OPTIMIZING LEGUME-RHIZOBIA SYMBIOSIS TO ENHANCE LEGUME GRAIN YIELD IN SMALLHOLDER FARMING SYSTEMS IN GHANA

BY

JACOB ULZEN

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BY

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(BSc. Agriculture (Hons); MSc. Soil Science)

A Thesis submitted to the Department of Crop and Soil Sciences, Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Kumasi, in partial fulfilment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

IN

SOIL SCIENCE

MAY, 2018.

DECLARATION

I hereby declare that this submission is my own work toward the PhD degree and that, to the best of my knowledge, it contains no material previously published by another person nor material which has been accepted for the award of any other degree of the University, except where due acknowledgment has been made in the text.

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Soybean and cowpea yields on smallholder farms in northern Ghana are far below the potential yield creating a huge yield gap. This thesis reports on a series of experiments aimed at improving the productivity of soybean and cowpea using effective and persistent strains, as key determinant, within an integrated soil fertility management framework to bridge the yield gap. The field experiments conducted mainly on smallholder farms at Kpachi, Kpalga, Tunayilli, Nyagli, Tanina and Busa in the northern part of Ghana addressed issues of persistence of *Bradyrhizobium* strains, the response to the application of phosphorus, organic manure, and *Bradyrhizobium* inoculation singularly or in various combinations using soybean and cowpea as legume hosts in four different studies. The economic benefits of these interventions were also evaluated using value cost ratio

(VCR). Four different *Bradyrhizobium* strains; 532 C (in Legumefix), USDA 110 (in Biofix) and BR 3267 and BR 3262 evaluated for their symbiotic effectiveness on soybean and or cowpea, significantly increased yield over the uninoculated control treatment at Nyankpala but not Nyagli. USDA 110 inoculation of soybean resulted in grain yield 1.5 fold that of 532 C. Strain BR 3267 increased grain yield of cowpea (>2 folds) relative to BR 3262. USDA 110 and BR 3267 were found to be economically profitable with VCRs of 8.7 and 4.6, respectively at Nyankpala. The persistence of *B. yuanmingense* strain BR

3267 and *B. japonicum* strain USDA 110 were monitored over a period of 296 days. At 296 days, the numbers of surviving cells of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 were $\log_{10}$ 1.9 and $\log_{10}$ 1.7, respectively. Native *Bradyrhizobia* population and soil moisture were the predominant factors that influenced the survival of the introduced strains. Addition of P and organic manure (fertisoil and cattle manure) improved cowpea response to *Bradyrhizobium* inoculation in a cross

factorial experiment. Yield increases of 1427 and 1278 kg ha^{-1} were obtained over the control (without an amendment) when fertisoil and cattle manure with P, respectively were applied in combination with *Bradyrhizobium* inoculant. The addition of P and *Bradyrhizobium* inoculant to organic manure was profitable with a VCR of 2. In a single non-replicate trial to test soybean response to P and/ or *Bradyrhizobium* inoculant (I), a greater yield response of 1371 kg ha^{-1} was obtained by I+P in the study locations in the Northern region. Both P and I significantly increased grain yield by 17 and 22% respectively over the control. In the Upper West region, yields were relatively low ranging from 128 (control) to 227 kg ha^{-1} (P+I) in the study locations. Nonetheless, a huge variability in soybean grain yield response to P and / or I was observed in individual farms. Soil nitrogen, phosphorus, cumulative rainfall, soil type, organic carbon, pH and texture explained 42-79 % of the variability in yield in the Northern and Upper West regions. About 75% of the farmers who applied inoculant alone obtained VCR $\geq$ 1 and 64% of the farmers who applied inoculant in combination with P had VCR $\geq$ 1. These results imply that *Bradyrhizobium* inoculation is an effective strategy for increasing grain yield of soybean and cowpea for smallholder farmers. Greater benefits were obtained when inoculants were applied in combination with P and/ or organic manure (fertisoil and cattle manure) and can thus be recommended as soil management option for farmers. These results have important implications for policy makers, government and nongovernment organizations in their quest to bridge the yield gap and improve livelihood for smallholder farmers.

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CHAPTER ONE

1.0 INTRODUCTION

Leguminous plants require high amount of nitrogen (N) for grain formation (Hungria and Kaschuk, 2014) but it is difficult for smallholder farmers with limited resources to supply the needed quantities. Most resource poor farmers tend to plant legumes without any major external input(s) thus obtaining low grain yields. Under such low soil N conditions, legumes depend on biological nitrogen fixation through symbiosis with native rhizobia to partially or fully meet their N requirement (Hungria and Kaschuk, 2014). However, most of the indigenous rhizobia cannot always meet all the N requirements of legumes even when promiscuous soybeans are planted (Sanginga *et al.*, 1996). This notwithstanding, agronomic and economic benefits of introduced *Bradyrhizobium* strains pertaining to BNF and yield enhancement have been demonstrated (Albareda *et al.*, 2009; Asei *et al.*, 2015; Martins *et al.*, 2003; Mpepereki *et al.*, 2000; Thuita *et al.*, 2011). Thus, using inoculant strains that are of proven superior symbiotic effectiveness can potentially bridge the existing yield gap of cowpea and soybean. Current yields recorded by cowpea and soybean farmers in northern Ghana is less than 1 t ha^{-1} and far below the potential yield of 2.5 t ha^{-1} or more depending on the crop variety (Adjei-Nsiah *et al.*, 2018; Dugje *et al.*, 2009a; Mensah, 2014)

In addition to effectiveness, *Bradyrhizobium* strains must be able to persist in between growing seasons without the host plant for considerable periods. However, little is known about the persistence of the strains under smallholder farm conditions; an attribute of the strain needed for the decision on whether or not repeated inoculation would be needed in subsequent cropping seasons. The absence of such baseline data on the effects of

environmental factors on the survival of introduced rhizobia in soils under field conditions has made it difficult to predict the fate of introduced strains. Most of the persistence studies in sub Saharan Africa have focused on greenhouse assessment of previously inoculated fields (Sanginga *et al.*, 1996; Zengeni *et al.*, 2006). However, it is widely known that conclusions from studies conducted in greenhouse or under laboratory conditions do not always reflect the reality of strain performance under field conditions (Pitkajarvi *et al.*, 2003). In parallel, very few studies have addressed the persistence of introduced strains in the field (Duodu *et al.*, 2005; Woomer *et al.*, 1992). For example, Woomer *et al.* (1992) predicted the persistence of introduced *Bradyrhizobium* under varying climatic conditions in Hawaii while Crozat *et al.* (1982) and Corman *et al.* (1987) studied the survival kinetics of *Bradyrhizobium* without considering the effects of the prevailing environmental conditions. Therefore, it is important to determine the survival rate under tropical environmental conditions in farmers' field and identify the major environmental factors (rainfall, soil moisture, temperature, relative humidity, sunshine and indigenous *Bradyrhizobium* population) that significantly influence the survival of strains. Such findings will further highlight the frequency of re-inoculation in subsequent seasons especially in areas where inoculant use is still an emerging science.

The soils in sub-Saharan Africa (SSA) exhibit wide variation in soil fertility within and between farms because of inherent and differential management practices (Giller *et al.*, 2011; Zingore *et al.*, 2007b). This possibly accounts for the varied responses when fertilizer or bio-fertilizer is applied (Falconnier *et al.*, 2016; Kihara *et al.*, 2016; Ronner *et al.*, 2016; Zingore *et al.*, 2007b). In a widespread testing of soybean response to rhizobia inoculation and or single superphosphate fertilizer on smallholder farms in Nigeria,

Ronner *et al.* (2016) observed a significant response of soybean to inoculation and phosphate fertilizer, which increased yield by 452 and 777 kg ha^{-1} respectively over the control. Although there was a general yield increase, variation among treatments and locations were high and were attributed to plant establishment, percentage sand, soil exchangeable magnesium, calcium, and potassium, total rainfall, pH, farm size, organic C and nitrogen (Ronner *et al.*, 2016). Kihara *et al.* (2016) attributed yield variation to limiting micronutrients. In order to make predictions and be able to accurately determine where rhizobia and phosphate fertilizer would lead to yield increase, it is important to test such inputs on large scale in the major legume growing areas of Ghana where environmental conditions also vary.

Adding high quality organic manure to *Bradyrhizobium* inoculant and phosphorus in the context of soil fertility management (SFM) could alleviate some of these limitations especially micronutrient deficiencies and improve yield accordingly (Palm *et al.,* 1997). Most soils in Ghana contain as low as 1.0% organic matter or even less (NSFMAP, 1998; Ulzen *et al.*, 2016). Organic manure enhances the survival of *Bradyrhizobium* through provision of organic carbon and improves crop response to inoculation (Zengeni *et al.*, 2006). Organic manure can also increase soil conditions such as water holding capacity leading to better plant growth and enhanced efficiency of *Bradyrhizobium* inoculant (Vanlauwe and Sanginga, 2004) since nirogen fixation depends on the plants'

carbohydrate and energy. Further, organic manure can cause the release of adsorbed P, thus enhancing the efficiency of applied P (Nziguheba *et al.*, 2016). However, legumes unlike cereals have received little research attention in the context of integrating organic and inorganic sources of fertilizer. There is therefore the need to evaluate these practices

especially in Ghana where the use of inoculant is being promoted among smallholder legume farmers.

The overall objective of this study therefore was to improve grain legume productivity through *Bradyrhizobium* inoculation within an appropriate soil fertility management (SFM) framework. The specific objectives were to:

i. evaluate the symbiotic effectiveness of introduced elite *Bradyrhizobium* strains; ii. determine the major environmental factors that affect persistence of introduced rhizobia strains under smallholder farmer conditions; iii. improve cowpea response to *Bradyrhizobium* inoculation through addition of phosphorus fertilizer and organic manure; iv. evaluate soybean response to *Bradyrhizobium* inoculation and phosphate fertilizer and the factors that influence their response on farmers' field;

v. determine the economic viability of using *Bradyrhizobium* inoculant, phosphorus fertilizer and organic manure singularly or in combination for soybean and cowpea production.

Hypotheses

The above specific objectives were formulated to test the following null hypotheses:

i. introduced elite *Bradyrhizobium* strains are more effective on soybean and cowpea under smallholder farmers condition; ii. the persistence of introduced *Bradyrhizobium* strains under smallholder farm conditions is influenced by environmental factors; iii. addition of phosphorus fertilizer and organic manure will improve cowpea response to *Bradyrhizobium* inoculation;

iv. response of soybean to *Bradyrhizobium* inoculation and phosphate fertilizer on farmer' field is highly variable and is influenced by edaphic and environmental factors.

v. the use of *Bradyrhizobium* inoculant, phosphorus fertilizer and organic manure either singularly or in combination is economically viable to smallholder farmers.

