alkaline soils due to adsorption complex that P formed after reacting with Ca^{2+}, thereafter reacting with the Ca^{2+} in the soil solution, and lastly with the Ca^{2+} on the surface of the calcite (Akinremi and Cho, 1991). The results are also in agreement with what Olsen *et al*. (1954) described as rich P solubility observed in sandy soils than in clay soils. Boukhalfa-Deraoui *et al*. (2015) stated that “available P in soils is affected by a number of parameters such as; soil pH, organic matter, limestone, and soluble salts”. Nonetheless, it is still essential to characterise available P in order to establish its activities to adjust soil fertility and make fertilizer recommendation. On the other hand, the interaction of incubation period with soil type had a significant effect on MBC. The less clayey We_1 soil with 18 % clay maintained its lower MBC compared to the rest of the soils (Table 11), owing it to the fact that sandy soils cannot retain water and drains quickly while clay loam preserve water and hold nutrients (Heritage *et al*., 2003). Biochar incubated soils had greater MBC in more clayey soils than sandy. Soil attachment to particles of biochar may contribute to micro-aggregation of

soil, and variability in the microbial responses to biochar with aggregates may lead to inconsistent, transient effect of biochar on microbial communities which may not even reflect on change in MBC (Rillig and Mummey, 2006). Similarly, interaction of incubation days with soil type significantly affected the urease activity and the available N, where a notably low U activity of 0.000 units (no activity) was recorded on Sd soil at 60 days of incubation. Several studies have demonstrated the role soil texture plays in carbon storage and nutrient retention (Najmadeen *et al*., 2010; Silver *et al*., 2000). From observations made by Raiesi (2006), fine textured soils showed aggregate stability, which acts as a media to greater nitrogen and organic carbon content. Nevertheless, an increase in organic carbon activates the U activity in soils (Mahata and Antil, 2004), which may explain the highest activation of U activity responding to interaction of soil type and incubation days (Appendix 1.). The effect of interaction of Hu soil with different incubation period had no significant effect on U activity, which is in agreement with a reported increase in enzyme activity with increased organic matter content as a result of less activity in sandy soils as compared to medium-fine sand (Wallenius *et al*., 2011 and Niemi *et al*., 2015). Furthermore, from the study it can be inferred that no great influence of soil type and incubation days' interaction on available N was detected. The differences in nutrient availability among soils alters the magnitude of microbes and makes it difficult to identify the individual effects (Kolb *et al*., 2009).

4.3.3.The effect of Interaction of incubation days with biochar rate, on selected soil chemical properties and bio-quality parameters

Biochar rate interaction with incubation days had significant effect on MBC and MBN. The significant increase of microbial community is considered beneficial in improving soil nutrient sources and overall fertility status, which will in turn sustain crop

production (Visser and Parkinson, 1989). According to Ding *et al.* (2016), improved microbial community occur by increasing nutrient availability, providing suitable shelter and ameliorating living conditions. However, Domene *et al.* (2014) found no significant change in microbial activity after biochar application. Therefore, there are high possibilities that increased microbial activity relied on easy mineralization of the organic content in biochar (Woolf and Lehmann, 2012). Enhanced nutrient (P and N) retention is due to an increasing shift in biological activities and microbial community (Lehmann *et al.*, 2011), thereby increasing plant nutrient availability in nutrient limited agroecosystem (Major *et al.*, 2010). The results of the study concur with the results of a 60 days' incubation study conducted by Botha (2016), which explained an increase in available N between day 1 to 7, as characterised by protein breakdown to form amino acid before being transformed to $NH_4^{+,}$ through the process of ammonification. In addition, increased available N could arguably be due to the composting nature of biochar which release exchangeable ammonium through biochar surface chemistry (Korner and Stegman, 1998). Similarly, available N and P increased with an increase in biochar rate interaction with incubation days. The higher available P concentrations is explained by Atkinson *et al.* (2010), who showed that soil microbes could be responsible for soil binding with biochar which in turn yielded nutrient retention. Moreover, the microbes could have broken down the organic P captured in phospholipids, nucleic acids and phytyl, and transformed it to phosphate ions, or organic complexation of Fe and Al, which reversed it from being fixed and unavailable (McBride, 1994). Soil urease activity decreased with increased incubation days and biochar rate interaction possibly because biochar may release toxic components such as ethylene which may inhibit some soil microbial activities (Hale *et al.*, 2012). The results of the study also demonstrated the weak effect of biochar to soil enzymatic activities, reflecting sensitivity of soil management practices (Niemi *et*

al., 2015). The latter is provoking further investigation on use of biochar for soil management. However, with the understood role of biochar to carbon sequestration, biochar rate interaction with incubation days had significantly increased SOC (Bruun *et al.*, 2011). An increased SOC is explained by fixed thermally stable carbon structure formed during pyrolysis with occurrence of series of cleavage and polymerization reactions (Spokas *et al.*, 2012).

4.3.4.The effect of Interaction of soil type with biochar rate, on selected soil chemical properties and bio-quality parameters

The effect of soil type interaction with biochar rate revealed a general decreased urease activity on sandy soils with increased biochar rate. The general decrease in urease activity in the soils indicates the sensitivity of urease to biochar amelioration in sandy soils (Ying *et al.*, 2011). Although urease is responsible for transformation of urea into ammonium and carbon dioxide (Dick, 1992), it is susceptible to many changes such as hydrocarbons by release of heavy metal and common oxidative urease damagers such as carbonyl and nitrotyrosine (Garcia-Gil *et al.*, 2000; Yang *et al*., 2018). Furthermore, diverse urease activity effects were observed in various soils and biochar rates. Though it was expected that there would be greater attachment of biochar when incubated in clay soils compared to sandy soils (Jaafar *et al*., 2015), there were no distinct differences among soils ameliorated. Biochar surface structure seem to attach both finer and lager soil particles (Jaafar *et al.*, 2014). It is still unclear whether soil type and biochar interaction could influence the microbial community directly or indirectly (Jaafar *et al.*, 2015). Hence, the variable response of soil microbes to biochar application and soil type (Rillig and Mummey, 2006). This observation might explain the available N fluctuations as reported by Han *et al.* (2004) that the catalytic efficiency of the enzyme reaction may vary from time to time

depending on the urea concentrations and soil organic matter levels. Furthermore, the study significantly revealed higher available N in sandy soils as compared to clay soils, of which Kolb *et al.* (2009) explained to be affected by correlations among soil factors (e.g. soil nutrients status, soil texture, and organic matter content) making it difficult to identify individual effects. However, inconsistent significant differences were observed on both the available P and β glucosidase. The differences in enzyme dynamics may be due to variation in soil chemical properties (Vepsäläinen *et al.*, 2004) and a deactivation of enzyme activity may be due to sorption or blocking of enzymes (Brantley *et al.,* 2015). According to Boukhalfa-Deraoui *et al.* (2015), soil P fixation was responsible for a decrease in the amount of available P generated or it is likely that the initial available P rapidly mineralised (Brantley *et al.*, 2015).

