EFFECTS OF SOIL AMENDMENTS AND RHIZOBIUM INOCULATION ON SOYBEAN NODULATION, GROWTH AND YIELD IN THE SEMI-DECIDUOUS FOREST AGRO-ECOLOGICAL ZONE OF GHANA

BY

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THIS THESIS IS SUBMITTED TO THE UNIVERSITY OF GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY IN CROP SCIENCE DEPARTMENT OF CROP SCIENCE COLLEGE OF BASIC AND APPLIED SCIENCES

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DECLARATION

I, Mohammed Alhassan hereby declare that except for the duly cited references of other researchers, this thesis I am submitting to the University of Ghana for Master of Philosophy Degree is my original research work carried out under supervision. Its entirety or part has not been previously presented elsewhere to another institution for the award of a degree.

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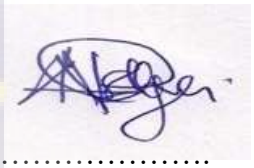

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DEDICATION

I dedicate this thesis to Almighty Allah for His protection and guidance throughout my education.

Special dedication also goes to my mother, Adama Mohammed of blessed memory.

May her soul continue to rest in perfect peace.

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ABSTRACT

Ghana's soybean cultivation is primarily restricted to the Guinea savanna and the forest/savanna transitional agro-ecological zones. Although soybean can be grown in the semi-deciduous forest zone, its productivity is limited due to low soil pH and limited nodulation. The study was conducted at the University of Ghana Forest and Horticultural Crops Research Centre at Kade in the semi-deciduous forest agro-ecological zone of Ghana between August and December, 2021.The objective of the study was to assess the combined effects of soil amendments, phosphorus fertilizer and rhizobium inoculation on soil chemical properties, nodulation, growth and yield of soybean. The experiment was laid in a split-split plot design with four (4) replications with main plot being soil amendments (No amendment, 2 tons/ha lime and 5 tons/ha rice husk biochar), subplot being P fertilizer at 0 and 20 kg P ha^{-1} and sub subplot with or without Rhizobium inoculation. Data on nodule number and effectiveness, shoot biomass, one hundred seed weight and grain yield were taken. Results from the study indicated that phosphorus application significantly influenced grain yield as grain yield was increased by about 60% due to P application. There was increase in soil pH from the initial 5.09 to 5.52 and 5.54 on plots that received biochar and lime respectively, 17 weeks after treatment application. The effect of inoculation on pH was also significant ($p < 0.05$). Rhizobium inoculation had significant effects on exchangeable K and Mg. The inoculated plots had exchangeable K and Mg values of 0.37 and 2.62 cmol $(_+)$/kg soil, respectively, while the values for the uninoculated plots were 0.33 and 3.31 cmol $(_+)$/kg soil for K and Mg respectively. Inoculation significantly influenced nodulation parameters such as nodule number, nodule effectiveness and nodule fresh weight of soybean. The application of rhizobium inoculant significantly ($p < 0.001$) increased nodule number and nodule effectiveness by 44 % and 45 % respectively, over plants that received no inoculants. The sole application of P fertilizer increased the number of nodules by 44 % compared to the plots that received no P fertilizer.

However, this did not translate intoincreased grain yield. The interaction between Rhizobium inoculation and Phosphorus fertilizer significantly affected dry shoot biomass of soybean. Treatment interaction between soil amendments and P fertilizer significantly influenced P-use efficiency. The results show that the three factors that were studied did not interact to significantly influence nodulation, growth and yield of soybean However, the three factors interacted to significantly enhance nodulation and improve P-use efficiency and some soil chemical properties (OC, Total N and exchangeable Ca and Mg). However, it is recommended that farmers can apply phosphorus fertilizer at the rate of 20 kg P/ha for increased grain yield of soybean on acid soil.

CHAPTER ONE

INTRODUCTION

Soybean [*Glycine max* (L.) Merrill] is an annual legume in the Fabaceae family and the genus Glycine Willd (Karnwal & Singh, 2009). The UN Food and Agriculture Organization classifies soybean as an oil seed rather than a pulse (Saxena & Vyas, 2016). Chinese traders along Africa's east coast introduced soybean to the continent in the nineteenth century (Ibrahim *et al.*, 2018). South Africa was the largest soybean producer in SSA in 2018/19 (1.17 million MT), followed by Nigeria and Zambia (Engelbrecht *et al.*, 2020). For the year 2019, total global soybean production was around 350 million tonnes, with Africa accounting for 3.1 million tonnes. Almost one-third of the latter (920,000 tonnes) was produced in West Africa (FAOSTATS, 2020). During the past ten years, production of soybean has increased steadily from 74,800 MT in 2008 to 176,670 MT in 2018 in Ghana (MoFA, 2019). Soybean is a key component of the predominantly cereal-based farming systems of the Guinea savannah agro-ecological zone of West Africa. It is an important component in poultry feed preparation. Soybean is also a source of household income and nutritional security, particularly among underprivileged households (Sanginga and Bergvinson, 2015). Because soybean contains high-quality protein, it can be used in place of expensive animal proteins to fight malnutrition. It contains all the necessary amino acids in addition to having a high protein level (FAOSTAT, 2009); explains why it has recently been advocated for large-scale production and usage as a meat alternative in Ghana (FAOSTAT, 2009). When grown in rotation with cereals, soybean reduces the amount of mineral nitrogen fertilizer required for cereal crops due to its symbiosis with nitrogen-fixing bacteria (NFB) (Sanginga 2003; Giller *et al.*, 2011). In Ghana, soybean is mostly cultivated in the five northern regions and the northern part of Volta region where about 90 % of the crop isproduced (MoFA, 2010). In recent times, soybean production is being expanded to the semi deciduous agro-ecological zone of Ghana where the soils are

relatively better because of its increasing demand. However, in this part of Ghana, soybean production is limited by low soil pH and limited nodulation (Adjei-Nsiah *et al.*, 2022). Recent research by Adjei-Nsiah *et al.* (2022) suggests that soybean could yield as high as 2.4 t ha^{-1} in the semi-deciduous agro-ecological zone with application of P fertilizer and rhizobium inoculation which is comparable to if not higher than yields obtained in the Guinea savanna agro-ecological zone under similar management practices (Adjei-Nsiah 2018, Adjei-Nsiah 2021; Ahiabor *et al.*, 2014). It is anticipated that the yield could even be higher if the pH is increased to slightly acidic to neutral since soybean performs well within that pH range. P availability, biological nitrogen fixation and microbial activity are all decreased in acidic soils, which has an impact on the growth and yield of soybeans (Ahiabor *et al.*, 2014). By adding rice husk biochar and conventional lime as soil amendments acidic soils can be corrected. Agricultural lime decreases Al and Mn toxicity that enhance plant roots system and promotes Ca, Mg, Mo, and P uptake (Brady and Weil, 2002). Biochar could be a panacea for the low pH and low P availability problem of Ghanaian soils. There is abundance of farm waste such as rice husk, cocoa pod husk and corncob which could be charred anaerobically to produce biochar. However, the use of these feedstock has received little attention by soybean farmers. Biochar enhances the organic matter content of the soil which has an impact on ion exchange capacity, plant nutrient retention, water holding capacity, bulk density and soil structure (Gaskin *et al.*, 2007). Biochar made from rice husk and cocoa pod husk burned at 450 ^{o}C had accessible P concentrations of 531 mg/kg and 3897 mg/kg (Sam, 2014). As a result, biochar could be used as a liming material with the added benefit of being a P source.

While several studies by Adjei-Nsiah *et al.*, (2018); (2021); and (2022), Ahiabor *et al.*, (2014), Ulzen *et al.*, (2016) and Ronner *et al.*, (2016), have demonstrated the beneficial effect of P-fertilizer application and rhizobium inoculation on the yield of soybean, information on the combined effect of liming, P-fertilizer application and rhizobium inoculation in the semi-deciduous forest agro-ecological zone of Ghana is scanty.

Increasing soybean yields in the semi -deciduous forest zone starts by reducing the soil acidity to a level at which the crop can produce its potential, followed by the increase and maintenance of soil fertility through application of P fertilizer and introducing external rhizobial population through inoculation. Phosphorus often regulates nitrogen fixation by legumes (Pérez-Fernández *et al.,* 2019), and this macronutrient is limited in Ghanaian soils (Buri *et al.,* 2010; Masso *et al.,* 2016). Seed inoculation with rhizobium strains improve nitrogen fixation in grain legumes (van Heerwaarden *et al.,* 2018; Adjei-Nsiah *et al.,* 2018; Ulzen *et al.,* 2018; Osei *et al.,* 2020; Ahiabor *et al.,* 2014; Asei *et al.,* 2015; Masso *et al.,* 2016; Ronner *et al.,* 2016). These inputs in addition to *Bradyrhizobium* inoculants will improve soil microbial activity, biological nitrogen fixation and chemical characteristics of acid soils.

The aim of the present research was to evaluate the combined effects of lime ($CaCO_3$), rice husk biochar, phosphorus fertilizer and *Bradyrhizobium* inoculants on soybean production in the forest agro-ecological zone of Ghana.

The specific objectives of the study were to:

1. Assess the effects of agricultural lime ($CaCO_3$), rice husk biochar, and Phosphorus fertilizer on the chemical characteristics of acidic soils.
2. Assess the effects of *Bradyrhizobium* inoculation, lime, rice husk biochar, and P fertilizer on soybean nodulation and P-use efficiency of soybean on acid soils.
3. Assess the effects of *Bradyrhizobium* inoculation, lime, rice husk biochar, and P fertilizer on growth and yield of soybean grown on acid soils.

The objectives were based on the hypothesis that;

1. The application of agricultural lime ($CaCO_3$) and rice husk biochar will improve soil chemical characteristics.
2. The addition of $CaCO_3$ and rice husk biochar will increase soil pH and hence enhance nodulation, and P-use efficiency of soybean on an acid soil.
3. The addition of *Bradyrhizobium* inoculant, lime, rice husk biochar and P fertilizer will increase growth and yield of soybean.

CHAPTER TWO

LITERATURE REVIEW

2.1 Origin and distribution of soybean

In general, researchers from all around the world stated that China is where soybeans originated. The annual wild soybean (*Glycine soja*) is a close relative of the soybean (*Glycine max*) that is currently cultivated in China. *Glycine soja* is only found in China, Japan, Korea and far Eastern Russia in East Asia, but China has the widest distribution, the highest numbers and the greatest variety of kinds (Qui and Chang, 2010). Based on observations that semi-wild soybeans are widely dispersed in northeast China but not in other areas, Fukuda (1933) theorized that northeast China is where the soybean originated. He added that different approaches to research and material collection may well have an impact on the fact that these wild soybeans are widely distributed in northeast China but are scarce in other areas.

While Wang (1985) studied the origin of soybean using ancient Chinese literature, inscriptions on bones and tortoise shells of the Shang dynasty based on which he also concluded that the earliest region for cultivating soybean was around the central or downstream of the Yellow Valley which was seconded by Chang (1989) based on his study of the relationship between the origin of agriculture and the origin of soybean. Hymowitz (1970) also believed that the origin of the soybean was the eastern part of northern China, which he referred to as winter wheat (*T. aestivum*). Literature related to soybean in past dynasties of China was collected by Guo (1993) who analyzed the arguments related to the origin of soybean and concluded that the origin of cultivated soybean is northeast China, but the exact origin of soybean remains unknown and therefore thought that these arguments are not conclusive. Asia has the longest history of growing soybean, with China having the largest cultivated area of the crop. Japan, North and South Korea and Indonesia are some of the countries that cultivate soybean. In Japan, most soybean varieties are large-seed types and are used as vegetable soybean which is called

'edamame'. A 100 fresh seed weight is greater than 70 g and a dry weight greater than 30 g, whereas in South Korea, varieties are small seed types; thus 100 seed weight is less than 15g (Qui and Chang, 2010).

According to reports by Hymowitz (1984), soybean cultivation began in the United States as early as 1765, when Samuel Bowen, an east India Company sailor imported soybean from China to Savenna (Georgia). The United States dispatched scientists to China, Korea, and Japan to gather soybean germplasm. Thousands of accessions of soybean were brought back from these nations and are now the main sources of soybean breeding in the United States.

In Africa, it was first introduced in 1857 (Shurtleff and Aoyagi, 2012) and later introduced by the Portuguese missionaries into Ghana in 1910, with major growing areas being, Bawku, Nakpanduri, Bimbilla, and Karaga. The main problem facing soybean farmers at that time was the loss of seed viability during storage (Plahar, 2006).

2.2 Morphological description of soybean

As an annual crop, soybean plants have a substantial taproot system, the majority of which is in the soil. The hypocotyls typically produce adventitious roots, while the taproots typically growinto the soil. The alternating trifoliate leaves are oblong and lanceolate in shape with a mucronate tip. They have long petioles and tiny stipules (Chaturvedi *et al.*, 2011). Soybean flowers emerge from auxiliary buds on the main stem and branches. A raceme of 2-35 papilionaceous flowers forms an inflorescence at each axil (Smith, 1995). Flowers typically self-pollinate, but in about 1% of cases, insects will cross-pollinate them (Chaturvedi *et al.*, 2011).Short-stemmed, 13–15 in clusters, 3–7 cm long, hairy, light brown in colour, and somewhat constrictive between seeds are the characteristics of mature pods (Chaturvedi *et al.*, 2011). Soybean cultivars can be divided into three categories based on their growth habits: determinate, semi-determinate, and indeterminate. In contrast to the indeterminate, which

experiences simultaneous vegetative and reproductive growth, the determinate plant's vegetative growth is almost finished when it starts to flower. After flowering periods, indeterminate stems on semi-determinates abruptly stop growing vegetatively (OECD, 2000). A thicket of hairs, which are typically fine in nature, mostly conceals the stems. The stem carries nutrients and water while also supporting the flowers and leaves. Depending on the variety and planting date, soybeans can grow to heights of 60–140 cm. They have erect, bushy growth patterns (Belfield *et al.*, 2011)

The stem, pods and leaves are covered in tiny, brown or gray-like hairs. Each leaf of a trifoliate has three to four unique structures. The seeds might vary in appearance, size, colour and form, but they are typically round or oval and have a cream seed coat. Flowers are normally pea-shaped, 5-6 mm long, and come in a range of colours, such as white, purple, and pink. Pods can be black, yellowish, or brown in colour. The fruit of the soybean is a hairy pod that contains three to four seeds (Rienke and Joke, 2005; Kumudini, 2010; Chaturvedi *et al.*, 2011). There are two stages in the growth and development of soybeans: the vegetative and reproductive phases. The fully unrolled leaf at the unifoliate node (V1), the first node above the unifoliate node (V2), three nodes on the main stem beginning with the unifoliate node (V3), and N nodes on the main stem beginning with the unifoliate nodes (V4) are all indicators of vegetative growth (Nand *et al.*, 2010).

2.3 Soil requirement

Soybean tolerates a variety of soil conditions, but thrives in warm, moist, well drained fertile loamy soils with adequate nutrients and good seed-to-soil contact for rapid germination and growth (Hans *et al.*, 1997; Addo-Quaye *et al.*, 1993). According to Ngeze (1993), soybean grows well in fertile sandy soils with pH between 5.5 and 7.0, and the crop can tolerate acidic soils better than other legumes, but it does not grow well in waterlogged, alkaline or saline soils. Keeping soil pH between 5.5 and 7.0 improves nutrient availability such as nitrogen and

phosphorus, microbes' ability to breakdown crop residues and symbiotic nitrogen fixation (Ferguson *et al.,* 2006). Rienke and Joke (2005) found that loamy textured soil produces higher yields, and that if the seeds germinate, they grow better in clayey soils.

2.3.1 Soil pH

The pH of agricultural soils determines the availability of nutrients, and thus the fertility and productivity of the soil. The concentration or activity of hydrogen ions in the soil is measured by pH. It determines how acidic or alkaline the soil is. The pH value decreases as the H^+ ion concentration rises, and thus soil acidity rises (USDA, 1999). The pH range between 6.1 and 7.3 on the pH scale is considered neutral in soil, unlike the pure system where neutrality is at seven (USDA, 1999). According to the USDA system (Table 2.1) provides descriptive terms for pH ranges in soils.

Table 2. 1: Descriptive Terms Associated with Soil pH

pH ranges	Descriptive Term
< 4.0	extremely acid
4.1 – 5.0	very strongly acid
5.1 – 5.5	strongly acid
5.6 – 6.0	moderately acid
6.1 – 7.3	neutral
7.4 – 8.0	slightly alkaline
8.1 – 9.0	alkaline
> 9.0	strongly alkaline

Source: USDA (1999).

Low soil pH causes conditions that stifle plant growth and development, resulting in stunted

growth. Soil acidity has the potential to harm legume growth by reducing nodule development and nitrogen fixation. The tolerance of different rhizobia strains to soil acidity varies more than that of the host plant (Mohammadi *et al.*, 2012).