1.1. Significance of the study

There is an urgent need to bridge the yield gap and increase on-farm income of smallholder legume farmers in SSA using *Bradyrhizobium* inoculant. Identifying effective *Bradyrhizobium* strains for cowpea and soybean and integrating with quality organic resources within a soil fertility management framework has the potential of increasing grain yield of cowpea and soybean.. Aside boosting cowpea and soybean production in the study areas, it will increase the income and consequently the livelihood of the farmers in the study locations.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1. Assessing the need to inoculate

In sub – Saharan Africa (SSA), where smallholder legume farmers rely solely on benefits from biological nitrogen fixation (BNF), grain yield of legumes are to a larger extent limited by the absence, low numbers, ineffective and partly effective native rhizobia (Giller and Cadisch, 1995; Thuita *et al.*, 2011). This implies that the number of ineffective native rhizobia must be overcomed through rhizobial inoculation to ensure that effective strains

with superior symbiotic characteristics are present in the rhizosphere (Lupwayi *et al.*, 2000). In many sub–Saharan African countries, inoculation with foreign *Bradyrhizobium* strains remains the norm. Very few countries have commercial facilities for inoculant production. Inoculation with introduced strains does not always result in the expected output. In view of this, promiscuous varieties of legumes were introduced to enable free nodulation by indigenous rhizobia. However, there have been reports that the nitrogen (N) demand of these promiscuous varieties are not usually met by the native rhizobia (Sanginga *et al.*, 1997). Woomer *et al.* (1992) argued that the use of promiscuous varieties with unkown nitrogen fixing abilities would be a poorer alternative to inoculation. They further highlighted the importance of using highly effective saprophytic competent strains in increasing biological nitrogen fixation in African cropping systems. Inoculation success has been reported in Europe, Australia and America (Albareda *et al.*, 2009; Martins *et al.*, 2003; Yates, 2004) and in some African countries such as Kenya

(Thuita *et al.*, 2011) and Zimbabwe (Mpepereki *et al.*, 2000). The major limitation, which however remains is the ability to obtain highly effective saprophytic strains, which can easily adapt to local conditions.

The need to inoculate primarily depends on the abundance of indigenous rhizobia and the soil N content (Revellin *et al.*, 1996; Thies *et al.*, 1991) and trials are set up with welldefined treatments to measure these indices. Date (2000) set up a trial with three basic treatments consisting of: inoculated treatment, non-inoculated with no fertilizer treatment and non-inoculated plants furnished with nitrogen fertilizer. The rationale behind the use of the inoculant was to overcome nitrogen limitation through improved symbiosis in biological nitrogen fixation; the non-inoculated treatment measured the symbiotic potential of the native rhizobia and the nitrogen treatment verified if the plant yield was

solely limited by nitrogen. Previously, need to inoculate trials were conducted for newly introduced legumes; however, it is important to do this on all legume fields if the full potential of rhizobia inoculation is to be realized.

2.2. Factors affecting legume response to rhizobia inoculation

2.2.1. Cropping history

Areas previously cropped with legumes may have high abundance of resident rhizobia through build up and release of rhizobia from nodule of plants, which could influence response to inoculation. The buildup of indigenous rhizobia limits the establishment of the introduced strains (Revellin *et al.*, 1996). Most probable number counts conducted by Revellin *et al.* (1996) on fields with soybean history revealed a population range of 10^1 to 10^6 cells g soil^{-1} in French soils and in Brazil, Vargas *et al.* (2000) reported a population size of 700 cells g soil^{-1}. Cropping history has been used as a basis for taking decisions on inoculation. This is essential because cropping history affects the size of the indigenous rhizobia population (Slattery *et al.*, 2004). However, merely looking at the numbers may not be sufficient for informed decision on inoculation (Makatiani and Odee, 2007); it would be of greater interest to evaluate the effectiveness and ability of the existing indigenous rhizobia to supply the nitrogen requirement of the legume to be planted. Cropping history also has influence on the nitrogen content of the soil as legumes may tend to have higher residual nitrogen than maize and this can affect nodulation.

2.2.2. Magnitude and effectiveness of indigenous rhizobia

The size of indigenous rhizobia has been reported to be the most predominant factor that obviates response to rhizobia inoculation. Thies *et al.* (1991) reported that response to rhizobia inoculation is inversely proportional to the native rhizobia present in the soil. In addition to rhizobia numbers, the effectiveness has been found to affect inoculation negatively (Makatiani and Odee, 2007). In Ghana, rhizobia populations and their effectiveness have been found to vary considerably among locations (Fening and Danso, 2002). At least 60 % of the soils in Ghana contain 1.3 x 10^3 cells of rhizobia capable of nodulating cowpea (Fening *et al.*, 2001). However, 68 % of these indigenous rhizobia that nodulate cowpea were classified as ineffective (Fening and Danso, 2002). The indigenous rhizobia are able to affect inoculation negatively mainly because they are persistent (Fening and Danso, 2002), well adapted to local conditions and therefore can compete successfully at the expense of exotic strains for nodule occupancy and nitrogen fixation. Since majority of these native rhizobia are ineffective, highly effective saprophytic competent strains must be introduced for increased yield. However, there have been reports of high mortalities of introduced strains due to competition from native strains and potential hazardous abiotic factors. To overcome this limitation and to boost the competitiveness of introduced strains, very large quantities should be applied in locations with very high but ineffective population of indigenous rhizobia (Triplett and Sadowsky, 1992).

2.2.3. Soil nitrogen availability in relation to nitrogen requirement of the legume crop

Nitrogen is an important nutrient element required in large quantities by many leguminous crops and its scarcity significantly affects grain yields (Danso, 1995). However, large

quantities of soil nitrogen have been found to hinder response to rhizobia inoculation hence the amount of nitrogen fixed from the atmosphere. Keyser and Li (1992) reported that high soil mineral nitrogen impair nodulation process and nodule functions. Low soil nitrate, on the other hand, has been found to favour rhizobia inoculation and consequently biological nitrogen fixation (Unkovich *et al.*, 2008). The effect of nitrogen on rhizobia inoculation has been the subject of many research activities. The actual mechanism underlying the inhibition of nitrogenase by nitrogen has however eluded rhizobiologists. Many have attempted to explain using different hypotheses. One of such hypotheses is the carbohydrate deprivation of the root nodules hence the inability of the rhizobia inside the nodule to obtain ATP (source of energy) for nitrogen fixation (Vessey and Waterer, 1992). It has also been suggested that increased rates of nitrogen causes resistance to oxygen diffusion in the root nodules; there exist a barrier to oxygen diffusion in the inner cortex of the root nodules (Vessey and Waterer, 1992). Other reports suggest that high levels of nitrogen affect rhizobia activity in the soil by inhibiting legume host production of lectin, which attracts the rhizobia towards the roots for effective infections. With this unresolved understanding of the major limitation, a big knowledge gap, which remains, is the inability of rhizobiologists to manipulate legume–rhizobia symbiosis in the presence of high nitrogen to obtain greater benefit of rhizobia inoculation.

Starter nitrogen has been introduced to boost plant growth in the early stages and ensure better symbiosis between the legume plant and the introduced rhizobia (Gan *et al.*, 2003; Yinbo *et al.*, 1997). Since the nitrogen demand of a leguminous crop increases during the podding stage, supplying enough nitrogen at that stage will boost grain production. The performance of highly effective rhizobia strains can be complemented by supplying

nitrogen to plants at flowering stage (Ezekiel-Adewoyin, 2015; Gan *et al.*, 2003). Time, rate and mode of application of nitrogen determine the extent of effect and whether that effect will be beneficial or disadvantageous to the introduced rhizobia. Da Silva *et al.* (1993) observed that foliar application of nitrogen was less suppressive on nodulation than direct soil nitrogen application.

2.3. Nutritional constraint on rhizobia affecting biological nitrogen fixation

In legume–rhizobia symbiosis, anything that affects the growth of the plant or rhizobia influences the symbiosis either positively or negatively. Nutrients that support plant growth, except nitrogen, if available tend to influence symbiosis positively. O'Hara (2001a) has reviewed nutritional constraints on root nodule bacteria extensively. Rhizobia needs nutrients to grow, to survive as saprophytes and in addition for gene regulation and nutrient storage. Major nutrients that affect rhizobia are carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorus (P), sulphur (S), potassium (K), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), molybdenum (Mo), nickel (Ni) and selenium (Se) (O'hara *et al.*, 1988). Among these, P, Ca, Fe and Mo affect nodule development and function most (O'Hara, 2001a).

Molybdenum is essentially required for symbiotic nitrogen fixation because of its role in the nitrogenase enzyme; C provides energy especially when rhizobia live as saprophytes while Mg is required to stabilize cell membranes (O'Hara, 2001a). Asei *et al.* (2015) applied inoculant and molybdenum and reported a greater yield increase of more than 200 % and 40 % compared to inoculant alone in Kpongo and Nyankpala, respectively in the Upper East and Northern regions of Ghana.

Iron (Fe) affects legume–rhizobia symbiosis since it forms an essential component of leghemoglobin and nitrogenase. However, the degree of the effect varies between legume and rhizobia species (Brear *et al.*, 2013). Iron deficiency affects nodule initiation and development (Brear *et al.*, 2013; Tang *et al.*, 1990). Battistoni *et al.* (2002) reported that Fe affects infectivity hence nodule occupancy as the Fe-sufficient inocula outperformed the Fe-deficient inocula under controlled conditions. Slatni *et al.* (2008) observed that nitrogen fixation was positively correlated with Fe concentration in root nodules. Tropical soils may be deficient in Fe and therefore it may be important for inoculant producers to supplement the inoculum with Fe as a means of improving its response.

Nitrogen fixation is a high energy driven process and phosphorus is among the elements that supply such energy for the process to occur (Keyser and Li, 1992). Phosphorus tends to be the single most important element in rhizobia nutrition and biological nitrogen fixation (Crews, 1993; O'Hara, 2001a). It is involved in every single activity and form part of the cell component of the rhizobia up to nitrogen fixation (O'Hara, 2001a).

Dissemination studies conducted extensively in legume growing areas in Ghana by COMPRO II and N2 Africa Projects revealed that phosphorus was limiting in these areas (Masso *et al.*, 2016). Application of inoculant alone in most cases did not increase yield but when P was applied in addition to inoculant, there was greater significant yield increase (Masso *et al.*, 2016). This emphasizes the importance of P in rhizobia–legume symbiosis. Plant growth and nodulation are improved in P deficient soils (Giller, 2001). Phosphorus forms part of the ATP, which is required to provide energy for the rhizobia for nitrogen fixation (Keyser and Li, 1992).

Toxicities of elements such as aluminum (Al) and manganese (Mn) affect survival rates of rhizobia as saprophytes and impair plant growth (FAO, 1984). Apart from Al preventing nodulation in leguminous crops, it also delays and suppresses nodulation (Bordeleau and Prévost, 1994).

2.4. Environmental constraints to biological nitrogen fixation and rhizobia survival in soils.

2.4.1. Soil pH

The symbiotic performance of rhizobia varies under a range of acidic conditions. A pH of a soil, either high or low affects nitrogen fixation (Bordeleau and Prévost, 1994; Hungria and Vargas, 2000). A pH > 8 causes salinity issues and a pH ≤ 5 makes nutrients such as P and Mo unavailable; induces Al and Mg toxicity, all of which affect nitrogen fixation (Bordeleau and Prévost, 1994; Giller, 2001). Low pH affects processes such as survival rate of rhizobia, infection of host legume by rhizobia, nodule establishment and development (Bordeleau and Prévost, 1994; Hungria and Vargas, 2000; Zahran, 1999). However, there are contrasting reports on legume– rhizobia symbiosis under low pH with some researchers reporting nodulation under low pH conditions, although this rarely occurs (Zahran, 1999). In contrast, Slattery *et al.* (2004) observed that as soil pH decreased, *Bradyrhizobium japonicum* population increased in Australian soils. A low soil pH has the tendency to affect the signal exchange between *Bradyrhizobium* and the host legume through reduction of secreted chemicals, reduction or modification of Nod factors (Hungria and Vargas, 2000; McKay and Djordjevic, 1993; Morón *et al.*, 2005) thereby affecting symbiosis.

