UNIVERSITY FOR DEVELOPMENT STUDIES

THE EFFECT OF BIOCHAR AND INOCULANT WITH COMPOST OR PHOSPHORUS ON SOIL FERTILITY AND SOYBEAN YIELD IN GUINEA SAVANNA ZONE OF GHANA

OSEI MICHAEL BANAHENE

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THESIS SUBMITTED TO THE DEPARTMENT OF AGRONOMY, FACULTY OF AGRICULTURE, UNIVERSITY FOR DEVELOPMENT STUDIES IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN CROP SCIENCE

AUGUST, 2020

DECLARATION

I hereby declare that this thesis is the result of my original work. No part of it has been presented for another degree in this university or elsewhere. All other references made from other research activities have accordingly been cited.

Candidate's Signature……...…… Date…….……

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Supervisors'

I hereby declare that the preparation and presentation of this thesis was supervised in accordance with the guidelines on supervision of dissertation laid down by the University for Development Studies.

Dr. Raphael Adu – Gyamfi ……. ……..

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(Co-Supervisor) Signature Date

ABSTRACT

The yield of soybean in Ghana stands at 1.45 t ha^{-1} out of an achievable harvest of 3 t ha^{-1} and among the contributory reasons for the low yields are inherent low soil fertility and lack of adequate indigenous bacteria in the soils used for soybean production. Amendment of the soil is believed to hold the key to improve yields of soybean. The objective of the study was to determine the effect of *Bradyrhizobium japonicum* inoculant (USDA 110 and USDA 136 stains) in combination with soil amendment on soybean yield and improvement of soil fertility. A plant house experiment was conducted in a 3 x 5 factorial combination and laid out in a randomized complete block design with three replications. The factors were *Bradyrhizobium japonicum* (3 levels) and soil amendment (5 levels). The treatments were biochar at a rate of 20 t ha^{-1}, biochar (10 t ha^{-1}) + compost (5 t ha^{-1}), biochar (10 t ha^{-1}) + rock phosphate (60 kg ha^{-1} P_20_5), biochar (10 t ha^{-1}) + triple superphosphate (60 kg ha^{-1} P_20_5) and the farmers practice, no amendment (control). These treatments were added to 5 kg of soil in a bucket and planted with Sung-pugun soybean variety inoculated with the two rhizobium strains. Non-inoculated control was kept. It was observed that the addition of compost to the biochar led to a higher plant height and grain yield than when TSP or Rock phosphate was added. The addition of *Bradyrhizobium japonicum* strain USDA 110 produced more nodules than that of *Bradyrhizobium japonicum* strain USDA 136, and the inoculants promoted more nodule development than the non-inoculant treatment. Soil amendment improved P, K and Na concentrations of the plant more than those on non-amended soil. Biochar + Compost recorded the highest Cation Exchange Capacity. The various soil amendment highly influenced the carbon concentration of the soil. The study concluded that the combination of biochar with compost improves the soil's biological, physical and chemical properties, which led to higher grain yield and has to be encouraged.

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DEDICATION

This dissertation is dedicated to my cherished parents, Opanin John Osei and Madam Rachael Adusei, my grandfather Opanin Abraham Adusei and my lovely wife Mrs Hannah Osei.

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Soybean (*Glycine max* L. Merrill) is a classified leguminous crop across the globe. It is innate to tropical and warm temperate areas of Asia, where it has been in cultivation for about 5000 years. Soybean is an ecologically important leguminous crop worldwide and common legume grown in Ghana (Plaher, 2006). According to Wilcox (2004) soybean became domesticated crop during the Zhou Dynasty in the Eastern half of Northern China. Moreover, the Center for Agriculture and Bioscience International (CABI) (2010) also confirmed that soybean originated from China and was the world's main producer and exporter throughout the first half of the 20th century. Plaher (2006) reported that soybean was made known to the people of Ghana formally known as Gold Coast in 1910 and was utilized by the people in the northern part, which lie within the Guinea and Sudan savanna agro-ecological zones and has since remained the leading producer in Ghana.

The crop grows from sea level up to 2000 m above sea level, from the equator to latitude 55° N and 55° S and under a warm condition of temperature, but the ideal temperature for growth and development is 30 °C. Also, for a better germination of seedlings, a seedbed temperature of 25 – 30 °C is optimal (Hartman, 2016). Again, depending on the variety and growing conditions, it can mature in 65 to 150 days after planting and requires about 500 mm of water during the rainy season. As reported by Dugje *et al*. (2009) soybean is more protein-rich (42-45 %) than any of the leguminous food grains in Africa and a good source of edible oil (20 – 25 %). Comparatively, soybean is more resistant to pests and diseases than other grain legumes such as groundnut and has a better storage quality (Dogbe *et al*., 2013).

The term biochar has ascended to depict the product from thermal treatment of organic materials, for example, municipal waste, wood shavings and crop residues in an oxygen-limited setting called pyrolysis (Bridgwater, 2003; Yavari *et al*., 2015). The stability of biochar in the environment is very high (Nguyen *et al*., 2008; Gollakota *et al*., 2016) with a mean residence time of 100 years (Swift, 2001; Domene *et al*., 2015; Lopez *et al*., 2018). Hence, various agronomic benefits have been stated as a result of applying biochar to cropping soil, especially in highly acidic poor soils (Novak, 2009). Adding biochar to the soil improves its qualities extending from biological, physical and chemical properties (Woods *et al*., 2006). Among its properties is the soil pH which is increased by biochar (Peng *et al*., 2011), thus decreasing lime requirement on acid soils and increase in cations exchange capacity (CEC) of soils (Chan and Tyler, 2007; Laird *et al*., 2010). Steiner *et al*. (2008) reported that nitrogen use efficiency (NUE) is increased as leaching of nitrogen is reduced due to the addition of biochar.

The addition of biochar to soil has been reported to improve microbial biomass because of the presence of labile C and promotes efficient enzymatic activities (Bruun *et al*., 2011; Luo *et al*., 2013). Again, the porous nature of biochar may provide refuge for microorganism away from predators (Pietikäinen *et al*., 2000) and store mineral nutrients and carbon substrates (Saito and Muramoto, 2002; Warnock *et al*., 2007).

Phosphorus is considered among the most important nutrients needed by plants and is involved in numerous energy transformations and chemical reactions, including biological nitrogen fixation. However, it is a finite, non-substitutional, non-renewable and geographically restricted resource. According to Grant *et al*. (2001), crop yield can increase from 50 – 100% due to phosphorus fertilizer application. Therefore, securing the long-term availability and accessibility of phosphorus is critical to worldwide food security. The signs of geopolitical constraints regarding phosphate rocks reserves are already apparent and likely to be more intense. However, Lombi *et al*. (2006) concluded

that a large percentage of phosphorus from chemical phosphate fertilizer is not available to plants because at least 70 – 90 % of phosphorus that enters the soil is fixed by iron and calcium in soils.

1.2 Problem statement

Due to declining soil fertility, soybean is considered among the lowest yielding crops in Africa. In Ghana, the production of soybean stands at 1.45 t ha^{-1} out of an achievable yield of 3 t ha^{-1} (Ministry of Food and Agriculture, 2015). According to Lawson *et al.* (2008), the main contributing factor to low crop yields in Ghana is low soil fertility, mainly due to the low content of organic matter, nitrogen and phosphorus. In northern Ghana, phosphorus is considered one of the most deficient soil nutrient elements, limiting soil fertility for legumes such as soybean production (Alenyorege *et al*., 2015). Researchers have made several attempts to improve the growth and yield of soybean through mineral fertilizers, compost and, in some cases, rhizobia inoculant (Asante, 1999). Rhizobia are reported to influence crop growth, yield, and nutrient uptake by different mechanisms. They fix nitrogen, help in promoting free-living nitrogen-fixing bacteria and increase the supply of other nutrients, such as phosphorus and iron (Saharan *et al*., 2011). The activities of this bacteria are affected by factors such as soil type, nutrient abundance, pH and soil moisture content.

1.3 Justification

The main contributing factor of low crop yield in northern Ghana is a result of low soil fertility due to the low content of organic matter, nitrogen and phosphorus (Lawson *et al*., 2008). The use of biochar and/ compost can retain water and nutrient, adjust soil pH, and provide a conducive habitat for soil microorganisms (Mitchell *et al*., 2015). These attributes of biochar and compost can enhance bacterial inoculants' activities, therefore

making it a choice for high yielding soybean production and improvement of savanna soils in Northern Ghana. Głodowska *et al*. (2017) demonstrated a prolonged bacterial viability rate when biochar was used as an inoculant carrier and attributed it to the chemical composition and porosity of the biochar as the factors that contributed to bacterial viability. This subsequently led to increased nodulation of soybean. The application of biochar in a study by Wang *et al.* (2016) improved soybean plant height, stem diameter, and leaf area during the flowering and podding phase and this is positively correlated with increasing biochar application rates. Quilliam *et al*. (2016) however, reported reduced nodulation in clover following the application of biochar. A recent report by Haering *et al*. (2017) and Akoto-Danso *et al*. (2018) showed the potentials of biochar and compost to improve the degraded soils of Northern Ghana. Also, the use of inoculants such as *Bradyrhizobium japonicum* strains (USDA 110 and USDA 136) are reported to influence crop growth, yield and nutrient uptake by different mechanisms (Saharan *et al*., 2011). Hence, using appropriate management strategies such as combining biochar and/compost with phosphorus and *Bradyrhizobium japonicum* requires adequate knowledge of the application to improve soil fertility and nutrient uptake in poor acidic soils.

1.4 Main objective

The main objective of the study was to determine the effect of *Bradyrihzobium japonicum* inoculant strains in combination with soil amendment on soybean yield and improvement of soil fertility.

1.5 Specific objective

- To determine the performance of soybean crop treated with Brady 110 and Brady 136 inoculants grown on biochar amended soil.

- To assess the effect of addition of compost or phosphorus to the biochar on crop performance
- To measure the fertility of the amended soil and nutrient content of the soybean plant grown on the amended soil.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Origin and distribution of soybeans

It is generally accepted that present soybean was natured some 6000–9000 years back from wild type in East Asia (Carter *et al*., 2004; Kim *et al*., 2012). It is believed to be indigenous to Asia, predominantly Japan, and China, from where it got to other parts in the world in the eighteenth century. Historically, the ancient Chinese over 5000 years ago utilized soybean not only as food but a substance or segment for medication (Norman *et al*. 1995). Center for Agriculture and Bioscience International (CABI) (2010) also confirmed that soybean cultivation began from China and was the world's main producer and exporter during many years in the twentieth century. In sub-Saharan Africa, soybean cultivation is mainly distributed along the savannah belt under a rain-fed system (Khojely *et al*., 2018). The first to have brought soybean into Ghana was the Portuguese missionaries around 1910 (Plahar, 2006) and was utilized by growers in the northern part of the country. However, the enthusiasm of the crop for food began around 1973 in Ghana (Shurtleff and Aoyagi, 2007). This early introduction did not survive as a result of the low yield (Mercer-Quarshie and Nsowah, 1975). Notwithstanding, genuine endeavours to develop the crop in Ghana began in the mid-1970s through the cooperation of the International Institute of Tropical Agriculture (IITA) and Ghana's Ministry of Food and Agriculture (MoFA) (Tweneboah, 2000).

The cultivation of soybean has gained attention worldwide of which the production in Africa represents 0.4 % to 0.6 % of the world’s production (FAO, 2008). According to FAO (2008), Nigeria is the leading producer in Africa, producing 437000 MT, South Africa (221000 MT), Uganda (166000 MT) and Zimbabwe (83,000 MT). However, a publication by world agricultural production.com (2020) reported that South Africa (1,425,000 MT) is the leading soybean producer in 2020, Nigeria (700,000 MT) being

second, followed by Zambia (285,000 MT), Zimbabwe (50,000 MT) and Uganda (30,000 MT).

2.2 Botany of soybean

Developed soybean, *Glycine max* (L.) Merr. is a diploidized tetraploid (2n=40), herb belonging to kingdom *Plantae*, phylum *Magnoliophyta*, class *Magnoliopsida*, order *Fabales*, family *Leguminosae*, the subfamily *Papilionoideae*, the tribe *Phaseoleae*, and the genus *Glycine* (Norman *et al*., 1995). Soybean comprises of two subgenera, *Glycine* which consist of seven long-lasting wild varieties limited to Southeastern Asia; and *Soja*, (Moench) involving the domesticated and economically important soybean, *Glycine max*, and its wild progenitor, *Soja soja* (Shurtleff and Aoyagi 2007) which grows wild in Korea, China, Russia and Japan. Linnaeus initially proposed the name *Glycine* in his first publication of Genera Plantarum; with the developed variety first showing up in the version, 'Species Plantarum', under the name *Phaseolus max* L. The combination, Glycine max (L.) Merr. was suggested by Merrill in 1917 and has been an accepted name for soybean plant since that time (Singh *et al*., 2007).

2.3 Morphological description

Soybean is a furry herbaceous plant which is an annual crop and depending upon the genotype, the plant can grow between a height of 30 to 183 cm. Depending on its growth habit, soybean has been put into two groups, one been determinate and the other one been indeterminate, which have six affirmed varieties developed in Ghana (CSIR and MOFA 2005; Lambon, 2016). The determinate genotypes are shorter in height and has fewer leaves but produce relatively more pods. In contrast, the indeterminate genotypes directly produce more pods on the stem and grow taller with more leaves. Moreover, the blooms are self- fertile, inconspicuous and small. They are either pink, white or purple borne in

the leave axils of the plant. Soybean development and advancement have been categorized into two fundamental stages: the vegetative and regenerative stages (Gary and Dale, 1997; Osman, 2011). The onset of seedlings with an unfolding of unifoliate leaves begins the vegetative stage which therefore develop trifoliate leaves, formation of nodes the main stem, the development of twigs and nodules. In contrast, the regenerative stage starts with the development of bud blossom, via full sprout blossoming, pod development to full growth.

The stalk, leaves, and pods are sheltered with fine brown or grey hairs. The leaves are trifoliate, having three to four leaflets per leaf. The fruitlet is a hairy pod that develops in bunches of three to five, every one of which is five to eight centimeters in length and it usually contains not more than four seeds per pod (Plahar, 2006). Soybean seeds vary in sizes, and seed coat. The seed coat colour ranges from cream, dark, brown and yellow to mottle. As stated by Gary and Dale, 1997 the developed soybean has a hard hull, that shield the cotyledons and hypocotyls from been harmed.

2.4 Soybean production

Soybean production is expanding quickly everywhere throughout the world because of the various advantages. As a native of Asia and foremost producer in the eighteenth century, soybean production is presently dominated by America. As per the data presented by Osman (2011), the total cultivated land used for soybean production in the world was 95.2 million hectares for every annum, and 212.6 million metric tons of soybean was produced across the globe annually. Oyatokun and Oluwasemire (2014) reported an increase of about 8 million tons of soybean grains amounting to 220 million metric tons of soybean grains produced per annum compared to the total production in 2011. The seven leading producers as at that time were the USA - 34 %, Brazil - 22 %, Argentina - 19 %, China - 9 %, India - 6 %, Paraguay - 4 %, Canada - 2 % and others - 4

% of the total production. Mawiya (2016) reported that 94 million hectares of land worldwide was cultivated to soybean and the U.S.A. accounted for more than 30 million, Brazil for nearly 22 million, Argentina recorded 15 million, China had 9.2 million, India had 8.2 million, Paraguay had 2.2 million and Canada cultivated on 1 million hectares, Ahlijah, (2017) reported that the world's production of soybean grain was about 260.6 million tons, of which United State of America accounted for 98.4 million tons, Brazil accounted for 68 million tons, Argentina accounted for 54.5 million tons, China accounted for 14.5 million tons, India accounted for 9.1 million tons, Paraguay also accounted for 6.7 million tons and the others accounted for 13 million tons. In connection to Sub-Saharan Africa, Masuda and Goldsmith, (2008) and IITA, (2009) reported that a field of 1.16 million hectares was used for the cultivation of soybean with normal harvest of 1.26 million tons of soybean grain in 2008, and Nigeria having the biggest territory of generation among the nations in Africa had 601 000 hectares, followed by South Africa with 150 000 hectares, Uganda also with 144 000 hectares, Malawi had 68 000 hectares and Zimbabwe with 61 000 hectares. However, things had not been the same since that time; and currently, South Africa is the leading producer of soybean in Africa, followed by Nigeria, Zambia, Zimbabwe and Uganda (Cornelius and Goldsmith, 2019).

2.5 Soybean production in Ghana

Ghana is very gifted with natural resources and agriculture accounts for one-fourth of Gross Domestic Product (GDP) and utilizes about 56 % of the active work force. Among the grain legumes, soybean has kept on being the most appreciated grain legume globally because of its significant source of protein and oil and its ability to fix atmospheric nitrogen to be able to grow on soils with low nitrogen concentrations (CIA, 2013; Wood, 2013). When soybean was brought to Ghana in 1910, it was utilized by livestock rearers in the northern part of Ghana. In the late 1960s and mid-1970s, research on soybean was

heightened by CSIR – Crop Research Institute and University of Ghana Agricultural Research Station. However, the interest declined because of loss of seed viability in storage, poor utilization of soybean at the family level, inadequate modern base for soybean processing and no market for the produce (Plahar, 2006). Additional concerns were brought up in this 21st century by researchers as they believed that they lack indigenous bacteria in Ghanaian soils. As indicated by MOFA (2006) and Dugje *et al*. (2009), soybean performs well on moderately well – drained loamy soil which has a pH ranging from 4.5 - 8.5. In the late 1980s to the 1990s, a public/private association methodology was accepted to campaign and promote soybean cultivation under the mandate of the Ministry of Agriculture. Once more, an Inter-Sectoral National Committee on soybean production and usage was created to advance and improve soybean. This advisory group involved the Ministry of Agriculture, Council for Scientific and Industrial Research (CSIR), Universities, Food Distribution Corporation, Farmers and Industries. Researchers in Nyankpala Agricultural Research Station of Crop Research Institute researched ways of improving yields and preserving the seeds to ensure viability. The Crop Research Institute worked with the International Soybean Program (INTSOY) of the University of Illinois to research the crop. The research effort was given momentum by the Grain and Legumes Development Board, Ghana/CIDA Grains Development Project of Crop Research Institute and University of Ghana Research Station, Kpong. Data available at that time showed that in the northern part of Ghana; where soybean production is very focused, average cultivated area of soybean per farmer was 3.4 acres with a minimum of 0.5 acres of land and a maximum of 80 acres of land while in the southern part, cultivation was still at the primary stages with the exception of Ejura Farms that cultivated soybean on 300 acres of land. According to the Ministry of Food and Agriculture (2015), through this gradual process, there has been an improvement in the

production of soybean in Ghana and with time, will meet its predicted value which is 3 tons ha^{-1}.