4.3.5.The effect of interaction of incubation days, soil type and biochar rate, on N, P and bio- quality parameters

Soil enzymes are the main mediators of biological processes in the soil as they are responsible for nutrient cycling, mineralization and organic matter degradation (Marx *et al.*, 2001). The results of this study showed that most enzyme activities were not affected significantly by the interaction of incubation days, soil types and biochar rates. Urease activity was the only enzyme affected significantly. Soil urease is involved in hydrolysis of C-N bonds of some amides and urea (Bremner and Mulvaney, 1978). Consequently, increased biochar rate would mean an increased urease activity (Tabatabai, 1994). However, Demisie and Zhang (2015), reported an indirect effect of biochar on urease activity through dissolution of organic carbon. On the other hand, Niemi *et al.* (2015) stated that biochar incubated soils had few or weak effects on soil enzyme activities. Therefore, biochar physiochemical properties

may have attributed to the effect of improved enzyme activity (Zimmerman *et al.*, 2011) resulting in degradable composition in biochar treated soils. However, Niemi *et al.* (2015) observed soil N immobilization after biochar addition, but biochar addition in this study did not reveal similar results as there were general fluctuation of soil nutrients and soil bio-quality parameters. Although minor significant effect on soil microbial activities were observed, biochar significantly affected available soil P and N, reflecting on the microbial activities in soil (Saarnio *et al.*, 2013)

4.4. Conclusion

The incubation study had hypothesized that biochar applied in different soil types (Sd, We_2, Hu and We_1) would affect soil bio-quality parameters (MBN, MBC, SOC, PA, PAGA, and DHA) and selected soil chemical properties (N and P). Certainly, the results of the study revealed various response of soil bio-quality parameters and selected soil chemical properties, after biochar application at different rates and in different soil types. Both the single and interaction effects of biochar rate, soil type and incubation days had variable significant effects on soil bio-quality parameters and selected chemical properties. Interestingly, biochar significantly affected and improved soil available N and P in different soil types. Although most of soil bio-quality parameters were not significantly affected after biochar addition in soil, there were some instances where bio-quality parameters such as U, SOC, MBC, and MBN were affected significantly by the interaction of incubation days with biochar rate, interaction of soil type with biochar rate which significantly affected U, and PAGA. While interaction of incubation rate with soil type had significantly affected U and MBC. Consequently, the study found inconsistent effects of biochar rate, incubation days and soil type on soil enzymes and other bio-quality parameters. Furthermore, the mechanism of microbial activity fluctuation and inhibition of enzymes need to be

determined. Biochar application rate, incubation days and soil type had variable effects on soil bio-quality parameters in the short-term study. Therefore, further prolonged studies (more than 120 day) is required for further evaluation of the effects of incubation period, biochar rate and soil type effects on soil nutrients ad bio-quality parameters.

5. References

Abbasi, M. K., and Anwar, A.A. (2015). Ameliorating effects of biochar derived from poultry litter and white clover residues on soil nutrient status and plant growth promotion - Greenhouse Experiments. PloS ONE. 10, pp. 137.

Akinremi, O.O., and Cho, C.M. (1991). Phosphatase and accompanying cation transport in calcareous cation-exchange resin system. Soil Science Society of America Journal. 55, pp. 959-964.

Anderson, C.R., Condro, L.M., Clough, T.J., Fiers, M., Stewart, A., Hill, R.A., and Sherlock, R.R. (2011). Biochar induced soil microbial community change: Implication for biogeochemical cycling for carbon, nitrogen, and phosphorus. Pedobiologia. 54, pp. 309-320.

Angst, T.E., and Sohi, S.P. (2013). Establishing release of dynamics for plant nutrients from biochar. GCB Bioenergy. 5, pp. 221-226.

Atkinson, C.J, Fitzegerald, J.D, and Hipps, N.A. (2010). Potential mechanisms for achieving agricultural benefits from biochar application of temperate soils. Plant and Soil. 337 (1-2), pp. 1-18.

Baaru, M., Mugendi, D.N., Bationo, A., Verchot, L., and Wanjohi, W. (2007). Soil microbial biomass carbon and nitrogen as influenced by organic inputs at Kabete, Kenya. Advances in Integrated Soil Fertility. Management in sub-Saharan Africa: Challenges and opportunities. pp. 827-832.

Bailey, V.L., Fansler, S.J., Smith, J.L., Bolton, H. (2011). Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. Soil Biology and Biochemistry. 43 (2), pp. 296-301.

Beck, T., Joergensen, R.G., Kandeler, E., Makeschin, F., Nuss, E., Oberholzer, H.R., and Sche, S. (1997). An inter-laboratory comparison of ten different ways of measuring soil microbial biomass C. Soil Biology and Biochemistry. 29 (7), pp. 1023-1032.

Blackwell, P., Riethmuller, G.P., and Collins, M. (2009). Biochar application to soil. Biochar for environmental management: Science and Technology. pp.206- 226.

Bonomelli, C., Gil, P.M., and Schaffer, B. (2019). Effect of soil type on calcium absorption and partition in young avocado (*Persea Americana Mill*) trees. Agronomy Journal. 9 (12), pp. 837- 838

Boukhalfa-Deraoui, N., Hanifi-Mekliche, L., and Mihoub, A. (2015). Effect of incubation period of phosphorus fertilizer on some properties of sandy soil with low calcareous content, Southern Algeria. Asian Journal Agricultural Research. 3 (9), pp. 123- 131.

Bot, A., and Benites, J. (2005). The importance of soil organic matter key to drought-resistant soil and sustained crop production. FAO soils bulletin.pp.80.

Botha, O.G. (2016). The effect of composted biochar on compost properties and mineralization. University of Stellenbosch. MSc Thesis. pp. 32-72.

Bouyoucos, C.J. (1962). Hydrometer method improved for making particle size analysis of soils. Agronomy Journal, 54, pp. 464-465.

Brantley, K.E., Savin, M.C., Brye, K.R., and Longer, D.E. (2015). Pine woodchip biochar impact on soil nutrients concentrations and corn yield in silt loam in the mid Southern U.S. Agronomy. 5, pp. 30-47.

Bray, R.H., and Kurtz, L.T. (1945). Determination of total, organic and available forms of phosphorus in soil. Soil Science. 59, pp. 39–45.

Bremner, J.M. (1960). Determination of nitrogen in soil by the Kjeldahl method. Agricultural Science. 55, pp. 11-33.

Bremner, J.M., and Keeney, D.R. (1965). Steam distillation methods for determination of ammonium, nitrate and nitrite. Methods of Soil Analysis. Chemical and Microbiological properties. 32, pp. 485-495.