2.4.2. Soil moisture

Soil moisture is known to have considerable effect on growth and vigor of host legumes and nitrogenase activity (Mohammadi *et al.*, 2012) and this has been reported as the major cause of nodulation failure (Hungria and Vargas, 2000). During moisture stress, the host legume closes its stomata, which are responsible for gas exchange with the atmosphere, to prevent further water deficit thus reducing the amount of photosynthates produced. This consequently reduces the amount of energy supplied to the rhizobia for fixing nitrogen resulting in nodulation failure. Soil moisture also affects processes such as synthesis of leghemoglobin and nodule function (Hungria and Vargas, 2000).

It is widely known that survival of rhizobia in soils depends partly on soil moisture. The growth and survival of rhizobia are severally affected at a water potential of 0.5-1.5 MPA (Hungria and Vargas, 2000). The effect of soil moisture on the survival of rhizobia is also dependent on the texture of the soil; in sandy soils, the effect is more pronounced than in clayey soil (Hungria and Vargas, 2000; Zengeni *et al.*, 2006). Under low moisture conditions, nodule efficiency is reduced which is compensated by enhanced nodule growth (Bordeleau and Prévost, 1994).

Nitrogen uptake and translocation are also essentially dependent on soil moisture. Emam *et al.* (2010) reported that the severity of soil moisture effect depends on the growth stage of the crop. Castellanos *et al.* (1993) further indicated that the moisture effect at the vegetative stage is more damaging to the plant than the reproductive stages.

It is worth noting that either excess or shortage of soil moisture is a problem and thus research aimed at harnessing optimum soil moisture is necessary. Rhizobia studies in arid conditions should consider the incorporation of organic manure or mulch to increase the moisture levels for enhanced effectiveness of the strain.

2.4.3. Soil temperature

Temperature like moisture affects every stage of nodule formation in legume rhizobia symbiosis (Hungria and Vargas, 2000). Survival of rhizobia is also severely affected by temperature. While low temperatures have been known to favour rhizobia survival, nodulation and nitrogen fixation are known to be affected by such temperatures (≥ 15 °C) (Abendroth *et al.*, 2006; Montanez *et al.*, 1995). Interestingly, different strains have different temperature ranges within which they function optimally and this has led different scientists to recommend different temperatures. For instance, Montanez *et al.* (1995) recommended 25 °C; Michiels *et al.* (1994) recommended 35-40 °C and Mpepereki *et al.* (1996), 28-47 °C depending on the growth rate of the rhizobia in question.

2.5. Soil fertility in sub-Saharan Africa (SSA)

Most agricultural soils in SSA including Ghana have low inherent soil fertility, which limits plant growth. The soil fertility status of low-income smallholder farmers is extremely poor as they tend to plant without any major external input. As a result, soil nutrient balances are often negative (Bationo *et al.*, 2006). Since soil fertility is critical in crop production, its decline has resulted in a biophysical constraint to crop production.

Thus, the major threat to food security is the inherent low fertility nature of soils in SSA. The nutrients that are mostly readily depleted are nitrogen and phosphorus; estimated losses of 660 kg N ha^{-1} and 75 kg P ha^{-1} respectively have been reported (Bationo *et al.*, 2006).

Considering the rate of decline in soil fertility and the rapid increase in population in SSA, there is an urgent need to address this constraint if meeting the food demands of the increasing population is to be achieved. Various means and methods have been proposed to address this issue but the methods are not excluded to application of inorganic fertilizers and organic fertilizers (Vanlauwe *et al.*, 2010). Due to varied reasons, these methods have not been able to substantially address the issue of soil fertility. For instance, most smallholder farmers do not have access to inorganic fertilizers and where they have, applying recommended rates of mineral fertilizers to meet the nutrient requirement of their crop is beyond their financial capacity (Jansa *et al.*, 2011). Blanket recommendation of these mineral fertilizers without taking into consideration the specific needs of geographical areas (Bationo *et al.*, 2006) has also led to low adoption rate of such technologies. In contrast, "micro –dosing" fertilizer technology has been recommended for smallholder farmers and has increased yields of millet and sorghum over 100% (Bationo *et al.*, 2006). Inability of farmers to generate large residues of high quality affected the adoption of organic manure technology (Mafongoya *et al.*, 2006).

A more sustainable approach based on locational demand is needed. Such an approach will be specific to and address the fertility issues of such location. For instance, erosion technology to address erosion prone fields could substantially increase yield. Integrated

Soil Fertility Management (ISFM) has been proposed (Vanlauwe *et al.*, 2010; Vanlauwe *et al.*, 2015) and has been the driving technology in increasing soil fertility and food production in SSA. However, this technology did not give much attention to biofertilizers as it emphasize on inorganic and organic fertilizers. While the inorganic and organic fertilizers are important, bio-fertilizers have the substantial potential to increase the availability of nutrients and are deemed environmentally friendly. Legumes have often been included in cropping systems in SSA but smallholder farmers have not receive maximum benefits in terms of biological nitrogen fixation because of the absence of effective and competitive *Bradyrhizobium* strains.

Redefining ISFM to include the use of biofertilizers will be a major step forward in combating soil fertility issues in SSA. It will be important to examine the interaction between the three factors especially in legume cropping systems using highly effective and competitive *Bradyrhizobium* strains.

2.6. Combined use of organic manure and inorganic fertilizer in the context of integrated soil fertility management

With increasing population and loss of agricultural land, meeting food demand for the current population while safeguarding the environment for future generations is important. In SSA, where soil negative nutrient balances are high, crop production depends essentially on external inputs such as organic and inorganic fertilizers (Chivenge *et al.*, 2011; Cobo *et al.*, 2002). The use of organic and mineral fertilizers in the context of ISFM in addressing soil fertility issues and increasing crop production for smallholder farmers has received massive research attention (Vanlauwe *et al.*, 2010). With good crop management and

favourable environmental conditions, combined application of inorganic and organic fertilizers can increase yield tremendously. However, the success depends on the availability and affordability of different types of inorganic fertilizer, the types, quality and quantities of organic material available and the ratio or proportion at which the two fertilizers are combined.

Available evidence establishes that combined application of N, P and millet glume-based compost increased pearl millet yield from 317 kg ha^{-1} in the control plot to 1574 kg ha^{-1} in Niger (Bachir, 2015). Similar combination of N, P and millet glume-based compost also increased cowpea yield from 365 kg ha^{-1} in the control plot to 1758 kg ha^{-1} in Niger (Bachir, 2015). Greater yields in excess of 1000 kg ha^{-1} were reported after combining cattle manure and mineral fertilizer (Badu, 2015). Chivenge *et al.* (2011) reported that combined application of organic and inorganic fertilizers resulted in yield increase of 114 % over the control. Sakala *et al.* (2000) reported that mineralization of organic resources is improved through combining them with mineral fertilizer. This may explain why grain yields obtained in plots with combined application of inputs are usually greater than those that received sole inputs (Mtambanengwe *et al.*, 2007; Murwira and Kirchmann, 1993; Nyamangara *et al.*, 2003). The mechanism underlying the yield increase is thought to be complex. However, the guiding principle is the direct and indirect hypotheses developed by Vanlauwe *et al.* (2001) and the organic resource quality classification by Palm *et al.* (1997). While mineral fertilizer supplies large portion of nitrogen to the plant, the organic manure, in addition to N, supplies micronutrients as well (Palm *et al.*, 1997). Organic manure has the ability to improve soil structure, increase water-holding capacity leading to better root growth and therefore ensures good growth of plants. It has also been reported

to reduce P sorption capacity of soils hence improving its availability (Nziguheba *et al.*, 2016; Palm *et al.*, 1997). The challenge is obtaining quality organic manure all the time and adjusting it to sites taking into consideration seasonal variation in climatic and soil conditions.

Replacing mineral fertilizer with effective *Bradyrhizobium* inoculant in the case of legume production will provide a cheaper alternative. However, the few studies that have attempted this, reported contrasting results. For example, Otieno *et al.* (2009) reported no significant difference in grain yield of grain legumes between farmyard manure and rhizobia inoculant. Zengeni *et al.* (2006) reported increase in grain yield after combining cattle manure and *Bradyrhizobium* inoculant. The differences may be due to the quality of the organic manure and efficiency of the *Bradyrhizobium* inoculant. In addition, prevailing conditions may differ from location to location. Further exploitation of such combinations including P sources because of its role in N fixation will be a further step in bridging the yield gap for smallholder farmers.

2.7. Added benefits of applying organic and inorganic manure

The added benefit of combined application of organic and inorganic manure is the extra yield gain after subtracting the individual yields. It is mostly used to determine the feasibility of interaction between organic and inorganic fertilizers. Usually positive values denote synergistic interaction and negative values indicate antagonistic interactions of the factors involved. Vanlauwe *et al.* (2001) reported added benefits of 488 and 579 kg grains ha^{-1} following the application of 45 kg ha^{-1} urea + 45 kg ha^{-1} organic manure and 90 kg ha^{-1} urea + 90 kg ha^{-1} organic manure, respectively. Okalebo *et al.* (2004) reported added

benefit of 684 kg grain ha^{-1} when wheat straw and soybean haulms with urea were applied and Badu (2015) reported added benefits of more than 1000 kg ha^{-1} grain yield after the combined application of NPK and organic manure. Antagonistic effect due to the application of organic and inorganic manure is rare and may only occur when very poor quality organic manure is used and/or when crop yields are limited by environmental factors (Vanlauwe *et al.*, 2001). Antagonistic effect of -250 kg ha^{-1} in maize was observed following the combined application of mineral fertilizer and organic manure (Mucheru *et al.*, 2004). The observed negative interaction was attributed to poor rainfall after germination. Antagonistic effect due to the combined application of mineral fertilizer, cattle manure and leucaena leaves has been reported in

Kenya (Mucheru *et al.*, 2004). This was attributed to the poor N quality of the leucaena. Adding organic resource materials to mineral nitrogen fertilizer resulted in negative added effect of -445 kg ha^{-1}. The combined application of millet glume-based compost, N and P resulted in negative added benefit of -216 to -494 kg ha^{-1} in millet and -68 to 463 kg ha^{-1} in cowpea (Bachir, 2015). It is apparent from the various studies reviewed under this discussion that to increase added benefits, in trials involving mineral and organic fertilizers, high quality manure is needed. In addition, various agronomic practices such as timely planting, weeding, and pest control and application of fertilizer must be adhered to ensure healthy crop production.

2.8. Agronomic use efficiency

Agronomic use efficiency is defined as increase in grain yield of a crop per unit of fertilizer applied (Chivenge *et al.*, 2011). One of the aims of combining organic and mineral

fertilizer in the context of ISFM is to maximize the efficient utilization of applied nutrient (Vanlauwe *et al.*, 2011). Agronomic efficiency is in two parts; the "capture efficiency" which depends on the nutrient supplied and the "conversion efficiency" which depends on the genotype of the plant (Vanlauwe *et al.*, 2011).