2.3.2 Causes of soil acidity

Soil acidity problems harm about a quarter of the world's farmland (Graham *et al.*, 2000). The effective treatment actions are necessary to understand the sources of acidity. Several natural and man-made causes contribute to soil acidity. Leaching of bases because of heavy rainfall, plant uptake of basic nutrients and decomposition of organic waste are examples of natural sources, whereas anthropogenic causes include the use of inorganic fertilizers, particularly ammonium-based fertilizers. Soil acidity rises in general when rainfall rises.

Soil acidity is determined by the concentration of H^+ ions in the soil solution; hence, a high H^+ ion concentration will result in a lower pH value and, as a result, a higher acidity. H^+ can be found in soils from a variety of sources, including:

i) Carbon dioxide released from plant roots and microbial respiration which combines with soil water to produce carbonic acid. This acid then dissociates to release H^+.

$$CO_2 + H_2O \rightarrow H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \qquad [2.1]$$

(ii) Decomposition of organic matter with concomitant releases of H^+.

(iii) Roots of plants also release H^+ and organic acids to lower the pH of soils.

(iv) Nitrification of ammonium

$$NH_4^+ + 2O^{2-} \rightleftharpoons NO_3^- + 2H^+ + H_2O \qquad [2.2]$$

(v) Hydrolysis of Al in soils releases large quantities of H^+ into soil solution as shown in the equations below.

$$Al^{3+} + H_2O \rightarrow Al(OH)^{2+} + H^+ \qquad [2.3]$$

$$Al(OH)^{2+} + H_2O \rightarrow Al(OH)^{+} + H^{+} \quad [2.4]$$

$$Al(OH)^{+} + H_2O \rightarrow Al(OH)_3 + H^{+} \quad [2.5]$$

A summary of the three reactions is thus

$$Al^{3+} + 3H_2O \rightarrow Al(OH)_3 + 3H^{+} \quad [2.6]$$

(vi) Oxidation of Sulphur compounds in soils leads to acidification as depicted in equation

$$S^0 + 3/2\ O^2 + H_2O \rightarrow H_2SO_4 \quad [2.7]$$

S^0 is elemental Sulphur

There are three general pools, or types of acidity: active, exchangeable and residual.

(i) Active Acidity

Active acidity is the concentration of hydrogen ions present in the soil solution, which can be measured by determining the pH value of a water suspension or soil extract. This concentration is influenced by carbonic acid (H_2CO_3), soluble organic acids, and acid salts formed through hydrolysis. The active acidity level plays a crucial role in the growth and development of plants and soil microorganisms (Pankova *et al.*, 2009).

(ii) Exchangeable Acidity

The hydrogen (H) and aluminum (Al) ions get adsorbed onto soil colloids. There is an equilibrium between the adsorbed and soil solution ions, which is known as exchangeable acidity. This allows for easy interchangeability between the two forms. In other words, it is the acidity caused by the exchangeable hydrogen and aluminum ions that can be easily dissolved in a simple salt solution like KCl (Getachew *et al.*, 2019).

(iii) Residual Acidity

It is the concentration of hydrogen ions attached to clay and organic matter and is measured as buffer pH in a buffer solution. The adsorbed H and Al ions pass into the soil solution and its acidity is also known as potential or adsorbed or reserve acidity. In an acid soil, most of the H^+ present is absorbed by the soil (Thomas, 1996).

2.3.3 Effect of soil acidity on nutrient availability and plant growth

Soil pH has a primarily indirect effect on plant growth because it affects chemical reactions and biological processes (Neina, 2019). Most crops absorb nutrients effectively when the soil pH is in the neutral range, according to Giller and Wilson (1991). Plant growth and development might be harmed if soil acidity is not appropriately regulated (Nduwumuremyi, 2013). The availability of nutrients such as nitrogen, molybdenum, phosphorus, and potassium are often affected as soil acidity rises (Brady and Weil, 2002). Solubilized rhizotoxic aluminum species in highly acidic soils, according to Kochian (1995), can inhibit root growth and function in most plants. Al toxicity, according to Pineros *et al.*, (2005), limits plant growth primarily through its negative effects on root growth and development. Aluminum toxicity also makes plants more susceptible to drought and limits their access to subsoil nutrients, preventing them from reaching their full genetic potential (Ownby and Popham, 1990). Aluminum toxicity, according to Giller *et al.*, (1998), reduces nutrient agronomic and recovery efficiencies.

Plant development is hampered by high soil acidity, which inhibits the growth and proliferation of soil microorganisms. The biological nitrogen fixation by bacteria that dwell in the nodules of legumes such as cowpea, peanut, and soybean is adversely affected by low pH (below 6) soils. According to Brockwell *et al.* (1995), the quantity of *S. meliloti* in soils with a pH less than 6 has fallen by roughly 10^{-3} *S. meliloti* in soils with a pH more than 7.0. Under low soil pH conditions, microbial growth and multiplication are inhibited, resulting in delayed organic matter decomposition (Zhao *et al.*, 2022).

Phosphorus availability is affected in very acidic soils. There is a large concentration of soluble Al^{3+} and Fe^{3+} in the soil in very severely acid conditions, such as in Oxisols (Hu *et al.*, 2022). Any additional P is precipitated as shown in equation [2.8]

$$Al^{3+} + H_2PO_4^- + 2H_2O \rightarrow 2H^+ + Al(OH)_2H_2PO_4 \quad [2.8]$$

Due to their large surface area exposed to the soil solution, the newly precipitated hydroxy phosphates are marginally soluble. The precipitated hydroxy phosphate matures over time, becoming less soluble and so unavailable to plants (Tisdale *et al.*, 1993). The effectiveness of P fertilizer use is reduced as a result of this. Kaolinites and aluminum and iron oxides are the principal clay minerals of Ghana's Oxisols, Ultisols, and Alfisols, which are the most common soil types (Nartey, 1998). These clay minerals exposed OH groups have a significant affinity to P. (Tan, 2010). The exposed OH groups in clay minerals become protonated at low pH and so retain additional P, as shown in equation [2.9].

$$Al(OH)_2^+ + H_2PO_4^- \rightarrow Al(OH)_2H_2PO_4 \quad [2.9]$$

The phosphate anion may also produce an inner sphere complex by replacing the clay's structural OH. Between the $H_2PO_4^-$ and the protonated OH, there is ligand exchange. While reversible, this reaction attaches the anion to the mineral too tightly, resulting in poor availability (Brady and Weil, 2002).

Low soil pH also lowers the availability of Mo and B, both of which are important for nodulation (Tisdale *et al.*, 1993). Basic nutrients including K, Ca, Mg, and Na become insufficient in plants due to their low solubility in acid soils. Because of the high solubility of these micronutrients in soil solution, Fe, Mn, Zn, and Cu are hazardous to plants in acidic soil conditions (Tisdale *et al.*, 1993). Particularly in mining areas and on soils where agrochemicals are used, high soil acidity causes considerable accumulation of hazardous heavy metals such as Hg, As, Pb, and Cd in crops (Sparks, 2003).

2.3.4 Control of soil acidity

As a solution, it has been suggested that soil acidity-tolerant crops be produced and planted. It is, however, time-consuming, and as a result, certain crop features are usually lost or suppressed. Breeding and the use of tolerant crops are primarily coping and/or adaptive strategies, not corrective ones (Curtin and Trolove, 2013). This has not proven very efficient due to the relatively slow decomposition of organic matter to release bases and the fact that organic matter functions as a buffer and thus resists changes in pH (Curtin and Trolove, 2013). As a result, liming has long been the most feasible method for correcting soil acidity and increasing soil productivity (Curtin and Trolove, 2013). According to Brady and Weil (2002), agricultural lime is any material that contains calcium and magnesium as cations in combination with anions such as carbonates, hydroxides, oxides, and silicates, and is used to elevate pH in acid soils. Liming components include calcite, dolomite, slaked lime, quick lime, and basic slag (Brady and Weil, 2002). In the series of events below, the methods by which calcite, the most often used agricultural lime, elevates soil pH when applied to wet soil are depicted.

(1) Water dissolves the material in the soil to produce Ca^{2+} and hydroxide (OH^-) according to the equation [2.10]:

$$CaCO_3 + H_2O \rightarrow Ca^{2+} + 2OH^- + CO_2 \qquad [2.10]$$

(2) The released Ca^{2+} substitutes for Al^{3+} and H^+ at the exchange sites of the soil.

(3) The OH^- produced from equation 2.10 reacts with Al^{3+} to form $Al(OH)_3$, and/or H^+ to form H_2O:

$$3OH^- + Al^{3+} \rightarrow Al(OH)_3 \qquad [2.11]$$

$$OH^- + H^+ \rightarrow H_2O \qquad [2.12]$$

The $Al(OH)_3$ formed precipitates out of the solution raising the soil pH.

High levels of Al^{3+} and H^{+} are lowered as a result of the combination of liming and OH^{-}. (Tisdale *et al.*1993). The release of excess OH^{-} from lime, which elevates the soil pH, is the most evident effect of liming. Liming, depending on the substance, nourishes the soil with two macronutrients: calcium and magnesium. The response rates of liming materials in soil are governed by particle size and surface area. The pH of the soil, the degree of mixing with the soil, and the chemical nature and content of the material all influence the reaction rate (Brady and Weil, 2002). Oxides and hydroxides, for example, react faster than carbonates due to their larger solubilities. There must be adequate moisture in the soil for the reaction to occur (Brady and Weil, 2002).

2.3.5 Soil acidity and its effects on soybean production

Phosphorus is precipitated or surface-adsorbed with Al and Fe as insoluble compounds, it is insufficient in soil solutions with low pH soils with high amounts of Al and Fe oxides (Kanyanjua *et al.*, 2002). The soil solution lacks a number of other vital cation-containing plant nutrients. Acidic soils affect soybeans both directly and indirectly.

These effects include plant root damage, which decreases water and nutrient uptake, a reduction in the availability of essential plant nutrients, the toxicity of aluminum and manganese (Mn), and soil microbe survival (Crawford *et al.*, 2008; Onwonga *et al.*, 2008). Several methods for correcting nutrient deficiencies can be used to enhance crop production in acid soils. Liming, organic matter addition, and mineral fertilizer application are some of these options (Onwonga *et al.*, 2010; Masarirambi *et al.*, 2012). Liming decreases Al^{3+} and H^{+} ions by reacting with water to produce OH^{-} ions, which then react with Al^{3+} and H^{+} in acid soil to produce $Al(OH)_3$ and H_2O. Lime increases pH by precipitating Al^{3+} and H^{+}, which boosts microbial activity and nutrient availability (Onwonga *et al.*, 2008).

As a leguminous crop, soybeans rely on microbial nitrogen fixation as a source of nitrogen. However, in acid soils, the population of rhizobia bacteria decreases, impairing nodulation

and nitrogen fixation. This has an adverse effect on crop nutrition and productivity. As a result, liming acid soils for soybean production increases the soil's microorganism development conditions. Because mineral fertilizers are easily available, they boost nutrient availability in the soil solution, while organic matter acts as a source of food for microorganisms, increasing their number and hence mineralization (Crawford *et al.*, 2008).

2.4 Factors that affect the growth and yield of soybean

Both biotic and abiotic variables influence the growth, development, and yield of the soybean plant. Soil microorganisms, soil water properties, and the availability of plant nutrients, as well as climate, insect and disease infestation, and cultivation and management strategies, are all elements to consider (Frempong-Manso *et al.*, 2019). Average temperatures of 20 to 30ºC are ideal for growing conditions. Temperatures below 20 °C and above 40°C are unsuitable for the crop. Likewise high temperatures can also have an impact on soybean yield. Changes in temperature affect the physiological, biochemical, metabolic and molecular functions of plants, according to Guy *et al.* (2018) which can hinder the growth and yield of soybean crops (Kotak *et al.*, 2017). The optimal rainfall is between 350 and 750 mm, evenly distributed throughout the growing season (Ngeze, 1993). The over-reliance on rainfall, which has become increasingly erratic (Dankwa *et al.*, 2021) has led to drought spells that negatively affect the growth and yield of soybean crops (MacCarthy *et al.*, 2017).

Soybeans may grow in a variety of soil types, but they thrive in warm, moist, fertile loamy soils with good drainage and sufficient nutrients (Hans *et al.*, 1997). Soybean thrives in fertile soils with a pH of 5.5 to 7.0 by Ngeze (1993). It is an acid-tolerant crop that outperforms all other legumes. It, on the other hand, does not grow well in alkaline, saline, or reduced soils. Soy beans can only endure a small amount of waterlogging (Norman *et al.*, 1995). The availability of nutrients such as nitrogen and phosphorus, microbial decomposition of crop wastes, and symbiotic nitrogen fixation are all improved by keeping soil pH between 5.5 and 7.0 (Ferguson

et al., 2006). Seeds of soya beans require enough moisture to germinate and grow.

2.5 Factors influencing nitrogen fixation in legumes

According to reports, grain legumes are capable of biologically fixing nitrogen in the range of 15-210 kg N/ha per year in Africa, and therefore play an important role in subsistence farmers' cropping systems (Dakora & Keya, 1997). They are also well-known for their capacity to establish symbiotic associations with N_2 fixing rhizobia bacteria (Zhang *et al.,* 2020). The overall N fixation process depends on numerous environmental factors that affect nodules formation, bacterial metabolism (Santachiara *et al.,* 2019) and plant growth (Aranjuelo *et al.,* 2014). Many environmental factors influence biological nitrogen fixation. Environmental factors such as saline and sodic soils, extreme soil pH (Acquino-Alves *et al.,* (2021), low nutrient availability (Divito and Sadras, 2014), mineral toxicity, extreme temperature (Alexander and Oliveira, 2013), soil water content (Munjonji *et al.,* 2018), soil mineral N (Torabian *et al.,* 2019), affect N fixation. Soil pH extremes, either low or high, can reduce rhizobial colonisation in the legume rhizosphere. Acidic soils, according to van Jaarsveld (2002), can reduce the amount of N_2 fixed.Under low soil pH conditions, nodulation and the amount of N_2 fixed are more strongly influenced than plant growth.

According to Panchali (2011), even the most effective rhizobium strains cannot form effective associations with the host plant in nodulation and N fixation under these conditions.

Agricultural management factors, in addition to environmental conditions, influence the percentage of N_2 obtained from the atmosphere. Some management factors that can influence plant growth and development include inoculation, phosphorus fertilizer application, genotype selection, and plant population selection (Ronner & Franke, 2012). The purpose of inoculation is largely determined by the presence and effectiveness of compatible rhizobia in the soil (Giller, 2001).

2.5.1 Soil moisture content

Soil moisture/water influences the growth of soil macro and microorganisms by mass flow, diffusion and nutrient content. Soil texture is significant because it determines how much water a particular soil can retain within its pore space; hence, soils with wide pores and pore spaces hold less water. As a result, soil aggregates with fewer inner pores, such as clayey loam and loamy sand, support the growth of rhizobia and other soil bacteria better (Turco & Sadowsky, 1995).

In general, the growth and establishment of rhizosphere microorganisms such as rhizobia are directly affected by soil water supply due to decreasing activity below critical tolerance limits. Soil water also has an indirect effect on plants by causing changes in plant growth, root exudates, and root architecture.

According to Boscari *et al.* (2002), low rhizobial population levels in dry seasons are most likely to blame for little or no nodulation of legumes in tropical soils. As a result, the impact of soil moisture/water on microorganisms, plant growth, vigour, and nodulation should not be underestimated. Rhizobia adapts to osmotic pressure in a variety of ways, primarily through the accumulation of organic and inorganic solutes in the intracellular space. *R. meliloti* is a typical example of a rhizobium that overcomes osmotic stress by accumulating compatible solutes such as K^+, trehalose, glycine, betaine, glutamate, dipeptide, N-acetyl glutminyl glutamine amide, proline, and proline betaine.

2.5.2 Soil temperature

The survival and proliferation of rhizobial strains in soils are both influenced by temperature. Temperature appears to have a different impact on rhizobia depending on the soil type and strain. Rhizobium strain for instance bacillus thuringiensis var. leguminosarum Bradyrhizobium sp. was outperformed by trifolii under temperature of the soil (Mohammadi

et al., 2012). High soil temperatures, according to Triplett and Sadowsky (1992), cause nodulation to be delayed or limited to subsurface soils. Drought and high temperatures both reduced plant dry mass and leaf area, especially when the two stresses were combined. The inhibitory effect of high temperatures on plant growth was caused by lower CO_2 and N_2 fixation rates as reported by Aranjuelo *et al*. (2007). High-root temperatures also lowered infection, N_2-fixation ability, and legume development, according to Hungria and Franco (1993), but this is also reliant on rhizobia strain type and strain-cultivar relationships (Arayankoon *et al.*, 1990). The ideal temperature correlations for various legumes and Rhizobia combinations are between 35 and 40°C for soybeans, peanut, and cowpea.