2.6 Food value of soybean

The cultivation of soybean has spread over the world because of its economic benefits and diverse usage. MOFA and CSIR have been supporting the production of soybean for its potential benefits, such as increasing income and its nutritional value to families involved in its value chain (Mbanya, 2011). Moreover, Gqaleni (2015) reported that both domestic animals and humans are being fed with soybean which is very healthful and has therapeutic properties, such as; reduction of different types of cancer, cardiovascular diseases, postmenopausal problems, diabetes and some neurodegenerative disorders. It is also used for soil preservation, green manure, compost, and nitrogen improvement of soils. Furthermore, it has been affirmed by Masuda and Goldsmith (2009) as a good source of feed for domesticated animals and fish kept in ponds. In addition, soybean is likewise utilized in soymilk production, cheese etc. Also, El Agroudy *et al*. (2011) reported that soybean contains 30 percent cholesterol-free oil, 40 percent protein and most of the important vitamins needed by human for healthy development. Dugje *et al*. (2009) reported that among the leguminous crops in Africa, soybean contains more protein than any of them. It has an average of 40 % protein content of which the seeds also comprise about 20 % oil when it is dried, and this oil is 85 % unsaturated and without cholesterol. Soybean can further be processed into flour, tufo, oil, yogurt, soy milk and tempeh (MoFA and CSIR, 2005). In view of its high protein content, it can accordingly be considered an astounding replacement for meat in developing nations, where resource-poor families find it difficult to afford animal protein-rich foods with animal protein which include eggs, meat, milk and fish as a result of its cost and frequent scarcity. Grant *et al*. (2001) also revealed that soybean contains a vital source of minerals, oil, carbohydrates and a lot of high-quality protein. Research has shown that the amount of

protein in soybean weighing one kilogram is proportionate to the quantity of protein in meat weighing three kilograms or two creates of eggs (60 eggs) or 10 liters of milk. Generally, the expense of getting one kilogram of soybean is considerably lower than acquiring a comparable quantity of meat or eggs (Asekabta, 2018). Soybean is highly digestible and also has a highly rich oil, odorless and colorless, which does not combine or coalesce easily.

Moreover, the cake acquired after oil extraction from soybean, as explained earlier is a significant feed for animals such as poultry birds due to its high protein content. According to Abbey *et al.* (2001), the extension in producing soybean has led to a significant development of poultry and other animal farming, which MoFA and CSIR (2005) affirmed. Additionally, the haulms as indicated by Dugje *et al.* (2009) after extraction of the seed, also give great feed to ruminants.

There are anti-nutritional substances that soybean is believed to contain and this diminish the nutritional benefit of the beans and are perilous to people's well-being and, therefore, should be detached before they can be taken in as food. However, this is not an issue since research by Ngeze (1993) and Allen *et al*., 2007 affirmed that these elements could be detached by basically soaking and or 'wet' warming the beans and this will remove a significant amount of the anti-nutritional chemicals making it safe to people. Conversely, soybean is also believed to have numerous medical advantages. Dugje *et al*. (2009) report that regular eating of soybean food can help avoid hormone-related cancers, such as breast and colon tumor. It also relieves menopausal signs because of the estrogen-like outcome of soy flavones. Studies also recommends that frequent eating and digestion of soybean products decreases the degree of cardiac infections by lessening fat, low density lipoprotein cholesterol, and avoiding plaque development in veins which may cause stroke or heart failure (Sirtori *et al*., 2009). Also, MOFA and CSIR (2005) reported that

foods with extraordinary good protein content and other nutritive values are valuable in treating nutrition deficiency disorders in youngsters and diabetics.

2.7 Moisture requirement for soybean

In both animals and plant life, water is an essential component required throughout their developing stages. Gerson *et al*. (2001) affirmed that due to variations in crop canopy with time, the water requirement of crop changes during the developing period. The critical element for water requirement, as per Van der Schrier (2011), is evaporation demand. Evapotranspiration (ET) is the loss of water from the soil by evaporation and transpiration from the plants. Numerous techniques have been recommended in the literature to estimate reference evapotranspiration (ET_o) from which crop water requirement can be determined. However, the determination of water prerequisites of crops using a lysimeter is arduous and very costly. Therefore, efforts have been made to compare the measured crop water requirement in the field with the projected crop water requirement by various techniques using agro-meteorological information (Allen *et al*., 2005, Allen *et al*., 2007).

Soybean water use continues fluctuating throughout the season (Jha *et al*., water). The report demonstrates that soybeans being local to tropical and warm temperate regions, require from 0.596 mm to 0.984 mm of water per acre of land every year from planting through to fully grown crop. This depends upon the planting date, maturity group, area, and weather. An exploration led by Jha *et al*. (2018) affirmed that soybean does not require water just for survival, but in other cases, soybean plant will require more water to produce higher yield and each time there is little water, decreases in yield quantity in some cases may happen.

Table 1: Example of soybean water use by growth stage

Growth stage	Water use (cm/day)
Germination and seedling	0.127 – 0.254
Rapid vegetative growth	0.254 – 0.508
Flowering to pod fill (full canopy)	0.508 – 0.762
Beginning maturity to harvest	0.127 – 0.254

Source: Tacker, P. and Vories, E. Arkansas. Soybean handbook. Chapter 8.

In other to obtain a higher yield, much attention ought to be paid to the crop phases where inadequate water is most critical, as Karam *et al*. (2005) and Payero *et al*. (2005) testified that the soybean plant is sensitive to water stress from the blossoming stage to grain maturity. Mundstock (2005) additionally detailed that the most crucial time for water or moisture by soybean plant is during flowering and early pod development stages and continues to be needed till physiological maturity is reached.

Also, Mourtzinis *et al*. (2018) affirmed that the most significant occasions for soybean plants to have enough water are during pod growth and seed fill. These are the periods where inadequate water can lead to a considerable low yield. The crops evapotranspiration and yield are greatly affected as a result of stress from water deficit. Again, inadequate water at this time can cause the abortion of flowers and pods, leading to fewer seed production and decreased yield. According to Foroud *et al*. (1993) and Ouda *et al*. (2008), as the soybean plant grows from flowering through seed formation, its capacity to withstand stressful conditions reduces and yield losses could increase. Given its prolonged root structure, the soybean plant can stand dry conditions before flowering, but appropriate moisture becomes vital once the buds are formed till the pods have developed seeds (Jha *et al*. 2018). According to Gabruch and Gietz (2014), the amount of water needed for soybeans ranges from 450-700 mm for every season, based on the weather and length of the developing time frame. Moreover, Jha *et al*. (2018) affirmed

that rain of 500 to 900 mm is essential for better yields and better seed quality, based on the growth conditions.

When soybeans approach maturity, there is the need to ensure that there is enough moisture to enable seeds to attain their maximum weight as lacking moisture at this stage can bring about a yield decrease of as much as 254 to 381 kg/acre of land (Mutwedu *et al*., 2020). It is essential to keep moisture sufficient but not excessive as at the later stages, there is still increasing seed size and weight. From blossom to complete seed formation in the pod, the soybean plant will utilize 20 to 30 mm of water every day. As a result, it is critical to supplement any shortage in rainfall with a water system or irrigation (Ewaid *et al*., 2019).

2.8 Fertilizer requirement for soybean

Maximum yield per unit area of soybean is achievable only when farmers meet the plant nutrient requirements. Other production factors such as soil type, fertilizer application rate can affect the fertilization process which fills in as an enhancement to the natural nutrients in the soil (Kuntyastuti, 2015). Indeed, even though one can utilize the best soybean genotypes and cultural practices, the yield will not be maximum capacity except if soil fertility is appropriately achieved.

According to Slaton *et al*. (2013), same as other crops, adequate fertility of the soil combined with a well-organized fertilization program is just one way of achieving high yields of soybean. Concerning fertilization, major reasons for the low output of soybean are inadequate multi-nutrient, for example, nitrogen, phosphorus, sulfur, zinc, iron, boron as farmers will in general supply just nitrogen and phosphorus to main crops, and often at a lower dose than prescribed. Sulphur deficiency is a result of the farmers' preference for diammonium phosphate (DAP) as a source of phosphorus instead of single superphosphate (SSP) (Weeks and Hettiarachchi, 2019). Furthermore, Mcgrath *et al*. (2013) reported that a well-arranged soil fertility program is a management technique that

leads to profitable soybean production. Information on what the supplements or nutrients do, which ones are required, the amount to apply, and when to apply them is a significant piece of a successful management strategy.

It is believed that high yielding soybeans need much nitrogen (N), phosphorus (P), and potassium (K), as well as a little sulfur (S) and some micronutrients. Even though soybean needs extensively less P and S than N or K, all are significant for plant growth and development. According to PaSricha and Tendon (1989) and confirmation by Salvagiotti *et al*. (2008) a soybean crop yielding 2.5 t seed use around 125 kg nitrogen, 23 kg phosphorus, 101 kg potassium, 22 kg Sulphur, 35 kg calcium, 19 kg magnesium, 0.192 kg zinc, 0.866 kg iron, 0.208 kg manganese and 0.074 kg copper from the soil for each hectare. Soybeans require reasonable measures of plant nutrient for significant returns.

2.9 Nitrogen fixation

Nitrogen is debatably the significant nutrient needed by plants, being a fundamental part of every amino acid and nucleic acids. However, the accessibility of nitrogen is restricted in numerous soils. According to Ferguson *et al*. (2010), the world's atmosphere comprises 78.1 % nitrogen gas (N_2), yet plants cannot utilize this type of nitrogen. However, nitrogen is needed for the biosynthesis of the fundamental building blocks of life; its existence is plenty in the earth's atmosphere, but its atmospheric form, dinitrogen (N_2), is relatively inert. Hence, before nitrogen can be used for organic processes, it must be fixed into further biologically available and accessible forms. Through science, it has been demonstrated that there are three basic types of nitrogen fixation and these comprise of atmospheric, biological, and industrial nitrogen fixation (Hoffman *et al*., 2014).

These three processes fixed around 1109 kg of nitrogen in the soil every year to be used by plants (Galloway *et al*., 2008). Among the three, biological nitrogen fixation has now gained much attention from researchers since it is simple and easy to practice. Also, using chemical fertilizers is often inefficient method, as Graham and Vance (2003) revealed

that 30 % - 50 % of added or used nitrogen fertilizers is lost through leaching, leading to serious environmental problems, for example, the eutrophication of waterways. Furthermore, the production of nitrogen fertilizer by the industrial fixation method produces a higher quantity of carbon dioxide, leading to global warming. Hence, there is a need to lessen our dependence on chemical nitrogen fertilizers and improve upon other alternative nitrogen inputs.

2.10 Biological nitrogen fixation

Natural or biological nitrogen fixation is one of the alternatives to the application of nitrogen fertilizer. Prokaryotes are made possible using an enzyme complex called nitrogenase, which results in atmospheric N_2 being reduced into a type of nitrogen that diazotrophic living organisms and plants can utilize (ammonia). According to De Bruijn (2015), the best recognized and broadly studied biological nitrogen fixation is the symbiotic collaboration between nitrogen-fixing "rhizobia" and legume plants. The rhizobia induce the production of particular structures called nodules on the roots or stems of some species of leguminous crops and fix nitrogen directly that can be utilized by the host plant; in return, the plant provides the vital energy source to the energy-intensive nitrogen fixation progression. As a leguminous crop, soybean provides nitrogen through a symbiotic association with nitrogen-fixing organisms (rhizobium) (Sarkodie-Addo *et al*., 2006; Nastasija *et al*., 2008). The microbes present in soybean root nodules fix nitrogen into the soil from the atmosphere, generally supplying most or all nitrogen required by the plant. Soybean cultivated on soils where -nodulated soybean has been grown recently will possibly not require inoculation; however, if there is a lack of rhizobium microorganisms, inoculation is prescribed (Darryl *et al*., 2004; Nastasija *et al*., 2008). The sum of nitrogen that a plant can fix depends upon the variety of the plant, the efficiency of Rhizobium bacteria, the type of soil and the condition of the atmosphere. Soybean, as reported by Mawiya, (2016) and Asekabta, (2018), can fix about 60 to 168

kg ha^{-1} of nitrogen every year when conditions are appropriate. The nitrogen requirements of soybean are met in a complex way, as it can use the nitrogen from the soil, as in the form of nitrate and can also use nitrogen from the atmosphere through the biological fixation of nitrogen.

The number of plants on the field is a major factor that influences the supply of nitrogen soybean is adding to a cropping system since the leguminous plants leave some measure of nitrogen in the soil through their residue. Shanahan *et al*. (2008) and Salvagiotti *et al*. (2009) detailed that soybean plants will efficiently use soil remaining nitrate and nitrogen mineralized from soil organic matter, acquiring 25 % to 75 % of plants nitrogen, with the balance provided from symbiotic fixation. The fixation of nitrogen using determinate soybean was improved from 200 to 280 kg ha^{-1}, when plant population was raised from 48,500 to 194,000 per hectare (Ennin and Clegg, 2001; Osman, 2011). According to Darryl *et al*., (2004) despite the procedure for N fixation being a biological one, it needs the existence of the correct type of soil microorganism that fix nitrogen, and must be regularly attracted or connected to the roots by chemical indications from the roots of the soybean. When the microbes or bacteria contact the root hairs, a rooting compound binds the microscopic organisms to the root hair cell wall. The microscopic organisms (bacteria) discharge a chemical that causes twisting and splitting of the root hair, enabling the microorganisms to attack the inside of the cells and change the plant cell structure to form nodules (Ibáñez *et al*., 2017). The microscopic organisms animate in colonies to about 10,000 CFU/g in a nodule called bacteroids. An enzyme, nitrogenase help the nitrogen fixation and this happens in a situation without oxygen, through an exchange compound, leghemoglobin. Dogan (2011) and Kasper (2019) affirmed that a pink-red colour inside the nodule is a sign of active fixation of nitrogen. The plant disposes off nodules that do not fix nitrogen and such nodules are either grey, white or green when cut opened. This might be because of ineffective rhizobium strain, deprived plant nutrition, pod

development or other plant stresses. According to Nastasija *et al*. (2008), nitrogen fixation is sometimes constrained by certain factors and amongst them are;

I. soil temperature below 16 ºC and above 27 ºC decrease microbe's action and slows the formation of the N-fixing relationship.

II. at the point when levels of nitrogen in the soil are high, there is reduction in both the number and activity of the nodules. In such a situation, the root does not attract the microbes or enable the plants to tolerate nodule and plant development, sacrificing nodule action.

III. when soil pores are occupied by water and not air, nitrogen fixation is constrained.

2.11 The mechanism of biological nitrogen fixation (BNF)

The biological change of N_2 to ammonia which is done through the activities of rhizobia bacteria is exceptionally vital and energy-consuming. N_2 is changed to ammonia (NH_3) through the consumption of ATP and redox equivalent, of which its by-product forms H_2 (N_2 +8H +8e-+16ADP + 16Pi). The enzyme that catalyzes the response is called nitrogenase and comprises the dinitrogenase reductase protein (Fe protein) and the dinitrogenase (MoFe protein), which genuinely catalyzes the decrease of N_2. The development of hydrogen gas is constantly supplemented by the development of ammonia and is an inefficient procedure (People and Craswell, 1992). However, some micro-organisms have hydrogenase that recovers this generally lost type of energy. In the terrestrial ecosystem, symbiotic, non-symbiotic or associative and free-living N_2 fixation are the three main strategies for N_2 fixation. Symbiotic systems contribute an estimated 70 % to N2 fixation, while non - symbiotic systems contribute with an estimated 30 % (Herridge *et al*., 2008). The terrestrial input, (that is to say the true origin and activities of human) of nitrogen from biological nitrogen fixation is estimated to be between the range of 240 - 280 t N/year (Galloway 1998; Abdul Aziz, 2013).

2.12 Symbiotic N_2 fixation

Bacterial of the kind rhizobium and their associate families together with leguminous plants make up a significant symbiotic association. Based upon the crop type, fixation through the microorganism (rhizobium) might happen on evolving root locks or hairs, at the intersection of the side roots or at the base of the stem. The appearance of bacterial nodulation genes and the stimulation of chemotaxis by the rhizobia as an initial phase of the association is done by the roots of the host plant by discharging a phenolic compound (flavonoids) which serves as an indicator to the rhizobia. Nod-gene initiation is essential to produce lectins (nod - factors) and attaching the bacterial onto the root hairs (Janczarek *et al*., 2015). The next stage is an attack of the rhizobia via the plant root hairs and also the meristem of the nodule. Within the segregating nodule, the cells of the rhizobia are crammed in symbiosomes and changed into bacteroids.

A mixture of nitrogenase, hemoglobin and the help of other enzymes are required for the change of the bacteroids for the nitrogen fixation (Martinez-Romero, 2006; Valentine *et al*., 2018). To fix nitrogen, carbon and energy provided from the plant to the bacteroids. These are dicarboxylic acids, for example, malate and succinate (Mitsch *et al*., 2018). The bacteroids therefore provide ammonium to the plant in return for the carbon and energy given by the plant and this ammonium diffuses over the peribacteriod membrane which is later modified into amino acid in the cytosol of the plant in the nodular tissue. The kind of nodules determines the form or procedure in which the nitrogen is transferred to the shoots from the roots, the indeterminate export amides (asparagine), while determinate which is a tropical legume forms nodules ureides, such as allantoin (Kumah, 2016; Wyman, 2018). Actinomycetes of the genus Frankia, starts an N_2 - fixing symbiosis in root nodules of an enormous number of non-leguminous woody dicotyledonous plant for example, Elaeagnaceae, Rhamnaceae (Clawson *et al*., 2004; Sellstedt and Richau, 2013) and the genera Casuarina and Alnus are examples of trees, (Nazaret *et al*., 1991; Benson

and Dawson, 2007). N_2 - fixing symbiotic interaction can also be seen on Gunnera plant species where cyanobacteria Nostoc is active or Anabaena with a fern called Azolla Anabaena (Bergman and Osborne, 2002; Franche *et al*., 2009).

2.13 Methods for assessing BNF

In order to observe an improvement in the fixation efficacy then conclude on its impact on a farming system, it is very important to measure the symbiotic biological N_2 fixation accurately in the legumes. As the most effective method of fixing nitrogen, biological nitrogen fixation can be measured using several procedures. (Sun *et al*., 2020)

The decision to use a specific strategy or procedure relies upon the nature and size of the test, the accessible assets, the type and the system being referred to. The few among them are: I) the total nitrogen difference (TND) methods, (ii) the xylem sap analysis, (iii) the N isotope methods and (IV) the acetylene reduction assay (ARA). In all the approaches mentioned above, there are confinements or limitations of which every process must be observed to decrease their impact on the symbiotic activity calculation.

2.14 Acetylene reduction assay (ARA)

Through observations, the ARA technique during the 1980s was generated as the N_2 fixing enzyme, nitrogenase, catalyzed the reduction of acetylene (C_2H_2) to ethylene (C_2H_4), according to Ngome (2006), is one of the regular strategies utilized for assessing biological nitrogen fixation. Since that time, the ARA procedure represents numerous estimations in legumes biological nitrogen fixation (Anglade *et al*., 2015). From the decapitated roots or entire plants, the quantity of ethylene formed by the removed nodules which has been incubated in an environment containing acetylene has at some point been changed into N_2 fixed by multiplying with a conversion factor of three (Adams *et al*., 2010; Ashworth *et al*., 2015).

These days, ARA is restricted to quantitative or measurable studies because:

(I) it requires interruption among singles, short-term capacities to get time-integrated measurement,

(II) multiplying by three as the conversion factor does not have any significant bearing all the time and enormous errors are possible to happen

(III) it is actually tough to recuperate the entire nodules during field situations to complete BNF appraisal (Ngome, 2009; Ngome *et al*., 2011).

The ARA is the greatest technique commonly used due to its ease and small cost involved, however, it is uncertain in the fact that the activity of the nitrogenase can be hindered by the product of ethylene by 50 % after 30 minutes (Calvo, 2010; Abdul – Aziz, 2013). In addition, it is unknown if the fixed N is merged into the plant (Ahilijah, 2017) then it must be utilized as an indirect strategy not giving complete values.