Agronomic N use efficiency up to 38 kg (kg $N)^{-1}$ has been reported for combined application of organic manure and mineral fertilizer in maize (Chivenge *et al.*, 2011). However, the agronomic N efficiency in sole application of either organic manure or mineral N fertilizer was not statistically different from the combined application indicating that yield increase was not necessary because of efficient utilization of applied nutrient (Chivenge *et al.*, 2011). Additions of higher rates of fertilizer decrease agronomic use efficiency (Cassman *et al.*, 2002; Chivenge *et al.*, 2011; Sanginga *et al.*, 2000). This is expected since agronomic use efficiency is inversely proportional to the amount of fertilizer added and directly proportional to yield increase. In view of this, adding higher quantities of fertilizer without corresponding yield increase will result in lower agronomic use efficiency. It is likely that higher agronomic values will be achieved in less fertile soils that respond to fertilizer application. Another way of achieving high agronomic use efficiency could be the use of genotypes with high nutrient conversion efficiency coupled with the use of quality organic resources.

2.9. Persistence (saprophytic competence) of rhizobia in soil

Crozat *et al.* (1982) defined saprophytic competence (persistence) as the ability of the rhizobia to survive in the soil in the absence of its host. Persistence of rhizobia is of great biological importance as it helps in predicting the N fixing ability of the legume*Bradyrhizobium* symbiosis (Woomer, 1990). In addition, for a strain to be selected

for inoculant production, it must be able to colonise the soil rapidly and adapt to the prevailing environmental conditions (Brockwell *et al.*, 1995). However, acquiring strains with such characteristics can take years. It therefore remains a continuous research precedence to support the effort of developing sustainable legume-rhizobia cropping systems for smallholder farmers (Howieson, 1995). In SSA where rhizobia inoculants are scarce due to few inoculant-producing facilities, determining the persistence of introduced strain under smallholder farm conditions with predictive models will be of great help in taking decisions on re-inoculation.

Rhizobia are facultative symbionts and are independent of their host in saprophytic state (Woomer *et al.*, 1988). In such state, rhizobia depend on the available C in the soil for their nutrition. In addition to the C source, the survival of the rhizobia depends on soil moisture, rainfall, soil temperature, native rhizobia population (Slattery *et al.*, 2001) and predators. Soil pH and texture also affect persistence of rhizobia in soils (Zengeni *et al.*, 2006). However, the responses of rhizobia to these stressful conditions vary among different genera and within species (Barthelemy-Delaux *et al.*, 2014; Woomer, 1990). Woomer *et al.* (1992) predicted the persistence of introduced rhizobia using climatic factors. Crozat *et al.* (1982) and Corman *et al.* (1987) studied the survival kinetics of rhizobia without considering climatic conditions. Other works on survival of rhizobia have been conducted under sterile conditions (Evans *et al.*, 1993). Most of the persistence studies in SSA have focused on greenhouse assessment of previously inoculated fields (Sanginga *et al.*, 1996; Zengeni *et al.*, 2006). Some other studies reported an initial decline in the number of introduced strains until equilibrium was attained and attributed it to

competition for space and nutrients (Brockwell *et al.*, 1987; Pitkajarvi *et al.*, 2003; Woomer *et al.*, 1992).

In addressing the predictability of survival of introduced rhizobia, several studies have used quantitative and qualitative means (DNA technology and Enzyme-Linked Immunosorbent Assay (ELISA)) including using predictive models under well-defined conditions in the past. Such models are highly recommended in SSA.

2.10. Economic benefits of rhizobia inoculants and fertilizers

In recent times, researchers have not been only interested in how an input elicits yield increase but also the profitability of such input. This helps to address the issue of whether or not and to what extent such interventions will be profitable to farmers (Roy *et al.*, 2006). Although there are many economic procedures for estimating benefits, the most commonly used are the value cost ratio (VCR) and the benefit cost ratio (BCR) (Dittoh *et al.*, 2012; Nziguheba *et al.*, 2010; Roy *et al.*, 2006). Value cost ratio is an indicator of gross returns whiles BCR indicates net returns. The VCR is a simple economic tool used to verify whether it is worth investing in a given technology based on cost recovery and potential profit (Masso *et al.*, 2016). The estimates are based on the cost of fertilizer, the value of gain in yield due to the use of the fertilizer and the rate of response. In general, there are indicators for determining the viability and profitability of an input based on some ratios. While Roy *et al.* (2006) considers a VCR ratio of 2 as viable and profitable,

Dittoh *et al.* (2012) consider VCR ratio in the range of 3 - 4 as viable and profitable. Dittoh *et al.* (2012) set a higher threshold due to high volatile output prices and production risks

from season to season. Obtaining a VCR within that range indicates that the amendment or fertilizer is truly profitable. A negative value or a value less than 2 is however, unanimously accepted as not viable and profitable. The use of rhizobia inoculants has been reported to be profitable in northern Ghana (Banka, 2016; Masso *et al.*, 2016; Ulzen *et al.*, 2016). Onduru *et al.* (2008) also reported that rhizobia inoculants are profitable. However, Asei *et al.* (2015) reported that the use of *Bradyrhizobium* inoculant alone was not profitable unless Teprosyn Mo was added to it. The difference in both reports could be as a result of the effectiveness of the different strains in fixing N towards grain yield production. In general, *Bradyrhizobium* inoculants are more

profitable than mineral fertilizers due to the higher cost of the latter. The VCR is indirectly proportional to cost, therefore the higher the cost, the lesser the VCR and the lower the profitability. Value cost ratio for *Bradyrhizobium* and fertilizer can be increased by maximizing activities that increase nutrient use efficiency such as applying appropriate rhizobia or fertilizer, using the most efficient method of application, improving water content and eliminating weeds that compete with plants for nutrients (Dittoh *et al.*, 2012).

2.11. Variability in yield response to soil amendment in smallholder farms in SSA

Soils in SSA are inherently low in nutrients especially nitrogen and phosphorus. These soils exhibit wide variation in soil fertility within and between farms because of inherent and differential management (Giller *et al.*, 2011; Zingore *et al.*, 2007b). This coupled with low use of chemical fertilizer hinders crop production and threatens food security (IFDC, 2006). Where fertilizer is applied, the response varies among the fertilizer treatments and locations (Falconnier *et al.*, 2016; Kihara *et al.*, 2016; Ronner *et al.*, 2016; Zingore *et al.*,

2007b). This has led to the broad classification of smallholder farms into responsive and non-responsive soils (Kihara *et al.*, 2016; Vanlauwe *et al.*, 2010). Various reasons broadly classified under management and environmental factors partly account for the yield variations in SSA (Ronner *et al.*, 2016). Falconnier *et al.* (2016) reported huge variability among soybean yields from 0.2–2.48 t ha^{-1} and cowpea from 0 – 1.02 t ha^{-1}. The observed variation was attributed to soil type, water-holding capacity, previous crop management, soil nutrients especially P and K (Falconnier *et al.*, 2016). Zingore *et al.* (2007a) who also observed wide variation in yield of maize from 0 – 3.0 t ha^{-1} in Zimbabwe attributed it to soil texture and differential fertility between home-fields and out-fields. Kihara *et al.* (2016) recently observed variations in yield of maize and sorghum in nutrient omission and diagnostic trials in five African countries. The yield variation was attributed to limitations in either N, P, K or organic carbon at the study locations. In a widespread testing of soybean on smallholder farms in Nigeria, Ronner *et al.* (2016) observed a significant response of soybean to inoculant and phosphate fertilizer. The variation in yield among treatments and locations were attributed to plant establishment, percentage sand, soil exchangeable Mg, Ca, and K, total rainfall, pH, farm size, organic C and N (Ronner *et al.*, 2016). It is evident from the numerous studies reported here that there is huge variation in yield among smallholder farmers. It is only through proper understanding of the causes and constraints that solutions can be developed to improve crop productivity in SSA. Crop diversification through cereal–legume rotations has been proposed as one of the means to reduce yield variability for smallholder farmers (Franke *et al.*, 2014). Furthermore, addressing limitations in secondary micronutrients and increasing soil carbon can improve response to fertilizer (Kihara *et al.*, 2016). There is the need to conduct cutting-edge research on a few smallholder farms that are variable in

nature to be able to predict for farmers with similar field characteristics. Usually not enough data are collected and as such, accurate predictions become difficult. The major challenge however remains the seasonal fluctuations in rainfall and temperature. The meteorological department can assist in overcoming such challenge by making precision predictions based on previous observations and communicating such information to farmers so that planting can be adjusted accordingly.

2.12. Legume technology delivery

Figure 2.2 is a hypothetical illustration of the major stakeholders in legume technology dissemination. The figure shows how the stakeholders can link up for a successful delivery. The researcher develops the protocol based on the knowledge of the major limitations causing low yields for smallholder legume farmers. The researcher develops a simple-to-do experiment, which is undertaken in partnership with Agricultural Extension Agents (AEAs) and farmers. The AEAs more or less act as liaison officers between the farmer and the researcher and communicates the challenges of the farmer to the researcher. The researcher trains the AEAs on the applications of the protocols involved in the technology. The AEAs in turn identify suitable farmers based on the description of the scientist and train them. The experiment is then carried out on the farmers' fields in parallel with the farmer practices and based on the results there maybe suggestions for refinement of the protocol leading to adoption of the technology. For sustainability of the technology, agro input dealers are vital. Farmers need to know where they can easily get the products tested (for example, inoculant or fertilizer) in the technology. Therefore, agro input dealers need to be trained by the researchers and AEAs on handling and storage of

the product so that the quality is not compromised before reaching the farmer, which can ultimately lead to rejection of the technology.

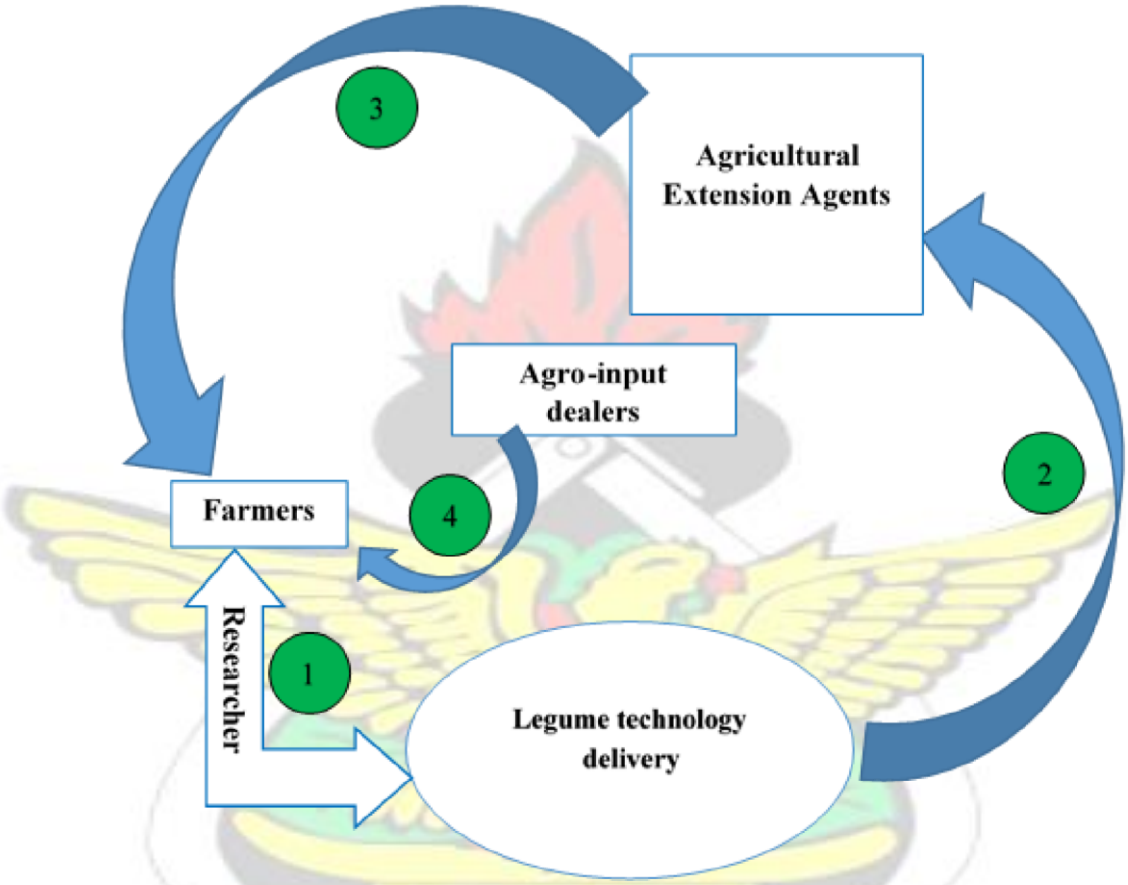


Figure 2.2. Legume technology delivery.