Bremner, J.M., and Mulvaney, R.L. (1978). Urease activity in soils. In Bums RG (ed.) Soil enzymes. Academic press. New York. pp. 149-196

Brookes, P. C., Landman, A., Pruden, G., and Jenkinson, D.S. (1985). Chloroform fumigation and the release of soil nitrogen: a rapid direct extraction method to measure microbial biomass nitrogen in soil. Soil Biology and Biochemistry. 17, pp. 837-842.

Bruun, E.W., Ambus, P., Egsgaard, H., and Hauggaard-Nielsen, H. (2011). Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. Soil Biology and Biochemistry. 46, pp. 73-79.

Bureau for Food and Agricultural Policy (BFAP). (2018). Small Scale Farmer's brief: Macro-economic Impact on profitability. South African Breweries Malting. Alberton.pp.1-19.

Casida, L. E., Klein, Jr., D. A. and Santoro, T. (1964). Soil dehydrogenase activity. Soil Science. 98, pp. 371-376.

Celik, I., Ortas, I., and Kilic, S. (2004). Effects of compost, mycorrhiza, manure and fertilizer on physical properties of a Chromoxerert soil. Soil and Tillage Research. 78 (1), pp. 59-56.

Chan, K.Y., and Xu, Z. (2009). Biochar: Nutrient properties and their enhancement. biochar for environmental management: Science and Technology. Earthscan, London, UK. pp. 67-84.

Chan, K.Y., Zwieten, L.V., Meszaros, I., and Downie, A. (2007). Agronomic value of green waste biochar as a soil amendment. Australian Journal of Soil Research. 45, pp. 629-634.

Chen, H., Teng, Y., Lu, S., Wang, Y., and Wang, J. (2015). Contamination features and health risk of soil heavy metals in China. Science of the Total Environment. 512, pp. 143- 153.

Chivenge, P.P., Murwira, H.K., Giller, K.E., Mapfumo, P., and Six, J. (2007). Long-term impact of reduced tillage and residue management on soil carbon stabilization: Implications for conservation agriculture on contrasting soils. Soil Tillage Research. 94, pp. 328- 337.

Clark, D.A., Piper, S.C., Keeling, C.D., and Clark, D.B. (2003). Tropical rain forest tree growth and atmospheric carbon dynamics linked to interannual temperature variation during 1984 – 2000. Proceeding of the national academy of sciences. 100 (10), pp. 5852- 5857.

Clough, T.J., and Condron, L.M. (2010). Biochar and the nitrogen cycle. Journal of Environmental Quality. 39, pp. 1218–1223.

Clough, T.J., Condron, L.M., Kammann, C., and Müller, C. (2013). A Review of biochar and soil nitrogen dynamics. Agronomy. 3, pp. 275-293.

Completely Randomized Design. (2008). The Concise Encyclopedia of Statistic.Springer, Newyork.

Conradie, W.J. (1994). Wingerdbemesting. Handelinge van die werksessie oor wingerdbemesting, gehou te Nietvoorbij op 30 September 1994. LNR-Nietvoorbij Instituut vir Wingerd- en Wynkunde, Stellenbosch.

Cornelissen, G., Martinsen, V., Shitumbanuma, V., Alling, V., Breedveld, G.D., Rutherford, D.W.., Sparrevik, M., Hale, S.E., Obia, A., and Mulder J. (2013). Biochar effect on maize yield and soil characteristics in five Conservation farming sites in Zambia. Agronomy. 3, pp. 256-274.

Cowie, B.P., and Singh, A. (2015). Long –term influence of biochar on native organic carbon mineralization in low-carbon clayey soil. Scientific reports. Pubmed. 4 (1), pp. 3687.

Czimczik, C.I., and Masiello, C.A. (2007). Controls on black carbon storage in soils. Global Biogeochemical Cycling. 21, pp. B3005, doi:10. 1029/2006GB0027998.

Deem, L.M and Crow, S.E. (2017). Biochar. Reference Module in Earth Systems and Environmental Sciences. pp. 1- 2.9op

DeLuca, T.H., MacKenzie, M.D., and Gundale, M.J. (2009). Biochar effects on soil nutrient transformation. In: Biochar for Environmental Management: Science and Technology. Earthscan. pp. 251–270.

Demir, K., Sahin, O., Kadioglu, Y.K., Pilbeam, D.J., and Gunes, A. (2010). Essential and non-essential element composition of tomato plants fertilized with Poultry Litter. Scientia Horticulturae. 127 (1), pp.16-22.

Demirbas, A. (2006). Production and characterization of biochar from biomass via pyrolysis. Energy Sources. 28, pp. 413-422.

Demisie, W., and Zhang, M. (2015). Effect of biochar application on microbial biomass and enzymatic in degraded red soil. Africa Journal of Agricultural Research. 10 (8), pp. 755-766.

Dempster, D.N., Jones, D.L., and Murphy, D.M. (2012). Clay and biochar amendments decreased inorganic but not dissolved organic nitrogen leaching in soil. Soil Research. 50, pp. 216–221.

Department of Agriculture, Forestry and Fisheries (DAFF). (2014). Food Security 2013. Republic of South Africa. Pretoria.pp.1-73

Department of Agriculture Land Reform and Rural Development (DALRRD). (2020). Economic Review of South African Agriculture. Republic of South Africa. Pretoria.pp.1-50.

Dick, R.P. (1992). Long term effect of agriculture system on soil biochemical and microbial parameters. A review. Agricultural Ecosystem and Environment. 40, pp. 25-36.

Ding, Y., Liu, Y., Liu, S., Li, Z., Tan, X., We2ang, X., Zeng, G., Zhou, L., and Zheng, B. (2016). Biochar to improve soil fertility. A review. Agronomy and Sustainable Development. 36, pp. 1-18.

Domene, X., Mattana, S., Hanley, K., Enders, A., and Lehmann, J. (2014). Medium term effects of corn biochar addition on soil biota activities and functions in temperate soil cropped to corn. Soil Biology and Biochemistry. 72, pp. 152-162.

Duku, M.H., Gu, S., and Hagan, E.B. (2011). Biochar production potential in Ghana. Renew Sustainable Energy. 15, pp. 3539-3551.

Du Plessis, J. (2003). Maize production. Department of Agriculture. Republic of South Africa. Pretoria.

Du Preez, C.C., Van Weesteen, C.W., and Mnkeni, P.N.S. (2011). Land use and soil organic matter in South Africa 1: A review on spatial variability and the influence of rangeland stock production. South Africa Science Journal. 107(5/6), pp. 1-8. Available online at http://www.sajs.co.za Accessed at 24/05/2014.

Dume, B., Mosissa, T., and Nebiyu, A. (2015). Effect of biochar on soil properties and lead (Pb) availability in a military camp in South West Ethiopia. African Journal of Environmental Science and Technology. 10 (2), pp. 63-73.

Edenborn, S., Edenborn, H., Krynock, R.M., and Zickefoose Haug, K.L. (2015). Journal of Environmental Management. 150, pp. 226-234.

Eivazi, F., and Tabatabai, M. A. (1988). Glucosidases and galactosidases in soils, Soil Biology and Biochemistry. 20, pp. 601–606.