2.5.3 Availability of phosphorus

Phosphorus availability in the soil during seedling development is critical for legume growth, N2 fixation, and grain production, and a lack of it can prevent nodulation (Giller, 2001). Nodule development and function are major P sinks, with nodules containing the most P in the plant (Sinclair and Vadez, 2002). P deficiency reduces nodule development, but P fertilizer application increases nodule number and dry mass, as well as N fixation (Sinclair and Vadez, 2002). Low soil phosphorus levels reported by Yakubu *et al.*, (2010), inhibited rhizobia population and root development in legumes, lowering N_2 fixation potential. Increased soil P input from 20 to 40 kg P_2O_5 ha^{-1} resulted in an increase in total plant growth and plant nitrogen content (Sinclair and Vadez, 2002; Magani and Kuchinda, 2009). African farmers' limited access to P fertilizers have contributed to low legume productivity (Sinclair and Vadez, 2002). All the cowpea types evaluated in the Sudan Savanna zone of Nigeria showed a strong response to applied P up to 60 kg P_2O_5/ha, according to Singh *et al.* (2011). Higher availability of P has been attributed to higher grain yield in cowpea as reported by Singh *et al*. (2011). According to Assuming-Brempong *et al*. (2013), growing legumes in Ghana's coastal savanna zone necessitates 90 to 120 kg P_2O_5/ha of phosphorus. According to Vesterager *et al.*, (2008), the amount of N fixed in a cowpea monocrop increased from 58 to 77 kg N/ha and in a cowpea-

intercrop increased from 30 to 43 kg N/ha as a result of P treatment in Tanzania's semi-arid zone. Nodulation has been reported to diminish as the amount of P and N applied increases, implying that readily available N has an antagonistic influence on the functions of Phosphorus (Vesterager *et al.*, 2008). This finding suggests that in the presence of easily available nitrogen in the soil, P treatment may not maximize biological nitrogen fixation.

2.5.4 Population of rhizobia strain in the soil

Rhizobia associate symbiotically with legume roots to fix atmospheric nitrogen. The more rhizobia populations grow, the more likely nodule infection becomes. The symbiotic properties of soil rhizobia populations and soil composition can differ (Martins *et al.*, 2003).

On the root of the same plant, nodules formed by multiple strains and species can appear (Moreira and Siqueira, 2006). The legume-rhizobia symbiosis can sustain agriculture in the tropics at moderate output levels if all environmental barriers to the symbiosis' proper functioning are removed (TSB-CIAT, 2004). Singleton *et al.* (1992) found that less than 60 % of tropical soils studied from Africa had less than 1,000 rhizobia/g soil and 47 % had less than 100 rhizobia/g soil, indicating that cowpea cross-inoculation was prevalent. Fening and Danso (2002) reported that 68 % of rhizobia samples collected from 20 Ghanaian soils were moderately effective in nodulating soybean. This suggested that Ghanaian soils are rich in the soil rhizobia which could boost the yield of leguminous crops with relatively low supplement of fertilizer.

2.5.5 Soil nutrient

The symbiosis, as well as the independent growth and survival of legume crops, are significantly influenced by the nutritional quality of the soil. With increasing legume age, nitrogen fixation declines, owing to an increase in soil nitrogen content hence lower yield. The

rate of N fertilizer and N_2 fixation have been found to have a negative exponential connection (Ledgard and Steele, 1992).

The attachment of rhizobia to root hairs, as well as nodulation and nodule formation, are all affected by calcium deficiency, with or without the confusing influence of low pH (Alva *et al.*, 1990). At the molecular level, calcium plays a critical role in symbiotic connections. An Al-induced Ca deficit has been blamed for poor nodulation of soybeans in acid soil (Biswas *et al.*, 2003). Several other nutritional variables influence the growth and survival of rhizobia in soils, in addition to macronutrients (Brockwell *et al*., 1995). Glutamate, glycerol, and organic matter supplementation of soil and inoculants has been proven to boost rhizobia survival and populations in soils, as well as early nodulation and N_2 fixation (Rynne *et al*., 1994). This finding reveals that, while rhizobia may certainly exist in soils, their efficacy can be improved by adding carbon, implying that they are C constrained in their natural condition.

2.6 Lime requirement

The amount of agricultural liming material needed to neutralize the undissociated and dissociated acidity in the range from the initial acid condition to a desirable neutral or less acid condition is referred to as a soil's lime need (McLean, 1971). Lime requirement is defined as the amount of liming material required to generate the highest economic yield of crops grown on low pH soils (McLean, 1971). There are a variety of practical methods for predicting the amount of lime to apply in order to achieve a sufficient level that eliminates Al toxicity to plant growth and development. Monitoring the concentration of exchangeable Al is one of the most frequent approaches for calculating the lime demand. Bell and Bessho (1993) claim that adding basic ions to soil, particularly Ca^{2+} ions, neutralizes exchangeable Al and promotes root growth. According to Hakim et al. (1989), the ideal lime rate for improving food crop yield on Ultisol is around 6 Mg $CaCO_3$/ha. Although numerous extraction solutions have been

developed to estimate the extractable Al, the KCl extraction method is often utilized (Oates and Kamprath, 1983).

2.6.1 Types of liming materials

Most limestone is mined and then processed into tiny particle sizes to improve the surface area and thus reactivity. Calcium carbonate and other impurities are common in limestone. Most of the lime put into the soil is made up of limestone. Calcitic limestone is ground limestone that has less than 6 % magnesium, while dolomitic limestone contains more than 6 % magnesium (Carey *et al*., 2009). Ground limestone is subjected to high temperatures to remove carbon dioxide, resulting in burnt lime (quicklime). After the heating process, calcium oxide is obtained. Magnesium oxide will only be present if it was part of the ground limestone before it was heated. Burnt lime reacts quickly with water to form hydrated lime ($Ca(OH)_2$) and releases a lot of heat, therefore it needs to be handled carefully (Carey *et al*., 2009). Because of its high solubility, calcium hydroxide is extremely reactive, and too much of it in the soil can quickly raise the pH above the desired level. Because of its caustic nature, it harms already-established plants in the field. Marls are made up of sea shells and calcium carbonate. Farmers in coastal locations use this type of equipment. Ground limestone and marls have a comparable reactivity (Carey *et al*., 2009).

2.6.2 Advantages of liming in acidic soils

Lime is a type of substance that contains carbonates, oxides, or hydroxides and is used to increase the pH of acidic soils while also neutralizing hazardous components. The pH of a soil is used to assess whether or not it should be limed (TSO, 2010). $CaCO_3$, Ca, Mg $(CaCO_3)_2$, Ca $(OH)_2$, CaO, and other liming minerals exist, and their neutralizing value and degree of fineness vary (TSO, 2010). Ca^{2+} and Mg^{2+} ions displace H^+, Fe^{2+}, Al^{3+}, Mn^{4+}, and Cu^{2+} ions from the soil adsorption site when lime is applied, resulting in an increase in soil pH. In

addition to boosting soil pH, lime also delivers substantial amounts of Ca and Mg, depending on the type of lime used. Increased availability of P, Mo, and B, as well as more favorable conditions for microbially mediated reactions like nitrogen fixation and nitrification, and, in some situations, enhanced soil structure, are all indirect impacts of lime (Nekesa *et al.*, 2005). For instance, lime application enhanced soybean root and shoot yields in Nigeria, according to Anetor and Akinrende (2006), as well as soybean grain yields in Brazil (Anetor & Akinrinde, 2006). In Croatia, Andric *et al.* (2012) found a 44 percent increase in soybean yield as a result of due to lime application. Furthermore, Nekesa *et al.* (2011) reported a good response of soybean grain yield to lime application, either alone or in combination with TSP fertilizer or Di-ammonium phosphate (DAP) in Western Kenya.

Liming is extensively used in the treatment or correction of soil acidity according to Kaitibie *et al.* (2002). Liming ensures maximum yields from numerous food crops grown on low pH soils. When lime is applied to the soil at the proper rate, it causes a variety of chemical and biological changes in the soil that are advantageous to assuring optimal productivity in acid soils. Liming reduces the quantities of soluble aluminum and manganese to levels that are non-toxic. The availability of Mn reduces as pH rises, which becomes a severe issue in many plants below pH 5.0 (Nduwumuremyi, 2013). Acid soils benefit from liming because it raises the calcium and magnesium levels. Because a large part of applied P fertilizer is biologically fixed to oxides of Fe, Al, and clay minerals, acid soils are often low in total and accessible plant phosphorus. Liming increases the amount of accessible P for plant uptake and use. The release of P from Al and Fe oxides increases soil accessible P concentration at pH ranges between 5.0 and 6.5 (Tan, 2010). Soil microbial characteristics can be utilized to determine soil quality (Brady and Weil, 2002). Soil acidity limits the activities of helpful bacteria, except for fungi, which can thrive across a wide range of pH. Liming boosts the multiplication and activity of most microorganisms, which speed up soil processes including organic matter decomposition and nutrient release (Brady and Weil, 2002). Liming also improves legume nitrogen fixation

in acid soils. It aids in the formation of phytohormones, increases root surface area, and improves the absorption of less mobile nutrients like P and micronutrients like Mo and B. (Brady and Weil, 2002).

Increasing soil pH enhances heavy metal complexation in soils (McBride, 1994). The key determinants of heavy metals bioavailability in soil are soil qualities such as nature and type of clay, organic matter status, redox potential, and soil pH, and so liming helps to reduce heavy metals availability to crops. Haynes (1983) found that calcium released from lime added to the soil increased plant resistance to a variety of pathogens, including *Erwinia* sp., *phytophthora* spp., *Ralstonia solani*, *Sclerotium rolfsii*, and *Fusarium oxysporum*. According to Haynes (1984), calcium combines with pectic chains to form rigid bonds that help plant cell walls resist enzymatic destruction by pathogens. Liming has been reported to lower soil N_2O emissions when soil moisture content is kept at field capacity, and it has been advocated as a mitigation method (Stevens *et al*., 1998). Soil pH has a potential impact on N_2O emission pathways and the conversion of N_2O to N_2, and it is suggested that liming could be a viable option for reducing N_2O emissions from farmland (Stevens *et al*., 1998).

2.7 Biochar

Biochar is a porous, high-carbon substance produced by thermal burning of biomass under restricted oxygen conditions and at quite high temperatures, often between 300 and 1000 degrees Celsius (Lehmann *et al*., 2003). Biochar is made by charring feedstocks including sawdust, animal manure, and crop leftovers to help recycle forestry and agricultural wastes (Lehmann *et al*., 2003).

Biochar is gaining popularity in agriculture as an environmentally beneficial amendment aimed primarily at mitigating climate change (Lehmann *et al*., 2003). Biochar, often referred to black carbon, is a carbon-rich substance with a significant specific surface area that has been shown to increase soil water and nutrient retention. Biochar, as soil amendment, reduces climate

change by capturing carbon from the atmosphere (Lehmann and Joseph, 2009). By increasing nutrient adsorption, water holding capacity and microbial activity, it also raises soil productivity, leading to increased crop yields.

2.7.1 Biochar production

Biochar is obtained by burning biomass with little or no oxygen. This makes it different from actual burning of biomass which involves naked flame and oxygen to oxidize the carbon in the biomass completely to carbon dioxide leading to the production of ashes and small amounts of carbon. Limiting oxygen accessibility leads to high carbon retention in the biomass. The yield of carbon in biochar is usually 50 % or less because the pyrolysis process also produces combustible gases and volatile compounds from the pyrolyzed biomass (Lehmann, 2007).

Heating of biomass under ambient temperatures results in dehydration. Moisture in the biomass is first driven off and this involves the provision of great energy due to high heat capacity of water and large quantity of energy needed to vapourize the water content (Taylor and Mason, 2010). Thus, fresh feed stocks are not ideal for biochar production. The moisture content of biomass should be between 10 and 15 % prior to pyrolysis. The torre faction phase in the thermal decomposition process starts when the biomass is dry the biomass is “roasted”, turning dark in colour due to chemical changes. Gases and other volatile compounds are released from the biomass. True pyrolysis starts when the temperature reaches 3000^{o} C, resulting in exothermic reactions. The feedstock fully readjusts itself to form solid biochar releasing volatile compounds and combustible gases (Taylor and Mason, 2010).

The features of the final products are greatly influenced by the rate of pyrolysis. The quality of the product is also dependent on the feed stock type (Taylor and Mason, 2010).

2.7.2 Biochar stability in the soil

The stability of a system influences how long it can maintain its soil and water quality (Lehmann, 2007). Biochar can last much longer in the soil environment than any other carbon-containing organic additive. Despite the rapid rates of mineralization common to organic matter in those conditions, traces of biochar have been identified in the soils of humid tropical climates such as the Amazon several years after application (Sombroek, *et al.*, 2003).

2.7.3 Structural composition of biochar

Thermal decomposition of cellulose in organic biomass between 250^{o} C and 350^{o} C leads to considerable loss of volatile compounds with a concomitant increase in aromatic C concentration. According to Demirbas (2004), water evaporates first, followed by hydrocarbons, tarry vapour, hydrogen gas, carbon monoxide and then carbon dioxide. Thereafter, there is the transformation of alkyl and O-alkyl aryl carbon (Baldock and Smernik, 2002). Consequently, a large mass of amorphous carbon matrix is formed. At a temperature of about 330^{o} C, there is lateral growth of graphene sheet, at the expense of the amorphous carbon phase and finally coalesce. At temperature above 600^{o} C, there is the elimination of most of the remaining non-carbon atoms and carbonization becomes the dominant process; a consequence of which is relative increase in carbon content. Carbonization can reach 90 % by weight in biochar produced from woody feed stocks (Demirbas, 2004).

According to Chan and Xu (2009), carbon content of biochar irrespective of type is between 172 and 905 g/kg, although organic carbon usually accounts for less than 500 g/kg for different materials. Total nitrogen content of biochar ranges from 1.8 to 56.4 g/kg depending on the type of biomass. The high total nitrogen content may not be available to crops as a result of complexation reactions with mineral nitrogen content less than 2 mg/kg. Studies have shown that the carbon-nitrogen ratio varies widely from 7 to 500. Total phosphorus and potassium

content of biochar fall between the ranges of 2.7 to 480 and 1.0 to 58.0 g/kg, respectively (Chan and Xu, 2009). Because of its oxidation and H^+ accretion from the soil solution in the first few weeks after the amendment to the soil result different properties of biochar vary as it ages in the soil. The feedstock used to make the biochar, the soil, and the current climate all affect how much its qualities change with time ((Cheng *et al.*, 2008: Heitkotter and Marschner, 2015).

2.7.4 Biochar as liming material and mode of application

Many studies on the pH of biochar have revealed that it is typically neutral to basic in soil reactivity. As a result, their use has been discovered to raise soil pH (Joseph *et al.*, 2010). The liming characteristic of biochar, according to Verheijen et al. (2010), is one of the most plausible processes driving increases in crop output when it is employed as a soil supplement. The addition of biochar to tropical soils reduced aluminum toxicity by lowering acidity levels (Verheijen *et al.*, 2010). Noble *et al.* (1996), reported the liming impact of agricultural wastes and other biomass when burned and applied to the soil. Farrell *et al.* (2013) and Masto *et al.* (2013) showed an increase in soil pH after application of biochar on different types of soils, which is attributable to the temperature during pyrolysis and type of feedstock.

Decarboxylation of organic anions, as shown by excess cations in biochar, consumes H^+ and thereby raises soil pH, according to Yuan *et al.* (2011). Alternatively, negatively charged functional groups on biochar surfaces, such as phenol, carboxyl, and hydroxyl, adsorb H^+ from soil solution, lowering its concentration and raising soil pH as a result (Brewer and Brown, 2012; Chintala *et al.*, 2014).

Biochar's silicates, carbonates, and bicarbonates form complexes with H+ ions, rendering the proton unavailable to the soil solution (Brewer and Brown, 2012; Chintala *et al.*, 2014). In acidic soils and soils with low organic matter levels, biochar has a greater impact on raising soil pH. (Stewart *et al.*, 2013). Due to the buffering potential of organic matter, soils with high organic matter content resist changes in pH when biochar is applied (Curtin and Trolove,

2013). This is because leaching of base cations is reduced as adsorption of H^+ ions to negatively charged functional groups of biochar, organic matter, and organo-mineral complexes is promoted (Chan *et al.*, 2007; Nelissen *et al.*, 2012; Taketani *et al.*, 2013). However, the intensity of this impact may be governed by the soil organic matter content, which is the principal determinant of soil cation exchange capacity (Brady and Weil, 2008).

The behaviour and fate of biochar materials in the soil and the environment is influenced by the method of its application to soils (Verheijen *et al.*, 2010). There are three main methods of biochar application viz; topsoil application, depth application and top-dressing.

2.7.5 Agronomic importance of biochar

The addition of biochar enhances plant growth and development, and crop yields, and increases the production of food and sustainability in marginal soils with low organic matter, inadequate water, and poor nutrient status (Lehman *et al.*, 2006). Due to the fact different soils react differently to the application of biochar, it takes some time to compare the responses of soils (Lehman *et al.*, 2006). The ideal biochar application rate depends on the soil type and the crop management system (Verheijen *et al.*, 2010). A unique strategy for creating a sink for atmospheric carbon dioxide in terrestrial ecosystems is the application of biochar (Lehman *et al.*, 2006). In addition to having a positive impact on emissions and greenhouse gas sequestration, the manufacture of biochar and its application to the soil has immediate advantages through improved soil fertility and greater crop output (Lehman *et al.*, 2006).