2.13. Factors affecting decomposition and mineralization of organic manure

The factors that affect the rate of decomposition and mineralization of organic manure have been well documented (Palm *et al.*, 1997). The major factors are but not excluded to soil temperature, soil moisture, soil microbes and the quality of the manure (Esse *et al.*, 2001; Fatondji *et al.*, 2009). The rate of decomposition is influenced by the mode of applications; organic resources that are buried decompose faster than those applied onto the surface of soil. Microbial activities that affect the decomposition and mineralization

of organic manure are regulated by soil moisture, temperature and aeration. In view of this, organic manure decomposition is expected to be high in soils with warm temperature, adequate moisture and sufficient aeration, and vice versa (Azeez and Van Averbeke, 2010). Termites have been reported to play a major role in organic manure decomposition (Esse *et al.*, 2001; Fatondji *et al.*, 2009). Upon the application of organic manure, different decomposer communities emerge based on the intrinsic properties of the manure (Cobo *et al.*, 2002), which enhances the rate of decomposition and nutrient release. The rate of decomposition is very important because it determines in principle the amount of nutrients that should be available for use by the plants.

The quality of organic manure is determined by carbon to nitrogen (C/N) ratio, lignin and polyphenol contents (Palm *et al.*, 1997). Nutrient release by organic manure with high C/N ratio is slow whereas nutrient release by organic manure with low C/N ratio is faster (Binh *et al.*, 2015; Kimani and Lekasi, 2004; Palm *et al.*, 1997). Fening *et al.* (2010) observed N immobilization of cattle manure in the first four weeks of decomposition in nutrient release studies due to high C/N ratio. Organic resources with high lignin and polyphenol contents cause temporal immobilization (Palm *et al.*, 1997). Therefore, the quality of organic manure can be improved by adding materials with high protein or nitrogen content. A decision support tool for management of organic resources based on the quality of organic resources has been developed (Palm *et al.*, 1997; Palm *et al.*, 2001). The decision support tool makes recommendations for appropriate use of organic resources considering the amount of nitrogen, lignin and polyphenol in the organic resources, compares with the ceiling and makes decisions on what to do with the organic resources. The decision support tool puts organic resources into four main classes.

Validation of such decision support tools is needed.

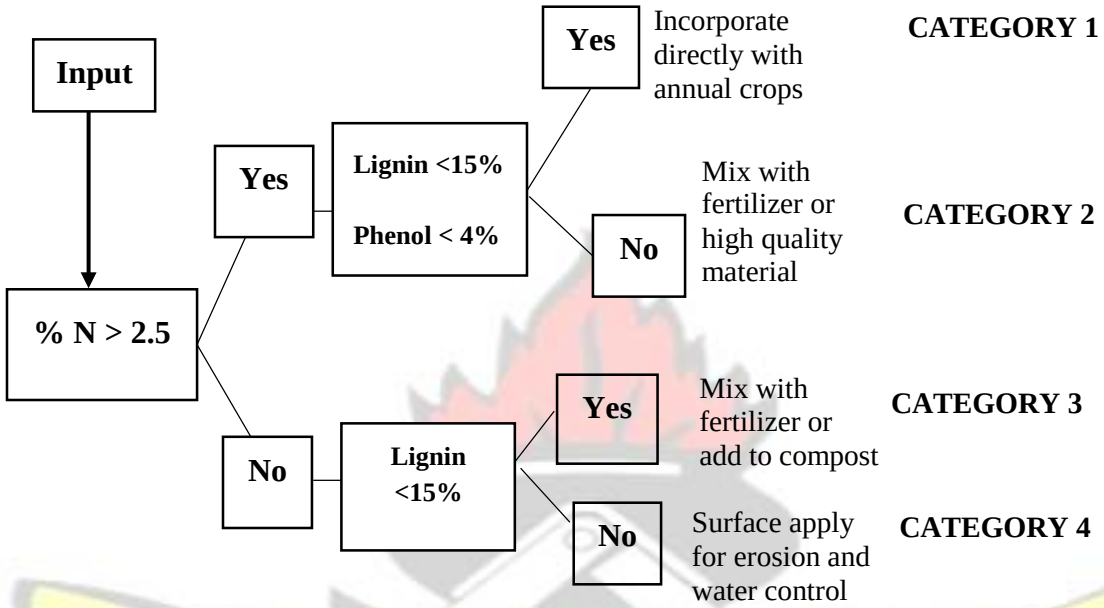


Figure 2.1. The Decision Support System for organic N management (Adapted from Palm *et al*., 2001).

2.14. Summary of literature review

Rhizobia inoculation remains one of the viable options for increasing grain yield on smallholder legume farms in SSA. It is evident from the available literature that many researchers have conducted studies on *Bradyrhizobium* inoculation especially soybean but little on cowpea. However, there are contradictory reports about the performance of introduced strains, although advances have been made. The poor performance of some of the tested inoculant strains in SSA has resulted in a continuous search for highly effective strains especially cowpea without much success. Equally, an important attribute of a strain but often neglected is the saprophytic competence (persistence). A strain must persist before entering into symbiotic relationship with a compatible host to exhibit its effectiveness. However, this aspect has received little attention in recent years. Studies on persistence would allow for predictions on the dynamics of introduced strains. Furthermore, decisions on rhizobia inoculations based on environmental and soil factors can be made with relative ease. Crop response to soil amendments such as P and or inoculant remains variable between locations and treatments. Studies that unravel the cause of variability will not only allow for yield increases but improved livelihood of smallholder farmers in SSA. Available literature indicates that soils in SSA are inherently low in fertility and hinder crop production. However, the principle behind combined application of organic manure and inorganic fertilizer can be harnessed to improve soybean and cowpea response to rhizobia inoculation. In recent times, assessment of applied treatments in terms of their economic benefits have gained attention since it represent the risk of adoption of a particular treatment. The current study attempts to bridge some of these knowledge gaps.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. General description of experimental sites

All the experiments were carried out in the Northern and Upper West regions of Ghana. The rainfall distribution in the study sites is unimodal with an annual rainfall of 1000 – 1200 mm and mean temperatures between 26 and 30 °C with little variation throughout the year. The rains last for 5 – 6 months starting from April or early May and reach its peak in August or early September whereas the dry periods last for 6 – 7 months starting from mid-November. The annual relative humidity, wind speed, sunshine hours and solar radiation of the area are 54%, 81 km day^{-1}, 7.9 h and 20.4 M J $m^{-2}day^{-1}$, respectively.

The field trials (including studies 1, 2 and 3) were set up at Kpalga (latitude 09 ˚ 26 ’45.8” N and longitude 000 ˚ 57 ’49.7” W with an elevation of 170 m above sea level), Kpachi (latitude 09 ˚25 ’48.5” N and longitude 000 ˚ 58 ’28.0” W with an elevation of 181 m above sea level), Tunayilli (latitude 09˚20'.398’ N and longitude 000˚59'.154' W with an elevation of 177 m above sea level) in the Northern region of Ghana and Nyagli (latitude 10 ˚08 ’54.1” N and longitude 002 ˚23 ’15.9” W with an elevation of 173 m above seal levels), Tanina (latitude 09˚53.126' N and longitude 002˚27.480' W with an elevation of 353 m above sea level) and Busa (09˚59.186' N and longitude 002˚20.370' W with an elevation of 345 m above sea level) in the Upper West region of Ghana (Figure 3.1). The soil types of the study locations are Acrisols (Kpalga, Kpachi and Tunayilli), Leptosols (Nyagli) and Lixisols (Tanina and Busa). The studies were conducted in the 2014 and 2015 cropping seasons. The fields had no known history of *Bradyrhizobium* inoculation and were planted to maize *(Zea mays* L.) in the previous cropping seasons.

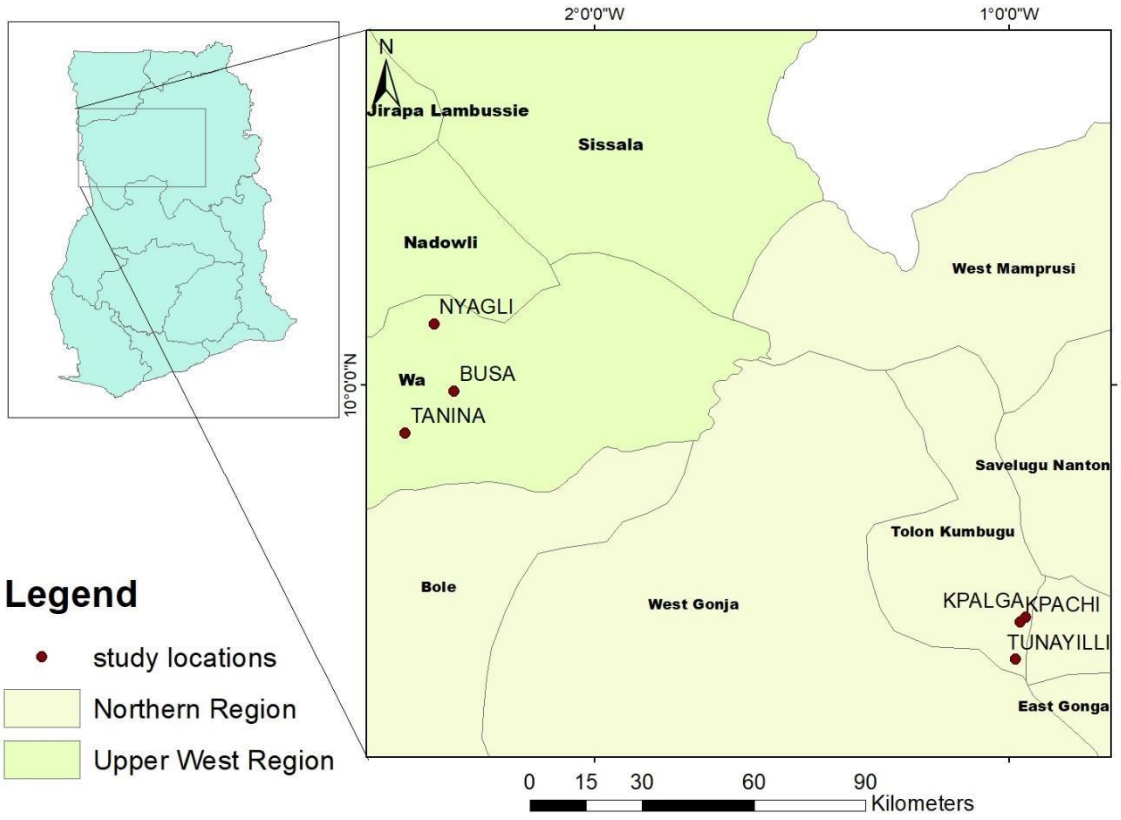


Figure 3.1. Map showing experimental sites in Northern and Upper West regions of Ghana.