2.15 Xylem sap analysis

The product of biological nitrogen fixation (essentially glutamate and ureids) are transported to different parts of the plant via the xylem as well as the N absorbed from the soil is either transported straight to the shoot as NO_3 or changed to amides before it is transported to long distance (Russelle, 2008). According to Ormeño-Orrillo *et al*. (2013), numerous legumes transport most of their fixed N as ureids and as indicated by Tegeder and Masclaux-Daubresse, (2018) the quantity of N in the xylem sap as ureids is directly proportional to the volume of N fixed. However, for legumes that are unable to transport their fixed N as ureids, biological nitrogen fixation can only be correlated to the quantity of amide N in the xylem sap (Lambon, 2016). According to Collier and Tegeder (2012) and a confirmation by Baral *et al*. (2016), Xylem sap analysis has a key disadvantage as the association involving the composition of the sap and the rate of BNF must be calibrated against an independent technique of measurement. However, the method is dependable and appears to be in line with ^{15}N isotope procedures.

2.16 Nitrogen balance and nitrogen difference method

The total nitrogen difference method is also one of the methods mostly used in estimating biological nitrogen fixation. Among the N balance method, the easiest among them is to grow a nitrogen fixing plant and a nearby non nitrogen fixing plant. The amount of nitrogen fixed at harvest is considered as the variation in plant tissue nitrogen among the two plants (Anglade *et al*., 2015). In spite of the fact that this strategy is cheap and simple to complete in setting up the field, it has been demonstrated to be exceptionally imprecise and undependable, as it mostly over– or under -estimates the effects of soil N in the system (McCauley, 2011; Paterson *et al*., 2016). The nitrogen difference (ND) technique is another method to replace the N balance technique in which accessible nitrogen levels in the soil under the crops, that is the leguminous crop and that of the non-leguminous crop are considered. By joining the soil nitrogen constituent, soil nitrogen alteration after the planting period and the respective difference in the uptake nitrogen obtained can be evaluated from the two crops, supposing soil nitrogen losses and its transformation are the same (Gastal, 2015).

One more presumption of the N difference method is the comparability of the root nitrogen between the crops involved. Because it is unusual or practical to efficiently harvest or pick plant roots from the field, nitrogen in the shoot and nitrogen in the root proportions are alike between the crops (Franklin *et al*., 2017). This idea is hard to approve in setting a field and root nitrogen, as well as root nitrogen been loss to the soil. This can indicate a huge difference in fixed N that is disregarded in nitrogen fixation estimate (Hall *et al*., 2015; Anderson, 2018). According to Giller, (2001) and also a confirmation by Chianu *et al*. (2012) this technique or method is particularly complex when managing intercropped legumes in light of the fact that, intercrop farming may disturb the capability of the legume and the crop used as a reference crop to access the nitrogen in the soil. This method is therefore prescribed for sandy soils or soils with low

nitrogen, since improving upon the nitrogen content of the soil equally upsurges the mistakes in biological nitrogen fixation estimate (Hsseini Bai *et al*., 2012; Alves and Urquiaga, 2012)

2.17 ^{15}N isotope methods and natural abundance

They are methods which are seen to be more valuable than the nitrogen balance techniques as they provide a yield-independent approximation of nitrogen fixation (Chalk *et al*., 2014). All the ^{15}N-isotopic methods depend on the naturally- occurring ^{15}N abundance in the air to enumerate nitrogen fixation. According to Bai *et al*. (2012), atmospheric ^{15}N concentrations are an even 0.3663 atom % worldwide, but due to the process of transforming the nitrogen that specially discriminates against or for^{15}N, biological material and soil will, in general, have ^{15}N concentration not quite the same as that of the atmosphere. The modification in ^{15}N concentrations using atmospheric ^{15}N is presented as δ^{15}N in parts per thousand (‰); therefore, the δ^{15}N of atmospheric N is 0 ‰. Numerous soils are improved and become more enhanced in ^{15}N after some time because of bacterial discrimination against the heavier ^{15}N isotope to suit the lighter ^{14}N isotope. (Nikolenko *et al*., 2018)

According to Chalk and Craswell, (2018), the level of soil ^{15}N enhancement in a field can differ significantly and both the biochemical factors and physical factor can have greater impact on it. The isotope dilution (ID) method can comparatively be regulated to favor this erraticism by including to the system a recognized amount of plant obtainable ^{15}N, typically with ^{15}N-known fertilizer, and adding a non-leguminous crop which does not fix nitrogen. The isotope dilution technique was been practiced frequently in the 1970s - 1990s preceding to advancement in isotopic mass spectrometry during the 1980s that prompted the improvement of the natural abundance method. The isotope dilution method may, in any case, be positive for soils wherever the δ^{15}N of plant obtainable nitrogen in

the soil is under 2 ‰ or anywhere highly-precision mass spectrometry analysis is not accessible.

The natural abundance technique evaluates the fixation of nitrogen by enumerating the change among a reference crop and a nitrogen fixing legume and with this, the total nitrogen from the air and the soil are obtained, after accounting for isotopic fractional process among ^{14}N and ^{15}N beyond ground sprout of the legume (the β esteem). The main believe of the natural abundance technique is that the leguminous crop and the non-nitrogen fixing crop are regaining similar pools of nitrogen in the soil. This method prefers the two crops been planted very close to each other and additionally, the two crops must have similar stature and rooting morphology (Scaccabarozzi *et al*., 2018). A subsequent assumption of this method is neither discernment nor indistinguishable separation among ^{14}N and ^{15}N in the plants' acceptance and metabolism of N (McCauley, 2011).

2.18 The response of soybean to rhizobia inoculation

Nitrogen is much needed by soybean for higher yield and biological nitrogen fixation has been assessed to provide up to 70 % of the nitrogen needed by the plant (Herridge *et al*., 2008). As testified by Salvagiotti *et al*. (2008) and Collino *et al*. (2015) averagely 50 - 60 % of nitrogen essential for soybean growth is obtained via biological nitrogen fixation. It has been testified that soybean reacts very well to inoculation once they are brought into territories where they are new and when the soils lack the right rhizobia (Van Kessel and Hartley, 2000; Hungria and Mendes, 2015) and also crop inoculation probably make it possible for higher yield in soils with insufficient inorganic N supply. O'Hara *et al*. (2002) and Tahir *et al*. (2009) affirmed that the response of soybean in terms of yield to inoculation is very mutable and influenced by characteristic field erraticism and by contrasts in ecological and edaphic conditions. Also, Masso *et al*. (2015) and Zahir *et al*. (2018) testified that, rhizobia inoculation brings about increment in nodulation in all agro-

ecologies which have a great impact on the grain yield as it was seen that the response was, however biggest when yields from control field ranged from 0.5 - 1.0 t/ha and when the nitrogen content of the soil varied from 0.05 and 0.15 %.

Active indigenous rhizobium strain is generally absent in numerous soils, except soybean is cultivated on that field for a minimum of five years. Lindstrom (2010) indicated that strains may fade totally without continuous application of inoculant or exchange in hereditary may deteriorate the valuable abilities of the newly added strains after some period though it has been shown that strain easily acclimatize to a new environment. An experiment was conducted on numbers of rhizobium inoculant in 52 and 55 agricultural fields. The experiment was associated with whether soybeans had been planted at the place within the preceding 13 years. Solomon *et al*. (2012) concluded that inoculating seeds using pertinent strains of microorganisms before planting was significant after the experiment particularly in territories where inoculant was used for the first time on the land. According to Denison, (2000) and Slattery *et al*. (2004), legumes response to inoculation importantly relies upon the rhizobia number previously formed in the soil, the accessibility of nitrogen in the soil and the management practice. Reaction to inoculation remains site explicit and relies upon the aspects above the efficiency and competition among the strain(s) utilized and host material(s) sowed.

As a result of applying the right rhizobia strain to legumes is the first choice to improve N_2 fixation; substantial variation in strains efficiency have been seen in various preliminaries as affirmed by Choudhry, (2012). According to Ahlijah *et al*. (2017) when the nitrogen content of the soil is adequate to satisfy what the crop needs, the host crop – rhizobia association fix just small N_2 to support. Therefore, less active rhizobia - host crop beneficial association might fix additional nitrogen once the request by the host for nitrogen is increased by the practice of the management and availability of adequate

nutrient. According to Tejera *et al*. (2006) nitrogenase action is an adaptable procedure that changes in accordance with the nitrogen request of the host.

The quantity of N_2 fixed turns out to be substantially more reliant on the request of nitrogen by the host crop than on the intrinsic capacity of the rhizobia to fix nitrogen (Bhattacharyya and Jha, 2012). Therefore, practices by the management that upsurge nitrogen request might likely be a further active means of improving upon the quantity of N_2 fixed by the leguminous crop compared to trying to improve the efficiency of the rhizobia – host crop association (Choudhry, 2012).

Through research rhizobia inoculation have proven to be very important in terms of nodulation, dry matter yield and total grain yield (Denton *et al*., 2017). Ezekiel-Adewayin, (2015) detailed that the use of rhizobia inoculation has a greater impact on the yield of legumes recording an average yield of 1,300 kg/ha in soybean and that nitrogen accumulation, nitrogen concentration, plant dry matter and grain nitrogen were similarly improved when the soybean seeds were inoculated. Additionally, an investigation using three different types of groundnut in a sandy loam soil detected that the application of rhizobia inoculant raised the amount of N_2 fixation by up to 46 % over the un-inoculated control.

It is as well accepted that differences in cultivars influence the fixation of nitrogen in several leguminous crop types and in certain crops, specific mixtures of cultivar and strain have been demonstrated to be particularly effective for nitrogen fixation (Allito *et al*., 2015). Many researches have confirmed variation on the interaction among strain and different species in soybean. Zerpa *et al*. (2013) observed a positive influence on the interaction between different varieties and strain on nitrogen fixation parameters though Allito *et al*. (2014) discovered a non-significant relationship.

2.19 Factors affecting nitrogen fixation in legumes

The development of active N_2 fixing symbiosis among leguminous plants and their N_2 fixing microbes rely upon several ecological influences and can be seriously affected by the farm management practice (Elliott *et al*., 2007). It has been reported that severe ecological conditions including mineral toxicity, salinity, unfavorable soil pH, extremely high or low levels of soil moisture, insufficient photosynthates, nutrient deficiency, extreme weather conditions and disease conditions can affect the plant development and growth (Singh *et al*., 2017). According to Katulanda, (2011), due to these factors, the persistent rhizobium strains are unable to actively complete root infection and the fixing of nitrogen in their full capacity. Drought conditions can poorly influence the nodule development of which the moisture stress can decrease the nitrogenase performance and nodule weight.

The cell wall of the nodule begins to deteriorate after the plant had under gone water stress for about 10 days leading to senescence of bacteroids. It has been confirmed that the aggregation of Na^+ reduces the development of the plant, development of the nodule, and the capacity of fixing nitrogen under salinity conditions (Aydi *et al*., 2008; Kouas *et al*., 2010). As per Niste *et al*. (2013), too much salinity can influence the early cooperation among the rhizobium and the nodule development directly in legumes and also the plant nitrogenase performance lessens drastically because of establishment of an unproductive nodule at high temperature (40ºC) (Oliveira, 2011; Hungria and Kaschuk, 2014).

However, in the leguminous rhizosphere rhizobia establishment may be diminished by low pH of the soil and nitrogen fixation can be repressed or hindered by low pH of the soil (Asamoah, 2015; Danso, 2017). Among the traits of extremely acidic soils (pH <4) are a low-level phosphorus, molybdenum and calcium alongside manganese toxicity and aluminum, which disturbs both the rhizobia and the crops. That is to say, when the pH of the soil is low, the plant growth is disturbed and nodulation and its fixation of nitrogen is

seriously affected. High alkaline (pH > 8) soils are also high in bicarbonate (HCO_3^-), chloride (Cl^-), sodium (Na^+) and borate (BO_3^-), which lessen the fixation of nitrogen (Siczek and Lipiec, 2011; Magadlela *et al*., 2014).

In addition to ecological factors, farm management practices also impact the atmospheric N_2. According to Ronner and Franke (2012), factors including the incorporation of inoculant, P - fertilization, variety selection, and plant density influence plant development and growth. According to Giller (2001), the need for inoculation depends on the effectiveness of the rhizobia and how well they matched in the soil. If the leguminous crop is promiscuous, mostly strains of rhizobia with which it can produce effective nodules are available, and with that it will occasionally respond to inoculation (e.g., soybean and Bambara beans). Among the leguminous crops, soybean is the most known crop that response to inoculation as most diversities of it are more precise and does not continuously nodulate with native rhizobia in Africa.

Furthermore, some diversities are more precise than others or are better adjusted to local ecological conditions. Indeterminate soybean species fix more N_2 because of their long period for development than determinate, or early maturing varieties. Ronner and Franke (2012) explained that phosphorus fertilizer improves nodulation, facilitating the nitrogen fixation efficacy and plant growth in soils where P is limited. In intercropping, legumes frequently need more nitrogen from N_2 fixation than legumes in a mono-cropping as cereals like millet or maize, developed as core crops, demand for more nitrogen. Therefore, as confirmed by Vesterager *et al*. (2008), with a smaller amount of N accessible in the soil, legumes in intercropping depend more on N_2 fixation. Naab *et al*. (2009) and Franke *et al*. (2018) stated that increasing plant number or population on a field demonstrates either positive for a percentage of nitrogen from N_2 fixation because of increment in race for soil N, or negative because of race for different plant food and moisture.

2.20 Response of soybean to phosphorus fertilizer application

Phosphorus is a nutrient needed by plants for many important activities such as seed and nodule formation, storing and transferring of energy, stimulation of root growth, blossoming and fruiting (Linkohr *et al*., 2002). Whitehouse (2010) and Lam *et al*. (2012) similarly affirmed that applying phosphorus on legumes could also increase the size of the leaf, the roots and the grain yield; concentration of nitrogen in the grain and tops; quantity and heaviness of root nodules and increment of acetylene lessening quantity of the nodules. According to Sinclair and Vadez, (2002), N_2 fixation in legumes is very sensitive to phosphorus deficiency and its inadequacy leads to a decreased in nodule mass and reduction in the production of ureide.

Additionally, nodules are a vigorous sink of P and nodule P attentiveness generally surpasses roots and shoots (Bargaz *et al*., 2011; Drevon *et al*., 2015). Consequently, nodule quantity and dry weight might be improved by treating soils with inadequate phosphorus with phosphorus fertilizer (Mei *et al*., 2012; Waluyo and Lie, 2016). However, Chrispin (2009) testified that applying 45 kg of phosphorus fertilizer per hectare improved dry matter (12.25 kg/ha) and grain yield (1.0 - 2 t/ha) yet, could not influence N_2 fixation showing that the leguminous plant been the host plant was more receptive to the phosphorus fertilization compared to the rhizobia.

Phosphorus fertilizer application and how it's managed under low accessible soil P status are significant in accomplishing high yield. The availability of phosphorus nutrient has been well-known to influence the operative of the BNF structure (Sahrawat *et al*., 2001; Spaepen *et al*., 2009) and for that matter, among the fundamental nutrient elements. The impact of P on interdependent nitrogen fixation in leguminous crops has gotten extensive attention. Jackson (2018) revealed that in soybean, the deficiency of P diminished the entire capacity of the nodule to function, its weight and its number. In agreement to Jackson (2018), remarkable increment in the growth of soybean, 100 - seed weight and

grain yield increase (83 - 124 kg/ha) because of supplementary levels of 90 and 100 kg P_2O_5 / ha was reported for by numerous researchers (Belete *et al*., 2018).

According to Nwoke *et al*. (2009), the difference in genotype development of soybean in soils with low P has been accounted for and various procedures have been projected to support plant growth in soils with low P levels. Despite the fact that the procedures shown by soybean genotypes in adjusting to low accessibility of P are not all that clear, the ability to identify and how to use phosphorus - efficient genotypes carefully with soluble soil or P fertilizer can ease phosphorus shortage within a few period (Attar, 2014; Liu *et al*., 2016).

The response of soybean to added phosphorus fertilizer relies upon the management factors and the state of the plant. Through the work of scientists, different rates of applying phosphorus fertilizer have been prescribed to improve upon the development, growth components and yield of soybean. In an investigation on soybean fertilizer with a locally adapted poor yielding, Malayan variety suggested that for optimum yield (2.0 t/ha), 20 kg ha^{-1} to 60 kg ha^{-1} of nitrogen and 30 kg ha^{-1} of phosphorus fertilizer must be applied. However, in an investigation including high yielding cultivars, Abbasi *et al*. (2010) revealed yield increments of 15 $kgha^{-1}$ for 40 kg P_2O_5 ha^{-1} and 35 $kgha^{-1}$ for 80 kg P_2O_5 ha^{-1}. Also, Ezekiel-Adewoyin, (2015) reported that increasing the rate of phosphorus up to 80 kg ha^{-1} and 125 kg P_2O_5 ha^{-1} improved the yield of soybean. Also, Belete *et al*, (2018) demonstrated that high amount of phosphorus fertilizer (90 kg P_2O_5 ha^{-1}) might be needed for effective production of soybean.

Mahamood *et al*. (2009) indicated that soil factors, for example, low amount of P leads to pod abortion and lessen yield in soybean. According to Bhuiyan *et al*. (2008), the fixation of nitrogen by leguminous crops, nodule formation and its development is influenced by inoculating seeds with rhizobium together with phosphatic fertilizer application and Gopalakrishnan *et al*. (2015) observed a similar pattern as some

leguminous crops were treated with rhizobium inoculant and realized an increment in nodule formation and most extreme development features when phosphatic fertilizer was applied.

2.21 Organic source of nutrients

Organic manure can be defined as resources that have been manufactured from one or more materials of a biotic nature (plant/animal) and/or unrefined mineral materials (lime, PR, etc.) that have been changed through controlled bacterial decay into a uniformed product with enough quantity of plant nutrients to be of worth as a fertilizer (Holm *et al*., 2010). Their sources of plant nutrients are used to changing extents in most nations. Also, they play an important role but not enough in nutrient influence by leaving aside their effect on the soil's biological and physicochemical properties. Moreover, they may be utilized in the form in which they are attained from the source or after changing degrees of processing. In most situations, the types of organic fertilizers in use in an area are determined by the organic resources that are locally obtainable or can be produced in the area. Among this organic manure or organic fertilizers are farm yard manure, compost, animal excreta (cow dung, poultry manure), crop residue etc. Crop residues describe the quantity of crop biomass left after the exclusion of the main produce (grain, fruit, etc.) from the field. Most crops produce large quantities of residues, e.g., hay, shoot, stubble, waste, and shells, which can have a lot of benefits such as sources of plant nutrients either right away or after decaying into compost. Farm yard manure is also another form of organic fertilizer that comprises a combination of dung and urine of ranch animals and their bedding material (litter). Compost is any biodegradable material (animal, crops, human and industrial waste) that has been mixed and decomposed through aerobic, anaerobic or partially aerobic decomposition and the action of micro-organisms. According to FAO (2003), compost is rated highest among all the organic fertilizers or

organic manure to be used as a supplement to the soil as it eliminates pathogens during its process of formation or decomposition through its high temperature requirement. Also, Buresh and Dobermann (2009), confirmed that the physical properties of the soil can be improved by the use of compost and among these properties are the soil's structure and its combination or aggregation, its capacity to hold water, biological properties which include increasing the population of microbes for biological actions and chemical properties which include the ability to hold nutrient through improved cation exchange capacity and increased capacity to resist changes in soil pH.