Ennis, C.J., Evans, A.G., Islam, M., Ralebitso-Senior, T.K., and Senior, E. (2012). Biochar: carbon sequestration, land remediation and impacts on soil microbiology. Critical Reviews in Environmental Science and Technology. 42 (22), pp. 2311-2364.

Faridullah, M.I., Yamamoto, S., Eneji, A.E., Uchiyama, T., and Honna, T. (2009). Recycling of chicken and duck litter ash as a nutrient source for Japanese mustard spinach. Journal of Plant Nutrition. 32, pp. 1082-1091.

Farnham, D.E., Benson, G.O., and Pearce, R.B. (2003). Corn perspective and culture. Corn: Chemistry and Technology. 5, pp.1-33.

Fauci, F., and Dick, R.P. (1994). Soil microbial dynamics: short- and long-term effects of inorganic and organic nitrogen. Soil Science Society of America. 58, pp. 801-806.

Fischer, D, and Glaser, B. (2012). Synergisms between compost and biochar for sustainable soil amelioration. Management of Organic Waste. 10, pp. 167- 197.

Freney, J.R., and Wetsleaar, R. (1967). The determination of nitrogen in soil with particular reference to nitrate. Division of Plant Industry and Technology. 23, CSIRO.

Fushimi, C., Araki, K., Yamaguchi, Y., and Tsutsumi, A. (2003). Effect of steaming rate on steam gasification of biomass. Thermogravimetric-mass spectrometric (TG-MS) analysis of gas evaluation. Engineering and Chemistry. 42, pp. 3929-3936.

Garcia-Gil, J.C., Plaza, C., Soler-Rovira, P., and Polo, A. (2000). Long term effect of municipal solid waste compost application on soil enzyme activities and microbial biomass in a long-term field experiment. Soil Biology and Biochemistry. 32, pp.1907-1913.

Ghaly, A. E., and Mahmoud, N. S. (2006). Optimum conditions for measuring dehydrogenase activity of *Aspergillus niger* using TTC. American Journal of Biochemistry and Biotechnology. 2 (4), pp. 186-194.

Gichangi, E.M., Itahari, J.K., Ikombo, B.M., Vankeilen, H., de Jager, Kariuki, A.M., Nguluu, S.N., and Wambua, J.M. (2002). Analysis of current nutrient management practices in semi-arid areas of Eastern Kenya. Proceeding of the International Symposium for Sustainable Dryland Agricultural Systems. pp. 263- 274.

Glab, T., Palmowska, J., Zaleski., T., and Gonde, K. (2016). Effect of biochar application on soil hydrological properties and physical quality of sandy soil. Geoderma.281, pp. 11-20.

Glaser, B., Lehmann, J., and Zech, W. (2002). Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal – a review. Soil Biology and Biochemistry. 35(4), pp. 219-230.

Grossman, J. M., O'Neill, B. E., Tsai, S. M., Liang, B., Neves, E., Lehmann, J. and Thies, J. E. (2010). Amazonian anthrosols support similar microbial communities that differ distinctly from those extant in adjacent, unmodified soils of the same mineralogy. Microbiology and Ecology. 60, pp. 192-205.

Gunes, A., Inal, A., Taskin, M.B., Sahin, O. (2014). Effect of phosphorus-enriched biochar and Poultry Litter on growth and mineral composition of lettuce (*Lactuca sativa* L.cv) grown in alkaline soil. Soil Use and Management. 30 (2), pp. 182-188.

Hailegnaw, N., Mercl, F., Pracke, K., and Szakova, J. (2019). Mutual relationships of biochar and soil pH, CEC, and exchangeable base cations in a model laboratory experiment. Journal of Soils and Sediments. 19, pp. 2405- 2416.

Hale, S.E., Lehmann, J., Rutherford, D., Zimmerman, A.R., Bachmann, R.T., Shitumbanuma, V., O'Toole, A., Sundqvisit, K.L., Arp, H.P., and Cornellisen, G. (2012). Quantifying the total and bioavailable polycyclic aromatic hydrocarbons and dioxins in biochar. Environmental Science and Technology. 46(5), pp. 2830.

Han, M.J., Choi, H.T., and Song, H.G. (2004). Purification and characterization of laccase from the white rot fungus *Trametes versicolor*. Journal of Microbiology. 43, pp. 555-560.

Harris, A., Rachid, G., Miraj, M., Arif, H., and Shan, H. (2007). On farm seed priming with zinc sulphate solution- A cost- effective way to increase the maize yields of resource poor- farmers. Field Crops Research. 110, pp. 119- 127.

Haytham, M.E. (2012). Effect of nitrogen sources on microbial biomass nitrogen under different soil types. International Scholar Research Network Soil Science. 4, pp. 1- 7.

Hazlenton, P., and Murphy, B. (2007). Interpreting soil test results: What do all numbers mean? - A guide to the interpretation of soil test results. European journal of Soil science. 58, pp. 1219-1220.

He, N., Wang, R., and Gao, Y. (2013). Changes in the temperature sensitivity of SOM decomposition with grassland succession: Implication for soil C sequestration. Ecology and Evolution Journal. 3, pp. 5045- 5054.

Heritage, J., Evans, E., and Killington, R. (2003). Microbiology in action. Cambridge University press.pp. 55 - 60.

Hooda, P.S. (2010). Assessing bioavailability of soil trace elements. Trace Elements in Soils. School of Geography, Geology and Environment, Kingston University London, UK. pp.227-267.

Horwath, W.R. (2005). The importance of soil organic matter in the fertility of organic production systems. Western Nutrient Management Conference. 6, pp. 244-249.

Inal, A., Gunes, A., Sahin, O., Taskin, M.B., and Kaya, E.C. (2015). Impact of biochar and processed poultry manure, applied to a calcareous soil, on the growth of mean and maize. Soil Use and Management Journal. 31, pp. 106-113.

Islam, R., and Wright, S.R. (2004). Soil microbial communities. Encyclopedia of Soil. 24, pp. 100-120.

Jaafar, N.M., Clode, P.L., and Abbot, L.K. (2015). Biochar-Soil interaction in four agricultural soils. Pedosphere. 25(5), pp. 729-736.

Jaafar, N.M., Clode, P.L., and Abbot, L.K. (2014). Microscopy observations of habitable space in biochar for colonization by fungal hyphae. Soil Journal of Integrated Agriculture. 13, pp. 483- 490.

Jean, A., and Wang, Y. (2013). Physical properties of soil. Journal of Science and Technology. 8 (1), pp.231-320.

Jien, S., Wang, C., Lee, T., and Lee, C. (2015). Stabilization of organic matter by biochar application in compost-amended soils with contrasting pH values and textures. Sustainability Journal. 7 (10), pp. 13317 – 13333.