According to Southavong *et al.* (2012) biochar might offer a quick fix for handling agricultural waste. The positive plant responses to the application of biochar have been attributed mainly to the direct supply of nutrients with very little consideration given to other biochemical factors that may affect nutrient availability (Lehmann *et al.*, 2003; Chan *et al.*, 2007; Van Zwieten *et al.*, 2007). The positive responses as a result of the application of biochar were attributed to either nutrient retention as in fertilizers or enhanced fertilizer-use efficiency and therefore can

be viewed as an indirect nutrient value of biochars. Rondon *et al*. (2007) and Van Zwieten *et al*. (2007) reported on how plants responded to biochar application-induced increases in pH or pH stability. According to Hoshi (2001), the capacity of biochar to regulate the pH of the soil was a contributing factor in the 20 % rise in height and 40 % increase in the volume of tea trees. The liming value of biochar is correlated with its capacity to preserve pH.

When biochar made from paper mill sludge was treated at a rate of 10 t ha^{-1} to acidic soil, Van Zwieten *et al.* (2007) saw a nearly 30 % to 40 % increase in wheat height. By neutralizing the deleterious effects of the exchangeable aluminum's presence, they came to the conclusion that the carbonates in the biochar enhanced wheat development.

In addition to toxin neutralization (Wardle *et al.*, 2008), improved soil physical properties, such as an increase in water-holding capacity (Eswaran *et al.*, 1980), and decreased soil strength, there have been other explanations for the positive responses to the application of biochar that are unrelated to plant nutrition (Chan *et al.*, 2007). Additionally, compared to a control that got the same amount of N without biochar, dry matter yield increased by 26 % when N fertilizer was also supplied at a rate of 100 kg N/ha in addition to biochar. The increased N fertilizer usage efficiency of radish following the application of biochar was related to the improved physical characteristics of the soil, which included decreased soil strength and increased water holding capacity. Lehmann *et al*. (2003) also observed that biochar has the capacity to retain fertilizer that had been applied and reduced leaching.

2.7.6 Importance of biochar in the environment

The process of capturing carbon that would otherwise be released into or remain in the atmosphere and storing it thereafter in plants and soils is known as carbon sequestration (FAO, 2008). Large quantities of carbon in biochar can be sequestered in the soil for thousands of

years (Lehmann *et al*., 2006). Marris (2006) stated that about 250 ha farms could sequester approximately 1900 tons of carbon dioxide per annum. The ability to store carbon in plant and soil systems means that there is a greater chance of reducing the greenhouse effect (Lal, 2004). According to Schmidt and Noack, (2000) biochar has recalcitrant carbon which can resist degradation. This characteristic of biochar makes it essential for major carbon sink. With respect to other terrestrial sequestration techniques, biochar has a higher potential to increase carbon storage time than afforestation (Ogawa *et al.*, 2006). Biochar application resulted in decreased emission of nitrous oxide and methane (Duku *et al*., 2011).

2.7.7 Biochar impact on soil performance and resource implications

Interactions among soils, biochar, micro-organisms and root of plants start occurring within a short time after biochar application (Lehmann and Joseph, 2009). Glaser *et al*. (2002) indicated that water retention in biochar amended soil is 18 % higher than in adjacent soils with little or no biochar amendment. The stable macro pore structure of biochar is in part responsible for improving a soil's water holding capacity (Brodowski *et al*., 2007).

Biochar has a very high capacity for cation sorption due to its huge specific surface area and strong cation exchange capacity (Gaskin *et al*., 2007). The specific surface area of biochar increases as temperature increases due to the creation of more micropores (Bird *et al*., 2008), and great quantities of carboxyl groups on the surfaces. Cheng *et al*. (2006) suggested that increases of carboxyl groups on char surfaces with time are in part due to either partial oxidation of open surfaces by biological and non-biological processes and/or chemisorption. The cation exchange capacity that biochar offers differs from that of soil organic matter due to the stability that it possesses. Given the incremental improvement in cation exchange capacity, there does not appear to be a cap on the amount of benefit that may be obtained through repeated addition. Water can be purified with biochar by removing nitrate and phosphate

(Mizuta *et al*., 2004; Eduah, 2009).

Having an established affinity for organic compounds, biochar may be able to loosely hold nutrients in a bio-available form, which is important for crop growth. It may also be able to bind hazardous substances in the soil (Yu *et al*., 2006). The indirect influence of biochar on the chemistry of the soil seems to arise from the amendment of soil pH. Studies show that terra preta sites have higher pH and phosphorus than surrounding soils. The ash component of biochar has more available forms of nutrients than uncharred biomass. The indirect effect of biochar on phosphorus availability in the soil and the mineral ash of its matrix containing phosphorus, potassium, and other potentially important micronutrients are essential in explaining its short- term influence on crop growth (Lehmann and Joseph, 2009).

According to Steiner *et al*. (2008), microbial activity in soil is enhanced on the addition of biochar. Sam (2014) also found that degradative abilities of heterotrophs were enhanced when cocoa pod husk biochar was amended to atrazine and paraquat contaminated soils. There is a sizable body of research that supports biochar's ability to stimulate local arbuscular mycorrhizal fungus, which has been linked to improved plant growth (Rondon *et al*., 2007). The microbial structure in soil that contains aged biochar is distinctively different from those in which fresh biochar has been amended (Kim et al., 2007). Microbial populations react initially with labile components of biochar on its amendment to soil and pyrolysis condensates seem to promote microbial activity in the soil (Steiner *et al*., 2008).

2.7.8 Biochar and nitrogen fertilizer interactions

One approach to improve the efficiency of fertilizer use is integrated crop management which uses organic manure and other organic resources (Fageria and Baligar, 2005). Organic matter added to soil decomposed faster in tropical environments compared to charred biomass.

When added to soil, biochar is more recalcitrant yet enhances the use of nitrogen from added inorganic fertilizers (Steiner *et al*., 2007; Widowati and Asnah, 2014). This is because the

application of biochar boosted the soil's cation exchange capacity, which decreased nitrogen loss (Chan et al., 2008) and also its capacity to inhibit ammonium transformation to nitrate released from fertilizer (Widowati and Asnah, 2014).

2.7.9 Negative effect of biochar on soil

McClellan et al. (2007) reported several instances of reduced plant growth as a result of biochar application due to the temporary high levels of pH and volatile nutrient imbalances associated with it when applied fresh. Mostly, biochar has an initial alkaline pH which is favoured for application to soils with low pH. When applied to alkaline soils, however, plants experience nutrient deficiency particularly basic cations and P. High pH of soils due to biochar addition may also cause NH_3 volatilization when ammonium-based fertilizers or organic manures are applied. Tars, resins and other transient compounds that are left on the surface of biochar just after production can impede plant growth (McClellan *et al.*, 2007)

Inaccessible to microbial and enzymatic degradation, biochar adsorbs substances like insecticides and organic waste (Kookana *et al.*, 2011; Zimmerman *et al.*, 2011). Some biochar products may be hazardous to plants and soil microfauna, according to Kookana *et al.* (2011).

2.7.10 Effects of biochar on soybean growth

Biochar has recently been the subject of in-depth study and is highly suggested for crop productivity. One of the crops on which the effects of biochar have been fairly assessed is soybean. For instance, Wang *et al.* (2016) found that soybean growth has improved during their investigation. Biochar might change the way soils were structured, as well as how well they could store water and absorb nitrogen. In comparison to areas lacking biochar, it was concluded that biochar increased soybean growth and gave the plants a more uniform growth during the reproductive periods. Following the application of biochar, Suppadit *et al.* (2012); Yooyen *et al.* (2015); Egamberdieva *et al.* (2016) reported an increase in nutrient uptake,

growth, dry matter, nodulation, and yield. Some soybean types respond favourably to rice straw biochar in terms of nodulation, growth, dry matter buildup, yield, and plant uptake of nutrients from the soil (Agbanu, 2017).

Thies and Rillig (2009) have suggested that the application of biochar can improve the activity of microorganisms in acidic soils by increasing the pH levels, which is conducive for soybean production. It has been observed that the combination of biochar with lime and other amendments significantly enhances soil water content, organic carbon (OC), nitrogen (N), phosphorus (P) and cation exchange capacity (CEC) (Agegnehu *et al*., 2016). Laird *et al*. (2010) reported that biochar is more effective than manure in reducing soil bulk density and enhancing water holding capacity. One of the main practical benefits of biochar is that it enhances grain yield while reducing the leaching of soil nutrients (Biederman and Harpole, 2012).

According to a study conducted by Arabi *et al*. (2018), soybean yield increased by 51% after the application of biochar. The study attributed this increase to the improvement in soil acidity caused by the biochar, resulting in an increase in the number of pods per plant and ultimately leading to higher grain yield. Another study conducted by Bayan (2013) reported a 62 % increase in the number of soybean pods per plant under the 2 % wheat straw biochar treatment compared to the biochar-free treatment. The study highlighted the positive effect of biochar on the growth and nodulation of soybeans.

2.8 Phosphorus

A vital plant nutrient that has been widely distributed in nature is phosphorus. For plant and animal life, phosphorus, nitrogen (N), and potassium (K) are crucial nutrients. Since phosphorus is a finite, non-renewable resource, its effective utilization is crucial, and its

shortage in soils significantly lowers crop yields. An essential component of plant bioenergetics is phosphorus. Phosphorus, a component of ATP, is necessary for the conversion of light energy to chemical energy during photosynthesis (ATP). P is employed in the phosphorylation process to change various enzymes' activities as well as for cell signaling. Many plant biomolecules could utilize ATP for biosynthesis, phosphorus is crucial for plant development and flower/seed formation. Phosphate esters are the raw materials used to make DNA, RNA, and phospholipids.

P is most frequently found in soil as the insoluble polyprotic phosphoric acid (H_3PO_4), but plants prefer to absorb it in the monovalent and divalent forms of $H_2PO_4^-$ and HPO_4^{2-} at pHs of 6-7, where each form accounts for 50 % of the total P. At a pH of 4-6, $H_2PO_4^-$ contains approximately 100 % of the total amount of P in solution (Black, 1968). Additionally, only 20 % of total P is represented by $H_2PO_4^-$ and 80 % is represented by HPO_4^{2-} at pH 8. Because of its low concentration in soil and high demand by plants and microbes, it is typically the limiting element. When mycorrhiza is present, phosphorus uptake by plants may increase. The deep purple colouring of plant leaves indicates phosphorus deficiency.

The division of photosynthates among the source, leaves, and reproductive organs is controlled by the quantity of P supplied throughout the reproductive growth phase, according to Marschner (1995), and this effect is critical for N-fixing legumes. Studies have revealed that a significant phosphorus shortage during early growth results in overall plant stunting, which often manifests as an unnatural coloring. The plants are often a dark bluish-green tint, with purple-tinged stems and leaves. Older leaves will exhibit the first signs of phosphorus deficiency since it is a very mobile nutrient in plants, and it may be transferred from older tissues to actively growing parts (McBride, 1994). Perennial crops will benefit from receiving fertilizer with a high phosphorus concentration because it may promote the development of strong roots. For the best agricultural yields, the phosphorus (P) content of the soil is essential. Additionally, phosphorus enables a plant's root development, energy storage, and transfer,

flower and fruit development, and early maturity. The majority of it, however, is in insoluble compounds that are not usually available to plants due to physico-chemical properties in most soils. The P- soluble chemicals have low solubility indices, are stationary, and are extremely reactive. Important activities including mineralization and immobilization of organic P molecules are a part of the phosphorus cycling in soils.

According to research, 0.2 µg/ml P was sufficient for ultimate growth, implying that a low P level in the soil solution is often suitable for normal plant growth (FAO, 1984: Barber, 1995). One way to address soil fertility constraints for sustainable agriculture in West Africa's Savanna regions is to create soil nutrient management technologies, based on a sufficient supply and viable share of inorganic and organic fertilizers.

According to Tisdale *et al*. (1985); Gupta (2011), phosphorus is a crucial plant nutrient for initial root development, energy transmission, photosynthesis, water use efficiency, nodulation, seed formation, size, and number, all of which contribute to high soybean grain yield. However, ongoing agriculture practices reduce soil P availability due to plant removal and Al^{3+} and Fe^{2+} ion fixation in acidic soils. P is supplied by the use of P fertilizers and organic sources to boost P availability. While organic sources of fertilizer take longer to break down and release nutrients, mineral fertilizers are easily accessible. For smallholder farmers, access to and the cost of mineral P fertilizers are significant obstacles (Buresh & Smithson, 1997).

In Chuka and Muthambi (Meru South District), Mugendi *et al.* (2010) discovered a notable rise in the weight of 1000 fresh soybean seeds and pods after applying 50 kg ha-1 P2O5. Also in Nigeria, the yield of soybean grains on acid soil was significantly boosted by applying P fertilizer at a rate of 30 kg P_2O_5 ha^{-1} (Mahamood *et al*., 2009). Following the application of 60 kg P_2O_5 ha^{-1}, Mabapa et al. (2010) found an increase in above-ground biomass and grain yields of soybean in South Africa. Following the application of P fertilizers in Nigeria, increased soybean grain yield and component levels have also been documented (Kamara *et al*., 2007; 2008; 2011). Numerous studies have shown how P affects N fixation and helps it to become

more stable. For instance, in the central Kenyan highlands, Mugendi *et al*. (2010) found that adding more P fertilizer up to 25 kg P_2O_5 ha^{-1} produced greater nodule fresh weight.

According to Ogoke *et al*. (2004), application of P fertilizer to Nigeria at rates of 30 kilogram P_2O_5 ha^{-1} and 60 kg P_2O_5 ha^{-1} greatly enhanced the number of nodules, whereas the largest quantity of N fixed was found at 26.4 kg P_2O_5 ha^{-1} (Amba *et al.*, 2011). Similarly, P application greatly influenced N fixation in Nigeria, according to Chiezey and Odunze (2009). Meanwhile, Lapinskas and Piaulokait-Motuzien (2006) discovered that lime added to inoculated seed fixed 106 kg N ha^{-1} when operating under acid soil in Lithuania.

2.9 Soil available nitrogen

The amount of N fixed in low-mineral-N soils is often high, but only when there is sufficient water and plant-growth-supporting nutrients (Unkovich *et al.*, 2008). The creation, establishment, and activity of nitrogenase are also inhibited by soils with high levels of nitrogen in the root area, according to a number of studies (Abdel-Wahab *et al.*, 1996; Peoples *et al*., 1995) because legumes absorb soil N with less energy than biologically fixing N from the atmosphere (Cannell and Thornley, 2000). N fixation is inhibited as soil mineral content N increases (Macduff *et al*., 1996).

According to Gan *et al*. (2004), applying starter N can sometimes boost nodule establishment and N fixation compared to not applying any mineral N. Depending on the crop type and environmental factors that affect crop growth, a starter N application that enhances BNF normally uses less than 4mM for NH_4^+ and 2mM for NO_3^-. This, according to Keyser and Li (1992), is due to the possibility that symbiotic legume-rhizobium does not create enough nitrogen during the early stages of growth to satisfy the N need for mineral nitrogen that is beneficial to early growth. N fertilizer can boost crop biomass and pod size by 16 % and 44 %, respectively, depending on whether it is administered during the vegetative or reproductive growth stages (Katulanda, 2011). Yinbo *et al*. (1997) found that timing and application rates

have a significant impact on legume response to N fertilizer application. The amount of plant N acquired from fixation is increased when N fertilizer is applied at the R5 (pod filling) stage (Yinbo *et al*., 1997).

2.10 Rhizobium Inoculation

Due to a smaller population of efficient and compactible rhizobium in the soil, nodules produced on the roots of legumes may not develop when they are first introduced to the soil. Therefore, it is crucial to add a suitable strain of rhizobium in areas where legumes have not been sown or where there are no local rhizobia populations (Ledgard and Steele, 1992). The process of inoculating legume seeds with enough of the right strain of rhizobia results in a quick and successful nodulation of that legume in the field (Sinha, 1997). Depending on the application method, inoculant formulations are available in a wide range of forms. Examples include concentrates that are liquid or frozen, inoculated granules, porous gypsum granules, and natural peat granules. The most popular kind of inoculant is peat-based, which is administered directly to seeds or in liquid form (Sham *et al*., 2005). Sedge peat and rhizobia broth culture are combined to create peat inoculant. A sufficient amount of moisture is added to promote rhizobia development and multiplication (Ledgard and Steele, 1992; FAO, 1984).