2.22 Biochar as an organic source of amendment

The word biochar has ascended to represent the kind of charcoal produced after the thermal action of natural feedstock, for example, crop residue, wood shavings, municipal waste or manure in an oxygen-limited area called pyrolysis (Bridgwater, 2003; Yavari *et al*., 2015). It is notable from charcoal and comparable resources by its proposed use for soil fertility while charcoal is referred to by its utilization as fuel (Lehmann and Joseph, 2009; Zhag *et al*., 2013). Apart from their purposes, one could not differentiate among carbon from biochar or charcoal (Tenenbaum, 2009; Lehmann and Joseph, 2015). The value of biochar depends on the feed stock and the production procedure utilized (Enders *et al*., 2012; Heitkötter and Marschner, 2015; Srinivasan *et al*., 2015) which influences its character and serviceability (Buresh and Dobermann, 2010; Hagner *et al*., 2016; Shaheen *et al*., 2019). By burning the natural material without oxygen between temperatures ranging from 300°C to 1000 °C, a greater part of the carbon turns out to be "fixed" which becomes more stable once it is added to the soil.

The stability of biochar in the environment is very high (Nguyen *et al*., 2008; Gollakota *et al*., 2016). Biochar has been assessed and reported to have been effective in the soil for about 10,000 years (Swift, 2001; Domene *et al*., 2015; Lopez *et al*., 2018). The Terra Preta soils which can be located in the Amazon Basin of America specifically south are

examples of stable biochar soils. An old practice by the Indians more than 2000 years back, add up biological waste to the soil which therefore altered into stable forms because of the anaerobic conditions. It has been confirmed that the soils are still good for farming with high fertility (International Biochar Initiative, 2007; Yang *et al*., 2017). As a result of the bulky quantities of biochar added to the soils, these soils contain much nutrients making it more fertile in spite of hundreds of years of leaching from substantial tropical rains.

2.23 Biochar production condition

The nature of biochar and how it is been applied to farming soil or carbon segregation is exceptionally influenced by the production temperature and the sort of biological resource utilized as feed stock (Gaskin *et al*., 2008; Kloss *et al*., 2012). Various elements, for example, lignin, cellulose and hemicellulose are broken down at various production temperatures. Every single organic material begins to experience thermal or heat decay at temperatures over 120 ˚C. In situations where biochar is made at low-temperature (< 300-400 ˚C), it has surface area which is low and is partially carbonized. However, the porosity of the biochar is increased when it is made under higher temperatures (400-600 ˚C) (Xie *et al*., 2015). When the surface area of biochar is improved under high temperatures, cation exchange capacity is diminished because of the damage of functional groups (Asirifi, 2017). According to Bruun *et al*. (2011) nitrogen, hydrogen and carbon which is more than 60 % are the few elements found in biochar and some few nutrient component comprising of potassium, calcium, silicon etc. Increment of the heat up to 800 °C during pyrolysis, was likewise seen to upsurge the carbon content to the detriment of other elements such as hydrogen and nitrogen (Lehmann and Joseph, 2009; Baronti *et al*., 2014).

2.24 Sources of biochar

Biochar is produced from numerous varieties of natural resources and can be done using various procedures bringing about products with different characteristics (Guerrero *et al*., 2005). It tends to be made from a broad scope of biomass resources, for instance, woods, farming waste including corn cobs and cocoa pods (Demirbas, 2004), green waste (Chan and Tyler, 2007), animal manure and further waste goods (Chan *et al*., 2008; Downie and Munroe, 2009; Lima *et al*., 2008). It can similarly be made out of the droppings of poultry birds (Revel *et al*., 2012), sewage muck (Khan *et al*., 2013), rice shell (Carter *et al*., 2013; Lu *et al*. 2014), wheat hay (Junma *et al*., 2014) and numerous different resources.

2.25 Basic characteristics of biochar

The characteristics of biochar in terms of its quality can be influenced by the structure of the biochar. The permeability of biochar and its surface area are the most critical part and play an important part in the determination of its possible end-use. The preliminary macro structure of a feed stock is similar to that of the subsequent biochar and this is mainly the circumstance for plant parts that are high in cellulose (Sohi *et al*., 2010). Pyrolysis eliminates mostly volatile compounds; in the biochar the macrostructure of the biomass is reserved in a larger quantity. On the other hand, there will be cracks in the macrostructure which is caused by the tension within the structure, and the emission of volatilized gases leads to minor or tiny holes to open in the material (Downie *et al*., 2009). In pyrolysis, the surface area and the permeability of the biochar in various temperatures is likely to have main influences on adsorption, the capacity to hold water and nutrient preservation capacity (Sohi *et al*., 2010). Bagreev *et al*. (2001) reported an upsurge in its permeability and thus surface area of biochar is related to the temperature of pyrolysis of which Boateng, (2007) confirmed that the surface area of biochar made was low utilizing switch grass; changing from 7.7 to 7.9 m^2 kg^{-1}. Another study expressing related preliminary outcomes, however, confirmed that, there was an increment in the surface

area of biochar when the pyrolysis temperature rises from 400 to 950 ºC (41 to 99 m^2 kg^{-1}) by a factor of three (Bagreev *et al*., 2001). These outcomes and that of Keiluweit *et al.* (2010) uncovered a wide propensity of increment in the surface area of biochar when pyrolysis temperatures were increased. Additionally, Keiluweit *et al*., (2010) indicated increment in its porosity and in this manner, the total carbon and volatile substance is decreased with surface area. In spite of the fact that the mechanisms involved in the improvement of the ability of the soil to hold water when modified with biochar is still not understood, it is very much observed that the surface area of soil units powerfully controls its capacity to hold water; sand holds onto little water and clay holds onto more water.

Adding biochar to the soils improved the surface area and may influence the capacity of the soil to hold water. It is commonly known that biochar has the ability to penetrate certain soils and improve on their capacity to hold water. It has been suggested that some biochar produced with temperatures not above 400 ˚C might be hydrophobic and this might constrain the efficacy of the biochar to store water (Day *et al*., 2005). Pyrolysis under low temperature conditions may produce biochar suitable to be used as a replacement of N fertilizer (Day *et al*., 2005), while biochar made at high temperatures are appropriate for adsorption activities, for example, decreasing the contamination of heavy metals in the soils (Sohi *et al*., 2010). On the contrary, Boateng (2007) demonstrated that biochar produced at 480 ºC had poor adsorption qualities without extra activation. Therefore, the permeability of the biochar and its surface area will probably not have impact on the quality or nature of the item over a longer period.

2.26 Biochar properties

Biochar is lightweight, very permeable with much quantity of carbon (Downie *et al*., 2009). The joined heterogeneity of the residue and the reaction of series of chemicals

taking place during the pyrolysis process produce a biochar that has exceptional chemical and structural features (Antal and Gronli, 2003; Demirbas, 2004). As reported by Sohi *et al*. (2009), it is significant to measure biochar properties like, pore-volume, pH, volatile or unstable compound content, ash remain content and water holding capacity, bulk density, and exact surface area in order to have a valuation for agricultural utilization of the material. According to Lehmann and Joseph, (2009) the chemical and structural make-up of biochar generally varied, except for pH, normally > 7. However, there are only few properties which can be found in all biochar and these include high content of carbon and the level of its aroma, partly clarify extremely the biochar's characteristic recalcitrance (Downie *et al*., 2009). Carbon, mineral substance (fiery remains or ashes), volatile matter and water content are usually observed as its significant components (Antal and Gronli, 2003).

Sohi *et al*. (2009) reported that with biochar, its chemical stability is to some extent represented by its greater aromatic structure and higher carbon content. The entire carbon content in biochar ranges from 172 g kg^{-1} to 905 g kg^{-1}, even though organic carbon usually represents < 500 g kg^{-1}, as Chan and Xu (2009) stated for different sources of this resource. According to Chan and Xu (2009), total N ranges from 1.8 to 56.4 g kg^{-1}, depending upon the feed stock. The ash from the biomass feed stock determines the content of the ashes of the biochar. Grass, husks of grains, straw remains, and manure usually produce biochar with much ash contents, which is not the same as woody feed stocks (Demirbas, 2004). Despite the level of nitrogen content in biochar, it may not be essentially valuable to plants once the nitrogen is usually available in a form which is inaccessible (usually nitrogen contents < 2 mg kg^{-1}) (Chan and Xu, 2009). As confirmed by Chan and Xu, (2009) the entire phosphorus and potassium in biochar depend largely on the feed stock, ranging from 2.7 – 48.0 g kg^{-1} for phosphorus and 1.0 - 58.0 g kg^{-1} for potassium.

However, overall potassium, nitrogen and phosphorus ranges in biochar are more extensive than reported in the write up for real fertilizers which are organic (Demirbas, 2004). Several minerals in the ashes of biochar are believed to happen as separate relations from the carbon matrix, except for potassium and calcium (Amonette and Joseph, 2009). As testified by Amonette and Joseph, (2009) mostly every mineral association includes two or more kinds of mineral. The pores in biochar have been classified into three groups (Downie *et al*., 2009), based on their inner diameter (ID): macrospores (ID > 50 nm), mesopores (2 nm < ID < 50 nm) and microspores (ID < 2 nm). The basic porosity of the biomass feed stock and its structure are reserved in the product formed from the biochar (Downie *et al*., 2009).

2.27 Biochar as soil amendment

Biochar as a soil amendment material is reported to last longer in the soil with other helpful properties and gave the assurance that sequestered carbon from the atmosphere and the quality of the soil can be improved by it, of which the terra preta is a good example (Lehmann *et al*., 2006). Numerous agronomic benefits have been reported with the use of biochar for farming, especially improving the fertility of the soil and the yield of crops (Novak, 2009). The addition of biochar to the soil influenced the soil physically, biologically and chemically (Woods *et al*., 2006) following preliminary field experiment done in tropical or semi-tropical regions with soils with high acidity. It was therefore assumed that the increase in crop yield found in such soils might be credited to a liming impact of biochar (Jeffrey *et al*., 2011). The International Biochar Initiative (IBI, 2013) reported that biochar can be utilized separately or mixed with other product like compost or manure, as an agent for fertilizing the soil, to advance resource use proficiency, remediation or defense against specific ecological contamination, and as a way for greenhouse gas decrease.

2.28 Biochar effect on soil chemical properties

Over the years, various soil properties have been reported to be affected by biochar. Among these is the soil pH which is increased by biochar (Chan and Tyler, 2007; Laird *et al*., 2010), therefore decreasing lime supplies and upsurges the capacity of exchanging cation in the soil (Novak *et al*., 2009; Peng *et al*., 2011). Steiner *et al*. (2008) reported that biochar reduces the leaching of nitrogen from the soil which therefore improves the nitrogen use efficiency (NUE). Adding biochar to the soil also diminished the contaminants of organic soil such as insecticides and also lessening of the mobility of heavy metals (Hilber *et al*., 2009). As a result of the high charge density per unit surface of the organic or biological matter, the amount of the soil's cation exchange capacity is increased (Atkinson *et al*., 2010). Biochar once again can decrease the acidity of the soil and raise the level of necessary elements, including calcium, Magnesium and potassium but diminishing aluminum accessibility (Deenik *et al*., 2011), while the water holding capacity of the soil can be enhanced by the high-surface-area (Gray *et al*., 2014).

2.29 Biochar effect on soil physical properties

The soil efficiency for the production of crop is directly affected by the physical conditions of the soil which helps in determining the ability of the soil to hold water, circulation of air in the soil and soil quality confinements for root movement (Benjamin *et al*., 2003). The addition of biochar to impermeable soils raises its porosity as a result of its molecule form and size, and this is because biochar mainly has a permeable inner structure (Laird *et al*., 2010). Herath *et al*. (2013) reported the soil's total porosity improvement by using biochar, yet these increments in porosity depended on the biochar utilized and the kind of soil where biochar was applied. The surface area of the soil increased as the soil porosity increased (Jessica and Peter, 2011). Also, the saturated hydraulic conductivity (K_s) and the penetrability of soil water was improved as the porosity of the soil increases (Asai *et al*., 2009). The bulk density (ρb) of the soil also

changes (Laird *et al*., 2010) and modifies the soil aggregate stability (Peng *et al*., 2011) with addition of biochar. Again, the application of biochar helped improved the soil to retain 15 % extra moisture content when compared with control treatment (Laird *et al*., 2010). As per Mukherjee *et al*. (2013) adding biochar also reduced the bulk density of the soil since the biochar has high permeability and once it is utilized in the soil, it prominently increases the pore volume to reduce the bulk density.

2.30 Biochar effect on soil biological properties

The soil is seen as compound societies of microorganisms that move in accordance with the characteristics of the soil, climatic and management factors, notably adding of organic manure (Thies and Rillig, 2009). As a result of the fractions of labile carbon and un-pyrolysed feed stock, the microbial biomass of the soil increases with the application of biochar (Bruun *et al*., 2011; Luo *et al*., 2013). On the other hand, Castaldi *et al*. (2011) reported that the microbial biomass of the soil is not affected by biochar and this according to Kuzyakov *et al*. (2009) is due to the recalcitrance of the biochar. The microbial biomass of the soil decreased as reported by Dempster *et al*. (2012) when there is an alteration in biochar as a result of its toxicity effect, while Lehmann *et al*. (2011) also testified that the rate at which biochar is applied and the soil type similarly influenced soil microbial biomass.

Among the reasons why soil microbial biomass vary as a result of biochar incorporation into the soil includes the enhancement of nutrients accessible in the soil (phosphorus, calcium and potassium), adsorption of noxious composites and enhanced water content of the soil and the change of pH status, as all of them can have influence on the actions of the microorganisms in the soil (Lehmann *et al*., 2011). The inward permeability of biochar may assist soil microorganisms keep away from predators (Pietikäinen *et al*., 2000) and reserve or keep mineral nutrients and carbon substrates (Saito and Muramoto, 2002; Warnock *et al*., 2007). Additionally, few inquiries have indicated that as a result of

biochar application, the microbial community makeup of the soil may change as reported in Amazonian Dark Earths (Terra Preta) (Steiner *et al*., 2008). The microbial biomass of such soils is more remarkable, and at times, of a greater diversity than the nearby or other areas (Kim *et al*., 2007).

2.31 Methods of biochar application

As a result of the erraticism of biochar kinds (from raw material), there are inadequate data accessible to agriculturalists on the appropriate way of biochar application and its potential request (Lehmann *et al*., 2006). On the other hand, with current study and the potential widespread biochar application, all things considered, the application procedure and specific tool can be developed for its application. There are various alternatives for biochar application and among them include applying through fluid slurries and scattering it on the field either manually or mechanically and deep banding with composts or manures. The majority among them have anyway not been examined, but field preliminary studies have incorporated and applied biochar into the soil through some sort of tillage. This method of use that decreases biochar's movement through soil erosion may bring about difficulties for the application in fields and no-tillage cultivation. According to Blackwell *et al*. (2009), approximately 30 % of biochar losses result from its surface application of the biochar and its handling in the commercial farming field. Therefore, techniques on timing and area of the application of biochar should be set up. As per Lehmann *et al*. (2015), the most prominent measure of biochar was assessed and concluded that high rate of biochar application (as much as 140 t C ha^{-1}) yield higher. This high volume of soil to accrue carbon from pyrolysis together with high stability of biochar brings about long-lasting carbon sequestration.

2.32 Impact of biochar application on agronomic parameters

The fertility of the soil is improved in multiple ways with the application of biochar; it either includes nutrient supplements to the soil or makes the nutrient uptake by plant

efficient (Lehmann *et al*., 2011). Also leaching of the nutrient might be reduced as the huge surface area brings about higher cation exchange capacity (Lehmann and Joseph, 2009). Numerous experiments have measured the effect of biochar on the yield of crops. The discoveries of the experiments have been very valuable as the results seems to be same in the harvest over the control to multiplying crop output because of application of biochar. A remarkable decline in leaching of applied fertilizers was also observed according to Lehmann *et al*. (2003) after the application of biochar to the soil.

Additionally, nutrients such as phosphorus, potassium and calcium uptake by the plant was improved. According to Steinbeiss *et al*. (2009), by raising the CEC, fertilizers applied might be adsorbed to the surface area and in this way utilized by plants more efficiently. A comparative observation was made by Steiner (2007), who testified a higher maize grain yield on the plot that utilized a combination of biochar and NPK fertilizer compared to the harvest of the others that received only NPK fertilizer. Asai *et al*. (2009) conducted research on the effectiveness of biochar on the yield of rice (*Oryza sativa* L.). Among the observations were: the response of nitrogen fertilizer was seen to be enhanced by the application of biochar; increased yield of rice and; enhanced xylem sap movement.

Oguntunde *et al*. (2004), experimented on a site with biochar and nearby site without biochar and discovered huge contrasts among them, the yield of maize grain was enhanced by 91 % as the biomass yield was also increased by 44 % on the biochar treated field. Hence, research have not yet confirmed any serious adverse outcomes from soil amendments with biochar.

CHARPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area

The experiment was carried out in a screen house at the Faculty of Agriculture experimental field, University for Development Studies at Nyankpala in the Tolon District of Northern Region. The area lies in the Guinea Savannah zone (0^{o} $58^{'}$ 42'W; 9^{o} 25' 14' N) and experiences semi-arid climatic conditions with unimodal rainfall. The rainfall ranges between 1000 – 1200 mm from April to November. The mean temperature for a night ranges from 20 to 22 °C and the mean temperature for a day range from 33 to 39 °C. The area has a relative humidity of 53 % - 80 % (Savannah Agriculture Research Institute (SARI) 2008).

3.2 Experimental design and treatments

The experiment was factorial combinations laid in a randomized complete block design with three replications. The blocking was against external factors like humidity which could not be controlled in the screen house. The positions of the replication were been changed every two weeks. The experiment consisted of two factors, rhizobium inoculant and soil amendment. The rhizobium had three levels which consisted of *Bradyrhizobium japonicum* strain USDA 110 (Brady 110), USDA 136 (Brady 136) and no inoculant. The soil amendment consists of five levels: Biochar, Biochar and Triple Super Phosphate, Biochar and Rock Phosphate, Biochar and Compost and control. Table 2 provides the treatment code with detailed treatment description. A bucket served as an experimental unit and there were 15 of them in a replication.

Table 2: Treatments and their detailed description

Treatment code	Detailed treatment description
NA + Brady 0	No soil amendment + no inoculant
NA + Brady 110	No soil amendment + Brady 110 inoculant
NA + Brady 136	No soil amendment + Brady 136 inoculant
B + Brady 0	Biochar (20 ton/ha) + no inoculant
B + Brady 110	Biochar (20 ton/ha) + Brady 110 inoculant
B + Brady 136	Biochar (20 ton/ha) + Brady 136 inoculant
B + CM + Brady 0	Biochar (10 ton/ha) + compost (5 ton/ha) + no inoculant
B + CM + Brady 110	Biochar (10 ton/ha) + compost (5 ton/ha) + Brady 110
B + CM + Brady 136	Biochar (10 ton/ha) + compost (5 ton/ha) + Brady 136
B + TSP + Brady 0	Biochar (10 ton/ha) + Triple super phosphate (60 kg/ha) + no inoculant
B + TSP + Brady 110	Biochar (10 ton/ha) + Triple super phosphate (60 kg/ha) + Brady 110
B + TSP + Brady 136	Biochar (10 ton/ha) + Triple super phosphate (60 kg/ha) + Brady 136
B + RP + Brady 0	Biochar (10 ton/ha) + Rock phosphate (60 kg/ha) + no inoculant
B + RP + Brady 110	Biochar (10 ton/ha) + Rock phosphate (60 kg/ha) + Brady 110
B + RP +Brady 136	Biochar (10 ton/ha) + Rock phosphate (60 kg/ha) + Brady 136

Table 3: Initial pH, electrical conductivity (EC), total carbon and concentration of nitrogen (N), phosphorus (P), iron (Fe), sodium (Na), calcium (Ca), Magnesium (Mg) and Aluminum (Al) concentration of biochar and compost amendment.