Jindo, K., Matsumoto, K., García Izquierdo, C., Sonoki, T., and Sanchez-Monedero, M. A. (2014). Methodological interference of biochar in the determination of extracellular enzyme activities in composting samples. Journal of Solid Earth. 6, pp. 919–935.

Karami, N, Clemente, R, Moreno-Jiménez, E, Lepp, N.W., and Beesley, L. (2011). Efficiency of green waste compost and biochar soil amendments for reducing lead and copper mobility and uptake to ryegrass. Journal of Hazard Matter. 191, pp. 41–48.

Karim, M.A., Higuchi, H., and Nawata, E. (2017). A participatory research experience on the introduction of Mugbean and short duration Aman Rice as summer crops in the Char lands of the Northern Bangladesh. Tropical Agricultural Development. 62(1), pp. 14-23.

Khan, H., Khan, M.A., and Husssain, I. (2008). Enzyme inhibition of the rhizomes of *Gloriosa superba* Linn (Cochicaceae). Journal of enzyme inhibition and medicinal chemistry. 22(6), pp. 722-725.

Khan, T.F and Huq, S.M.I. (2014). Effect of biochar on the abundance of soil Bacteria. British Microbiology Research Journal. 4(9), pp. 896-904. Available online at http://www.sciencedomain.org/review-history.php?iid=489&id=8&aid=4478 Accessed at 22/06/2014.

Khodadad, C.L.M., Zimmerman, A.R., Green, S.J., Uthandi, S., and Foster, J.S. (2011) Taxa-specific changes in soil microbial community composition induced by pyrogenic carbon amendments. Soil Biology and Biochemistry. 43, pp. 385-392.

Kim, I., Yu, Z.Q., Xiao, B.H., and Huang, W.L. (2007). Sorption of male hormones by soils and sediments. Environmental Toxicology and Chemistry. Pubmed. 26 (2), pp. 264-270.

Kolb, S.E., Fermanich, K.J., and Dornbush, M.E. (2009). Effect of charcoal quantity on microbial biomass and activity in temperate soils. Soil Science Society of America Journal. 73, pp. 1173-1181.

Korner, I., and Stegman, R. (1998). Influence of biowaste composition and composting on the nitrogen dynamics during composting and on nitrogen contents in compost. International Society for Horticultural Science. 469, pp. 97- 110.

Kristin, M.M. (2011). Biochar amendment to forest soils: effect on soil properties and tree growth. MSc. thesis. College of Graduate Studies. University of Idaho.

Krull, E.S., Lehmann, J., Skjemstad, J., and Baldock, J. (2008). The global extent of black C in soils. Grassland: ecology, management and restoration. Science publisher. Ink, New York. pp. 13-17.

Kumar, S., Mast, R.E., Ram, L.C., Sarkar, P., George, J., and Selvi, V.A. (2013). Biochar preparation from *Parthenium hysterophorus* and its potential use in soil application. Journal of Ecology and Engineering.55, pp. 67- 72.

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., and Xu, X. (2009). Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labelling, Soil Biology and Biochemistry. 41, pp. 210–219.

Laird, D., Flemimg, P., Wang, B., Horton, R., and Karlen, D. (2010). Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma. 158, pp. 436-442.

Laird, D.A., Brown, R.C., and Amonette, J.E. (2009). Review of pyrolysis platform of coproducing bio-oil and biochar. Biofuels, By-products and Bio refining. 3(5), pp. 119-167.

Lehmann J., and Rondon, M. (2006). Bio-Char soil management on highly weathered soils in the humid tropics. Biology Approach Sustain Soil System. 36, pp. 517.

Lehmann, J., and Joseph, S. (2009). Biochar for environmental management: Science and Technology. Earthscan. pp. 1-405.

Lehmann, J., da Silva, Jr. J.P., Steiner, C., Nehls, T., Zech, W., and Glaser, B. (2003). Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant and Soil. 249, pp. 343-357.

Lehmann, J., Rilling, M.C., Thies, J., Hockaday, W.C., and Crowley, D. (2011a) Biochar effect on soil biota. A review. Soil Biology and Biochemistry. 43, pp. 1812-1836.

Lehnmann J., Rillig M.C., Thies J., Masiello C.A., Hockaday W.C., and Crowley D. (2011b) Biochar effects on soil biota- A review. Soil Biology and Biochemistry. 43, pp. 1812-1836.

Lemanowicz, J., Siwik-Ziomek, A., and Koper, J. (2019). Enzymatic variation of soils exposed to the impact of the soda plant in terms of biochemical parameters. International Journal of Environmental Science and Technology. 16, pp. 3309- 3316.

Lentz, R.D., and Ippolito, J.A. (2012). Biochar and manure effect calcareous soil and corn silage nutrient concentration and uptake. Journal of Environmental Quality.41, pp. 1033-1043.

Li, Y., Zhang, W., Zheng, D., Zhou, Z., Yu, W., Zhang, L., Feng, L., Liang, X., Guan, w., Zhou, J., Chen, J., and Lin, Z. (2014). Genomic evolution of Saccharomyces cerevisiae under Chines rice wine fermentation. Genome Biology. 6 (9), pp. 2516-26.

Liang, B., Lehmann, J., Solomon, D., and Kinyangi, J. (2006). Black carbon increases cation exchange capacity in soils. Soil Science Society of America Journal. 70, pp. 1719-1730.

Logan, M.J., Jardine, T., Miller, T., and Bunn, S.E. (2008). Lipid corrections in carbon and nitrogen stable isotope analyses: comparison of chemical extraction and modelling methods. Journal of Animal Ecology. 77, pp. 838-846.

Lynch, J. (2009). Improving bean production in drought-prone, Low fertility Soils of Africa and Latin American- An intergraded approach. Pennsylvania State University as Lead University- Project. 11, pp.1-26.

Mabapa, P.M., Ogola, J.B.O., Odhiambo, J.J.O., Whitbread, A., and Hargreaves, J. (2010). Effect of phosphorus fertilizer rates on growth and yield of three soybeans (*Glycine max*) cultivars in Limpopo province. Africa Journal of Agricultural Research. 5(19), pp. 2653-2653.

Mahata, M.K., and Antil, R.S. (2004). Effect of organic matter and level of organic carbon on urease activity of selected surface soil contaminated with lead. Environment and Ecology. 22, pp. 314-318.

Maru, A., Ahmed, O., and Charles, P.W. (2015). Coapplication of chicken litter biochar and urea only to improve nutrients use efficiency and yield of *Oryza SativaL*. cultivation on a tropical acid soil. The Scientific World Journal. pp. 853- 943.

Major, J., Rondon, M., Molina, D., Riha, S., and Lehmann, J. (2010). Maize yield and nutrition during 4 years after biochar application to a Colombian savanna oxisol. Plant and Soil. 333(1-2), pp. 117-128.

Makhaga, N.S., Odhiambo, J.J.O., Whitbread, A.M., and Hargreaves, J.G. (2011). Simulating the effects of nitrogen and phosphorus fertilizers on maize biomass and

grain yield in Vhembe district, Limpopo province Combined Congress Book of Abstracts. pp. 166.