The main objective of inoculation is to increase the quantity of the desired strain of rhizobia in the rhizosphere because this is necessary for optimizing nodulation and N fixation, which enhances BNF and grain yields of soybean (Lupwayi *et al*., 2000). In addition to preventing crop failure, inoculation also helps prevent issues that are less directly related to production, such as growing crops that are N deficient (Deaker *et al*., 2004). The most advantageous agronomic technique for maximizing legume output was found to be inoculation with a desired rhizobium (Gudni and Graig, 2003). However, inadequate competitiveness with native strains, unfavourable environmental circumstances, and other related factors limits their quantity and viability (Batilan and Johnson, 1995). Under ideal conditions, legumes can fix 200 kg N/ha per

year (Giller, 2001). Only the existence of productive and efficient rhizobial strains—which may be native or introduced—allows for the benefit of legume-N. Prior to planting, it is important to inoculate seeds since this can increase nodulation and N fixation by establishing a significant rhizobial population in the rhizosphere. The most important of these was a deficiency in bacteria necessary for soybean nodulation (Khidir, 1997). According to reports, the crop should be infected with the right rhizobium strain for higher yields (Hardson and Atkins, 2003). An effective substitute for nitrogen fertilization has been found as soybean inoculation. Due to its lower cost and advantages in terms of plant growth and seed quality, rhizobium inoculation has also been discovered to offer enormous potential as a fertilizer substitute. Although adding a small amount of N fertilizer to the soil improves nodulation, nodule development and N fixation are greatly reduced when large amounts of N are added to the plant (Ahmed, 2013). Only 6 % of natural rhizobia populations in Ghanaian soils, according to research by Fening *et al.* (2002), are highly successful, with the rest 68 % and 26 % being only moderately and ineffectively effective. In Yamgambi Congo, infected soybeans produced 80–300 % more yields in another field study (Shurleff and Aoyagi, 2009).

2.11 Soybean nodulation, biological nitrogen fixation (BNF) and BNF-related factors

Like other legumes, soybeans collaborate with soil microbes to fix nitrogen through root nodules. In both natural and agricultural systems, the rhizobium-legume connection is significant. The formation of root nodules, which act as both a habitat and a reliable source of food for bacterial symbionts that also provide anaerobic conditions (such as low oxygen) for nitrogen fixation, is caused by the infection of legume roots with nitrogen-fixing bacteria of the Rhizobiaceae family in low nitrogen soils (Eckardt, 2006). The three (3) key stages of the root nodule's formation are pre-infection, nodule initiation, and differentiation. When rhizobial symbionts are in the pre-infection stage, flavonoids produced by legume root hairs serve as chemotaxis and also cause the expression of nod genes (Eckardt, 2006). The gene's expression

leads to the creation of a protein called nod factor, which sets off a series of sequential events including the curling of root hair around rhizobia-invading organisms, their entry into plants via the infection thread, and the beginning of cell division in the cortex of the root, which denotes the early stages of nodule formation. In the soil layer directly surrounding plant roots, when microbial activity is at its peak, rhizobia colonize and grows when flavonoids are present (Hopkins and Hurner, 2004). *Bradyrhizobium japonicum*, a nitrogen-fixing bacterium (rhizobia), coexists symbiotically with soybean plants (Sarkodie-Addo *et al*., 2006). One of the most ideal nitrogen fixation systems is the relationship between rhizobium bacteria on root nodules and the soybean plant. When the soybean plant is inoculated with the soybean *bradyrhizobium*, it may fix 57.94 kg/ha of nitrogen (Shurleff and Aoyagi, 2009). Rhizobium bacteria, which are present in the roots of some legume plants, fix atmospheric N_2 to produce NH_4^+, which is then transformed into amino acids that plants may use. Through the process of denitrification, rhizobia may use nitrate in the soil and transform it into gaseous N oxide. A variety of rhizobium and *bradyrhizobium* strains exhibit this (Ledgard and Steele, 1992; Russelle, 2008). Both the properties of the rhizobium and the host plant have a significant impact on the amount of fixed N. Enhanced rhizobium strains is often chosen in improved BNF under particular circumstances, however the chosen strains must be able to thrive in the field, which is based on elements like their capability and efficacy (Ledgard and Steele, 1992). An essential tool for boosting agricultural output and soil fertility is inoculation (Keyser and Li, 1992).

The total N and grain yield of soybean cultivars have been observed to increase when rhizobium-inoculated seeds are used. N requirements for soybeans are complex since the crop can take both N from the soil and N from the environment (Mrkovack *et al*., 2008). In the early phases of growth, nitrogen fixation ability is modest, but it rises quickly afterwards. N fixation rises at the R3 and R4 phases and starts to fall at the R5 stage, which also happens to be when N demand peaks. The R5 stage is marked by a high requirement for photosynthates from

nodules and pods, which promotes thylakoid membrane energization (Diaz *et al.*, 2009). The nitrogen fixation process is influenced by the host plant's physiological conditions. The development and activity of nitrogen-fixing plants are adversely affected by other environmental factors. Host legume vigour is constrained by nutrient deficiencies, temperature extremes, salt, plant diseases, mineral toxicity, an unfavorable pH, grazing, insufficient or excessive photosynthesis, and soil moisture (Zahran, 1999). The impact of salinity on legumes might be critical due to their low tolerance to salinity and the high sensitivity of the symbiotic nitrogen fixation to stress. Saline conditions have a strong influence on the growth of nodules and rhizobial infection of root hair (Rao *et al.*, 2002). Saline circumstances do not impair rhizobia's ability to colonize roots, but they do have an impact on the growth, development, and effectiveness of fully developed nodules (Rao *et al.*, 2002). Acidic soils are detrimental to the development, survival, and nitrogen fixation of microorganisms as well as the interaction between rhizobium and legumes (Bordeleau and Prevost, 1994). Extreme soil pH can greatly diminish the number of rhizobia that colonize the rhizosphere (van Jaarsveld *et al.*, 2002).

Extremely low amounts of calcium and phosphorus, as well as manganese and aluminum toxicity, are present in highly acidic soils (pH 4), which have an impact on rhizobia and host plants. In low pH soils, nitrogen fixation and nodulation are negatively impacted (Bordeleau and Prevost 1994). Temperature changes have an impact on nitrogen fixation and nodulation because rhizobium strains' ability to compete in their native environment is greatly reduced (Bordeleau and Prevost, 1994). High temperatures are one of the limiting factors affecting nitrogen fixation in the tropics and subtropics. Nitrogenase activity is greatly reduced due to ineffective nodule formation at high temperatures (Hungria and Franco, 1993).

Moisture stress influences the morphology of rhizobia strains. Some strains have been reported to take on irregular shapes when exposed to low water levels. Moisture stress causes a reduction in nodulation and infection in legumes (Zahran, 1999).

Drought and soil moisture both have an impact on nodulation. Drought has an impact on the

number, weight, and size of nodules. The amount of N fixed depends heavily on soil moisture availability and decreases with water stress (Guriqbal, 2010). The three main factors associated with the effects of drought on BNF are carbon scarcity, oxygen limitation, and nitrogen metabolism regulation (Ladrera *et al*., 2007). Sinclair *et al*. (2007) also stated that N_2 fixation combined with soil dryness reduces yield due to insufficient N for protein production, which is a critical seed product. Rasmo *et al*. (2003) reported that bacteroides senescence occurred after exposing common bean to ten (10) days of moisture stress due to cell wall degradation of nodule. Drought, for example, has a negative impact on nodule weight and nitrogenase activity.

2.12 Benefits of soybean

Soybean is a pulse and oilseed crop that has a high yield potential. The seed has 20 % edible oil and 40 % protein. It has a 5 % protein level and contains lysine, a substance that is uncommon in grains (Joshi, 2015). With minimal agricultural inputs, the crop can be grown effectively in many Nigerian states (Dudge *et al*., 2009). The most economical, easily digestible, and nutrient-dense oil source in the bean family is soybean. It is consumed as soy milk, tofu, a curd that resembles cheese, soya kebabs, and soy sauce throughout East Asia (Encyclopedia Britannica, 2008). It is one of the few plants that offers a complete protein since it includes all eight essential amino acids for a healthy human body (Soytech, 2015). Low serum cholesterol levels and a reduced risk of cancer have both been associated with soy and other legumes. For postmenopausal women, it has also been demonstrated to be a successful substitute for hormone replacement treatment (Graham and Vance, 2003). Numerous industrial uses exist for soybeans. Lecithin-containing oil and protein are separated into two components of the crushed soybean (meal or flour). Doughnut milk, meat pies, pancakes, and soy sweets can all be made with soybean flour. The oil can be used to create anti-corrosive compounds, soaps, shampoos, and detergents, lubricants, diesel fuel, hydraulic fluids, disinfectants, fungicides, and herbicides, as well as cosmetics, waterproof cements, and metal casting agents

(Adu-Dapaah *et al*., 2004: Graham and Vance, 2003). Paints, textile coatings or weather proofing, and paint removers are some of the additional industrial uses for soybeans (Shurleff and Aoyagi, 2009). To fix atmospheric nitrogen, soybeans, like other legumes, form symbiotic relationships with nitrogen-fixing bacteria. Soybeans, in particular, form a symbiotic relationship with *Bradyrhizobium japonicum* and *Sinorhizobium* species. This feature, along with its oil, protein, and wide environmental adaptation, ensures that it will continue to be an important food crop around the world (Keyser and Li, 1992).

Soybean is a key ingredient in the production of poultry meal in Ghana. The crop has the potential to boost farmers' revenue, especially when produced as a cash crop. It can also eradicate striga, an endemic parasitic weed that lowers agricultural produce yield and quality (Dudge *et al.*, 2009). In cattle feed all over the world, it serves as a source of protein. A tonne of crude protein yields 440–480 kg of high-quality, highly digestible protein (FAO, 2009; Dudge *et al*., 2009). In the late reproductive stage (after R6 and before R7), when the pods are still green and the seed fill is 80–90 % developed, vegetable soybean is harvested (MoFA and CSIR, 2005; Diep *et al*., 2002). It has more protein than beef and fish, both of which have a protein level of around 18 %. Because soybean products are low in cholesterol, high in fiber, vitamin B1 and B2, calcium, and phosphorus, and free of cholesterol, they are in great demand for health reasons (Greenberg and Huartung, 1998). Cattle, sheep, and goats are fed on the haulms that result from the seed extraction process (Dudge *et al*., 2009). Shelled beans, fresh, frozen, or a combination of the three can be picked and planted (Diep *et al*., 2002). The crop has the potential to relieve poverty and undernourishment, especially among pregnant women and children (Malema *et al*., 2006; IITA, 2009). The standard of living can be raised by the production of soybean, and the health of both humans and animals can be improved by the superior quality of soybean meal (Malema, 2007).

CHAPTER THREE

MATERIALS AND METHODS

3.1 Site description

The research was conducted at the Forest and Horticultural Crops Research Centre (FOHCREC), Kade of the University of Ghana in the Denkyembuor district of the Eastern region of Ghana (latitude 6°09 and 6°06′N and longitude 0°55′ and 0°49′ W). The center is 135.9 meters above sea level and is situated in Ghana's semi-deciduous forest agro-ecological zone. The area experiences a Bi-modal rainfall pattern with peaks in June and October, a brief gap in August, and a dry spell from December to March. The FAO-UNESCO classifies the soils at the trial site, which are primarily forest Ochrosols formed from precambium phyllitic rocks, as Acrisols and are deep, well-drained soils (Ahn, 1961; FAO-UNESCO, 1998). Kade typically receives about 112.69 mm (4.44 inches) of precipitation and has 233.17 rainy days (63.88 %) annually (Akosah, *et al.*, 2021). The temperature of the area is between 25 and 38 °C (Ofosu-Budu, 2003).

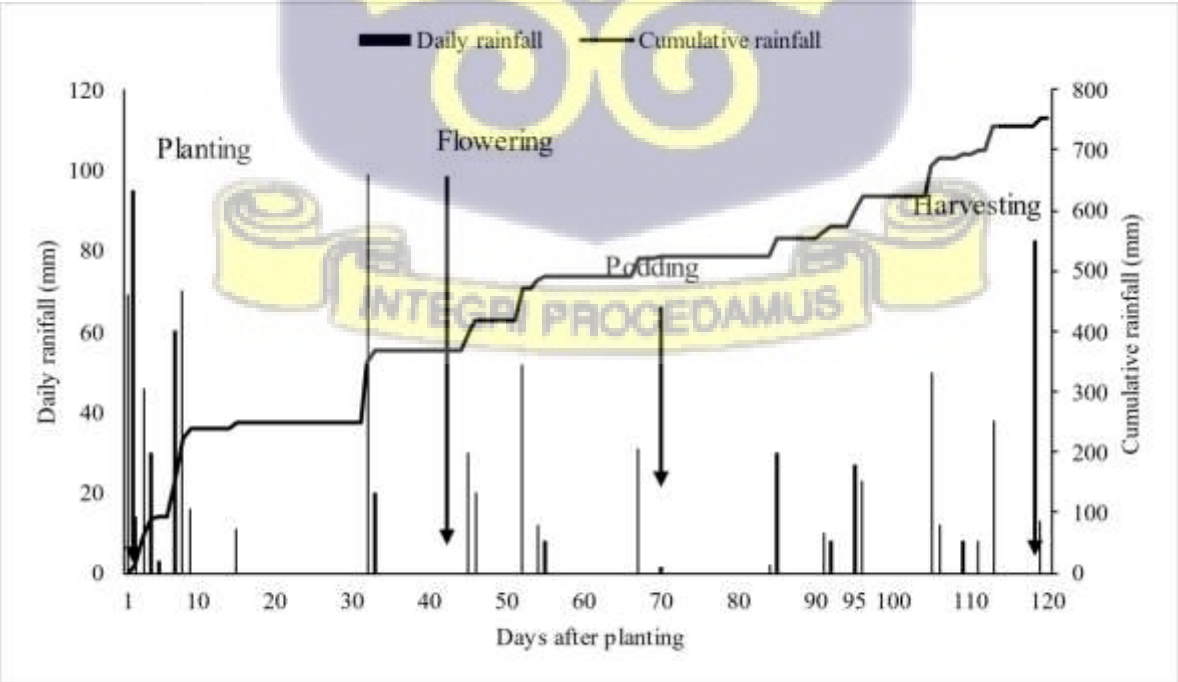

Figure 1: Daily and cumulative rainfall during the growing season in Kade, Ghana.

3.1.1 Soil characteristics

Soil samples were taken from the experimental plot to a depth of 0-20 cm using an auger, for initial soil characterization. Twenty samples, selected at random from various areas of the plot, were combined, sub-sampled, air-dried, crushed, and sieved using a 2 mm sieve to remove ironstone, twigs, and plant roots to create a composite sample.

The soil samples were analyzed for the physical and chemical properties like Total N, Available P, pH, Organic Carbon, Organic Matter and Exchangeable Ca, Mg and K. The methodologies used for the soil analysis are discussed under section 3.10.

3.2 Experimental design and field layout

The factorial experimental design was arranged in a split-split plot design with four (4) replications. Jenguma variety of soybean was used for the trial. The treatment combinations included 5 tons/ha of biochar treatments (rice husk biochar), 2 tons/ha of lime ($CaCO_3$), two levels of Phosphorus fertilizer (0 and 20 kg P ha^{-1}), and *Bradyrhizobium* inoculation and control treatment (No inoculation).

Land preparation was done by ploughing and harrowing using tractor and fields were well levelled before lining and pegging were done. The fields were demarcated into plots before treatment application. The experimental treatments were replicated four times giving a total of 48 experimental units. Eight weeks after applying the soil amendments, three seeds were sown in drilled holes, which were later thinned to two seedlings per stand three weeks after planting (3WAP). Each plot had ten rows, each measuring six metres, spaced 50 cm apart. Within each row, the soybean was spaced 10 cm apart.

3.3 Soil liming

Liming was done by applying granulated calcium carbonate ($CaCO_3$) (Omya Calciprill 110, Germany) eight weeks prior to planting. Liming at the rate of 2 tons per hectare was done by

drilling at a depth of 10 cm and covering with soil to avoid being blown away by wind.

The Omya Calciprill 110 granulated $CaCO_3$ used had the following compositions: diameter of 2 mm -6 mm; Calcium Carbonate 91 %; Magnesium carbonate 2 % (51 % calcium oxide (CaO) = 36 % Ca (Total); 0.9 % Magnesium oxide (MgO) = 0.6 % Mg (Total) and H_2O < 2 %.

3.4 Biochar and Phosphorus amendment

Biochar obtained from rice husk was used as an additional liming material in this study. The rice husk biochar was charred in a furnace at pyrolysis temperatures of 700 ºC at FOHCREC, Kade. The charred rice husk was spread and dried under the sun for five days and sieved for chemical characterization. The rice husk biochar was analyzed for pH, Total N, Available P, Exchangeable K, Ca and Mg; Organic carbon.

Table 3.1: Chemical properties of rice husk biochar used in the study.

Property	rice husk biochar
Total N (%)	1.27
P (%)	0.09
K (%)	1.38
Ca (%)	0.80
Mg (%)	0.41
OC (%)	48.14
pH	6.85
C: N	38.01

The rice husk biochar at the rate of 5 tons per hectare was applied in drills at 10 cm depth and covered with soil to prevent being eroded by water or wind, two (2) weeks prior to planting.

Phosphorus fertilizer (Triple Super Phosphate) at two levels (0 and 20 kg P ha^{-1}) were also applied one week after planting in drills 10 cm away from the plant.