Parameter	Unit	RB	Compost
pH *(CaCl2)*	-	8.2	9.04
EC	$mS\ cm^{-1}$	1.03	2.16
C	*%*	40.58	25.4
N	*%*	0.45	1.78
P	$g\ kg^{-1}$	0.68	2.35
K	$g\ kg^{-1}$	4.02	5.33
Fe	$g\ kg^{-1}$	0.78	10.12
Na	$g\ kg^{-1}$	0.81	1.05
Ca	$g\ kg^{-1}$	1.74	36.88
Mg	$g\ kg^{-1}$	0.82	1.86
Al	$g\ kg^{-1}$	0.45	5.80

3.3 Screen house construction

A screen house was constructed at the Horticulture Department's experimental field. The structure was built from wood and fenced with wire mesh. A transparent polythene sheet was used for roofing the structure to prevent rainwater and reduce sun intensity.

Plate 1: Construction of screen house **Arranged pots under screen house**

3.4. Soil filling and treatment application

The potting soil for the experiment was collected from a farmland at Zegyuri, a community near Tamale. The soil for the experiment was classified as Petroplinthic Cambisol with 45.7 % sand, 48.40 % silt and 5.90 % clay. At a depth of 20 cm, the soil contained 33 mmol kg^{-1} effective cation exchange capacity (ECEC), while total nitrogen (N) and soil organic carbon (SOC) were 0.4 and 4.1 g kg^{-1}(Asirifi *et al*., 2021). A soil of 5 kg was weighed into a plastic basin and mixed thoroughly with the respective soil amendments (Table 2) before transferring into the plastic buckets (height 18 cm and of diameter 20 cm), which served as experimental unit. The quantities for the various amendments were calculated using a bulk density of 1.6 gcm^{-3} of the soil.

3.5. Biochar production

Biochar was produced from rice husk using the muffle furnace at the Agssip laboratory at the University for Development Studies. The rice husk was placed in a metallic

container and kept in the furnace. A temperature of 500 °C was set and left for 2 hours to produce the rice husk biochar.

3.6. Germination test

In order to determine the viability of the soybean seeds (Sung pugun variety) purchased from Savanna Agriculture Research Institute, a germination test was performed. Twenty (20) seeds were randomly selected, covered with thin layer of soil and watered twice daily for germination. After few days, 18 seeds germinated out of the 20 seeds sown, representing 90 %.

3.7. Seed inoculation and sowing

Soybean seeds were inoculated with USDA 110 (Brady 110) and USDA 136 (Brady 136) inoculant using the Slurry method outlined by Woomer *et al*. (1994). Twenty (20 g) of sugar was dissolved in 80 ml of distilled water as a binding agent. Two bowls were filled with 1 kg of soybean seeds each and the sugar solution was sprinkled on it to moisten the seeds to help stick the inoculant to the seeds. Five (5 g) of the USDA 110 and USDA 136 inoculant were poured on the seeds and stirred gently until the seeds became black, indicating that the seeds were coated with the inoculant. The inoculated seeds were dried under shade for 15 minutes after which they were sown.

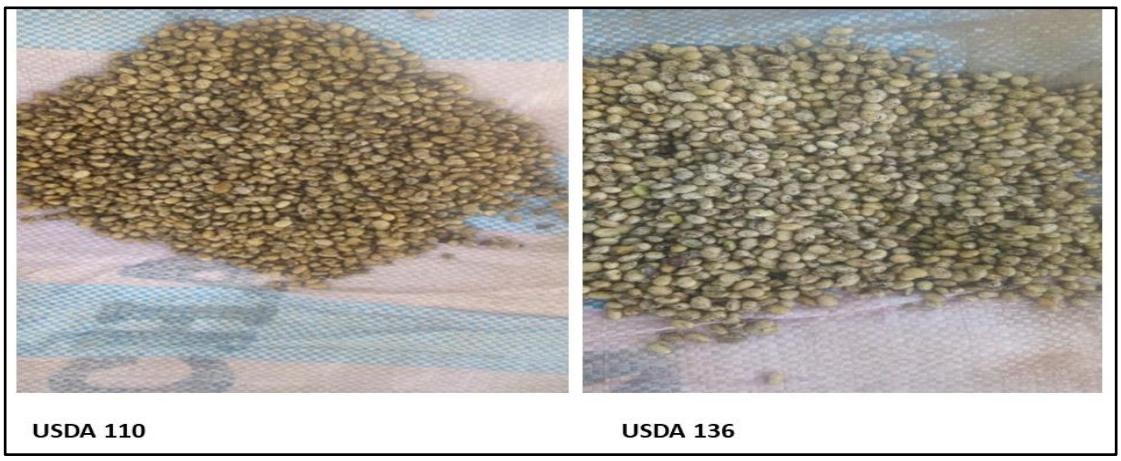

Plate 2: Inoculated soybean seeds with two strains of Brady rhizobium

3.8. Planting and weeding

Soybean seeds were planted on the 13th of April, 2019, three seeds per hole and thinned to two per stand two weeks after planting. Weeds were controlled by hand picking. A hoe was used in clearing the surroundings.

Plate 3: Arranged experimental buckets under shed constructed (Replication one)

3.9. Application of insecticide

Spraying of insecticide was done using a knapsack sprayer three weeks after germination, pre-flowering, flowering and podding of the pods. A systemic insecticide known as Chlorpyrifos (D-Ban Super®) was used for the first and second spraying at three weeks after germination and pre-flowering to control soybean aphids (*Aphis glycines*). The third spraying was conducted using contact and a systemic insecticide Bifenthrin (Akatemaster®) to control the leaf miner (*Odontota horni*) and soybean stem borer (*Dectes texanus texanus*). At podding and browning of pods, seven- day each after the third spraying, the fourth and the fifth spraying was conducted to control green stink bug (*Chinavia hilaris*) and brown stink bug (*Halyomorpha halys*).

3.10. Agronomic data collection

Growth and yield indicator parameters were measured and these included:

plant height, number of nodules, number of effective nodules, pod length, number of seeds per pod and grain weight

3.11. Plant height

A meter rule was used to record plant height every two weeks for ten weeks starting from 27th April 2019. The plant height was measured in centimeters from the base of the plant on the soil's surface to the youngest expanding leaf.

3.12. Number of nodules

One plant from each experimental pot was uprooted at the plant's flowering stage, and the nodules were carefully washed in a basin of water to remove clamp soils. Nodules were detached from the roots of the plant gently and counted.

3.13. Number of effective nodules

All counted nodules were again cut open using a razor blade. The pink-coloured nodules were grouped, counted and recorded as effective nodules per plant.

3.14. Pod length

After harvest, three pods from each plant were selected randomly and measured with a meter rule. Its average was calculated and recorded as the pod length in centimeters.

3.15. Number of seeds per pod

Ten pods were arbitrarily chosen from each experimental unit and threshed. Then, the seeds from the ten pods were counted and averaged, representing the seed per pod.

3.16. Grain weight

When the pods were fully matured and dried, the pods were harvested from the various experimental units by hand picking. The soybean pods were dried for three days, bagged and threshed. They were winnowed and the seeds were weighed using a weighing scale.

3.17. Soil and plant sampling for further analysis

After the experiment, 200 g of soil sample was taken from each treatment bucket for pH, EC, CEC, C, N, P nutrient analysis. The samples were air-dried and then sieved with a 2 mm sieve to remove plant debris. The above-ground biomass of the soybean was harvested and oven-dried at 50 °C for 72 hours. It was then milled using a milling machine. Both the soil and plant samples were packed and transported to Ruhr University of Bochum, Germany for analysis.

3.18 Soil parameters and analysis after experiment

3.18.1 Total elemental analysis

The soil elemental analysis was done using Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). A computer software was used to support the ICP-OES which uses electromagnetic ratio for the identification of the elements contained in a sample released from the excited atoms molecules and their ionized form. Preceding to the ICP-OES, the soil was digested for 15 minutes with a microwave using concentrated nitric acid. Some of the ground soil sample (0.250 g) was weighed together with 10 ml concentrated nitric acid into an inert Teflon microwave vessel and left in a fume hood chamber overnight. The vessels were gently fastened and digested in a microwave (MARS express, CEM, Kamp-Lintford) set at 1600 w for 15 minutes and 120 °C. Water of 10 ml was added to the content after it was left to cool to room temperature. Using cellulose membrane filter paper of 0.2 microns the processed samples were filtered before the ICP-OES reading was done. Among the elements measured were potassium (K), phosphorus (P), nitrogen (N) copper (Cu), aluminum (Al), sodium (Na), zinc (Zn), iron (Fe), calcium (Ca), nickel (Ni), chromium (Cr), cobalt (Co), mercury (Hg) and lead (Pb).

3.18.2 Electrical conductivity and pH

A ratio of 5:1 of the soil to 0.01 M calcium chloride was used in determining the electrical conductivity and the pH of the soil samples. A suspension was formed by weighing 5 g

of the soil sample and 25 ml 0.01 M $CaCl_2$ into a 50 ml glass beaker. It was therefore left to settle for an hour after stirring with a rod. A buffer of pH 7 and 4 and 2 mS electrical conductivity was used in calibrating the pH and electrical conductivity meters before the reading was taken.

3.18.3 Carbon to nitrogen (C: N) ratio

For C: N ratio determination, the plant samples and soil samples were first ground to a fine size of 0.02 mm using a milling machine and a ball mill (Pulverisette 7, Fritsch GmbH, Idar-Oberstein) respectively. About 1 g of the soil sample, 0.12 g of plant sample and a standard of 250 mg aspartic acid were weighed into stainless steel crucibles. The measurement was performed by C / N analyzer (Vario MAX cube, Elementar, Hanau). The C / N analyzer works on the principle of combustion, gas separation and gas detection. The samples were first combusted at a high temperature between 950 to 1200 °C. Gas separation followed, where carbon dioxide or helium carrier gas pushes combustion gases through the analyzer. Carbon, hydrogen and sulfur combustion gases are confined in separate columns then released while Nitrogen gas flows directly into the columns. Gas detection was made by thermal conductivity detector utilizing a Wheatstone bridge circuit to compare the relative thermal conductivity differences between the carrier gas and the analyte gas.

3.19 Elemental analysis of plant

The above-ground biomass of the plant in each pot was cut with a sharpened knife, kept in an envelope and oven-dried for 72 hours at a temperature of 50 °C. The milled samples were packaged in a zipper storage bag for further analysis. The elemental analysis was done by using the Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES). A computer software is used to assist the ICP-OES which uses electromagnetic ratio to find the elements contained in a sample released from the excited atoms molecules and their ionized form. Preceding to the ICP-OES, the milled plant sample was digested

for 15 minutes with a microwave. About 10 ml of concentrated nitric acid was added to 0.125 g of the plant sample which has been milled and kept in an inert teflon microwave vessel in a fume hood chamber and the mixture was left overnight. The mixture in the vessel was firmly closed and digested in a microwave (MARS express, CEM, Kamp-Lintford). The microwave was set to operate under 120 °C with a power of 1600 w and stayed for 15 minutes. Ten (10) millilitres of water were added to the digested mixture, after which it was allowed to normalize to room temperature in the vessel. A 0.2 microns cellulose membrane filter paper was used to filter the digested samples before ICP-OES reading. The measured elements include potassium (K), carbon (C), iron (Fe), nitrogen (N), chromium (Cr), phosphorus (P), Titanium (Ti), mercury (Hg), cobalt (Co), lead (Pb), magnesium (Mg), aluminium (Al), calcium (Ca) and sodium (Na).

Plate 4: Packaged plant and soil samples to Ruhr University of Bochum, Germany after production

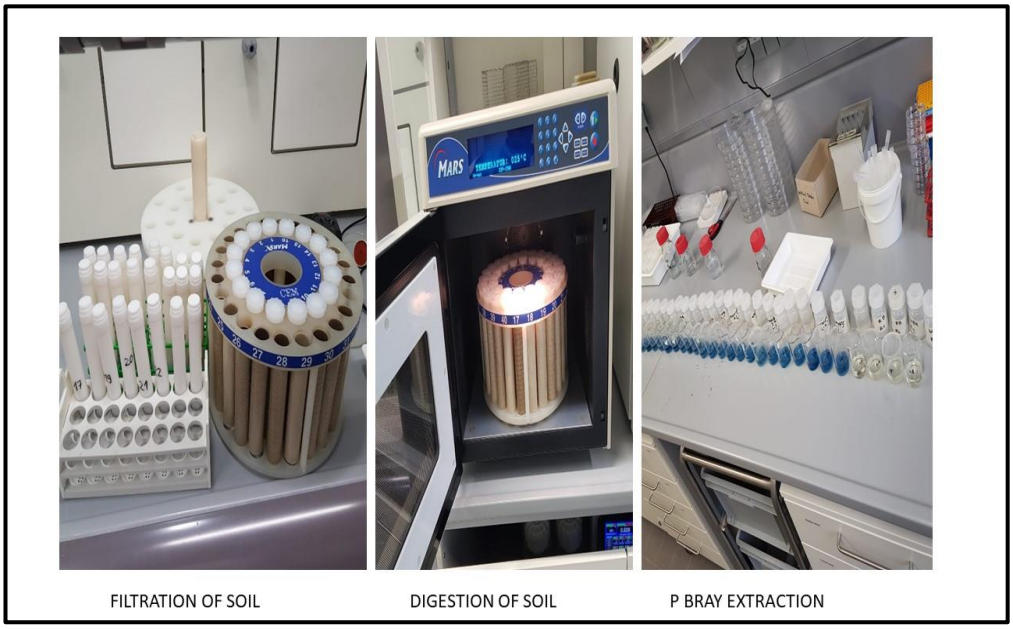

Plate 5: Soil preparation for P extraction

3.20 Data analysis

A two-way ANOVA was performed to test the effect of inoculant and soil amendments on soil fertility and soybean performance. The statistical analyses were carried out using GENSTAT 18 statistical tool and the means were separated by the least significant difference (LSD) at 5 %.

CHAPTER FOUR

4.0 RESULTS

4.1 Plant height at two weeks interval after planting

The various soil amendment had no significant influence on the plant height at two weeks ($P > 0.487$) but had a significant impact at four weeks ($P < 0.001$), six weeks ($P < 0.0001$) and eight weeks ($P < 0.001$) after planting. However, the inoculant had no significant influence on plant height. The interaction between the soil amendment and the inoculant also could not influence the plant height significantly. At week two, there was no difference between the various amendment. Statistically, there was no difference between the soil amended with biochar and non-amended soil as weeks passed, but changes were observed when the second level of the amendment was added. Biochar + compost produced higher plant height than when rock phosphate or triple supper phosphate was added. By week eight, differences could not be found among the amendments. However, there was a significant difference between the amended and non-amended soil (Figure 1).

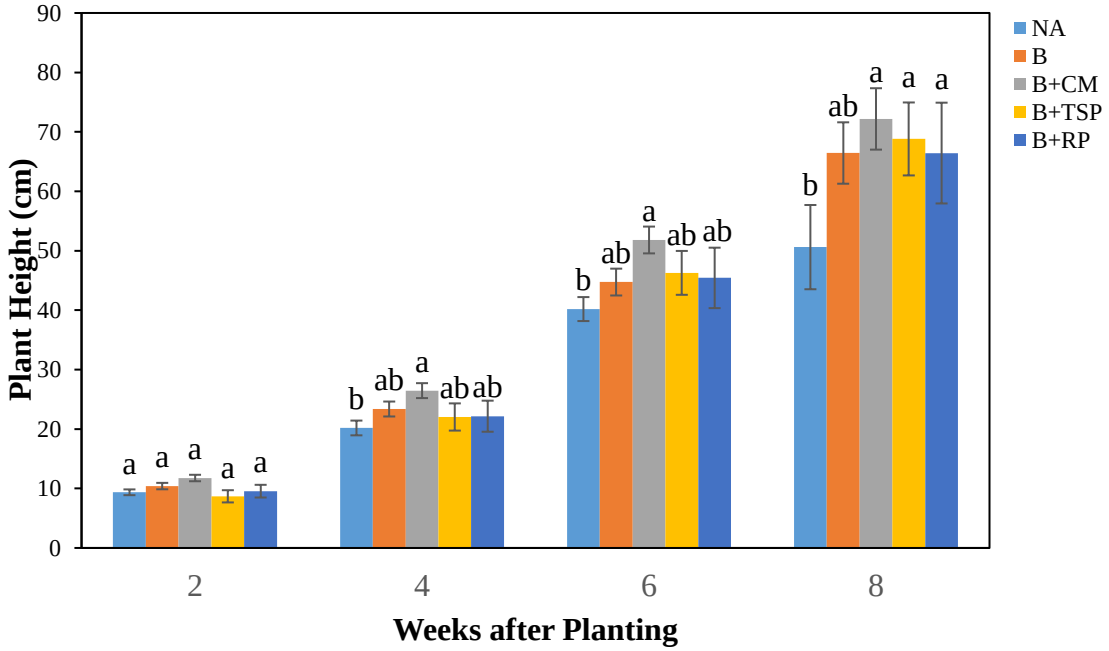

Figure 1: Effect of soil amendment and rhizobium inoculant on soybean plant height at two-week intervals after planting. Error bars represent standard error of mean (SEM)

4.2 Nodule number

Both the inoculants and the soil amendment applied had a significant ($P < 0.0001$) influence on the number of nodules produced. The inoculants and soil amendments also had a significant interaction effect ($P < 0.001$) on nodule production. In the non-amended soils, the non-inoculated treatment recorded a lower nodule number than the inoculated treatments (Figure 2). In soils amended with biochar, Brady 110 strain produced a larger number of nodules than that produced by strain 136. Indeed, there was no significant difference between strain 136 and the non-inoculated treatment (Figure 2). When compost was added to the biochar, there were no significant differences between the two inoculated treatments and the control in nodules production. When phosphorus was added to the biochar, the two strains of *Brady rhizobium* produced statistically the same number of nodules significantly higher than the non-inoculated treatment.