3.2. Weather data

Weather data such as rainfall, soil temperature, relative humidity and sunshine hours over the period of the experiment for each site were downloaded from www.awhere.com.

3.3. Soil sampling and preparation

Unless otherwise stated, all soil sampling and laboratory analysis reported in this section were carried out on both soybean and cowpea fields. Ten core samples were taken from each plot at a depth of 0 - 20 cm using an augur (Eijkamp, Netherland). The soil samples were bulked and thoroughly mixed to obtain composite samples from which subsamples were taken for chemical analysis and most probable number count of rhizobia. The

samples were sieved with a 2 mm mesh sieve to remove stones, broken sticks and other debris before the physical and chemical analyses.

3.4. General laboratory analyses

3.4.1. Determination of soil physical properties

3.4.1.1. Particle size analysis

Fifty–one grams of air-dried soil was weighed into a 1L screw lid-shaking bottle. Hundred millilitres distilled water was added and swirled gently to mix thoroughly. Twenty millilitres of 30% H_2O_2 was added, followed by 50 ml of 5% sodium hexametaphosphate and drops of amyl alcohol and swirled gently. It was then shaken on a mechanical shaker for 2 h and the content transferred into a 1L sedimentation cylinder. The first hydrometer reading was recorded after 40 seconds and the first temperature reading was also taken with the help of a thermometer. The 1L sedimentation cylinder with its content was allowed to stand undisturbed for 3 h and the second hydrometer and temperature readings recorded respectively. The particle size was determined following the steps outlined by Bouyoucos (1962)

Calculation

$$\% \text{ Sand} = 100 - [H_1 + 0.2 (T_1 - 20) - 2] \times 2$$

$$\% \text{ Clay} = [H_2 + 0.2 (T_2 - 20) - 2] \times 2 \%$$

$$\text{Silt} = 100 - (\% \text{ Sand} + \% \text{ Clay})$$

where
H_1 = 1st hydrometer reading at 40 seconds

T_1 = 1st temperature reading at 40 seconds

T_2 = Temperature reading at 3 hours

H_2 = 2nd hydrometer reading at 3 hours

-2 = Salt correction to be added to hydrometer reading

0.2 (T - 20) = Temperature correction to be added to hydrometer reading.

3.4.2. Determination of soil chemical properties

3.4.2.1. Soil pH

This was determined using the Jenway 3510 pH meter (England) in a 1:2.5 soil to distilled water ratio. A 10 g air-dried soil was weighed into a 100 ml beaker. To this, 25 mldistilled water was added from a measuring cylinder, stirred thoroughly for 20 minutes. The soil – water suspension was allowed to stand for 15 minutes. After calibrating the pH meter with buffer solution at pH 4.0 and 7.0, the pH was read by immersing the electrode

into the upper part of the suspension.

3.4.2.2. Soil organic carbon

The modified Walkley and Black procedure as described by Nelson and Sommers (1996) was used to determine organic carbon. The procedure involves a wet combustion of the organic matter with a mixture of potassium dichromate and sulphuric acid after which the excess dichromate was titrated against ferrous sulphate. One gram of soil was weighed into a conical flask. A reference sample and a blank were included. Ten millilitres of

0.166 *M* (1.0 *N*) potassium dichromate solution was added to the soil and the blank flask. To this, 20 ml of concentrated sulphuric acid was carefully added from a measuring cylinder, swirled and allowed to stand for 30 minutes on an asbestos mat. Distilled water

(250 ml) and 10 ml concentrated orthophosphoric acid were added and allowed to cool. One milliliter of diphenylamine indicator was added and titrated with 1.0 *M* ferrous sulphate solution.

Calculation:

$$\% \text{ Organic C} = \frac{M \times 0.39 \times mcf\ (V_1 - V_2)}{g}$$

where:

M = molarity of the ferrous sulphate solution

V_1 = ml ferrous sulphate solution required for blank titration

V_2 = ml ferrous sulphate solution required for sample titration

g = weight of air – dry sample in grams mcf = moisture correction factor (100 + % moisture) / 100

0.39 = 3 x 0.001 x 100 % x 1.33 (3 = equivalent weight of C)

1.3 = compensation factor for the incomplete combustion of organic matter

3.4.2.3. Total nitrogen

The Kjeldahl method involving digestion and distillation method as described by Bremner and Mulvaney (1982) was used to determine the total nitrogen. Ten grams of soil sample was weighed into a Kjeldahl digestion flask and 10 ml distilled water was added to it.

After 30 minutes, 5 ml concentrated sulphuric acid and selenium mixture were added, shaken carefully and digested for 3 hours until a colourless solution was observed.

The digest was diluted with 50 ml distilled water and allowed to cool. The digest was made to 100 ml with distilled water and shaken well. A 10 ml aliquot of the digest was transferred into the reaction chamber and 20 ml of 40% NaOH solution was added followed by distillation. The distillate was collected over 4% boric acid. Using bromocresol green as an indicator, the distillate was titrated with 0.02 *N* HCl solution. A blank distillation and titration were also carried out to take care of nitrogen traces in the reagents as well as the water used.

Calculation:

14 g of N contained in one equivalent weight of NH_3

$$\text{Weight of N in the soil} = \frac{14 \times (A - B) \times N}{1000}$$

where:

A = volume of standard HCl used in the sample titration

B = volume of standard HCl used in the blank titration

N = Normality of standard HCl

Weight of soil sample used, considering the dilution and the aliquot taken for distillation

$$\frac{10\text{ g} \times 10\text{ml}}{100\text{ ml}} = 1\text{g}$$

Thus, the percentage of nitrogen in the soil sample is,

$$\% \text{ Total N} = \frac{14 \times (A - B) \times N \times 100}{1000 \times 1}$$

Note:

When N = 0.1 and B = 0
% Total N = A x 0.14

3.4.2.4. Available phosphorus

The readily acid – soluble forms of phosphorus were extracted with Bray No. 1 solution as outlined by Olsen *et al.* (1982). Phosphorus in the sample was determined on a spectrophotometer (210 VGP Buck scientific) by the blue ammonium molybdate with ascorbic acid as a reducing agent. A 5 g soil was weighed into 100 ml extraction bottle and 35 ml of Bray 1 solution (0.03 *M* NH_4F and 0.025 *M* HCl) was added. The bottle was placed in a reciprocal shaker and shaken for 10 minutes and filtered through Whatman No. 42 filter paper. An aliquot of 5 ml of the filtrate was pipetted into 25 ml flask and 10 ml colouring reagent (ammonium paramolybdate) was added followed by a pinch of ascorbic acid. After mixing well, the mixture was allowed to stand for 15 minutes to develop a blue colour. The intensity of the colour was measured using a 21D spectrophotometer at 660 nm wavelengths. The available phosphorus was extrapolated from a standard curve.

A standard series of 0, 1.2, 2.4, 3.6, 4.8, and 6.0 mg P L^{-1} was prepared by pipetting respectively 0, 10, 20, 30, 40 and 50 ml of 12.0 mg P L^{-1} in 100 ml volumetric flask and made to volume with distilled water.

Calculation:

$$P\ (mg\ kg^{-1}) = \frac{(a - b) \times 35 \times 15 \times mcf}{g}$$

where:

a = mg P L^{-1} in the sample extract

b = mg P L^{-1} in the blank

g = sample weight in grams

mcf = moisture correction factor

35 = volume of extraction solution

15 = final volume of the sample solution

3.4.2.5. Extraction of exchangeable cations

Calcium, magnesium, potassium in the soil were determined in 1.0 *M* ammonium acetate (NH_4OAc) extract. A 10 g sample was transferred into a leaching tube and leached with a 250 ml of buffered 1.0 *M* ammonium acetate (NH_4OAc) solution at pH 7. Hydrogen plus aluminum were determined in 1.0 *M* KCl extract as described by Page *et al.* (1982).

3.4.2.6. Determination of exchangeable calcium and magnesium

A 25 ml portion of the extract was transferred into a conical flask and the volume made to 50 ml with distilled water. Potassium ferrocyanide (1 ml) at 2%, hydroxylamine hydrochloride (1 ml), potassium cyanide (1 ml) at 2% (from a burrette), ethanolamine buffer (10 ml) and 0.2 ml Eriochrome Black T solution were added. The mixture was titrated with 0.01 *M* ethylene diamine tetra acetic acid (EDTA) to a pure turquoise blue

colour. A 20 ml 0.01 *M* EDTA in the presence of 25 ml of 1.0 *M* ammonium acetate solution was added to provide a standard blue colour for titration. The titre value was recorded. The titre value of calcium was subtracted from this value to get the titre value for magnesium.

Calculation:

$$Ca + Mg\ (cmol\ (+)\ kg\ ) = \frac{0.01 \times (V^1 - V^2) \times 1000}{0.1 \times W}$$

where:

V_1 = ml of 0.01 *M* EDTA used in the sample titration

V_2 = ml of 0.01 *M* EDTA used in the blank titration

W = weight in grams of air – dry soil extraction

0.01 = concentration of EDTA used

3.4.2.7. Determination of exchangeable potassium

Potassium in the percolate were determined using flame photometry as described by Sparks *et al.* (1996). A standard series of potassium were prepared by diluting 1000 mg/l for both potassium solutions to 100 mg L^{-1}. This was done by taking 25 mg portion of each into one 250 ml volumetric flask and made to volume with water. Portions of 0, 5, 10, 15 and 20 ml of the 100 mg L^{-1} standard solutions were put into 200 ml volumetric flasks respectively. Hundred millilitres of 1.0 *M* NH_4OAc solution was added to each flask and made to volume with distilled water. The standard series obtained was 0, 2.5, 5.0, 7.5, 10.0 mg L^{-1} for

potassium. Potassium were measured directly in the percolate by the flame photometry at wavelengths of 766.5.

Calculations:

$$\text{Exchangeable K (cmol kg}^{-1}\text{ soil)} = \frac{(A - B) \times 250 \times mcf}{(10 \times 39.1 \times g)}$$

where:

A = mg L^{-1} K or Na in the diluted sample

B = mg L^{-1} K or Na in the blank sample

g = air – dried sample weight of soil in grams

mcf = moisture correction factor

3.4.2.8. Determination of Cu, Fe and Mn by Diethylenetriamine pentaacetic acid (DTPA) extraction.

Ten grams air dried soil was weighed into plastic bottles for each of the elements above. Hundred millilitres DPTA extractant was added and shaken for 2 hours and filtered with Whatman No. 42 filter paper. The values were read using Atomic Absorption Spectrophotometer with the appropriate standards.