3.5 Rhizobium inoculation

Bradyrhizobium japonicum strain USDA 110 present in 1010 cells g^{-1} of the rhizobium inoculant (Nodumax) was employed in the study. The inoculant was obtained from the International Institute of Tropical Agriculture in Ibadan, Nigeria. The "Jenguma" variety of soybean seeds were moistened in a basin with Gum Arabica solution before the inoculant was added at a rate of 5 g per kilogram of seeds. The mixture was thoroughly stirred and consistently swirled until an even coating was achieved. To ensure that the inoculant adhered sufficiently to the surface of the seeds before planting, the seeds were then spread out on a sack in the shade and was air-dried for 30 minutes.

3.6 Soybean planting and cultural practices

Inoculated seeds were planted early morning to prevent direct sun exposure, which could have reduced the inoculant's quality. To prevent contamination, the uninoculated seeds were planted first but on the same day as the inoculated ones. Three manual hoe weedings were done to control the weeds. To regularly control diseases and insect pests, Mancozeb 80WP fungicide (Agrithane) and insecticide Emamectin Benzoate (Attack) were sprayed.

Seedlings were sprayed with an Emamectin Benzoate solution one week following their emergence. One week after emergence, seedlings were sprayed with an Emamectin Benzoate insecticide at the concentration of 250 mL/15L to control caterpillars and leaf miners. Cypermethrin 36 g at the rate of 35 mL/15L was used to control grasshoppers. Spraying was

done with Attack (permethrin (25g/L) + pirimiphos-methyl (475g/L) once every week till the plants were 6 weeks old.

Manual irrigation was carried out when the rains ceased just after planting using water can for uniform distribution. The irrigation was done early morning and late evening till plant establishment when it was reduced to three days intervals. This was done to provide water for the sown seeds to enhance uniform germination. All cultural practices recommended for growing soybean were applied equally to all plots. The crops were grown for a maximum of 120 days.

3.7 Measurement of grain yield and growth parameters

3.7.1 Nodule number and effectiveness

Ten plants were randomly selected from each plot and uprooted with a spade. The plants were washed with clean water to separate the roots from the soil and expose the nodules. The nodules were taken from the roots and counted. To assess nodule efficiency, the nodules were cut open using a knife. The effectiveness was assessed using a hand lens. Reddish or pink nodules were declared effective. Nodules were sampled once at the commencement of podding.

3.7.2 Nodule fresh weight

The nodules removed were counted and freshly weighed to determine nodule fresh weight.

3.7.3 Shoot biomass

Shoot biomass was sampled at the start of podding. Ten plants whose nodules were taken and counted were packaged in black polythene bags and labelled. They were sun dried for two weeks until all attained constant weight. The dry shoot weights were taken with an electric weighing scale.

3.7.3 One hundred seed weight

This parameter was determined by counting 100 seeds from each plot and after oven drying, their weights measured with the use of Standard electronic scales and recorded.

3.7.4 Harvest index

Plants harvested at maturity with their pods attached were sun-dried for 10 days before being weighed. The total weight of the biomass was recorded. The pods were then separated from the plants, and the seeds were threshed. The total weight of the seeds was recorded. This was then divided by the total biomass weight to calculate the harvest index, which was expressed as a percentage (Donald & Hamblin, 1976).

$$\text{Harvest Index (H.I)} = \frac{Grain\ yield}{Biomass\ yield}\ x\ 100 \qquad [3.1]$$

3.7.5 Grain yield

All of the remaining plants on the recorded plants with tags were harvested by hand-pulling them out of the ground after they reached full maturity, and the pods were typically dried. The pods were then detached from the plants, weighed, shelled, and the grains were separated from the husks. The grains were further dried on a concrete platform to moisture content of 13 % using a moisture meter and then weighed.

3.7.6 Haulm weight

At maturity, all recorded plants with tags from each of the 48 plots were harvested and dried under the sun for five days. The shoot biomass of the plants, which includes the leaves, pods and grains was weighed using a digital weighing scale. After threshing, the grains from each plot were weighed using an electric balance, and the values were subtracted from the total shoot weight of each plot to determine haulms weight.

3.8 Agronomic P-use efficiency

The Agronomic P-Use efficiency (APUE) defined as the amount of Phosphorus used in producing one kilogram of grain was determined by using the following formula: (Yuen and Pollard, 1953).

$$\text{Agronomic P-use Efficiency (APUE)} = \frac{Yp - Yc}{Fert\ a} \qquad [3.2]$$

Where Yp = treated plots

Yc = control plots

Fert a = amount of fertilizer applied

3.9 Rainwater-use efficiency

Rainwater-use efficiency (RWUE) indicates yield attained by a treatment per millimeter of rain water received during the study period.

Rainwater-use efficiency was calculated by dividing the grain yield (kg/ha) by cumulative rainfall (mm) from sowing to harvest (Sharma *et al.*, 2013).

$$\text{RWUE (kg/ha/mm)} = \frac{Grain\ yield\ (kg/ha}{Cummulative\ rainfall\ (mm) from\ sowing\ to\ harvest} \qquad [3.3]$$

3.10 Final soil chemical analysis

Soil samples were taken from each of the 48 plots after harvest along planting lines where the amendments were applied. Ten samples were taken diagonally along the planting lines at a depth of 0-20 cm using an auger. Soil samples were air -dried and sieved for analysis of chemical properties like Total N, Available P, pH, Organic Carbon, Organic Matter and Exchangeable Ca, Mg and K.

3.10.1 Soil pH

The soil water ratio of 1:2:5 was used to determine the pH of the soil, which equals 10 g of air-dried soil to 25 ml of distilled water. A 50 ml beaker was filled with 10 grams of air-dried soil. The suspension was rapidly agitated for 20 minutes after being given 10 milliliters of distilled water. The suspension of soil and water was allowed to stand for 30 minutes, by which time the majority of the suspended clay would have separated from the suspension. With blanks at pH 7 and 4, the pH meter was calibrated. In the partially settled dispersion, a pH meter electrode was placed, and the pH readings were read and recorded (Page *et al.*, 1982; and Black, 1965).

3.10.2 Total Nitrogen by Kjeldahl Method

The total nitrogen was determined using the Kjeldhal digestion method (Kjeldahl, 1883). Two grams of the air-dried soil were weighed into a 50 ml Kjeldhal flask after sieving througha 2 mm screen. 5 ml of strong sulphuric was applied after distilled water was used to moisten the weighed soil. After digesting, the fluid was allowed to cool. Using distilled water, the digested solution was placed into a 50 ml volumetric flask after cooling. In a conical flask, 5 mL of the digestsolution and sodium hydroxide solution were distilled to produce 2 % boric acid. A 0.0012 M HCl solution was used to titrate the distillate, and the end point changed from green to red.

3.10.3 Available P determination

The soil's available phosphorus was determined using the Bray 1 method (Bray and Kurtz, 1945). 50 ml of the Bray 1 solution (0.03 N NH_4^+ F^+ 0.025 N HCl) and five grams (5 g) of soil sample were put in a centrifuge container. The suspension was agitated for five minutes on a mechanical shaker, allowed to sit overnight to settle, and then filtered through No. 42 Whatman filter paper into a 100 ml volumetric flask and diluted to the required level. Using Watanabe and Olsen's (1965) molybdate-ascorbic acid technique, the amount of available phosphorus in the filtrate was calculated as follows:

A 50 ml volumetric flask filled with distilled water was used to contain ten (10) mL aliquots of the filtrate in duplicates. P-nitrophenol indicator was used to change the pH, and a few drops of 4 N NH_4OH were added till the solution turned yellow. The solutions were diluted in distilled water to a volume of 40 ml, and then 8 ml of reagent B, which consisted of 12 g of ammonium molybdate, 0.29 g of antimony tartrate, 140 ml of concentrated H_2SO_4, and 1.056 g of ascorbic acid, was added. Shaking was used to thoroughly combine the solutions, and they were let to stand for 15 minutes to stabilize the color (The colour changed to blue of varying shades depending on the concentration of the P in each sample). 8 ml of reagent B and distilled water were mixed to produce a blank solution. As mentioned earlier, a 25 mg L^{-1} standard P solution was used to calibrate the spectrophotometer. Using a spectrophotometer with a wavelength of 712 nm, the Philips PU 8620 was used to measure the intensity of the blue color. The spectrophotometer was used to measure the P concentration, and the following calculation was made:

$$\text{Mg P kg}^{-1}\text{ soil} = \frac{(Spectrometer\ reading - blank\ reading)\ x\ vol.of\ extract}{Vol.of\ aliquot\ x\ sample\ weight\ (g)} \quad [3.4]$$

3.10.4 Organic carbon determination

The amount of organic carbon in the soil was measured using the Walkley and Black (1934) wet combustion method. A 0.5 g sample of a 0.5 mm sieved soil was placed in a 250 ml Erlemeyer flask along with ten (10) ml of an IN potassium dichromate ($K_2Cr_2O_7$) solution and twenty (20) ml of concentrated sulphuric acid (H_2SO_4). After the solution had been allowed to cool for 30 minutes, the flask was carefully spun to make sure the dirt was completely covered. Additionally, 10 mL of orthophosphoric acid and 200 mL of distilled water were added. The unreduced $K_2Cr_2O_7$ that was still in solution after the oxidation of the oxidizable organic material in the soil sample was titrated with 0.2 N ammonium ferrous sulphate solution after adding 2 ml of barium diphenylamine sulphate indicator.

3.10.5 Soil exchangeable bases

Ten grams of soil was weighed into an extraction flask, and 100 mL of a pH 7 1N ammonium acetate solution was added. After shaking the combination for an hour, Whatman No. 42 filter paper was used to filter the mixture's contents. For the measurement of Ca^{2+}, Mg^{2+}, K^{+}, and Na^{+}, aliquots of the extract were employed. Using the Atomic Absorption Spectrometer, the quantities of Na and K in the extracts were measured after the photometer was calibrated with a standard solution containing 10 ppm of Na and K.

$$\text{K (Cmol/kg soil)} = \frac{G \; x \; vol \; of \; extract \; x \; 103 \; x \; 102 \; x \; H}{weight \; of \; soil \; x \; 106 \; x \; I} \qquad [3.5]$$

Where I = atomic mass

G = AAS reading (µg/L)

H= charge of exchangeable base

3.11 Statistical analysis

Data collected during the experiment at 10 weeks after planting (WAP) for nodule number, nodule dry weight, 100 seed weight, grain yield, haulms weight, shoot biomass at the start of podding, Agronomic P-use efficiency and rainwater-use efficiency were subjected to analysis of variance (ANOVA) using GENSTAT software 12th Edition. Means were separated by Least Significant Difference (LSD) at 5 % probability.

CHAPTER FOUR

RESULTS

4.1 Physico-chemical characteristics of the soil and rice husk biochar

Results of the initial soil analysis for the experimental area are presented in Table 4.1. The soil of the experimental site was strongly acidic, moderate in N and exchangeable Ca and Mg, and low in organic matter (Hoskins, 1997; Hazelton and Murphy, 2007). The total nitrogen present in the rice husk biochar was greater (1.27 %) than that of soil (0.24 %). The pH of the soil was 5.09 while that of the rice husk biochar was 6.85. The Available P in the soil from the study area was higher (10.59 mg kg^{-1}) compared to rice husk biochar (0.09 mg kg^{-1}). The soil particle analysis indicated that silt dominated (53 %), followed by sand (36 %), and clay the least (11 %). Calcium recorded the highest (3.56 cmol $_{(+)}$ kg^{-1}) exchangeable cations. Low Potassium was observed (1.00 cmol $_{(+)}$ kg^{-1}).

Table 4.1: Initial physical and chemical properties of 0-20 cm layer of soil and rice husk biochar used for the experiment.

Soil Property	Soil	Rice husk biochar (RHB)
pH (1:25 H_2O)	5.09	6.85
Total Nitrogen (%)	0.24	1.27
Organic carbon (%)	1.32	48.14
Available P (mg kg^{-1} soil)	10.59	0.09
Sand (%)	36	
Silt (%)	53	
Clay (%)	11	
	Exchangeable cations	
Calcium (Ca^{++}) ($cmol_{(+)}$ kg^{-1})	3.56	
Potassium (K^{+}) ($cmol_{(+)}$ kg^{-1})	1.00	
Magnesium (Mg^{++}) ($cmol_{(+)}$ kg^{1})	1.32	

4.2 Effect of rhizobium inoculation, soil amendments, and phosphorus on soil chemical properties 120 days after treatment application.

The results of the residual effects of the treatment on soil physical and chemical properties taken 120 days after application are presented in Table 4.2. In general, there was increase in soil pH from the initial 5.09 to 5.52 and 5.54 on plots that received biochar and lime, respectively, seventeen weeks after treatment application, although these differences were not

significant ($p > 0.05$). The effects of inoculation on pH were however significant ($p < 0.05$).

The results (Table 4.2) show that the interaction effects of rhizobium inoculation and soil amendment (R × S), rhizobium inoculation and phosphorus (R × P), soil amendment and phosphorus (S × P) and rhizobium inoculation, soil amendment and phosphorus (R × S × P) treatments on soil total nitrogen were significant ($p < 0.05$).

The sole application of inoculant, soil amendments and Phosphorus had significant effects on soil organic carbon (SOC) ($p < 0.05$) (Table 4.2). Soil organic carbon was significantly higher on the inoculated plots than on the uninoculated plots, while the plots that were amended with lime had higher organic carbon content than the biochar plots but was not significantly different from the control plots. However, surprisingly plots that received P had lower P content than the plots that did not receive any P. Except for soil amendment and Phosphorus interaction which were not significant ($p > 0.05$), all other treatment interactions had significant effects ($p < 0.05$) on organic carbon (Table 4.2)

Rhizobium inoculation had significant effects on exchangeable K and Mg (Table 4.2). The inoculated plots had exchangeable K and Mg values of 0.37 and 2.62 cmol $(_+)$/kg soil, respectively, while the values for the uninoculated plots were 0.33 and 3.31 cmol $(_+)$/kg soil for K and Mg respectively. However, rhizobium inoculation did not have any significant effecton exchangeable Ca.

All treatment interaction effects were not significant for exchangeable K ($p > 0.05$) (Table 4.2). Meanwhile, rhizobium inoculation and soil amendments interacted (R × S) to significantly ($p < 0.05$) influence exchangeable Ca and Mg. The results also show that, the interaction effectsof soil amendment and phosphorus (S × P) and rhizobium inoculation, soil amendment and phosphorus (R × S × P) were significant for exchangeable Ca and Mg. Rhizobium inoculation and Phosphorus (R × P) did not significantly influence exchangeable Ca and Mg.

Table 4.2: Effects of rhizobium inoculation, soil amendment, and phosphorus on soil chemical properties

Treatment	pH	Total N (%)	OC (%)	Avail P (mg kg^{-1})	Exchangeable cations (cmol $(+)$ kg^{-1})		
					K	Ca	Mg
Rhizobia (R)							
RH+	5.46	0.172	1.36	8.30	0.37	4.67	2.62
RH-	5.58	0.15	1.29	7.21	0.33	4.91	3.31
Soil Amendment(S)							
Biochar	5.52	0.16	1.28	7.11	0.35	4.49	2.57
Lime	5.54	0.16	1.36	7.02	0.37	4.75	3.15
No Amendment	5.51	0.17	1.34	9.12	0.34	5.13	3.18
Phosphorus(P)							
P+	5.53	0.16	1.28	6.4	0.36	4.69	2.86
P-	5.52	0.16	1.37	9.11	0.34	4.89	3.07
P-Values(0.05)							
R	*0.018*	*<0.001*	*0.003*	*0.423*	*0.012*	*0.196*	*<0.001*
S	*0.865*	*0.002*	*0.019*	*0.359*	*0.327*	*0.019*	*0.004*
P	*0.73*	*0.959*	*<0.001*	*0.05*	*0.529*	*0.28*	*0.184*
R×S	*0.233*	*0.008*	*0.019*	*0.135*	*0.386*	*<0.001*	*0.003*
R×P	*0.097*	*0.012*	*0.041*	*0.42*	*0.523*	*0.288*	*0.191*
S×P	*0.335*	*<0.001*	*0.064*	*0.275*	*0.066*	*<0.001*	*<0.001*
R×S×P	*0.843*	*<0.001*	*0.041*	*0.454*	*0.22*	*<0.001*	*<0.001*

4.3 Effect of rhizobium inoculation, soil amendments, and phosphorus on nodule number, nodule effectiveness and nodule fresh weight.

The results indicate that there was no interaction between rhizobium inoculation, soil amendment and Phosphorus fertilizer on nodule number after treatments application on soybean (Table 4.3).

The application of rhizobium inoculant significantly ($p < 0.001$) increased nodule number and nodule effectiveness by 44 % and 45 % respectively, over plants that received no inoculation. (Table 4.3). Lime and biochar effects resulted in 41 % and 62 % increase in nodule number over the control, although there were no significant ($p > 0.05$) effects on nodule number. The sole application of P fertilizer increased the number of nodules by 44 % compared to the plot that received no amendment.