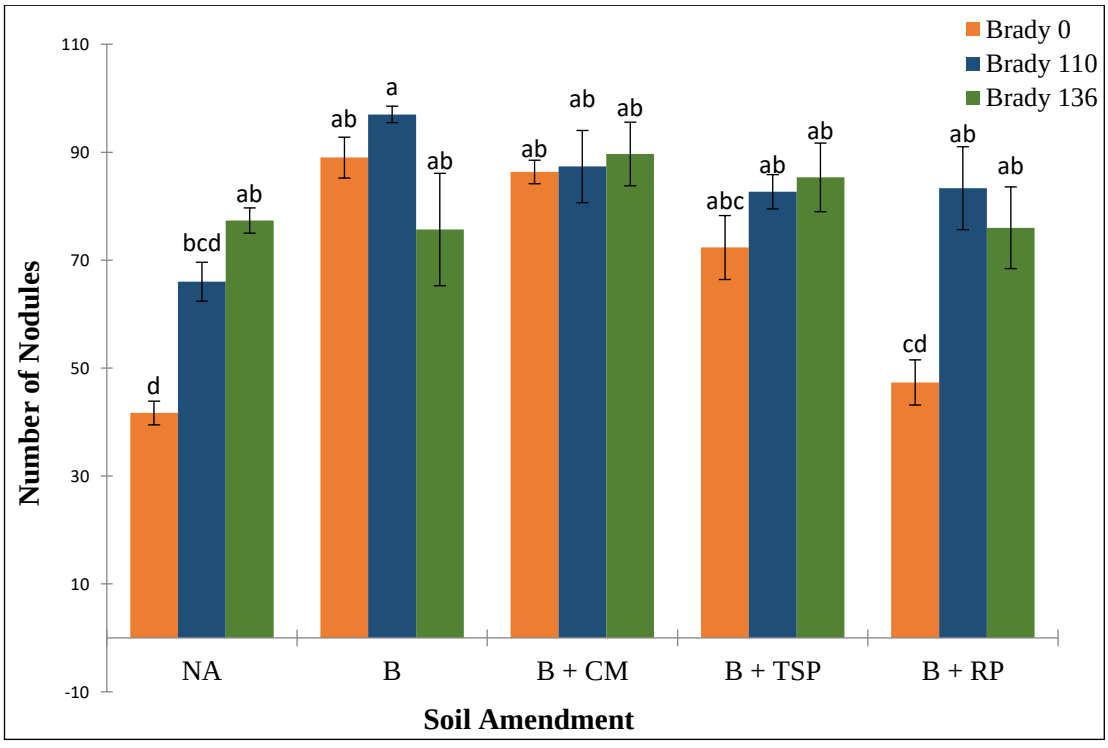

Figure 2: Effect of soil amendment and rhizobium inoculant on nodulation of soybean plant. Error bars represent SEM

4.3 Effective nodules

The inoculant application had a significant influence ($P < 0.008$) on the effectiveness of the nodules produced by the plants. Similarly, the nodules' effectiveness was also affected significantly ($P < 0.032$) by the various soil amendment. Moreover, the interaction between the inoculant and the soil amendment also had a significant impact ($P < 0.011$) on the effectiveness of the nodules produced (Appendix 6). The pattern observed for nodule number repeated in the effective nodule number. In the untreated soil or non-amended soil, the non-inoculated treatment recorded a lower number of effective nodules than the inoculated treatments. In soils amended with biochar, Brady 110 strain recorded a greater number of effective nodules than Brady 136 strain. Statistically, there was no difference between the non-inoculated treatment and Brady 136 strain. When compost was added to the biochar, there were no significant differences among the inoculated treatments and the control in effective nodules produced (Figure 3). However, Brady 110 strain recorded a larger number of effective nodules than the Brady 136 strain. When phosphorus was added to the biochar, the two strains of Brady rhizobium produced statistically the same number of effective nodules significantly higher than the non-inoculated treatment.

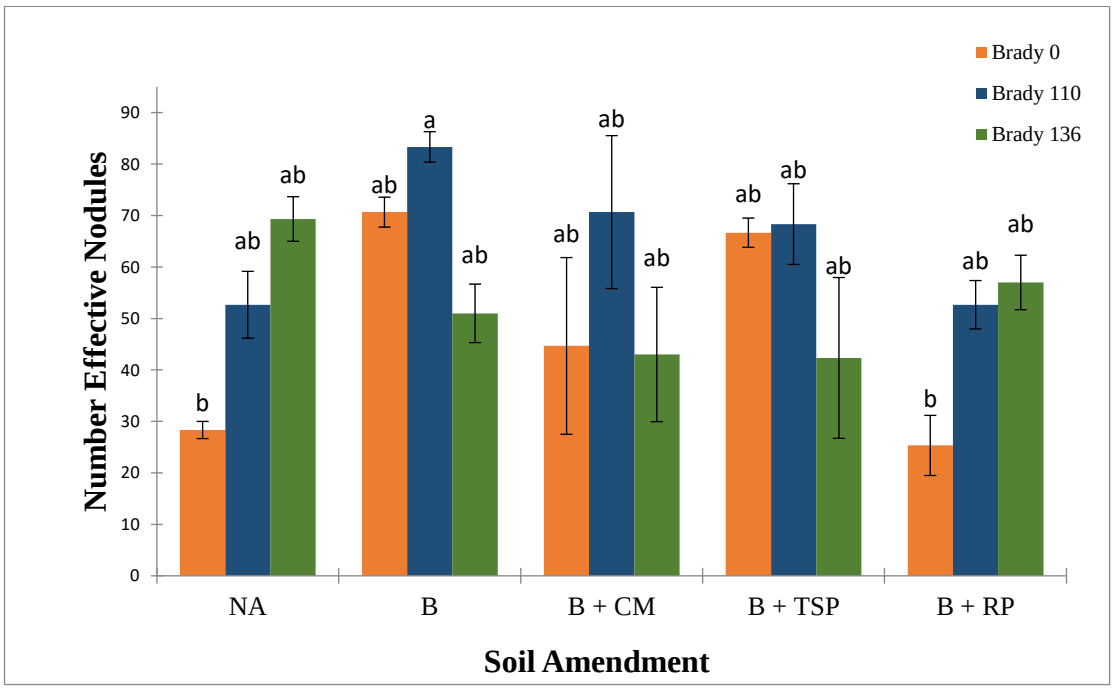

Figure 3: Effect of soil amendment and rhizobium inoculant on the effectiveness of nodules on soybean plant. Error bars represent SEM

4.4 Pod length

The soil amendment had a significant ($P < 0.0026$) influence on the pod length of the soybean plant (Figure 4). The pod length was again influenced significantly ($P < 0.0013$) by the inoculant applied. However, the interaction between soil amendments and the inoculant applied had no significant impact on the pod length of the plant. The inoculated seeds provided longer pods than the non-inoculated seeds.

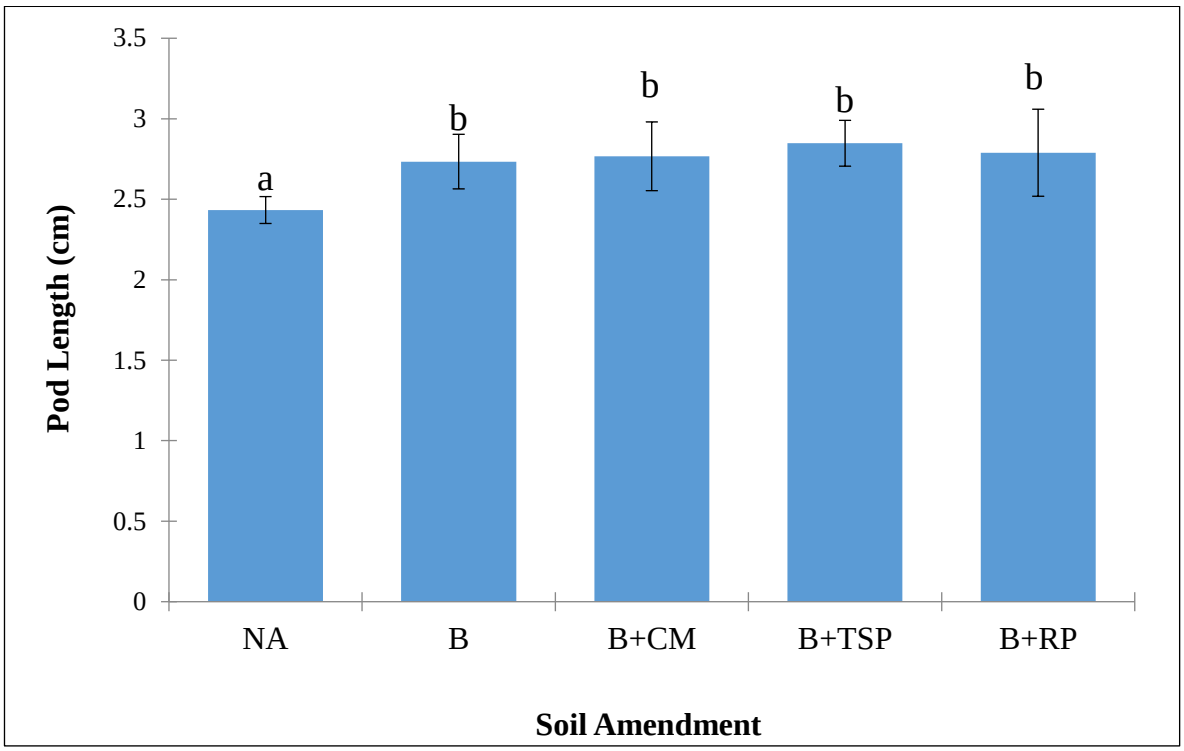

Figure 4: Effect of soil amendment and rhizobium inoculant on the pod length of soybean. Error bars represent SEM

4.5 Seeds per pod

The application of the inoculant could not have a significant (P > 1.00) impact on the number of seeds per pod. Also, the various soil amendment had no impact (P > 0.22) on the number of seeds per pod. Again, the interaction between the inoculant and the soil amendment could not influence the number of seeds per pod significantly (P > 1.00).

4.6 Grain yield

The inoculant application had a significant (P < 0.0001) impact on the grain yield of the soybean. Similarly, the grain yield was significantly (P < 0.0001) affected by the soil amendment. Also, the interaction between the inoculant and the soil amendment had a significant (P < 0.033) impact on the grain yield (Appendix 9). The addition of biochar significantly improved grain yield over non-amended soil. It could be seen that the addition of compost or phosphorus to the biochar led to higher grain yield (Figure 5). Looking at the interaction of the amendment with inoculants, it was observed that in the

non-amended soils, there were no significant differences among the inoculated treatments in grain yield. In soils amended with biochar, Brady 110 strain produced a higher number of grains. However, there was no significant difference between strain 136 and the non-inoculated treatment except biochar with rock phosphate amended soil (Figure 5). When compost was added to the biochar, there was a significant difference between strain 110 and strain 136. Buckets treated with strain 110 yielded better than that of strain 136. When phosphorus was added to the biochar, there was a significant difference among the two strains of Brady rhizobium, of which strain 110 produced higher grains than that produced by strain 136. In general, in the amended soils, the *Bradyrhizobium* strain 110 performed significantly better than strain 136. In most treatments, the strain 136 was not significantly different from non-inoculated treatment.

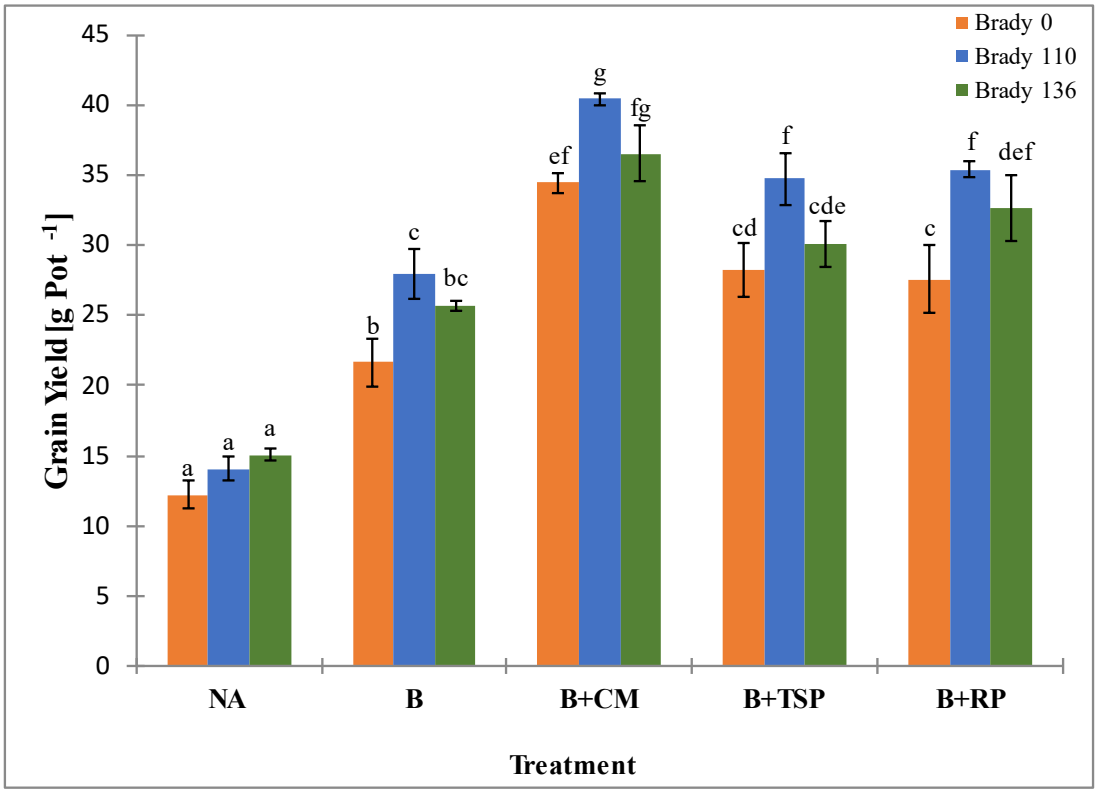

Figure 5: Effect of soil amendment and rhizobium inoculant on the grain yield of soybean. Error bars represent SEM

4.7 Plant nutrient concentration

4.7.1 Phosphorus

The amount of phosphorus was more in plants grown on the amended soil than the non-amended soil in some cases (Table 3). Phosphorus was seen to be more in plants grown on biochar + compost amended soil with 1.42 g kg^{-1} followed by plants grown on biochar + triple super phosphate having 1.15 g kg^{-1} with Brady 110 and Brady 0, respectively. However, the least amount of phosphorus was recorded in a plant grown on biochar + rock phosphate having 0.72 g kg^{-1} with Brady 136 (Table 3).

4.7.2 Potassium

Plants grown on biochar + triple super phosphate amended soil had more potassium than the other plants with 17.80 g kg^{-1} followed by plants grown on biochar only recording 15.81 g kg^{-1} with Brady 110. The least amount of potassium was seen in soil amended with biochar + compost, recording 10.88 g kg^{-1} under Brady 136 (Table 3).

4.7.3 Sodium

Reference to Table 3, the amount of sodium was more in plants grown on soil amended with biochar + triple super phosphate having 1.61 g kg^{-1} followed by plants grown on biochar + compost recording 1.46 g kg^{-1} all with Brady 136. The least amount of sodium was recorded in plants grown on soil amended with biochar + rock phosphate recording 0.50 g kg^{-1}.

4.7.4 Aluminum

The amount of aluminum was found to be more present in plants grown on biochar + compost amended soil with Brady 110 recording 1.42 g kg^{-1} followed by plants planted on biochar + triple super phosphate recording 1.15 g kg^{-1} with Brady 0 and plants planted on biochar + triple super phosphate with Brady 136 recording 1.13 g kg^{-1} (Table 3). Among soils that are non-amended, plants on soils with Brady 136 recorded the least amount of aluminum. However, in general, the least amount was recorded by plant grown

on soil amended with biochar + rock phosphate with Brady 136 having 0.72 g kg^{-1} (Table 3).

4.7.5 Calcium

Plants grown on soils amended on biochar + compost with Brady 136 and biochar + compost with Brady 110 recorded the highest values of 14.76 g kg^{-1} and 13.84 g kg^{-1} for calcium after the experiment respectively (Table 3). The plant grown on non-amended soil with Brady 110 recorded the least amount of calcium.

4.7.6 Magnesium

The maximum amount of magnesium was recorded in plants grown on soil amended with biochar + compost with Brady 0 having 4.17 g kg^{-1} followed by biochar + compost with Brady 110 at 4.08 g kg^{-1} and biochar + rock phosphate with Brady 110 recording 4.07 g kg^{-1} (Table 3). Among all, plants grown on biochar with Brady 0 amended soil had the least magnesium of 3.43 g kg^{-1}.

4.7.7 Iron

Iron was found to be more in plants grown on soil amended with biochar + compost with Brady 136 having 0.81 g kg^{-1} followed by plants on biochar + rock phosphate amended soil with Brady 110 recoding 0.79 g kg^{-1} (Table 3). However, it was seen that plants on soil amended with biochar + triple super phosphate with Brady 110 had the minimum amount of iron of 0.33 g kg^{-1}.

Table 4: ...t of soil amendment on plant nutrient concentration after production

T...nents	Al g/kg	Ca g/kg	K g/kg	Fe g/kg	Mg g/kg	Na g/kg	P g/kg
N... Brady 0	0.97 ± 0.2^{a}	13.23 ± 0.77^{a}	13.70 ± 0.57^{bc}	0.68 ± 0.04^{b}	3.58 ± 0.12^{a}	0.53 ± 0.04^{a}	0.97 ± 0.06^{ab}
N... rady110	0.86 ± 0.17^{a}	10.72 ± 0.78^{a}	15.24 ± 0.12^{ab}	0.45 ± 0.01^{cd}	3.52 ± 0.13^{a}	0.76 ± 0.06^{a}	0.86 ± 0.02^{a}
N... rady136	0.84 ± 0.12^{a}	13.19 ± 0.71^{a}	13.02 ± 0.79^{bc}	0.86 ± 0.02^{a}	3.52 ± 0.24^{a}	0.73 ± 0.04^{a}	0.84 ± 0.03^{a}
P... dy 0	1.07 ± 0.01^{a}	11.72 ± 1.03^{a}	12.71 ± 1.47^{c}	0.63 ± 0.07^{bc}	3.43 ± 0.27^{a}	0.60 ± 0.06^{a}	1.07 ± 0.05^{ab}
P... dy 110	0.90 ± 0.01^{a}	12.31 ± 0.9^{a}	15.81 ± 0.43^{ab}	0.42 ± 0.04^{abc}	3.80 ± 0.34^{a}	0.61 ± 0.03^{a}	0.90 ± 0.05^{a}
P... dy 136	0.95± 0.01^{a}	12.96 ± 0.43^{a}	12.49 ± 1.43^{c}	0.52 ± 0.04^{c}	3.55 ± 0.04^{a}	0.69 ± 0.01^{a}	0.95 ± 0.19^{ab}
B... +Brady0	0.98 ± 0.01^{a}	13.15 ± 1.18^{a}	15.68 ± 1.38^{ab}	0.41 ± 0.01^{cd}	4.17 ± 0.18^{a}	1.46 ± 0.18^{b}	0.98 ± 0.02^{a}
B... +Brady110	1.42 ± 0.01^{a}	13.84 ± 0.20^{a}	13.00 ± 1.62^{bc}	0.42 ± 0.02^{cd}	4.08 ± 0.20^{a}	0.67 ± 0.03^{a}	1.42 ± 0.09^{b}
B... +Brady136	0.76 ± 0.02^{a}	14.76 ± 1.66^{a}	10.88 ± 0.82^{d}	0.81 ± 0.05^{a}	3.75 ± 0.19^{a}	0.65 ± 0.07^{a}	0.76 ± 0.05^{ab}
B... P+Brady 0	1.15 ± 0.21^{a}	12.40 ± 1.09^{a}	17.80 ± 0.34^{a}	0.45± 0.07^{cd}	3.88 ± 0.12^{a}	1.61 ± 0.07^{b}	1.15 ± 0.19^{a}
B... P+Brady110	1.04 ± 0.01^{a}	10.99 ± 0.93^{a}	13.31 ± 0.76^{bc}	0.33 ± 0.01^{d}	3.68 ± 0.17^{a}	0.70 ± 0.04^{a}	1.04 ± 0.19^{ab}
B... P+Brady136	1.13 ± 0.11^{a}	13.74 ± 0.77^{a}	14.39 ± 0.49^{b}	0.57± 0.08^{c}	3.84 ± 0.31^{a}	0.51 ± 0.04^{a}	1.13 ± 0.04^{ab}
B... -Brady 0	0.86 ± 0.01^{a}	13.15 ± 1.1^{a}	12.88 ± 0.94^{c}	0.43 ± 0.03^{cd}	3.91 ± 0.11^{a}	0.50 ± 0.04^{a}	0.86 ± 0.07^{a}
B+RP+Brady 110	0.81 ± 0.01^{a}	13.21 ± 0.72^{a}	13.21 ± 0.88^{bc}	0.79 ± 0.06^{ab}	4.07 ± 0.10^{a}	0.88 ± 0.02^{a}	0.81 ± 0.07^{a}
B+RP+Brady 136	0.72 ± 0.01^{a}	12.04 ± 0.94^{a}	13.66 ± 1.19^{bc}	0.66 ± 0.01^{b}	4.05 ± 0.27^{a}	0.59 ± 0.05^{a}	0.72 ± 0.05^{a}

4.8 Soil Carbon: Nitrogen

The application of the various soil amendment had a significant ($P < 0.0001$) impact on soil carbon but had no significant influence ($P < 0.733$) on the soil nitrogen. However, the carbon to nitrogen ratio was generally affected significantly ($P < 0.0001$) by the various soil amendment. It was seen that the carbon content was more in soil treated with biochar only (0.98 %), and the least carbon content was seen in soil without amendment (0.59 %), both under Brady 136. Nitrogen was also seen to be more in soil amended with biochar only (0.078 %) and the least was obtained in soil amended with biochar + triple supper phosphate (0.07 %), all under Brady 110. Indeed, considering the carbon to nitrogen ratio, biochar amended soil with Brady 136 inoculated seed gave a higher carbon-nitrogen ratio while the control recorded the lowest soil carbon-nitrogen ratio. The complete data is shown in table 4.