Manning, P. (2012). The impact of nitrogen enrichment on ecosystems and their services. Soil Ecology Ecosystem Services. pp. 256–269.

Marx, M.C., Wood, M., and Jarvis, S.C. (2001). A microplate fluorimetric assay for the study of enzyme diversity in soils. Soil Biology and Biochemistry. 33, pp. 1633-1640.

Mbagwu., J.SC., and Piccolo., A. (1997). Effect of humic substance from oxidized coal on soil chemical properties and maize yield. Humus Substance and Soil. 11, pp. 921- 925.

McBride, M.B. (1994). Environmental chemistry of soils. The Amazon book review. Technology and engineering. Oxford University Press. pp.1- 406.

Meliani, A., Ahmed, B., and Mederbel, K. (2012). Microbial diversity and abbandance in soil: Related to plant and soil type. American Journal of Plant Nutrition and Fertilization Technology. 2 (1), pp. 10- 18.

Mensah, A.K., and Frimpong, K.A. (2018). Biochar and/or Compost applications improve soil properties, growth, and yield of maize grown in acidic soils in Ghana. International Journal of Agronomy.7, pp. 1- 8.

Miller, J.O. (2016). Soil pH and nutrient availability. Digital Repository at the University of Maryland (DRUM). 301, pp. 314- 1328.

Mohawesh, O., Coolong, T., Aliedeh, M.A., and Alqaraleh, S. (2018). Greenhouse evaluation of biochar to enhance soil properties and plant growth performance under arid environment. Bulgarian Journal of Agricultural Science. 24(6), pp. 1012-1019.

Mondini, C., Fornasier, F., and Sinicco, T. (2004). Enzymatic activity as a parameter for the characterization of the composting process. Soil Biology and Biochemistry. 36 (10), pp. 1587- 1594.

Musumuvhi, T., Odhiambo, J.J.O., and Mzezewa, J. (2018). Biochar and poultry litter effects on selected soil physical and chemical properties of maize (*Zea* mays) in a dry environment. South Africa, MSc.Thesis. University of Venda. pp.1-84.

Najmadeen, H.H., Mohammad, A.O., and Mohamed-Amin, H.H. (2010). Effects of soil texture on chemical compositions, microbial population and carbon mineralization in soil. Egypt Journal of Experiment and Biology (Bot). 59, pp. 59-64.

Ndor, E., Jayeoba, O.J., and Asadu, C.L.A. (2015). Effect of biochar soil amendment on soil properties and yield of sesame verities in Lafia, Nigeria. American Journal of Experimental Agriculture. 9(4), pp. 1- 8.

Nelson, D.W, and Sommers, L.E. (1975). Organic carbon estimation in soils: Analytical protocols and their implication. Rubber Science Journal. 26, pp. 45- 54.

Niemi, R.M., Heiskanen, I., and Saarnio, S. (2015). Weak effects of biochar amendment on soil enzyme activities in bare or *Phleum pratense* soil. Boreal Environment and Research. 20, pp. 324- 334.

Odhiambo, J.J.O., Ogola, J.B.O., and Madzivhandila, T. (2010). Effect of green manure legume-maize rotation on maize grain yield and weed infestation levels. Africa Journal of Agricultural Research. 5(8), pp. 618-625.

Odugbenro, G.O., Liu, Z., and Sun, Y. (2019). Dynamics of C and N in a clay loam soil amended with biochar and corn straw. Indian Journal of Agricultural Research. 10, pp. 18805

Okalebo, J.R., Gathua, K.W., and Woomer, P.L. (2002). Laboratory methods of soil and plant Analysis: A Working Manual; TSBF-KARI, Nairobi, Kenya.

Olasekan, A.A., Agbede, T.M., Ejue, W.S., and Aboyeji, C. (2019). Biochar, poultry litter and NPK fertilizer: sole and combine application effects on soil properties and ginger (*Zingiber officinale* Roscoe) performance in a tropical Alfisol. Open Agriculture. 5, pp. 30- 39.

Olsen, S.R., Cole, C.V., Watanabe, F.S., and Dean, L.A. (1954). Estimation of available Phosphorus in soil by extraction with sodium bicarbonate. United States Department of Agriculture. 939, pp. 1-18.

O'Neill, B., Grossman, J., Tsai, M.T., Gomes, J.E., Lehmann, J., Peterson, J., Neves, E., and Thies, J.E., (2009). Bacterial community composition in Brazilian anthrosols and adjacent soils characterized using culturing and molecular identification. Microbiology and Ecology. 58, pp. 23-35.

Paliwal, R.L. (2000a). Introduction to maize and its importance. In FAO, 2000.Tropical maize improvement and production. Rome, Italy.

Paliwal, R.L. (2000b). Tropical maize: Improvement and production. FAO Plant production and protection series. Rome, Italy. 28.

Paz-Ferreiro, J., Gasc´o, G., Guti´errez, B., and M´endez, A. (2012). Soil biochemical activities and the geometric mean of enzyme activities after application of sewage sludge and sewage sludge biochar to soil. Biology, Fertilizer and Soils. 48, pp. 511-517.

Peake, L., Freddo, A., and Reid, B.J. (2014). Sustaining Soils and Mitigating Climate Change Using Biochar. Journal of Sustainable Science and Technology. pp. 7, pp.109-126.

Peech, M. (1965). Lime requirements. In Black, C.A. (ed). Methods of soil analysis. American Society Journal of Agronomy. Madison.9, pp. 927-932.

Phares, A.C., Osei, B., and Tagoe, S. (2017). Effects of biochar and poultry litter on the composition of Phosphorus Solubilizing Fungi and soil available Phosphorus concentration in Oxisol. Journal of Agriculture and Ecology Research International. 12, pp. 1-15.

Pholo, M. (2009). Morphologycal, physiologycal and yield response of maize (*Zea mays L*.) to seed treatment. Department of Soil, Crop and Climate Sciences. University of Free State. Bloemfontein. pp.1-182.

Pietikäinen, J., Kiikkilä, O., and Fritze, H. (2000). Charcoal as a habitat for microbes and its effect on microbial community of the underlying humus. Oikos. 89, pp. 231–242.

Piotrowska, A., and Koper, J. (2010). Soil β-glucosidase activity under winter wheat cultivated in crop rotation systems depleting and enriching the soil in organic matter. Journal of Elements. 15(3), pp. 593–600.

Rahimzadeh, M.R., Kazemi, S., and Moghadamnia, A.A. (2015). Cadmium toxicity and treatment: An update. Caspian Journal of Internal medicine.8 (3), pp. 135- 145.

Raiesi, F. (2006), Carbon and N mineralization as affected by soil cultivation and crop residue in a calcareous wetland ecosystem in central Iran. Agricultural Ecosystem and Environment. 112, pp. 13-20.