Soybean treated with the rhizobium inoculants significantly produced more effective nodules than the uninoculated plants (Table 4.3). The individual application of inoculant increased nodule effectiveness by 11 % compared to the plants that did not receive inoculants. Phosphorus application alone resulted in 45% increase in nodule effectiveness over plots that received no P fertilizer. The addition of lime and rice husk biochar as soil amendments recorded 39 % and 70 % increase respectively, in nodule effectiveness over plants that did not receive soil amendments. Despite these increases, there were no significant ($p > 0.05$) influence of soil amendments and phosphorus on nodule effectiveness. In the analysis of variance (Table 4.3), there was no interaction effect among all the treatments on nodule effectiveness.

The nodule fresh weight from inoculated plants was higher than non-inoculated plants (Table 4.3). The application of rhizobium inoculant had significant effect ($p < 0.05$) on nodule fresh weight. Lime and rice husk biochar treatment respectively resulted in higher nodule weight, but the differences were not significant ($p > 0.05$). None of the interactions were significant.

Similarly, plants that received P fertilizer had higher nodule fresh weight than plants that did not receive P fertilizer but the difference was not significant.

4.4 Effects of Rhizobium inoculation, soil amendments, and phosphorus on dry shoot biomass

The application of phosphorus significantly ($p < 0.05$) increased dry shoot biomass of soybean by 28% as compared to the plants that did not receive phosphorus fertilizer (Table 4.3). The results indicate that there were significant ($p < 0.05$) interaction effects between rhizobium inoculants and Phosphorus on dry shoot biomass. The other interactions (Soil amendment × Phosphorus), (Rhizobia × Soil amendment) and (Rhizobia × Soil amendment × Phosphorus) had no significant ($p > 0.05$) effect on the above parameters measured.

Table 2.3: Effects of rhizobium inoculation, soil amendment, and phosphorus on nodule number, nodule effectiveness, nodule fresh weight, and dry shoot biomass

Treatment	Nodule number	Nodule effectiveness	Nodule fresh weight (mg)	Shoot biomass (kg/ha)
Rhizobia (R)				
RH+	7.35	6.80	5334.00	1353.00
RH-	1.03	0.59	1117.00	1139.00
Soil Amendments (S)				
Biochar	4.39	3.76	3625.00	1341.00
Lime	5.05	4.62	3476.00	1228.00
No amendment	3.12	2.71	2575.00	1169.00
Phosphorus (P)				
P+	4.94	4.37	3617.00	1398.00
P-	3.44	3.02	2833.00	1095.00
P- values (0.05)				
R	*<.001*	*<.001*	*0.005*	*0.427*
S	*0.203*	*0.167*	*0.388*	*0.737*
P	*0.152*	*0.122*	*0.224*	*0.024*
R×S	*0.416*	*0.244*	*0.958*	*0.489*
R×P	*0.352*	*0.281*	*0.243*	*0.031*
S×P	*0.588*	*0.549*	*0.266*	*0.132*
R×S×P	*0.774*	*0.565*	*0.195*	*0.142*

4.5 Effects of rhizobium inoculation, soil amendments, and phosphorus on haulm weight and one hundred seed weight

None of the treatment interactions had a significant effect on one hundred seed weight. The results show that phosphorus fertilizer application significantly ($p < 0.0.5$) influenced haulm weights of soybean (Table 4.4). P fertilizer increased soybean haulm yield by 44 % relative to no P-fertilizer. Inoculation alone did not affect haulm weight. The application of soil amendments did not significantly ($p > 0.05$) influence haulm weight of soybean. None of the treatment interactions had significant effect on soybean haulm weight.

The application of rhizobium did not significantly ($p > 0.05$) affect one hundred seed weight of soybean, even though, rhizobium inoculated plots recorded higher mean one hundred seed weight of 11.77 g as against 10.84 for non-inoculated plots (Table 4.4). This represents an increase of 9.23 %. Similarly, the sole application of soil amendments and P fertilizer, had no significant ($p > 0.05$) effects on one hundred seed weight, even though one hundred seed weight was increased by 12 % due to the application of P fertilizer.

4.5 Effects of rhizobium inoculation, soil amendments, and phosphorus on grain yield of soybean

All treatment interactions did not have significant ($p > 0.05$) effects on soybean grain yield (Table 4.4). The effect of rhizobium inoculation on grain yield was not significant ($p > 0.05$) although grain yield was increased by 46 % compared to the plants that did not receive inoculants (Table 4.4).Application of soil amendments did not also significantly ($p > 0.05$) affect yield although limeand biochar amended plots recorded approximately 100 % and 14 % increases in grain yield respectively, as compared to the control. Phosphorus application however significantly ($p < 0.05$) influenced grain yield as yield was increased by 60 % relative to the

plants that did not receive Phosphorus (Table 4.4).

Table 4.4: Effects of rhizobium inoculation, soil amendments, and phosphorus on total seed weight, one hundred seed weight, haulm weight and grain yield.

Treatment	Haulm weight (kg/ha)	One hundred seed weight (g)	Grain yield (kg/ha)
Rhizobia (R)			
RH+	991.00	11.77	138.00
RH-	441.00	10.84	94.70
Soil Amendments (S)			
Biochar	742.00	11.59	122.70
Lime	726.00	11.32	118.50
No amendment	681.00	11.01	107.80
Phosphorus (P)			
P+	845.00	11.95	143.30
P-	588.00	10.66	89.40
P- values (0.05)			
R	*0.092*	*0.612*	*0.175*
S	*0.884*	*0.875*	*0.444*
P	*0.018*	*0.204*	*0.013*
R×S	*0.643*	*0.656*	*0.142*
R×P	*0.728*	*0.466*	*0.256*
S×P	*0.665*	*0.677*	*0.847*
R×S×P	*0.865*	*0.267*	*0.776*

4.6 Effects of rhizobium inoculation, soil amendments and phosphorus on harvest index (HI), P –use efficiency, and rainwater-use efficiency

The effects of rhizobium inoculation, soil amendment, and phosphorus fertilizer on Harvest Index (HI), Phosphorus-Use efficiency and rain-water Use efficiency are shown in Table 4.5. The HI for plots inoculated with *Bradyrhizobium* was higher than that of non-inoculated plots but the difference was not significant. There was also no significant difference in the effects of soil amendments and Phosphorus fertilizer on harvest index respectively. Meanwhile, harvest index increased by 25 % and 23 % due to the application of rhizobium inoculation and P fertilizer, respectively (Table 4.5).

From the analysis of variance, the effects of soil amendment alone had significant ($p < 0.05$) influence on P-use efficiency. The application of rhizobium inoculant alone and P fertilizer alone did not elicit significant difference in P-use efficiency. The results show that, application of P fertilizer alone significantly ($p < 0.05$) influenced rainwater-use efficiency (Table 4.5)

Interactions between soil amendment and P-fertilizer were significant for P-use efficiency of soybean (Table 4.5). The other interactions (Rhizobium × Phosphorus), (Rhizobium × Soil amendment) and (Rhizobia × Soil amendment × Phosphorus) had no significant ($p > 0.05$) effects on the parameters measured (Table 4.5).

Table 4.5: Effects of rhizobium inoculation, soil amendment and phosphorus on harvest index (HI), P - use Efficiency and Rainwater-Use efficiency

Treatment	Harvest index	P-Use efficiency	Rainwater-use efficiency (kg/ha/mm)
Rhizobia (R)			
RH+	1.40	2.97	0.15
RH-	1.12	2.00	0.11
Soil Amendments (S)			
Biochar	1.30	4.20	0.14
Lime	1.27	1.74	0.13
No amendment	1.20	1.52	0.12
Phosphorus (P)			
P+	1.39	3.01	0.16
P-	1.13	1.96	0.10
P- values (0.05)			
R	*0.220*	*0.685*	*0.175*
S	*0.844*	*0.043*	*0.444*
P	*0.267*	*0.368*	*0.013*
R×S	*0.117*	*0.612*	*0.142*
R×P	*0.853*	*0.225*	*0.256*
S×P	*0.271*	*0.044*	*0.847*
R×S×P	*0.975*	*0.978*	*0.776*

CHAPTER FIVE

DISCUSSION

This chapter focuses on discussing relevant findings from this research work and made comparisons with references to related work conducted elsewhere. The discussion makes key inferences from the results and relates it to other research works all over the world.

Soil and biochar characterizations

The soil of the experimental site was strongly acidic with a pH of 5.09, moderately high in N and exchangeable Ca and Mg, and low in organic matter (Hoskins, 1997; Hazelton and Murphy, 2007). The low pH of 5.09 might be due to the nature of the inherent parent material and/ or leaching and hence justification for the investigation into the possible use of rice husk biochar and lime as soil amendments in this study. Soil pH is one of the most critical soil chemical characteristics that affects nutrient absorption and microbial activity (Liu and Hanlon, 2012). Low soil pH inhibits rhizobium growth and root infection, resulting in symbiotic failure and a low soybean yield. Extreme soil pH may reduce rhizobia colonization around the root zone of the legume, limiting the amount of nitrogen fixed (Nisa et al., 2012). The soil might have a low organic carbon content, which is due in part to the soil's low organic matter and high temperature, which accelerates the decomposition of organic matter (Agboola and Aiyelari, 2000).

The pH of the rice husk biochar was 6.85 which was slightly neutral. The pH of the biochar was a result of the release of bases due to high charring temperature as corroborated by Struebel *et al.*, (2011). High pyrolysis temperatures release organic acids and phenolic substances through the cracking of hemicellulose and cellulose and in effect, these acids react with basic cations in the feedstocks to form alkaline salts which increase the pH of biochar (Streubel, 2011; Shinogi and Kanri, 2003). The organic carbon content of the biochar was high which

was similar to that reported by Zanzi (2001) who reported that high heating rates of biochar was found to provide a shorter time for the dehydration reactions and the formation of less reactive anhydrocellulose, leading to the production of biochar with a higher carbon yield. The available P in the RHB was also high due to the release of inorganic orthophosphate ions that interacted with the released Ca to create Ca-P compounds that are not readily soluble, significantly increasing the biochar's total P content (Lehman, 2007). Shenbagavalli and Mahimairaja (2012) reported that charring of biomass can greatly enhance P availability from plant tissue by cleaving organic phosphorus bonds, to form soluble P salts in the charred materials. Rice husk biochar generally has higher total P content due to the higher inherent P content in the feedstock.

5.1 Effect of rhizobium inoculation, soil amendments and phosphorus on soil chemical properties.

The growth and development of plants require optimal soil conditions, which are a crucial prerequisite. The physical-chemical characteristics of the soil, such as its minerals, water, soil organic matter, and air, improve the health of plants and encourage their root hairs to assimilate nutrients (Chen, 2006).

It was observed in this research that there was an increase in mean soil pH from lime ($CaCO_3$) and rice husk biochar (RHB) soil amended plots. Application of rice husk biochar (RHB) and lime were observed to increase soil pH which positively increased the yield. The low yield of the control could be attributable to low soil pH, which prevented the growth and proliferation of rhizobium strains that aid in nodulation. According to research, most soil microorganisms flourish in soil with a pH of 5.5. (Zenni *et al.*, 2017). These soil bacteria boost soil aeration, resulting in enhanced soil structure and an increase in soybean output.

The current study also showed significant influence of rhizobium inoculation on pH, total N,

exchangeable K and Mg and organic carbon. Again, soil amendments also significantly enhanced exchangeable Ca and Mg. The increase in total nitrogen observed in the study could be related to the amount of nitrogen from the applied biochar. Cross and Sohi (2011) explained that biochar mineralization occurs in the soil, leading to the release of labile compounds such as nitrogen. It is usually rapid at first and progresses slowly over time. Plant-based biochar has been shown to contain small amounts of mineral nitrogen, but it accounts for the majority of the total nitrogen pool (Cui *et al.,* 2017). The increase in organic carbon content following biochar addition is probably due to additional C added by the biochar. Biochar is reported to contain a large amount of recalcitrant C that is not easily decomposed though not entirely inert. It undergoes mineralization and release labile fractions of C into soil. The observed increase in organic carbon could also be related to positive priming effect which stimulates the mineralization of native soil organic carbon. Singh and Cowie (2014) espoused that biochar supports the proliferation and metabolic activities of microbes to mineralize native carbon.

The pH was an important soil characteristic and a major determinant of soil fertility. Acidity and alkalinity are closely associated with the physico-chemical elements of the soil-plant system (Nanganoa *et al.*, 2020; Mak-Mensah *et al.*, 2021). Previous study by Nanganoa *et al.*, (2020) found that an increase in soil acidity accelerated the loss of essential elements like K, Ca, and Mg; and decreased the cation exchange capacity, thereby affecting the geochemical cycle of soil nutrients.

Available P of the soil taken 120 days after treatments application was significantly ($p = 0.05$) affected by the applied Phosphorus fertilizer. The application of P-fertilizer was responsible for the increase in available P of the soil.

5.2 Effects of rhizobium inoculation, soil amendments, and phosphorus on grain yield

The overall grain yield of this study was very low compared to many studies which reported greater yield response of soybean to combined application of inoculant and P-fertilizer(Adjei-Nsiah *et al.*, 2022; Ulzen *et al.*, 2018, Ronner *et al.*, 2016; Masso *et al.*, 2016; Adjei- Nsiah *et al.*, 2018; Kyei-Boahen *et al.*, 2017; and Ulzen *et al.*, 2020). The low yield reported in this study could be attributed to the erratic rainfall pattern during the flowering and podding stages of the crop as evidenced in Figure 1. Elsewhere, Kamara *et al*., (2011) found reduced grain yields of up to 74.05%, and reduced number of pods by 57.89 % as a result of relativelylow rainfall observed. Although yield was generally low, the study confirms the response of soybean to P fertilizer as grain yield was increased by 60 % over plots that receive no P-fertilizer. Grain legume response to P fertilization has been reported by several authors (Kamara *et al.*, 2007; Mahamood *et al.*, 2009; Kamanga *et al.*, 2010; Karikari *et al.*, 2015). P fertilizer is known to play important roles in many processes in legumes such as energy transfer, nodulation, atmospheric nitrogen fixation, flower initiation, fruit development, and seed formation (Beegle and Durst, 2002; Krasilnikoff *et al.*, 2003; Ndakidemi and Dakora, 2007; Nyoki *et al.*, 2013). In this study, plants inoculated with *Bradyrhizobium* resulted in46 % increase in mean grain yields over non-inoculated plants although this did not translate into significant yields. This supports Argaw's (2014) findings that genotype and seed inoculation enhanced seed yield in comparison to control. According to Shrivastava et al. (2000), seed inoculation with B. japonicum increased yield on average by 11% compared to control. According to Ahmad and Mohammad (2007), rhizobium inoculation led to improved soybean growth. It has been discovered that inoculating seeds prior to sowing can aid in promoting soybean growth and development (Wafaa et al., 2002).

It was naturally expected that the applications of lime, biochar, P fertilizer and inoculated seeds would increase the grain yield of soybean significantly. However, the soybean yield did not

respond to biochar and lime applications. This may be due to the fact that the treatment effects of the soil amendments could not be realized in the first year of production. Again, water (rainfall) is a major environmental factor that impacts plant growth. Moisture stress due to erratic rainfall pattern is known to be a major constraint to crop production. Hartfield and Prueger (2015) reported that, under water deficit conditions, there was reduced biomass and grain yield of soybean. Another study attributed soybean yield reduction under water stress conditions during the reproductive period to accelerated leaf senescence and shortening of the seed filling duration (Dong *et al.*, 2019).

5.3 Effects of rhizobium inoculation, soil amendments, and phosphorus on nodule number, nodule effectiveness, nodule fresh weight, one hundred seed weight and dry shoot biomass

The plants treated with *Bradyrhizobium* inoculants showed significant ($P < 0.01$) effect on the number of nodules and nodule effectiveness. The nodule weight of the inoculated plants was significantly higher than the un-inoculated plants. In this current study, the application of P resulted in remarkable shoot biomass and grain yield of soybean. Kumaga and Ofori (2004) and Devi *et al.* (2012) reported that the availability of phosphorus from applied fertilizer led to an increase in nodule number of soybean. Nodule count and nodule effectiveness were significantly increased by inoculation. Nodules contribute to fixing nitrogen into the soil through a process called symbiotic nitrogen fixation (SNF). The process, SNF is regulated by soil conditions (Moron *et al.*, 2005) which influences the survival, infection and activity of the rhizobia. Rhizobial inoculation, according to Argaw (2014), significantly improved nodule number and dry weight compared to control. Additionally, significant responses of soybean to inoculation and a larger nodule count when compared to the control were found by Okereke *et al.* (2004) and Tahir *et al.* (2009). The study also showed that only a small number of nodules were seen on the uninoculated soybean plants. Inoculation of soybean plants with

Bradyrhizobium japonicum enhanced the number of nodules and the effectiveness of the nodules, the number of seeds per plant, and the weight of the seeds, according to Dahmardeh *et al.* (2010) and Morad *et al.* (2013). In this study, it was discovered that inoculating soybeans increased the nodule count by 613 % but did not enhance yield. *Bradyrhizobium japonicum*-inoculated soybean cultivars showed significant differences ($p < 0.05$) in nodule count and seed weight, according to a study by Hungry and Bohrer (2000). P-fertilizer and rhizobium inoculation interacted significantly to affect shoot biomass. This is in accordance with Ahiabor *et al.* (2014) who reported that soybean shoot biomass increased significantly with the combined application of phosphorus and Rhizobium inoculants.