Table 5: Soil Carbon, Nitrogen and their ratio after harvest

Treatment	C (%)	N (%)	C: N
NA+ Brady 0	0.630 ± 0.34^{ab}	0.067 ± 0.003^{a}	9.375 ± 0.30^{ab}
NA+ Brady 110	0.613 ± 0.03^{a}	0.072 ± 0.006^{a}	8.465 ± 0.34^{a}
NA+ Brady 136	0.597 ± 0.03^{a}	0.067 ± 0.002^{a}	8.893 ± 0.32^{a}
B+ Brady 0	0.925 ± 0.01^{ef}	0.077 ± 0.001^{a}	12.067 ± 0.18^{de}
B +Brady 110	0.835 ± 0.04^{de}	0.078 ± 0.004^{a}	10.744 ± 0.14^{c}
B + Brady 136	0.980 ± 0.05^{f}	0.075 ± 0.003^{a}	13.099 ± 0.18^{e}
B + CM + Brady 0	0.794 ± 0.06^{cd}	0.073 ± 0.006^{a}	10.887 ± 0.17^{c}
B +CM + Brady 110	0.747 ± 0.05^{bcd}	0.072 ± 0.005^{a}	10.297 ± 0.25^{bc}
B +CM + Brady 136	0.691 ± 0.03^{abc}	0.066 ± 0.004^{a}	10.467 ± 0.11^{bc}
B + TSP +Brady 0	0.763 ± 0.05^{cd}	0.074 ± 0.005^{a}	10.312 ± 0.16^{bc}

B +TSP +Brady 110	0.757 ± 0.01^{cd}	0.07 ± 0.002 ^{a}	10.860 ± 0.25^{c}
B +TSP +Brady 136	0.776 ± 0.07^{cd}	0.074 ± 0.005$_{a}$	10.532 ± 0.16^{c}
B + RP +Brady 0	0.785 ± 0.03^{cd}	0.074 ± 0.003^{a}	10.567 ± 0.16^{c}
B + RP +Brady 110	0.825 ± 0.04^{de}	0.075 ± 0.004^{a}	10.996 ± 0.28^{dc}
B + RP +Brady 136	0.820 ± 0.06^{de}	0.076 ± 0.006^{a}	10.821 ± 0.05^{c}

4.9 Plant Carbon, Nitrogen and their ratio

In general, the soil amendment did not significantly affect the plant carbon and nitrogen and the carbon-nitrogen ratio. Plant grown on soil treated with biochar + compost of which the seed was inoculated with Brady 110 inoculant recorded (43.02 %) and (2.31 %) as the highest carbon and nitrogen respectively. The carbon-nitrogen ratio ranged from 18.58 to 26.53, of which plant grown on soil treated with biochar only and biochar with rock phosphate recorded the least and the highest carbon-nitrogen ratio respectively.

Table 6: Total C, N and C: N at maturity of soybean (above ground biomass) as affected by soil amendment and inoculant

Treatment	C (%)	N (%)	C: N
NA + Brady 0	42.47 ± 0.30	1.90 ±0.16	22.64 ± 1.81
NA + Brady 100	42.72 ± 0.56	1.85 ± 0.20	23.50 ± 2.07
NA + Brady 136	40.58 ± 2.20	1.81 ± 0.23	22.84 ± 1.80
B + Brady 0	42.35 ± 0.96	2.29 ± 0.13	18.58 ± 0.63
B + Brady 110	42.74 ± 0.55	2.06 ± 0.14	20.93 ± 1.13
B + Brady 136	41.99 ± 0.81	1.94 ± 0.23	22.33 ± 3.02
B + CM +Brady 0	42.85 ± 0.19	2.03 ± 0.23	21.63 ± 2.10
B + CM +Brady 110	43.02 ± 0.31	2.31 ± 0.17	18.82 ± 1.32

B + CM +Brady 136	41.74 ± 0.93	1.67 ± 0.21	26.01 ± 4.11
B + TSP +Brady 0	42.51 ± 0.62	2.10 ± 0.10	20.35 ± 1.24
B + TSP +Brady 110	42.99 ± 0.13	2.23 ± 0.38	20.67 ± 4.10
B + TSP +Brady 136	42.45 ± 0.76	2.04 ± 0.36	22.57 ± 5.03
B + RP +Brady 0	42.46 ± 0.42	1.83 ± 0.23	23.77 ± 2.50
B + RP +Brady 110	36.94 ± 5.12	1.70 ± 0.10	26.53 ± 2.55
B + RP + Brady 136	41.99 ± 0.36	1.63 ± 0.19	26.35 ± 2.73

4.10 Soil pH and Electrical Conductivity

The soil amendment had no significant influence ($P > 0.523$) on the soil pH as well as the inoculant applied ($P > 0.242$). The interaction between the inoculant and the soil amendment also had no significant influence ($P > 0.919$). The soil pH among all treatments ranged between 5.0 and 5.31 (Table 6). The highest acidic soil was obtained from soil treated with biochar in combination with triple super phosphate fertilizer (5.0) inoculated with Brady 136. The least acidic soil was obtained from non-amended soil (5.316) without inoculant. Both the soil amendment ($P > 0.08$) and the inoculant ($P > 0.48$) had no significant influence on the electrical conductivity of the soil. The interaction between the soil amendment and the inoculant also had no significant influence ($P > 0.07$) on the electrical conductivity of the soil. The electrical conductivity (EC) also ranges between 73.5 and 106.3 µS cm^{-1} (Table 6). In combination with Brady 110, it was observed that biochar recorded the least electrical conductivity while the highest EC was obtained from biochar +compost + Brady 110 inoculant.

Table 7: pH and Electrical Conductivity as affected by soil amendment

Treatment	pH	Electrical Conductivity (EC) [µS cm^{-1}]
NA + Brady 0	5.316 ± 0.07	75.82 ± 5.46[a]
NA + Brady 100	5.162 ± 0.07	84.03 ± 8.99[a]
NA + Brady 136	5.174 ± 0.09	83.07 ± 10.58[a]
B + Brady 0	5.117 ± 0.07	87.82 ± 7.46[a]
B + Brady 110	5.186 ± 0.12	73.57 ± 13.83[a]
B + Brady 136	5.123 ± 0.04	93.05 ± 8.06[a]
B + CM +Brady 0	5.182 ± 0.05	98.32 ± 12.31[a]
B +CM +Brady 110	5.187 ± 0.03	106.37 ± 15.99[a]
B +CM +Brady 136	5.076 ± 0.14	100.13 ± 23.73[a]
B + TSP +Brady 0	5.180 ± 0.02	74.00 ± 8.91[a]
B +TSP +Brady 110	5.188 ± 0.04	90.45 ± 16.82[a]
B +TSP +Brady 136	5.009 ± 0.03	82.07 ± 23.76[a]
B + RP +Brady 0	5.087 ± 0.07	73.08 ± 10.82[a]
B + RP +Brady 110	5.179 ± 0.16	81.35 ± 12.90[a]
B +RP + Brady 136	5.071 ± 0.09	84.93 ± 5.04[a]

4.11 Cation Exchange Capacity

The Cation Exchange Capacity (CEC) of the soil at the end of the experiment was analyzed. The various soil amendment had a significant influence ($P < 0.002$) on the soil's cation exchange capacity. However, the interaction between the soil amendment and the inoculant could not influence the CEC significantly. The soil amendment led to a higher CEC than that

of the non-amended soil. Statistically, when the soil was amended the were no difference among the amended soils.

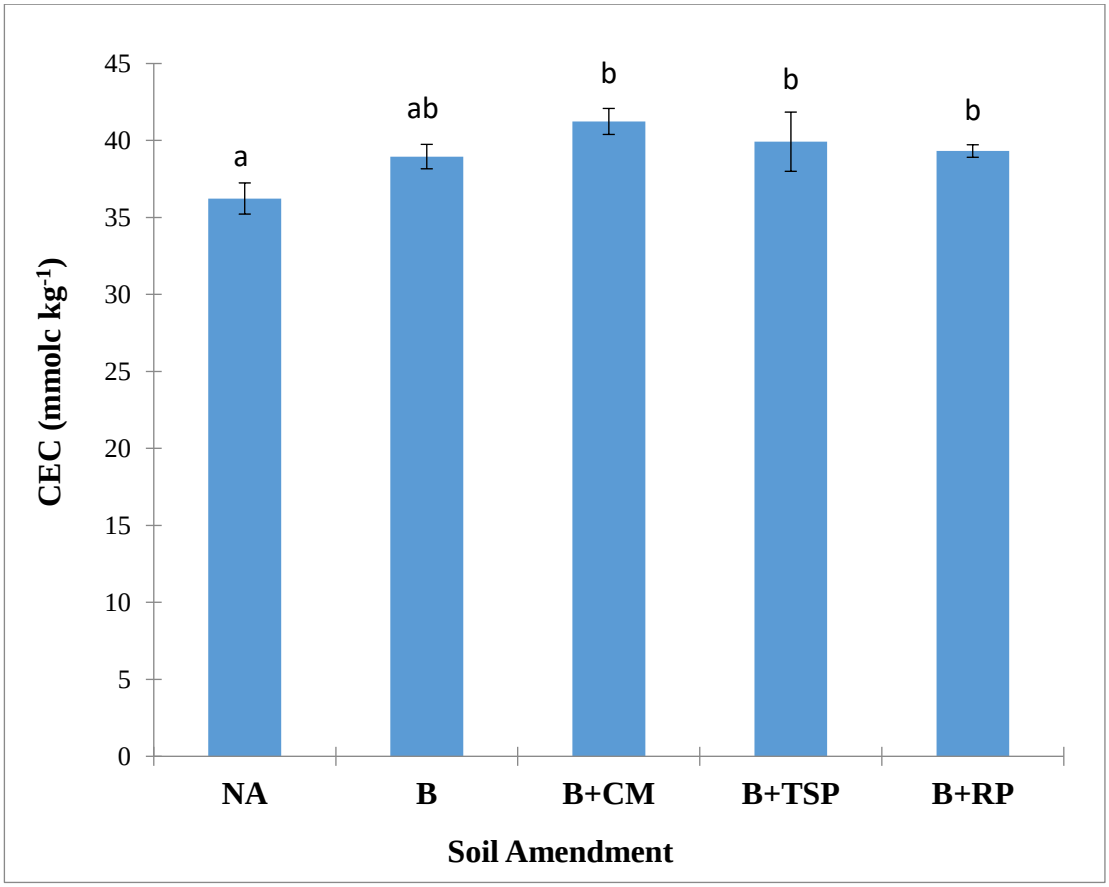

Figure 6: Effect of soil amendment on cation exchange capacity. Error bars represent SEM.

CHAPTER FIVE

5.0 DISCUSSION

5.1 Plant Height

The plant height, which determines the growth form, competitive vigor and the reproductive size of a plant was measured from two to eight weeks after planting. The sung pugun soybean plant height was influenced by the soil amendments; biochar, compost, rock phosphate, and triple super phosphate except for the first two weeks. In this study, there were no differences in the plant height growth at the early stages in response to the various soil amendment. As weeks went by, the amended soils with compost and phosphorus increased the plant height more than non-amended soil. Among the amended soils, compost added to biochar increased the plant height more than that of the two phosphate types, triple super phosphate and rock phosphate. Previous studies (Turuko and Mohammed 2014; Mitchel *et al*. (2015) have shown that biochar stimulated plant growth and increased fertilizer efficiency, especially when biochar is combined with organic fertilizers such as compost. The ability of biochar + compost to improve the fertility of the soil by supplying adequate nutrient to improve plant growth has been observed by Turuko and Mohammed (2014). According to Mitchel *et al*. (2015), biochar has the ability to retain water and nutrient and compost, as reported by Adeyemo and Agele (2010), supplies nutrients to the soil and has a lasting effect more than inorganic fertilizers because they are slow releasers of nutrient. This attribute may have contributed to the improved soybean height under biochar + compost pots than their corresponding biochar + phosphorus counterpart. Also, soils fertilized with compost had higher contents of soil organic matter. More so, soil organic matter improves soil fertility by providing nutrients through mineralization and acting as a habitat for soil microorganisms (Fischer and Glaser 2012). These microorganisms play an essential role in decomposing

organic matter and contribute to the cycling of nutrients, leading to the availability of nutrients for plant use. The application of biochar helps to increase crop productivity through increasing soil nutrient supply, microbial activity and decreasing nutrient leaching (Steiner *et al*., 2008; Major *et al*., 2010; Liu *et al*., 2013; Ventura *et al*., 2013; Graber *et al*., 2015). These attributes of both compost and biochar factored into the rapid growth of plant grown on soils amended with biochar and compost.

In our study, the benefits of inoculation were not felt in terms of plant height. A similar result was observed by Ohsowski *et al*. (2018), who reported that biochar + compost was more beneficial than other amendment combinations but biochar + compost with inoculant were not more beneficial to plant growth than those without inoculant and this could be due to the existence of indigenous rhizobia strains in the soil.

5.2 Nodulation and their effectiveness

Legume nodules are very complex organs, having several interacting processes that operate at diverse levels, including, at least, carbon metabolism, oxygen supply, cellular redox and transmembrane transport (Udvardi and Poole, 2013; Van Hameran *et al*., 2013; Esfahani *et al*., 2014). In this research, soybean responded positively to inoculant regarding nodule formation in both the amended and non-amended treatments. The addition of *Brady rhizobium* inoculant might have increased the bacteria population responsible for legume-bacteria symbiosis for biological nitrogen production, as a result of improved number of nodules in the inoculated pots. A comparable report was made by Lu *et al*. (2017), who also testified higher nodules count when *Brady rhizobium* was added to highly degraded soils. Considering the two strains of *Brady rhizobium*, Brady 110 produced more nodules than Brady 136. This result is in line with other researchers such as Kumaga and Etu-Bonde,

(2004) and Aliyu *et al*. (2013), who testified that inoculation with *Bradyrhizobium japonicum* strain 110 inoculant increased nodulation of soybean than any other Brady rhizobium strain. This may be due to Brady 110 being a good senser of flavonoids secreted by the roots of the host plant, which makes them attach themselves to the root hairs to facilitate nodule formation. Comparatively, soil amendment further increased the number of nodules by 31.34 % more than the non-amended pots. According to Bargaz *et al*. (2018) other nutrient sources that may be contained in the soil amendment materials are carbon, phosphorus, potassium, iron, manganese etc., and they help to increase the efficiency of the bacteria inoculant. Among the soil amendment, the sole application of biochar gave the highest nodule count especially in Brady 110. Biochar may have adjusted the soil's physical and chemical properties to enhance the microbial inoculant activities and therefore increase effective nodulation. Other authors (Quilliam *et al*., 2013; Palansooriya *et al*., 2019) suggested that biochar may increase microbial activity by providing habitat for microorganisms and altering microbial mediated processes in soil. The addition of compost to biochar did not perform better than the sole biochar amended soil as the effectiveness of the nodules decreased by 22.8 %. According to Adeli *et al*. (2005), the use of compost in legume crops, including cowpea, soybean reduces symbiotic N_2 fixation, which is the main factor for nodule production. Compost provides the crops with enough nitrogen and nitrogen from biological nitrogen fixation is not an urgent need of the crop since crop pays for it in the form of carbohydrate. This attribute of compost may have contributed to the decrease in the nodules produced and the effectiveness of the nodules.

5.3 Pod Length

It is known that the pod length of the plant can determine the number of seeds in each pod (Osei, 2017). Also, it can be another critical factor that can also be based on to conclude on the potential grain yield. The results revealed that the soil amendment and the inoculant all had a positive effect on the pod length of the plant. This could be as a result of the various amendments applied to the soil. The addition of biochar to the soil improves the carbon concentration of the soil as it is a carbon-based material. Soil microbes use this carbon as a form of energy to mineralized nutrients in the soil, making them available for plant use. Also, biochar improves nutrient retention and the soil's water holding capacity. The addition of compost to the soil improves the soil's organic matter, which enriched the soil with nutrients, making it fertile for plant growth. (Buresh and Dobermann, 2009) Moreover, compost is a slow releaser of nutrients and as a result, released nutrient into the soil for a more extended period to be used by the plants (Mitchell *et al*., 2015). Adding phosphatic fertilizer to the soil improved the phosphorus content of the soil, which the plant needed to perform its functional activities such as cell division, energy transformation and increase its yield attributes such as the number of leaves, pod length etc. These attributes of the various amendments helped improved the pod length of plants grown on the amended soils than those on the non-amended soils. Considering the inoculant application, the inoculated plants performed better than the non-inoculated plants in terms of both the pod length and the number of seeds per pod. Among the inoculated seeds, Brady 136 improved the pod length 1.75 % more than Brady 110.