Ramaru, J., Mamabolo, Z., and Lekgoro, J. (2000). Improving soil fertility management in South Africa: Learning through participatory extension approaches. Management of African soils. 19, pp. 15-20.

Rao, S., Meunier, F., Ehosioke, S., Lesparre, N., Kemna, A., Nguyen, F., Garre, S., and Javaux, M. (2019). Impact of maize roots on soil-root electrical conductivity: A simulation study. Vadose Zone Journal. 18 (1), pp. 1-20.

Riedel, T., Hennessy, P., Iden, S.C., and Koschinsky, A. (2015). Leaching of soil-derived major and trace elements in an arable topsoil after the addition of biochar. European Journal of Soil Science. 66(4), pp. 823- 834.

Rillig, M.C., and Mummey, D.L. (2006). Mycorrhizas and soil structure. New Phytol. 1717, pp. 41-53.

Romheld, V., and Kirk, E.A. (2010). Research of potassium in agriculture: Needs and prospects. Plant and Soil. 335, pp. 155- 180.

Rondon, M.A., Lehmann, J., Ramirez, J., and We2rtado, M. (2007). Biological nitrogen fixation by common beans (*Phaseolus vulgaris L*.) increases with bio-char additions. Biology, Fertilizer and Soils. 43, pp. 699-708.

Saarnio, K., Teinila, K., Saarikoski, S., Carbone, S., Gilardoni, S.,Timonen, H.,Aurela, M., and Hillamo, R. (2013). Online determination of Levoglucosan in ambiet aerosols with particle-into-liquid-sampler-high-performance anion-exchange chromatography-mass spectrometry. Atmosphere Measures and Technology.6, pp. 2839- 2849.

SAS Institute Inc. (2013). Base SAS® 9.4 Procedures Guide: Statistical Procedures, Second Edition. Cary, NC: SAS Institute Inc.

Schulz, H, Dunst, G, and Glaser, B. (2014). No Effect level of co-composted biochar on plant growth and soil properties in a greenhouse experiment. Agronomy. 4, pp. 34-51.

Shah, T., Khan, S., and Shah, Z. (2017). Soil respiration, pH and EC as influenced by biochar. Soil and Environment. 36(01), pp. 77- 83.

Sheng, M., Tang, M., Chen, H., and Yang, B. (2008). Influence of arbascular mycorrhizae on photosynthesis and water status of maize under salt stress. Mycorrhiza. 18 (6-7), pp. 287- 289.

Sika, M.P. (2012). Effect of biochar on chemistry, nutrient uptake and fertilizer mobility in sandy soil. South Africa, MSC. Stellenbosch University. pp. 71.

Silver, W.I., Ostertag, R., and Lugo, A.E. (2000a). The potential for carbon sequestration through reforestation of abandoned tropical agricultural and pasture land. Ecology and Restoration. 8, pp. 394-407.

Silver, W.L, Neff, J.C., Mcgroddy, M., Veldkamp, E., Keller., M., and Junior, R.C.O. (2000b). Effect of soil texture on below ground Carbon and nutrient storage in lowland Amazon Forest ecosystem. Ecosystems. 3(2), pp.193- 209.

Simonsson, M., Hillier, S., and Oborn, I. (2009). Changes in clay minerals and potassium fixation capacity as a result of release and fixation of potassium in long term experiments. Geoderma. 151(3-4), pp. 109- 120.

Sivakumaran, S., Dunlop, S., Cailleb, S., Deurera, M., Simpsonc, R., McIvora, I., Jeyakumard, P., Greena, S., Millsa, T., Vogeler I., and Clothiera B. (2010). Charcoal (biochar) as a carbon sequestration approach and its effect on soil's functions. Proceedings of the 19th World Congress of Soil Science, Soil Solutions to Change the World. pp. 22-25.

Smith, J.L., Collins, H.P., and Bailey, V.L. (2010). The effect of young biochar on soil respiration. Soil Biology and Biochemistry. 42, pp. 2345-2347.

Sohi, S.P., Krull E., Capel L.E., and Bol, R. (2010). A review of biochar and its use and function in soil. Agronomy. 105, pp. 47-82.

Soil classification working group (1991): A taxonomic system for South Africa. Pretoria, South Africa: Department of Agricultural development. CN Macvicar.

Soinne, H., Hovi, J., Tammeorg, P., and Turtola, E. (2014). Effect of biochar on phosphorus sorption and clay soil aggregate stability. Geoderma. 219 – 220, pp. 162- 167.

Spokas, K.A. (2010). Review of stability of biochar in soils: predictability of O:C molar ratios. Carbon Management. 1, pp. 289- 303.

Statistix Institute. (2013). Analytical software version 10.0 package for windows.

Steiner, C., Garcia, M., and Zech, W.J. (2007). Terra preta nova: A tribute to win Sombroek. Plant and Soil. Springer. 291, pp. 275- 290.

Swaine, M., Obrike, R., Clark, J.M., and Shaw, L.J. (2013). Biochar alteration of the sorption of substrates and products in soil enzyme assays. Applied Environment and Soil Science. 2013, pp.1-5.

Syuhada, A.B., ShamsWe2ddin, J., Ishak, C.F., and Abdu, A. (2016). Biochar as soil amendment: Impact on chemical properties and corn nutrient uptake in Podzol. Canadian Journal of Soil Science.4, pp. 96 - 100.

Tabatabai, M. A. and Bremner, J. M. (1971). Michaelis constants of soil enzymes, Soil Biology and Biochemistry. 3, pp. 317–323.

Tabatabai, M.A. (1994). Soil enzymes. methods of soil analysis. Soil Science Society of America Journal. 5, pp. 775-883.

Trumbore, S. (2000). Age of soil organic matter and soil respiration: radiocarbon constraints on belowground C dynamics. Ecology and Application. 10, pp. 399–411.

Uras, U., Carrier, M., Hardie, A.G., and Knoetze, J.H. (2012). Physico-chemical characterization of biochar from vacuum pyrolysis of South African agricultural wastes for application as soil amendments. Analytical Application and Pyrolysis. 3, pp. 2794 - 2800.

Van Averbeke., W, and Khosa., T.B. (2007). The contribution of smallholder Agriculture to nutrition of rural households in semi- arid environment in South Africa. Water South Africa. 33, pp. 412- 418.

Van Wambeke, A. (1992). Soils of the Tropics: properties and appraisal. McGraw-Hill, New York.pp.353.

Vance, E. D., Brookes, P. C., and Jenkinson, D. S. (1987). An extraction method for measuring microbial biomass C. Soil Biology and Biochemistry. 19, pp. 703–707.

Vepsäläinen, M., Erkomaa, K., Kukkonen, S., Vestberg M., Wallenius, K., and Niemi, R.M. (2004). The impact of crop plant cultivation and peat amendment on soil microbiological activity and community structure. Plant Soil. 264, pp. 273-286.

Visser, S., and Parkinson, D. (1989). Microbial respiration and biomass lodgepole pine stand acidified with elemental sulpWe2r. Canadian Journal of Forest Research. 19, pp. 955-961.