3.5. Enumeration of rhizobia population in the soil and inoculant

The enumeration of rhizobia in soils and inoculants was conducted using the most probable number count method (Vincent, 1970) where host legumes nodulate in the

presence of their homologous rhizobia along serial soil dilution until nodulation no longer occurs (Woomer *et al.*, 1997). Cowpea or soybean was used as the trap host depending on the test crop for field evaluation. Uniform clean cowpea seeds of good viability were surfaced sterilized with 95% alcohol and 3% hydrogen peroxide and rinsed in several changes of sterilized distilled water as described by Somasegaran and Hoben (2012) . The seeds were pre -germinated in Petri dishes that contained moist sterile tissue and incubated at 28 °C. Upon the emergence of the radicle, seedlings were transferred aseptically to plastic growth pouches (Mega International, USA) containing 50 ml of Broughton and Dilworth N-free plant nutrient solution (Broughton and Dilworth, 1970) with the help of forceps. The growth pouches were arranged on a wooden rack and kept at the greenhouse for one week prior to inoculation. Ten steps, ten-fold dilutions and six steps, five-fold dilutions were prepared for the *Bradyrhizobium* strains and the soil samples, respectively. Each growth pouch with a well-developed seedling was inoculated with 1 ml of the diluent replicated four times. At each replication, pipette tips were changed to prevent contamination. The plants were irrigated with sufficient N – free nutrient solution as and when required. Pattern of nodulation was assessed after twentyeight days based on the presence or absence of root nodules. Population estimates were assigned to the results using MPNES software (Woomer *et al.*, 1990).

3.6. Inoculant preparation

The BR 3267 and BR 3262 are industrial strains imported from Brazil as slant cultures. The strains were sub cultured on yeast mannitol agar (YMA) incubated at 28 °C. The Broth cultures were then prepared in yeast extract mannitol broth and placed in an orbital

incubator at a temperature of 28 °C at 125 rpm until it became turbid. Peats imported from IITA-Ibadan, Nigeria, were bagged (50 g peat/bag) and gamma radiated at Ghana

Atomic Energy Commission (GAEC). Using a 20 ml sterile syringe with 18 gauge needle, 50 ml of the *B. japonicum* broth cultures were withdrawn from the broth and introduced into 50 g peat aseptically under the laminar flow cabinet as described by Somasegaran and Hoben (2012). The needle hole was sealed with sterilized paper tape and the peatbased inoculum labelled accordingly. The bag was gently massaged until the peat absorbed the inoculum evenly. The freshly prepared inoculants were then incubated at 28 °C for two weeks to cure (Somasegaran and Hoben, 2012). Direct cell count by the drop plate method (Somasegaran and Hoben, 2012) was done to verify the colony forming units in the cured inoculants.

3.7. Source of planting materials

Soybean and cowpea seeds were obtained from the International Institute of Tropical Agriculture (IITA), Ghana. The rhizobia inoculants; Biofix was obtained from MEA, Kenya, Legumefix from Becker Underwood, UK, Nodumax from IITA and the cowpea strains BR 3267 and BR 3262 were obtained from EMRAPA through Savannah Agricultural Research Institute, Ghana. Decomposed cattle manure were obtained from the study area. Fertisoil (a commercially prepared compost) was obtained from DeCo, Ghana.

The soybean cultivar (Jenguma) is a medium maturing variety (105-110 days maturity) and takes 45 days to attain 50 % flowering. Cowpea cultivar (Songotra) is semi erect and takes 80-89 days to reach full maturity (medium maturing variety) (Dugje *et al.*, 2009b).

3.8. Study 1. Symbiotic effectiveness and economic benefits of introduced *Bradyrhizobium* strains.

3.8.1. Field preparation, layout, inoculation and sowing

The field was ploughed and harrowed to a depth of 15 cm and divided into plots before planting. Each plot measured 6 x 3 m with an alley of 2 m between plots and 3 m between blocks. Soybean cultivar, Jenguma, and cowpea cultivar, Songotra, were used for this study. Five grams of each of the *Bradyrhizobium* inoculants was added to 1 kg of seeds using the two–step method, which involves adding a sticker to the wet seeds before the inoculant. Gum Arabic was used as a sticker in this study at a ratio of 1.5 g to 15 ml clean lukewarm water. Inoculated soybean and cowpea seeds were air dried for 30 minutes and manually sown at a spacing of 75 cm x 10 cm.

3.8.2. Treatments and experimental design

The study was laid out in a randomized complete block design with 5 replications and 4 treatments for each crop. The treatments were; Legumefix with *B. japonicum* strain 532 C (Becker Underwood, UK), Biofix with *B. japonicum* strain USDA 110 (MEA, Kenya), uninoculated control, and uninoculated with applied N in the form of urea at a rate of 100 kg N ha^{-1} for soybean and strains BR 3262 and BR 3267 (EMBRAPA, Brazil), uninoculated control and uninoculated with applied N at a rate of 100 kg N ha^{-1} in the form of urea for cowpea. The urea was split applied; 50 kg N ha^{-1} at one week after planting and the other half at 50 % flowering (R_3 growth state). Each treatment received a basal application of 30 kg P ha^{-1} and 30 kg K ha^{-1} as triple super phosphate and muriate of potash, respectively.

3.8.3. Harvesting and data collection

Nodulation and shoot biomass were assessed at the R_3 stage (Fehr *et al.*, 1971) for both soybean and cowpea. The plants were cut at about 5 cm above the soil level. The roots of the plants were carefully dug out, collected into polythene bags, together with detached nodules, and transported to the laboratory. The roots were put in a 1 mm mesh sieve and washed under running tap water to remove adhered soil. The nodules were gently removed, washed and counted. Shoot and nodules were oven dried at 60 °C for 72 h. Shoot dry matter was measured after harvesting the pods at maturity (R_8 stage) (Fehr *et al.*, 1971). The shoot dry matter was measured from 1 m^2 square plot in the case of cowpea. The dry weight was used to estimate the dry matter yield in kilogram per hectare.

At physiological maturity (R_8 stage) (Fehr *et al.*, 1971), both soybean and cowpea were harvested. Pods were manually pricked from the cowpea plants on three harvesting periods. The pods were air dried, threshed and winnowed. For the soybean, the whole plant were harvested and further dried before threshing and winnowing. The grains were oven dried at 60 °C for 72 h. The grains were weighed with a standard electronic balance and recorded. The dry weights were used to estimate the grain yield per hectare.

3.8.4. Economic analysis

Return on investments for using the *Bradyrhizobium* inoculants were calculated using value cost ratio. The value cost ratio (VCR) was calculated based on the adopted equation from Nziguheba *et al.* (2010) as follows:

$$VCR = \frac{(Y^B - Y^C)\, P^G}{(Q_B - P_B)}$$

where YB is the grain yield from treated plots, YC is the grain yield from uninoculated control plots, PG is the unit price of grain yield, PB is unit price for inoculant or fertilizer,

and QB is the quantity of inoculant or fertilizer. The dollar to cedi exchange rate as at the time of this study was USD $ 1 to GH¢3.60. An inoculant with a positive VCR was considered economically viable. A VCR value greater than or equal to a threshold of 3 - 4 was considered profitable (Dittoh *et al.*, 2012).

3.8.5. Statistical Analysis

The data obtained from Kpachi and Kpalga were pooled together (herein referred to as Nyankpala) because the interaction between strains and locations was not significant. The data from all the study locations were subjected to Analysis of Variance (ANOVA) using GenStat statistical software version 12. Significant differences were assessed at 5% ($p = 0.05$) level of significance. Where there was significant difference, means were separated using the Fishers protected least significance difference (LSD) procedure. Orthogonal contrast was used to compare individual pairs of treatments. Multivariate Analysis of Variance (MANOVA) was used to assess the contributions of a strain's inoculation to grain yield.

3.9. Study 2. Persistence of *Bradyrhizobium* under field conditions.

3.9.1. Experimental setup

The field was ploughed and harrowed to a depth of 15 cm. The *Bradyrhizobium. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 as peat-based inoculum were manually introduced into an area measuring 2 m x 3 m. The inoculum was manually incorporated into the soil using a hoe that was pre- sterilized with 95 % ethanol. Measures

were taken to ensure that there was no legume on the plots that could have influenced the persistence of the strains throughout the study.

3.9.2. Soil sampling

Soil samples were collected from each site at 0, 21, 42, 81, 142 and 296 days after incorporation of the peat based inoculant using an auger (Eijkamp, Netherland). Five core soil samples were thoroughly mixed and composite samples taken for enumeration of *Bradyrhizobium* population. The soil auger (Eijkamp, Netherland) were surfaced sterilized with 95% ethanol between sampling from sites. The samples were kept in refrigerator at 4 °C prior to cell count.

3.9.3. Soil moisture measurement

The Trase system (Model 6050X1 Santa Barbara California 93105 USA) which uses the Time Domain Reflectometry (TDR) was used to measure instantaneous volumetric water content from the individual plots. Metal waveguide of 15 cm was used to measure the volumetric water content. At each measurement, the TDR was set at zero, the metal waveguides inserted into the waveguide connector before pushing it into the soil. It is inserted in a way such that there is no space between the surface of the soil and the waveguide connector. The value displayed on the TDR is recorded as the volumetric water content at that instant. Three measurements were made on each plot at different points and averaged as the final volumetric moisture content per plot.

3.9.4. Enumeration of rhizobia numbers

The estimation of *Bradyrhizobium* numbers were carried out according to the procedure described under section 3.5. The test crops were soybean for Biofix and cowpea for BR 3267.

3.9.5. *Bradyrhizobium* strains used in this study

The *B. yuanmingense* strain BR 3267 is a cowpea elite strain imported from Brazil (EMBRAPA, Brazil) as slant culture whiles *B. japonicum* strain USDA 110 specifically for soybean was imported from (MEA, Kenya) as peat-based Biofix inoculant.

3.9.6. Proximate analysis of carrier materials used for the inoculants

The proximate analysis of the carrier materials of the inoculant was done following the procedures described by AOAC (1990). A 2 g of the carrier materials were weighed into a moisture can and oven dried at 105°C until constant weight was attained. The moisture content was then determined. The carrier materials were ashed in muffle furnace at 550°C for 4 h to determine the ash content. Crude protein was determined using the Kjeldahl method described under section 3.4.2.3. The crude fat was determined using petroleum ether (b.p. 40-60°C). The fiber was determined using sulphuric acid solution (1.25 %), sodium hydroxide solution (1.25%), antifoam reagent (Octyl alcohol) and ethyl alcohol (95%).The percentage carbohydrate was determined by adding nitrogen free extract and fiber.

3.9.7. Data analysis and fitting of regression models

The MPN data was log transformed to minimize the variation associated with the enumeration technique before fitting the models. Rhizobia population (log_{10}) were counted over time and fitted into various decline functions using non-linear regression procedures such as hyperbolic, exponential, logistics, Gompertz and exponential decline.

The best-fit model was chosen based on the "r" and the Akaike information criterion corrected (AICC) values (Leggett *et al.*, 2017; Owusu-Ansah *et al.*, 2017). The AICC value describes the amount of information that is lost in fitting the model. The model with the least information lost is considered the best-fit model. Once the general non-linear regression function was selected, individual environmental factors (soil moisture, native rhizobia population, annual rainfall, soil temperature, relative humidity and sunshine) were regressed singularly against the introduced *Bradyrhizobium* population (log_{10}) using curveExpert Professional software version 2.5.1 (Hyams, 2016). All the environmental factors were combined and regressed against the introduced strains using the multivariate non-linear regression function in XLSTAT version 19.7. Boxplots were constructed for each location using Microsoft Excel to compare the survival rates of the introduced strains in the different locations The decline rate from the various locations were pooled together because there was no significant difference between them (appendix 1) and analysed using ANOVA function in XLSTAT version 19.7.