5.4 Grain Yield

Shahid *et al*. (2009) have postulated that the final grain yield is a collective contribution of its various growth and yield parameters, which are influenced by various agronomic practices and ecological circumstances. In this study, soil was amended at two levels; biochar with compost or phosphorus and planted with inoculated seeds. The results revealed that the soil amendments and the inoculants significantly impacted the soybean grain yield. The amendment of the soil led to a higher yield than the non-amended soils. The introduction of the second level amendment, compost or phosphorus, led to improved grain yield. The results revealed that the addition of compost to the biochar led to higher grain yield than when TSP or Rock phosphate was added. This is as a result of compost having the ability to release nutrients to the plants at the right time or being a slow releaser of nutrients to plants. The combination of biochar with compost among the treatments improved soil fertility, which increased the grain yield. This is in line with Ganeshamurthy and Sammi Reddy (2000), who stated that compost application significantly influenced dry matter production and grain yield of soybean. According to Nyende (2001) and Olupot *et al*. (2004), this influence of compost can be attributed to the fact that compost increases the dissolution of nutrients, particularly phosphorus, in the soil and contains a high amount of organic matter, which increases the moisture retention of the soil. Deksissa *et al*. (2008) and Adeyemo and Agele (2010) also reported that, apart from the capability of compost to release nutrients, compost and biochar improve the physical properties of soil as soil bulk density, water holding capacity, infiltration and aeration. Compost and biochar provide an organic nutrient source and organic matter which improves soil conditions such as water and nutrient holding capacities, pH, cation exchange capacity, micronutrient concentrations (Mutuo *et al*., 2000; Vanlauwe *et al*., 2001; Zingore *et al*., 2008) Even though, the addition of compost to biochar appeared to have

increased grain yield over phosphorus application, the presence of phosphorus fertilizers brought vast improvement in yield over biochar only and the non-amended soils. In the weathered acidic soils in Sub-Saharan Africa, phosphorus is fixed (Bationo and Mokwunye 1991; Nakamura *et al*., (2013), making P application relevant. The presence of biochar in phosphorus applied soils brought synergistic interaction that culminated in P for growth and yield. The study revealed that it did not matter whether rock phosphate with lower solubility or TSP is used. Both, in the presence of biochar benefit the crop. The importance of inoculation was felt on amended soils. In the non-amended soils, yield was very low and did not matter the strain of Brady inoculant used. Indeed, the benefit of inoculation was not observed in non-amended soil. On the amended soils, Brady 110 strain proved superior over Brady 136. The importance of inoculation has been made by Solomon *et al*. (2012) and Ahlijah *et al*. (2017), who submitted that inoculation of seeds with pertinent strains of microorganisms before planting was significant, particularly in territories where legume crops were going to be grown for the first time on the land. Ahiabor *et al*. (2014) and Rechiatu *et al*. (2015) reported significant increases in soybean grain yield after inoculation of soybean in the Northern savanna zones of Ghana. Before that, Van Kessel and Hartley (2000), Hungria and Mendes, (2015), have reported that soybean reacts most powerfully to inoculation when brought into new territories where soils lack appropriate rhizobia. In our study, the benefits of inoculation were not felt on non-amended soils, and this could be due to the presence of indigenous rhizobia strains in the soil. Legumes like cowpea, groundnut and soybean have been grown in the soil used over many years. Pre-existing Rhizobia populations in the soil might have been inimical to the introduced strain. However, the benefit of the inoculation was felt when the soils were amended with biochar and others.

5.5 Plant nutrient concentration

The nutrient concentration of the soybean variety (Sung pugun) was considered after the experiment. This parameter was considered in order to obtain the amount of nutrient stored in the plant. Higher nutrient accumulation was obtained from plants grown on the amended soils than the non-amended soils. The addition of biochar, compost and phosphorus fertilizer to the soils might have improved the soil fertility and made nutrients available for plant use. According to Arif *et al*. (2017), biochar integration with organic and inorganic fertilizer enhanced crop productivity and soil quality. Considering nutrients such as potassium, phosphorus, aluminum, iron, magnesium and calcium, an average of 13.2, 0.84, 0.84, 0.45, 3.52 and 10.72 g kg^{-1} were obtained as the amount of nutrients reserved in the soybean plant respectively when the soil was not amended. When the soil was amended, an average of 17.8, 1.42, 1.42, 0.79, 4.17 and 14.76 g kg^{-1} was obtained as reserved nutrients in the plant respectively. The various amendments improved the cation exchange capacity and enriched the soil with nutrients necessary for essential plant nourishment. Also, the amended soil provides favourable conditions for bacterial proliferation, which increased microbial composition in soil. These attributes may have contributed to P, K, Na, Al, Fe and Mn in the amended soils. The addition of biochar to the soil may also induce changes in nutrient availability. It may provide additional N (Atkinson *et al*., 2010) and P (Olmo *et al*., 2016) or soil organic carbon (Munera-Echeverri *et al*., 2020) sources for microbial proliferation in the soil to improve the fertility of the soil for better plant growth. According to Chan *et al*. (2007), biochar can include mutable concentrations of alkalinity that is straightly added as calcium (Ca), potassium (K), hydroxides into the soil. Even though, Okalebo and Woomer (2005) testified that nutrient such as potassium might be lost from manure through leaching, biochar and compost, according to Lehmann *et al*. (2011) and Mitchell *et al*. (2015) have the ability

to retain water and nutrients. Moreover, it can also adjust soil pH to offer a conducive habitat for soil microorganism for the mineralization of organic matter for a better plant growth. As a result, adequate nutrients are made available in the soil to be absorbed by plants, making it a choice for high yielding soybean production and improvement of savanna soils in Northern Ghana.

5.6 Plant carbon and nitrogen concentration of soybean biomass

Carbon (C) and nitrogen (N) concentrations of soybean biomass were measured in the study due to its potential as feed for animals and composting material. Plant C and N in general play a fundamental role in maintaining ecosystem structure and function (Talgre *et al.* 2017). Carbon forms the substrate and energy source of physiological and biochemical processes, whereas nitrogen is an essential component of plant proteins and nucleic acids (Yuan and Chen 2015). Although not statistically different, the study revealed an increase of up to 12 % in nitrogen concentration of soybean grown on soil with biochar and compost application. According to Atilio and Causin (1996) plant, nitrogen metabolism is controlled by the plant's nitrogen demand and the nitrogen concentration supplied to the soil. The primary source of nitrogen to plant is soil (Fageria and Baligar 2005), which are transformed into the plant system through nitrogen fixation, nitrification and mineralization (ammonification). Ahmad *et al*. (2013) discussed bacterial nitrogen fixation as another important nitrogen source to plant, especially in leguminous plants through inoculant such as Rhizobium. As similarly observed in the soil, Brady rhizobium *japonicum* did not affect the plant nitrogen concentration, probably due to the short duration of the study. The addition of phosphorus fertilizer stimulated the nitrogen content of soybean biomass; however, TSP was more beneficial to plant nitrogen concentration with an average percentage of 2.13 compared to 1.72 of plants grown on RP amended soil. The finding supports Rivaie (2008) report, which

explained that TSP is a readily inorganic phosphorus source made available to the plant immediately after application, whiles dissolution of RP occurs slowly.

In general, the carbon concentration of plant tissue is derived from a photosynthetic process where carbon dioxide is converted to carbohydrate in the presence of water from the soil. Hence, the addition of soil amendment materials and inoculation did not influence plant carbon concentration. The soil amendments and inoculant did not influence the carbon and nitrogen ratio (C: N) of the plant as equally observed in the carbon and nitrogen content independently. The revealed C: N of soybean biomass which was between 18 and 26 in the study, is, according to Brady and Weil (2010) a near perfect balance of 1:24 being recommended C: N concentration to satisfy N requirement for decomposing microorganisms, thus eliminating any period of net N immobilization.

5.7 Soil carbon

Carbon plays a significant role in the soil ecosystem, providing energy to mediate biological activities and nutrient cycling. In general, about 80 % of the terrestrial ecosystem carbon worldwide is hosted in the soil (Lal, 2008; Le Quere *et al*., 2016). The addition of biochar in this study significantly increased the soil carbon concentration regardless of the inoculant applied. This is explained by the fact that, biochar is a carbon-based material and therefore adds direct carbon to the soil. Several studies have equally reported improved carbon concentration following biochar application to poor acidic soils (Mensah and Frimpong, 2018). Further, the carbon in biochar is more stable and, therefore, can sequester atmospheric carbon to the soil (Mašek *et al*., 2019). Although the co-application of biochar and compost was also beneficial than non-amended control, it was about 18.5 % less compared to soil with the sole application of biochar. This finding agrees with Wu *et al*. (2017) who reported increased carbon content when biochar combined with other organic amendments like

compost and manure. A possible explanation might be that compost supplies a more labile carbon which will readily be mineralized and taken up by either the plant, microorganism or emitted to the atmosphere in the form of carbon dioxide. The results again revealed that biochar + phosphatic fertilizer improved the carbon concentration than the non-amended soil but could not perform better than biochar + compost amended soil. The addition of compost improved the organic matter content of the soil. This result is in line with Singh *et al*. (2015), who reported that compost's application provides higher organic matter, which improves soil organic carbon than the application of inorganic fertilizers.

5.8 Soil nitrogen

Nitrogen is the most abundant element in the atmosphere. In agricultural crop production, nitrogen is usually the most limiting crop nutrient for plant growth (Ohyama, 2010). In this study, the amendment of the soil led to higher nitrogen than the non-amended soils. The soil amendment increased the soil nitrogen 7.4 % more than the non-amended soil. This could be as a result of the addition of biochar to the amended soils. It is believed that most of the nitrogen in the soil has been lost through leaching. However, the addition of biochar to the soil has the ability to improve the water holding capacity of the soil preventing the nutrients from leaching. Considering the amended soil, the co-application of biochar + compost improved the soil nitrogen more than biochar + rock phosphate or triple super phosphate. The addition of compost to the soil adds nitrogen to the soil but this nitrogen needs to be mineralized before plants can use it. This process takes time to release the nitrogen, making compost a slow releaser of nutrient, making nitrogen available in the soil for a longer period. Even though the co-application of biochar + rock phosphate or triple super phosphate improved the nitrogen content of the soil more than the non-amended soil, much research has

not been done on the principles on how phosphatic fertilizers affect the nitrogen content of the soil.

5.9 Soil pH and electrical conductivity

Soil pH is the measure of the acidity or alkalinity of the soil. After this experiment, it was observed that statistically, the soil pH was not affected by the soil amendments. Similarly, biochar could not affect the soil pH positively in a study that involves biochar application at a rate of 20ton ha^{-1} in the acidic soils of Northern Ghana (Haring *et al*., 2017). Although pH was not significantly affected in the study, biochar addition caused an increased in the soil pH. This could be attributed to the liming potential of biochar as a result of the higher inherent pH of biochar. Also, the treatment could not influence the soil pH due to the lower production temperature, lower pH and electrical conductivity of rice husk biochar. This is in line with observation made by Shetty and Prakash (2020) who reported that rice husk biochar decreased soil pH under the effect of different biochar on acid soil and growth parameters of rice plant. The soil pH ranged between 5 to 5.3 which indicates that, the soil was strongly acidic. Depending on the soil test categories, a soil with pH between 5.1 – 5.4 is acidic (Schroder *et al*., 2011). According to Walker *et al*. (2018), the higher the electrical conductivity, the more salt buildup around the root zone of the plant which can easily cause injury to the plant, reduction in crop yield and also causing long term damage to the soil by changing the soil structure. The various soil amendment and the inoculant could not affect the electrical conductivity significantly. The electrical conductivity per this experiment ranged between 73.08 – 106.37 mSm^{-1}. The amendment of the soil with biochar led to a higher electrical conductivity than the non-amended soil. The introduction of the second level amendment, phosphorus or compost increased the electrical conductivity of the soil. The results further revealed that the addition of compost to the biochar led to a higher electrical

conductivity than when the rock phosphate or triple supper phosphate were added. This could be attributed to the addition of the compost which adds nutrients and salt to the soil. The inoculation had no significant impact on the electrical conductivity of the soil.

5.10 Cation Exchange Capacity

Cation Exchange Capacity (CEC) is a soil chemical property which is a degree of the soil's capacity to hold positively charged ions. Also, the ability of the soil to hold or store cations is as a result of the soil's cation exchange capacity. The results revealed that the soil's Cation Exchange Capacity was highly influenced when the soil was amended than the non-amended soil. This improvement of the CEC could be attributed to the addition of biochar to the soil due to its high specific surface area due to its porous structure. Also, the slow oxidation of biochar in the soil could increase the number of the carboxylic group, increasing the CEC of the soil. This result is in line with Mia *et al*. (2017), who reported that the CEC increased with aging due to the increased carboxylation of carbon through abiotic oxidation of biochar. Even though, the amended soil improved the CEC of the soil, the co-application of biochar + compost increased the CEC of the soil 3.31 % and 4.94 % more than biochar + triple super phosphate and biochar + rock phosphate respectively. This improvement may be due to the addition of compost, which can boost the soil's cation exchange capacity. A simple explanation might be that compost accumulates extra electrons and form anions which are negatively charged particles. These anions attract and hold positively charged particles called cations by raising the soil's CEC making nutrients less likely to leach from the soil.

CHAPTHER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

From the results obtained, it can be concluded that:

- *Brady rhizobium japonicum* inoculation was efficient in biomass production and grain yield of soybean when compared with the non-inoculated seeds. Statistically, there was not much difference between the two *Brady rhizobium* strains. However, Brady 110 recorded a slightly higher nodule number than seeds that were inoculated with Brady 136.
- The plant morphology and growth indicator parameters including plant height, the number of leaves, nodule numbers and effectiveness were significantly increased by both the lone application of rice husk biochar and its combination with compost and phosphorus. These increased growth parameters further resulted in a substantial improvement of the total grain yield of soybean compared to the unamended control.
- Plant nutrient concentrations of soybean tissue were influenced by soil treatment but not by inoculation. The combination of biochar and compost greatly influenced the nutrient in the soybean tissue than when it was combined with TSP and RP. The concentration of calcium and potassium formed the highest amount of nutrient in the soybean tissue followed by magnesium and aluminum respectively.
- Biochar improved soil quality by increasing the soil's organic carbon, total nitrogen, and cation exchange capacity. Although not significant, the pH was relatively higher in the biochar amended soil. In all soil parameters, combining the biochar with the other amendments; that is compost, triple superphosphate and rock phosphate proved more beneficial to soil fertility than when the biochar was applied alone.

6.2 Recommendations

Biochar applied alone and in combination with compost improved the soil fertility, leading to higher grain yield. This is the first option of recommendation to soybean farmers. However, where compost availability is limited, phosphorus fertilizer can be used in combination with biochar.

Bradyrhizobium japonicum (Brady 110 and 136) helped to improve nodulation and by extension nitrogen biosynthesis. In some cases, the Brady 110 was better than 136. The study recommends the two strains, but where the two are available Brady 110 is more beneficial.

The extension service of the Ministry of Food and Agriculture should educate farmers on the production and use of biochar, especially soybean farmers to improve their soil quality and yield.

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APPENDICES

Appendix 1: Analysis of variance for plant height two weeks after planting

Source	DF	Sum of squares	Mean squares	F	Pr > F
Amendment	4	3.943	0.986	0.892	0.487
Inoculant	1	1.125	1.125	1.019	0.325
Amendment*Inoculant	4	0.253	0.063	0.057	0.993

Appendix 2: Analysis of variance for plant height four weeks after planting

Source	DF	Sum of squares	Mean squares	F	Pr > F
Amendment	4	161.483	40.371	7.449	**0.001**
Inoculant	1	1.496	1.496	0.276	0.605
Amendment*Inoculant	4	1.549	0.387	0.071	0.990

Appendix 3: Analysis of variance for plant height six weeks after planting

Source	DF	Sum of squares	Mean squares	F	Pr > F
Amendment	4	351.018	87.755	11.088	**0.0001**
Inoculant	1	6.256	6.256	0.790	0.385
Amendment*Inoculant	4	1.515	0.379	0.048	0.995

Appendix 4: Analysis of variance for plant height eight weeks after planting

Source	DF	Sum of squares	Mean squares	F	Pr > F
Amendment	4	1369.848	342.462	23.145	**< 0.0001**
Inoculant	1	73.633	73.633	4.976	**0.037**
Amendment*Inoculant	4	7.687	1.922	0.130	0.970

Appendix 5: Analysis of variance for nodule number

Source	DF	Sum of squares	Mean squares	F	Pr > F
Amendment	4	4780.311	1195.078	15.827	**< 0.0001**
Inoculant	2	2206.533	1103.267	14.611	**< 0.0001**
Amendment*Inoculant	8	2955.022	369.378	4.892	**0.001**

Appendix 6: Analysis of variance for effective nodules

Source	DF	Sum of squares	Mean squares	F	Pr > F
Amendment	4	2911.467	727.867	3.046	**0.032**
Inoculant	2	2683.600	1341.800	5.616	**0.008**
Amendment*Inoculant	8	5943.733	742.967	3.110	**0.011**

Appendix 7: Analysis of variance for pod length

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Inoculation	2	0.763708	0.381854	8.338639	0.001319
Soil amendment	4	0.950838	0.23771	5.190919	0.002673
Inoculation: Soil amendment	8	0.1742	0.021775	0.475507	0.863647

Appendix 8: Analysis of variance for seeds per pod

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Inoculation	2	4.79E-30	2.39E-30	2.69E-29	1
Soil amendment	4	0.533333	0.133333	1.5	0.227169
Inoculation: Soil amendment	8	1.98E-29	2.48E-30	2.79E-29	1

Appendix 9: Analysis of variance for grain yield

Source	DF	Sum of squares	Mean squares	F	Pr > F
Amendment	4	2860.248	715.062	306.582	**< 0.0001**
Inoculant	2	242.974	121.487	52.087	**< 0.0001**
Amendment*Inoculant	8	46.658	5.832	2.501	**0.033**

Appendix 10: Analysis of variance for plant nutrient concentration

Source	DF	Sum of squares	Mean squares	F	Pr > F
Soil amendment	5	0.103	0.021	0.750	0.593
Inoculation	2	0.061	0.030	1.109	0.343
Soil amendment*Inoculation	7	0.177	0.025	0.923	0.503

Appendix 11: Analysis of variance for plant carbon nitrogen ratio

Source	DF	Sum of squares	Mean squares	F	Pr > F
Soil amendment	5	0.000	0.000	1.390	0.256
Inoculation	2	0.000	0.000	0.464	0.633
Soil amendment*Inoculation	7	0.000	0.000	0.358	0.919

Appendix 12: Analysis of variance for soil carbon

Source	DF	Sum of squares	Mean squares	F	Pr > F
Soil amendment	5	0.427	0.085	15.441	**< 0.0001**
Inoculation	2	0.004	0.002	0.323	0.726
Soil amendment*Inoculation	7	0.048	0.007	1.238	0.314

Appendix 13: Analysis of variance for soil nitrogen

Source	DF	Sum of squares	Mean squares	F	Pr > F
Soil amendment	5	0.000	0.000	1.390	0.256
Inoculation	2	0.000	0.000	0.464	0.633
Soil amendment*Inoculation	7	0.000	0.000	0.358	0.919

Appendix 14: Analysis of variance for soil carbon nitrogen ratio

Source	DF	Sum of squares	Mean squares	F	Pr > F
Soil Amendment	5	28.972	5.794	0.813	0.550
Inoculation	2	5.258	2.629	0.369	0.695
Soil amendment*Inoculation	7	62.183	8.883	1.246	0.310

Appendix 15: Analysis of variance for pH

Source	DF	Sum of squares	Mean squares	F	Pr > F
Soil amendment	5	0.088	0.018	0.853	0.523
Inoculation	2	0.062	0.031	1.486	0.242
Soil amendment*Inoculation	7	0.052	0.007	0.358	0.919

Appendix 16: Analysis of variance for electrical conductivity

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Inoculation	2	262.0619	131.031	0.74399	0.483779
Soil amendment	4	1583.216	395.8039	2.247362	0.087457
Inoculation: Soil amendment	8	2866.519	358.3148	2.034501	0.076127

Appendix 17: Analysis of variance for cation exchange capacity

Source	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Inoculation	2	79.51254	39.75627	8.051857	0.001588
Soil amendment	4	101.8188	25.45471	5.155355	0.002778
Inoculation: Soil amendment	8	89.9213	11.24016	2.276476	0.069075