Vithanage, M., Herath, I., Almaroai, Y.A., Rajapaksha, A.U., Huang, L., Sung, J., Lee, S.S., and OK, Y.S. (2017). Effect of carbon nanotubeand biochar on bioavailability of Pb, Cu, and Sb in multi-metal contaminated soil. Environment Geochemistry and Health Journal. 39, pp.1409- 1420.

Walkley, A., and Black, I.A. (1934). An examination of the different method for determining soil organic matter, and a proposed modification of the chromic acid titration method. Journal of Soil Science. 37, pp. 29-38.

Wallenius, K., Rita, H., Mikkonen, A., Lappi, K., Lindstrom, K., Hartikainen, H., Rateland, A., and Niemi, R.M. (2011). Effect of land use on the level, variation and spatial structure of soil enzyme activities and bacterial communities. Soil Biology and Biochemistry. 43, pp. 1464- 1473.

Wang, I., Grulkoski, I., and Jour, A. (2018). Biochar application in soils. Scientific reports. 8(1), pp. 7281.

Wardle, D.A., Nilsson, M.C., and Zackrisson, O. (2008). Fire-derived charcoal causes loss of forest humas. Science journal. 320, pp. 629- 630.

Warnock, D. D., Lehmann, J., Kuyper, T. W., and Rillig, M. C. (2007). Mycorrhizal responses to biochar in soil concepts and mechanisms. Plant Soil. 300, pp. 9-20.

White, D.C. (1997). Determination of the sedimentary microbial biomass. Extractable lipids phosphates. Oecologia. 40, pp.51- 62.

Whitman, T., and Lehmann, J. (2009a). Pyrogenic organic matter effects on soil bacterial community composition. Soil Biology and Biochemistry. 138, pp. 13- 16.

Whitman, T., and Lehmann, J. (2009b). Biochar – one way forward soil carbon in offset mechanisms in Africa. Environmental Science and Policy. 12, pp. 1024-1027.

Woolf, D., and Lehmann, J. (2012). Modelling the long term response to positive and negative priming of soil organic carbon by black carbon. Biogeochemistry. 111, pp. 83-95.

Wu, M., Hongling, Q., Chen, Z., and Wu, J. (2011). Effect of long-term fertilization on bacterial composition in rice paddy soil. Biology and Fertility of Soils. 47(4), pp. 397-407.

Wu, W., Yang, M., Feng, Q., McGrouther, K., Wang, H., Lu, H., and Chen, Y. (2012). Chemical characterization of rice straw- derived biochar for soil amendment. Biomass and Bioenergy. 47, pp. 268-276.

Xiao, Q., Zhu, L., Shen, Y., Li, S. (2016): Sensitivity of soil water retention and availability to biochar addition in rainfed semi-arid farmland during a three-year field experiment. – Field Crops Research. 196, pp. 03-10.

Xu, X., Thornton, P.E., and Post, W.M. (2013). A global analysis of soil microbial biomass carbon, nitrogen and phosphorus in terrestrial ecosystems. Global Ecology and Biogeography. 22 (6), pp. 737- 749.

Yang, G., Wagg, C., Veresoglou, S.D., Hempel, S., and Rillig, M.C. (2018). How soil biota drive ecosystem stability. Trends in Plant Science. 23, pp. 1057- 1067.

Yamato, M., Okimori, Y., Wibowo, I. F., Anshori, S., Ogawa, M. (2006): Effects of the application of charred bark of Acacia mangium on the yield of maize, cowpea and

peanut, and soil chemical properties in South Sumatra, Indonesia. – Soil Science and Plant Nutrition. 52(4), pp. 03-10.

Ying, X., Xin-qing, L., Zhi-hong, Z., Hong-guang, C., Bin, F., and Like, Z. (2011). Adsorption and kinetics of ammonium from aqueous medium onto biochar. Earth Environment. 39, pp. 511- 516.

Yoo, G., and Kang, H. (2011). Effects of biochar addition on greenhouse gas emissions and microbial responses in a short-term laboratory experiment. Journal of Environmental Quality. 10, pp. 2134 - 2140.

Yuan, B., and Yue, D. (2012). Soil microbial and enzymatic activities across a chronosequence of Chinese pine plantation. DeveloPLent on the Loess Plateau of China. Pedosphere, 22, pp. 1-12.

Yuan, J., and Xu, R. (2010). The amelioration effects of low temperature biochar generated from nine crop residues on an acidic Ultisol. Soil use and Management. 27 (1), pp. 110- 115.

Zang, U., Goisser, M., Häberle, K., Matyssek, R., Matzner, E., Borken, W. (2014): Effects of drought stress on photosynthesis, rhizosphere respiration, and fine-root characteristics of beech saplings: A rhizotron field study. Journal of Plant Nutrition and Soil Science. 177(2), pp. 03-10.

Zheng, W., Papiernik, S.K., Guo, M., and Yates, S.R. (2010). Construction of reactive surface barrier to reduce 1, 3 Dichloropropene emissions. Environmental Toxicology and Chemistry. 24, pp. 1867-1874.

Zhu, X., Chen, B., Zhu, L., and Xing, B. (2017). Effect of mechanism of Biochar-microbe interactions in soil improvement and pollution remediation: A review. Environmental Pollution. 227, pp. 98- 115.

Zimmerman, A., Gao, B., and My, A. (2011). Positive and negative carbon mineralization priming effects among a variety of biochar amended soils. Soil Biology and Biochemistry. 43, pp. 1169- 1179.

APPENDIX

Appendix 1. Labserve soil test interpretation, pH, K and available P

pH (Water) (**cmol kg^{-1}**)	**Interpretation**
< 5.4	Strongly acidic
5.5- 6.4	Moderately acidic
6.5- 6.9	Slightly acidic
7	Neutral
7.1- 7.5	Slightly alkaline
7.6- 8.3	Moderately alkaline
> 8.4	Strongly alkaline

Total K (mg kg^{-1})	**Description**
<20	Very low
20- 40	Low
40- 120	Medium
120- 240	High
>240	Very high

Available P (mg kg $^{-1}$)	**Description**
<15	Extremely low
15- 20	Very low
20- 80	Optimum range
80- 140	High
>140	Very high

Appendix 2. Labserve soil test interpretation, Total N, Ca, Mg, Zn, Cu, Fe, Mn, CEC and Organic matter

Parameter	Unit	Optimum range
Total N	Mg kg $^{-1}$	20- 40
Ca	Mg kg $^{-1}$	> 200
Mg	Mg kg $^{-1}$	> 60
Zn	Mg kg $^{-1}$	2- 10
Cu	Mg kg $^{-1}$	1- 10
Fe	Mg kg $^{-1}$	10- 250
Mn	Mg kg $^{-1}$	10- 250
CEC	Cmol kg $^{-1}$	>10
Organic Matter	%	> 0.75