5.4 Effects of rhizobium inoculation, soil amendments and phosphorus on harvest index (HI), P-use efficiency (PUE) and rainwater-use efficiency (RWUE)

Harvest index (HI), which relates the economic yield to the total dry matter yield was foundnot to be statistically influenced by Rhizobium inoculation, soil amendments and Phosphorus fertilizer. Although studies by Adjei-Nsiah *et al.* (2021) and Adjei-Nsiah *et al.* (2022) have shownhigher P use efficiency in soybean when treated with P-fertilizer, our current study suggests very poor P- use efficiency which could be attributed to poor rainfall. The poor P-use efficiency in the present study implies that the added TSP was not used up by the plants, due to inadequate rainfall during the cropping season. Soil amendment which comprised $CaCO_3$ and rice husk biochar, on the other hand, significantly influenced P-use efficiency. This could be attributed to the fact that the additional amount of P in the rice husk biochar was used up.

The results showed that biochar amended plot had 176 % increase in P-use efficiency (PUE) over un-amended plots. The increase in PUE with soil amendment application could be the result of the additional nutrient made available by biochar. This shows additional P was taken up by the plant. This is similar to the observation of Muhammad *et al.* (2017).

CHAPTER SIX

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The study was conducted to test the hypothesis that the combined effect of soil amendments, rhizobium inoculation and phosphorus fertilizer application will increase soybean nodulation, growth and yield and soil physical and chemical properties in the semi-deciduous forest agro-ecological zone of Ghana. However, the findings suggest that while there is no evidence that combination of the three factors will improve soybean growth and grain yield on acid soils, the results indicate that the combined application of rhizobium inoculant, soil amendments and P fertilizer could enhance nodulation and improve P-use efficiency and some soil chemical properties (total N, OC and exchangeable Ca and Mg) on acid soils. The study also revealed that application of phosphorus at 20 kg ha^{-1} could increase grain yield of soybean by about 60%.

6.2 Recommendations/ Suggestions

1. It is recommended that farmers can apply phosphorus fertilizer at the rate of 20 kg P/ha for soybean grain yield enhancement on acid soil.

2. This study needs to be repeated in future under optimum rainfall condition to ascertain the full effects of the treatments.

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APPENDICES

ANALYSIS OF VARIANCE (ANOVA) TABLES GROWTH AND YIELDPARAMETERS

Appendix 1. ANOVA Table for Average nodule number

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	45.19	15.06	6.67	
Block*Rhizobia stratum					
Rhizobia	1	478.80	478.80	211.91	<.001
Residual	3	6.78	2.26	0.27	
Block*Rhizobia*Soil amendment stratum					
Soil Amendment	2	30.86	15.43	1.83	0.203
Rhizobia * Soil Amendment	2	15.95	7.98	0.94	0.416
Residual	12	101.43	8.45	0.70	
Block*Rhizobia*Soil amendment*P stratum					
P	1	27.00	27.00	2.24	0.152
Rhizobia*P	1	11.02	11.02	0.91	0.352
Soil amendment*P	2	13.21	6.60	0.55	0.588
Rhizobia*Soil amendment*P	2	6.27	3.19	0.26	0774
Residual	18	217.00	12.06		
Total	47	953.51			

Appendix 2. ANOVA Table for Average Nodule Effectiveness

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	31.616	10.539	5.19	
Block*Rhizobia stratum					
Rhizobia	1	463.763	463.763	228.58	<.001
Residual	3	6.087	2.029	0.29	
Block*Rhizobia*Soil amendment stratum					
Soil Amendment	2	29.540	14.770	2.08	0.167
Rhizobia * Soil Amendment	2	22.578	11.289	1.59	0.244
Residual	12	85.135	7.095	0.85	
Block*Rhizobia*Soil amendment*P stratum					
P	1	21.870	21.870	2.63	0.122
Rhizobia*P	1	10.268	10.268	1.24	0.281
Soil amendment*P	2	10.286	5.143	0.62	0.549
Rhizobia*Soil amendment*P	2	9.799	4.899	0.59	0.565
Residual	18	149.398			
Total	47	840.339			

Appendix 3. ANOVA Table for Grain Yield

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	116360	38787	5.37	
Block*Rhizobia stratum					
Rhizobia	1	22544	22544	3.12	0.175
Residual	3	21659	7220	6.63	
Block*Rhizobia*Soil amendment stratum					
Soil Amendment	2	1892	946	0.87	0.444
Rhizobia * Soil Amendment	2	5024	2512	2.31	0.142
Residual	12	13062	1089	0.24	
Block*Rhizobia*Soil amendment*P stratum					
P	1	34884	34884	7.58	0.0013
Rhizobia*P	1	6339	6339	1.38	0.256
Soil amendment*P	2	1538	769	0.17	0.847
Rhizobia*Soil amendment*P	2	2362	11.81	0.26	0.776
Residual	18	82819	4601		
Total	47	308483			

Appendix 4. ANOVA Table for Harvest Index

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	7.1649	2.3883	6.04	
Block*Rhizobia stratum					
Rhizobia	1	0.9435	0.9435	2.39	0.220
Residual	3	1.1859	0.3953	1.71	
Block*Rhizobia*Soil amendment stratum					
Soil Amendment	2	0.0794	0.0397	0.17	0.844
Rhizobia * Soil Amendment	2	1.1922	0.5961	2.58	0.117
Residual	12	2.7717	0.2310	0.39	
Block*Rhizobia*Soil amendment*P stratum					
P	1	0.7792	0.7792	1.332	0.267
Rhizobia*P	1	0.0208	0.0208	0.04	0.853
Soil amendment*P	2	1.6674	0.8337	1.41	0.271
Rhizobia*Soil amendment*P	2	0.0296	0.0148	0.02	0.975
Residual	18	10.6660	0.5926		
Total	47	26.5007			

Appendix 5. ANOVA Table for Haulms weight

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	3962544	1320848	2.18	
Block*Rhizobia stratum					
Rhizobia	1	3630572	3630572	6.00	0.092
Residual	3	1815942	605314	4.69	
Block*Rhizobia*Soil amendment stratum					
Soil Amendment	2	32110	16055	0.12	0.884
Rhizobia * Soil Amendment	2	118353	59176	0.46	0.643
Residual	12	1549365	1291114	1.10	
Block*Rhizobia*Soil amendment*P stratum					
P	1	792979	792979	6.73	0.018
Rhizobia*P	1	14697	14697	0.12	0.728
Soil amendment*P	2	98162	49081	0.42	0.665
Rhizobia*Soil amendment*P	2	34387	17193	0.15	0.865
Residual	18	2120109	117784		
Total	47	14169220			

Appendix 6. ANOVA Table for Nodule weight

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	34174506	11391502	2.78	
Block*Rhizobia stratum					
Rhizobia	1	213405502	213405502	52.12	0.005
Residual	3	12282506	4094169	0.81	
Block*Rhizobia*Soil amendment stratum					
Soil Amendment	2	10325004	5162502	1.02	0.388
Rhizobia * Soil Amendment	2	434254	217127	0.04	0.958
Residual	12	60468775	5039065	1.08	
Block*Rhizobia*Soil amendment*P stratum					
P	1	7371169	7371169	1.59	0.224
Rhizobia*P	1	6757502	6757502	1.45	0.243
Soil amendment*P	2	13247588	6623794	1.43	0.266
Rhizobia*Soil amendment*P	2	16636004	8318002	1.79	0.195
Residual	18	83622788	4645710		
Total	47	458725598			

Appendix 7. ANOVA Table for Shoot biomass

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	533665	177888	0.27	
Block*Rhizobia stratum					
Rhizobia	1	550837	550837	0.84	0.427
Residual	3	1963718	654573	1.67	
Block*Rhizobia*Soil amendment stratum					
Soil Amendment	2	245302	122651	0.31	0.737
Rhizobia * Soil Amendment	2	596008	298004	0.76	0.489
Residual	12	4703296	391941	2.16	
Block*Rhizobia*Soil amendment*P stratum					
P	1	1100496	110496	6.06	0.024
Rhizobia*P	1	997633	997633	5.50	0.031
Soil amendment*P	2	823501	411751	2.27	0.132
Rhizobia*Soil amendment*P	2	790952	395476	2.18	0.142
Residual	18	3267612	181534		
Total	47	15573022			

Appendix 8. ANOVA Table for One Hundred seed weight

Source of variation	d.f (m.v)	s.s	m.s	v.r	F prob.
Block stratum	3	71.34	23.78	0.74	
Block*Rhizobia stratum					
Rhizobia	1	10.22	10.22	0.32	0.612
Residual	3	96.38	32.13	3.25	
Block*Rhizobia*Soil amendment stratum					
Soil Amendment	2	2.67	1.34	0.14	0.875
Rhizobia * Soil Amendment	2	8.62	4.31	0.44	0.656
Residual	12	118.56	9.88	0.87	
Block*Rhizobia*Soil amendment*P stratum					
P	1	19.92	19.92	1.75	0.204
Rhizobia*P	1	6.35	6.35	0.56	0.466
Soil amendment*P	2	9.10	4.55	0.40	0.677
Rhizobia*Soil amendment*P	2	32.65	16.32	1.44	0.267
Residual	16 (2)	181.88	11.37		
Total	45 (2)	532.78			

Appendix 9. ANOVA Table for Total Seed weight

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	181812	60604	5.37	
Block*Rhizobia stratum					
Rhizobia	1	35225	35225	3.12	0.175
Residual	3	33842	11281	6.63	
Block*Rhizobia*Soil amendment stratum					
Soil Amendment	2	2957	1478	0.87	0.444
Rhizobia * Soil Amendment	2	7850	3925	2.31	0.142
Residual	12	20410	1701	0.24	
Block*Rhizobia*Soil amendment*P stratum					
P	1	54506	54506	7.58	0.013
Rhizobia*P	1	9904	9904	1.38	0.256
Soil amendment*P	2	2402	1201	0.17	0.847
Rhizobia*Soil amendment*P	2	3691	1845	0.26	0.776
Residual	18	129405	7189		
Total	47	482004			

Appendix 10. ANOVA Table for Rainwater-use efficiency

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	0.145264	0.048421	5.37	
Block*Rhizobia stratum					
Rhizobia	1	0.028144	0.028111	3.12	0.175
Residual	3	0.027039	0.009013	6.63	
Block*Rhizobia*Soil amendment stratum					
Soil Amendment	2	0.002363	0.001181	0.87	0.444
Rhizobia * Soil Amendment	2	0.006272	0.003136	2.31	0.142
Residual	12	0.016307	0.001359	0.24	
Block*Rhizobia*Soil amendment*P stratum					
P	1	0.043549	0.043459	7.58	0.013
Rhizobia*P	1	0.007913	0.007913	1.38	0.256
Soil amendment*P	2	0.001919	0.000960	0.17	0.847
Rhizobia*Soil amendment*P	2	0.002949	0.001474	0.26	0.776
Residual	18	0.103391	0.005744		
Total	47	0.385110			

Appendix 11. ANOVA Table for P-Use use efficiency

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	96.12	32.04	0.56	
Block*Rhizobia stratum					
Rhizobia	1	11.42	11.42	0.20	0.685
Residual	3	171.32	57.11	6.73	
Block*Rhizobia*Soil amendment stratum					
Soil Amendment	2	70.51	35.26	4.16	0.043
Rhizobia * Soil Amendment	2	8.67	4.34	0.51	0.612
Residual	12	101.82	8.49	0.54	
Block*Rhizobia*Soil amendment*P stratum					
P	1	13.40	13.40	0.85	0.368
Rhizobia*P	1	24.87	24.87	1.58	0.225
Soil amendment*P	2	117.28	58.64	3.73	0.044
Rhizobia*Soil amendment*P	2	0.71	0.35	0.02	0.978
Residual	18	283.35	15.74		
Total	47	899.48			

ANALYSIS OF VARIANCE (ANOVA) TABLES FOR SOIL CHEMICAL AND NUTRIENT CHARACTERISTICS

Appendix 12. ANOVA Table soil pH

Source of variation	d.f (m.v)	s.s	m.s	v.r	F prob.
Block stratum	3	0.14117	0.04706	1.64	
Block*Unit*stratum					
Rhizobia	1	0.17867	0.17867	6.23	0.018
P	1	0.00348	0.00348	0.12	0.730
Soil amendment	2	0.00839	0.00420	0.15	0.865
Rhizobia*P	1	0.08404	0.08404	2.93	0.097
Rhizobia * Soil Amendment	2	0.08753	0.04376	1.53	0.233
P*soil amendment	2	0.06502	0.03251	1.13	0.335
Rhizobia*P*Soil amendment	2	0.00986	0.00493	0.17	0.843
Residual	32 (1)	0.91817	0.02869		
Total	46 (1)	1.48909			

Appendix 13. ANOVA Table Soil Total Nitrogen

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	0.00007706	0.00002569	0.36	
Block*Unit*stratum					
Rhizobia	1	0.00600769	0.00600769	84.07	<.001
P	1	0.00000019	0.00000019	0.00	0.959
Soil amendment	2	0.00105537	0.00052769	7.38	0.002
Rhizobia*P	1	0.00050052	0.00050052	7.00	0.012
Rhizobia * Soil Amendment	2	0.00079212	0.00039606	5.54	0.008
P*soil amendment	2	0.00438162	0.00219081	30.66	<.001
Rhizobia*P*Soil amendment	2	0.00677404	0.00338702	47.40	<.001
Residual	33	0.00235819	0.00235819		
Total	47	0.02194681			

Appendix 14. ANOVA Table Soil Organic Carbon

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	0.013338	0.004446	0.83	
Block*Unit*stratum					
Rhizobia	1	0.053935	0.053935	10.06	0.003
P	1	0.100193	0.100193	18.68	<.001
Soil amendment	2	0.047728	0.023864	4.45	0.019
Rhizobia*P	1	0.024345	0.024345	4.54	0.041
Rhizobia * Soil Amendment	2	0.048283	0.024141	4.50	0.019
P*soil amendment	2	0.032141	0.016071	3.00	0.064
Rhizobia*P*Soil amendment	2	0.037951	0.018975	3.54	0.041
Residual	33	0.176957	0.005362		
Total	47	0.534870			

Appendix 15. ANOVA Table Soil Exchangeable K

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	0.016049	0.005350	1.32	
Block*Unit*stratum					
Rhizobia	1	0.028470	0.028470	7.02	0.012
P	1	0.001645	0.001645	0.41	0.529
Soil amendment	2	0.009384	0.004692	1.16	0.327
Rhizobia*P	1	0.001692	0.001692	0.42	0.523
Rhizobia * Soil Amendment	2	0.007943	0.003971	0.98	0.386
P*soil amendment	2	0.024035	0.012018	2.96	0.066
Rhizobia*P*Soil amendment	2	0.012887	0.006444	1.59	0.220
Residual	33	0.133891	0.004057		
Total	47	0.235997			

Appendix 16. ANOVA Table Soil Exchangeable Mg

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	0.3774	0.1258	0.45	
Block*Unit*stratum					
Rhizobia	1	5.6444	5.6444	20.12	<.001
P	1	0.5167	0.5167	1.84	0.184
Soil amendment	2	3.7373	1.8686	6.66	0.004
Rhizobia*P	1	0.5002	0.5002	1.78	0.191
Rhizobia * Soil Amendment	2	3.9317	1.9658	7.01	0.003
P*soil amendment	2	4.8282	2.4141	8.61	<.001
Rhizobia*P*Soil amendment	2	6.8445	3.4222	12.20	<.001
Residual	33	9.2555	0.2805		
Total	47	35.6358			

Appendix 17. ANOVA Table Soil Exchangeable Ca

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	1.9050	0.6350	1.74	
Block*Unit*stratum					
Rhizobia	1	0.6348	0.6348	1.74	0.196
P	1	0.4408	0.4408	1.21	0.280
Soil amendment	2	3.2686	1.6343	4.48	0.019
Rhizobia*P	1	0.4256	0.4256	1.17	0.288
Rhizobia * Soil Amendment	2	11.7589	5.8795	16.11	<.001
P*soil amendment	2	10.1501	5.0751	13.90	<.001
Rhizobia*P*Soil amendment	2	19.1143	9.5572	26.18	<.001
Residual	33	12.0448	0.3650		
Total	47	59.7431			

Appendix 18. ANOVA Table Soil Available P

Source of variation	d.f	s.s	m.s	v.r	F prob.
Block stratum	3	62.09	20.70	0.97	
Block*Unit*stratum					
Rhizobia	1	14.09	14.09	0.66	0.423
P	1	88.48	88.48	4.13	0.050
Soil amendment	2	45.18	22.59	1.06	0.359
Rhizobia*P	1	14.29	14.29	0.67	0.420
Rhizobia * Soil Amendment	2	91.08	45.54	2.13	0.135
P*soil amendment	2	57.53	28.77	1.34	0.275
Rhizobia*P*Soil amendment	2	34.59	17.29	0.81	0.454
Residual	33	706.15	21.40		
Total	47	1113.50			