3.10. Study 3. Improving cowpea response to *Bradyrhizobium* inoculation through the addition of phosphorus fertilizer and organic manure.

3.10.1. Field preparation, layout, inoculation and planting

The fields' preparation, layout, inoculation and planting were carried out as described under section 3.8.1.

3.10.2. Treatment structure and experimental design

The study was a 2 x 2 x 3 factorial experiment arranged in randomized complete block design with three replications. The treatments used were +/- *Bradyrhizobium* inoculant, two levels of phosphorus (30 kg P ha^{-1} and 0 kg P ha^{-1}), two sources of manure (fertisoil, cattle manure) and a control (no manure). Phosphorus was applied in the form of triple super phosphate. Fertisoil and cattle manure were applied at a rate of 5 tons ha^{-1}. Fertisoil contains high nitrogen and other nutrients in sufficient quantities than cattle manure. However, farmers use cattle manure, hence the comparison between the two in this study.

3.10.3. Harvesting and data collection

Harvesting and data collection on nodulation, shoot biomass and grain yield were done according to the procedures described under section 3.8.3.

3.10.4. Decomposition and nutrient release patterns of fertisoil and cattle manure

One hundred and eighty grammes of cattle manure and fertisoil were weighed separately into litterbags. The litterbags were then tied to pegs in two parallel lines in the field in a randomized arrangement, replicated 3 times. Each replication consisted of 10 litterbags. The litterbags (20 × 30 cm) were made from nylon mosquito nets (1.0 mm mesh size) as described by (Tetteh, 2004). Two litterbags from each replication were sampled at intervals of 2, 4, 6, 8 and 10 weeks to monitor and determine the dry matter disappearance from cattle manure and fertisoil at each location (Anderson and Ingram, 1994). At each

sampling time, the remaining material in each litterbag was cleaned of sand manually with a soft brush. Fresh weight of the remaining organic material (both cattle manure and fertisoil) was recorded and oven dried at 65 ºC to a constant weight (approximately 48 h). After oven drying, samples from the two litterbags were bulked and mixed to obtain a composite sample. A ten-gramme sub-sample was then taken from the composite sample, ground to less than 1 mm particle size and analysed for phosphorus, potassium organic carbon, magnesium and calcium. The sample for total nitrogen was not oven dried. Equations previously used by Gnankambary *et al.* (2008) were used to compute the percentage dry weight, nutrient release and decomposition of cattle manure and fertisoil at each sampling time.

$$R\ (\%) = 100 \times \frac{M_t}{M_o}$$

where,

M_t = dry weight of remaining cattle manure or fertisoil at time t

M_o = initial dry weight of cattle manure or fertisoil in the litterbag.

$$\text{Nutrient release } (\%) = 100 \times \frac{C_o \times M_o - C \times M_t}{C_o \times M_o}$$

where,

C_o = initial concentration of the nutrient (N, P, K or C) cattle manure and fertisoil C_t = concentration of the nutrient (N, P, K or C) in the decomposing cattle manure and fertisoil at sampling time t

M_o = dry weight of remaining cattle manure and fertisoil at time t

M_t = initial dry weight of cattle manure and fertisoil in the litterbag.

To describe the decomposition pattern and calculate decomposition rate constants (k), data for each organic material was modelled using a single exponential model as described by Olson (1963).

$$M = M_{t\,o.}\, e^{-kt}$$

where:

Mt = dry weight of remaining cattle manure and fertisoil at time t Mo

= initial dry weight cattle manure and fertisoil in the litterbag.

k = decomposition factor

3.10.5. Determination of lignin content of fertisoil and cattle manure

The acid detergent fiber (ADF) method as described by Anderson and Ingram (1994) was used to determine the lignin content. After the alcohol and dilute sulphuric acid extraction, 2 ml of 72 % sulphuric acid was added to the organic manures and shaken for 4 hours. The solution was transferred into a 100 ml Erlenmeyer flask with 40 ml distilled water, boiled for 2 hours and filtered. Sugar which represents cellulose was determined in the hydrolysate. The residue was washed with water, dried at 60 °C for 48 hours, weighed and then ashed in a muffle furnace. The lignin content of the residue was considered as the loss in weight on ignition.

3.10.6. Determination of polyphenol content of fertisoil and cattle manure

The Folin-Denis method as described by Anderson and Ingram (1994) was used to determine the polyphenol content. One gram each of dried, milled and sieved cattle manure and fertisoil were weighed into 50 ml separate conical flasks. Ethanol (20 ml) was

added to the organic materials and heated to 60 °C to extract the polyphenol. The extraction was repeated after the alcohol extract was decanted into another flask. After the third extraction, the volume of the extract was made to 50 ml by adding ethanol. Standard solutions of acid with concentrations of 0, 20, 40, 80 and 100 mg L^{-1} tannic acid were prepared. The samples and tannic acid standards were subjected to colour development. Absorbance values of the standard and sample solutions were read on a spectrophotometer at a wavelength of 760 nm. A standard curve was obtained by plotting absorbance values against concentrations of the standard solutions and used to determine sample solution concentrations.

Calculations:

mg kg^{-1} polyphenol = graph reading × sample dilution × aliquot dilution where:

sample dilution = final volume/weight of sample = 50/1 aliquot dilution = 50/1 (1 ml of initial 50 ml extract was put in a 50 ml flask and made to the 50 ml mark with ethanol. i.e. 50/1)

3.10.7. Agronomic P use efficiency and added benefit

Agronomic use efficiency following the formula of (Vanlauwe *et al.*, 2011) was calculated as

$$P\text{ - }AE = \frac{(Y_F - Y_C)}{F_{appl}}$$

Where, Y_F and Y_C refer to grain yields (kg ha^{-1}) in the fertilizer P and control plot treatments respectively, and F_{appl} is the amount of fertilizer P applied.

Added benefits were calculated based on the formula of Vanlauwe *et al.* (2002) as follows:

$$AB = Y_{comb} - ((Y_{ino} - Y_{con}) + (Y_{om} - Y_{con}) + (Y_{phos} - Y_{con}) + Y_{con})$$

Where:

AB denotes added benefit ; Y_{comb}, Y_{con}, Y_{ino}, Y_{om}, and Y_{phos} are the grain yields obtained in combined application of all the inputs, control treatment, rhizobia inoculant alone, organic manure alone, and phosphorus alone, respectively.

3.10.8. Economic analysis

The profitability of investing in the treatments; *Bradyrhizobium* inoculant, fertisoil, cattle manure and phosphorus (TSP) were determined through the value cost ratio (VCR) (Roy *et al.*, 2006).

$$VCR = \frac{\text{Value of extra crop produced due to treatment (\$ ha}^{-1})}{\text{Cost of treatment (\$ ha}^{-1})}$$

Prices of *Bradyrhizobium* inoculant, fertisoil, cattle manure and phosphorus were 6 US$ ha^{-1}, 4 US$ ha^{-1}, 4 US$ ha^{-1}, and 26 US$ ha^{-1} respectively. Cattle manure is not sold on the market; therefore, the cost of sampling for 50 kg (4 US$ ha^{-1}) was estimated as the cost price. A kilogram of cowpea cost 0.6 US$ on the market. The dollar to cedi exchange rate as at the time of this study was USD $ 1 to GH¢3.60. A VCR value ≥ 2 was considered profitable (Roy *et al.*, 2006).

3.10.9. Statistical Analysis

The data obtained from each experimental site was pooled together. The data was transferred to SISVAR software version 5.6 for analysis of variance (ANOVA) (Ferreira, 2008). Where there was significance difference, means were separated using Scott Knott at 5% probability. Stepwise regression was performed with Minitab version 17 to determine the most contributing growth parameter to grain yield. T-Test was used to compare the amount of nutrients released by fertisoil and cattle manure.

3.11. Study 4. On-farm evaluation of soybean response to *Bradyrhizobium* inoculation and/ or phosphorus fertilizer.

3.11.1. Study area

Agronomic trials for testing the response of soybean to *Bradyrhizobium* inoculant and phosphorus fertilizer were set up in Northern region (Savelugu – Nanton and Gushiegu - Karaga districts) and Upper West region (Sissala West, Sissala East and Wa municipal) during the 2015 cropping season. The names of the districts under each region where the experiment was conducted with dominant soil types are mapped in Figures 3.3 and 3.4. The general rainfall distribution, relative humidity, wind, sunshine hours and solar radiation are described under section 3.1.

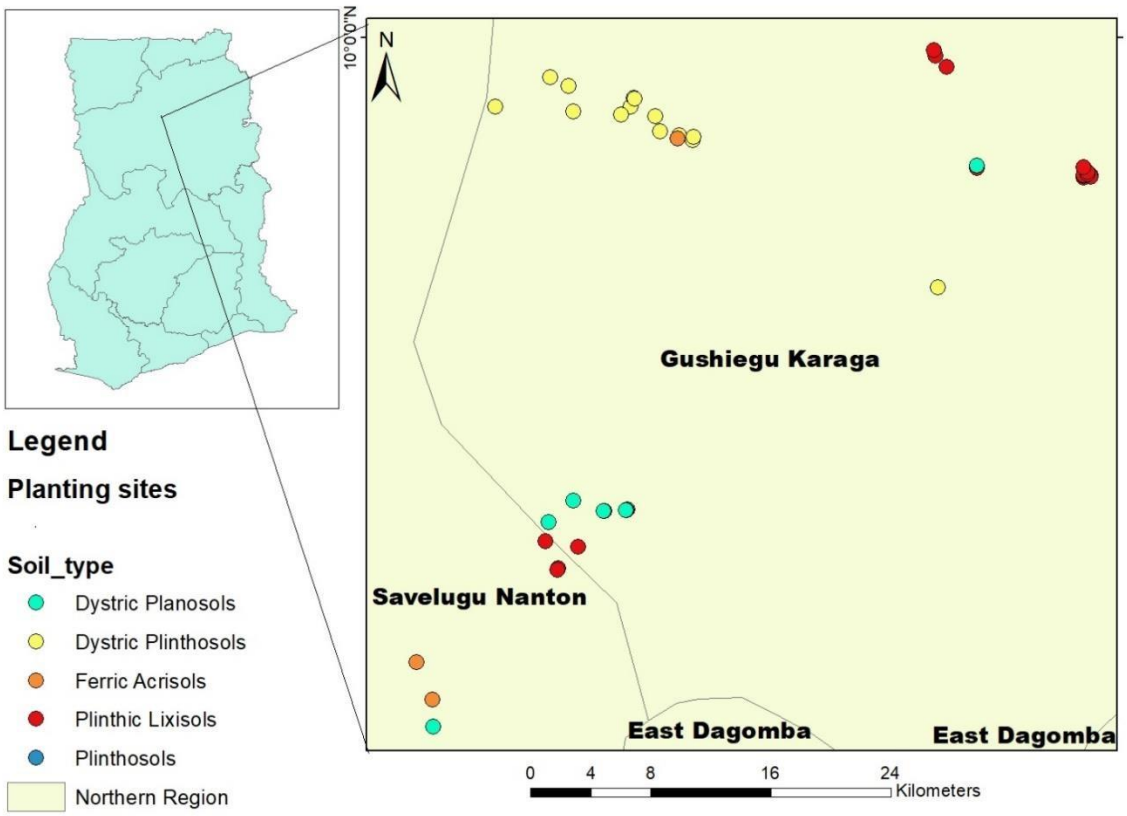


Figure 3.2. Map showing study locations with dominant soil types in the Northern region.

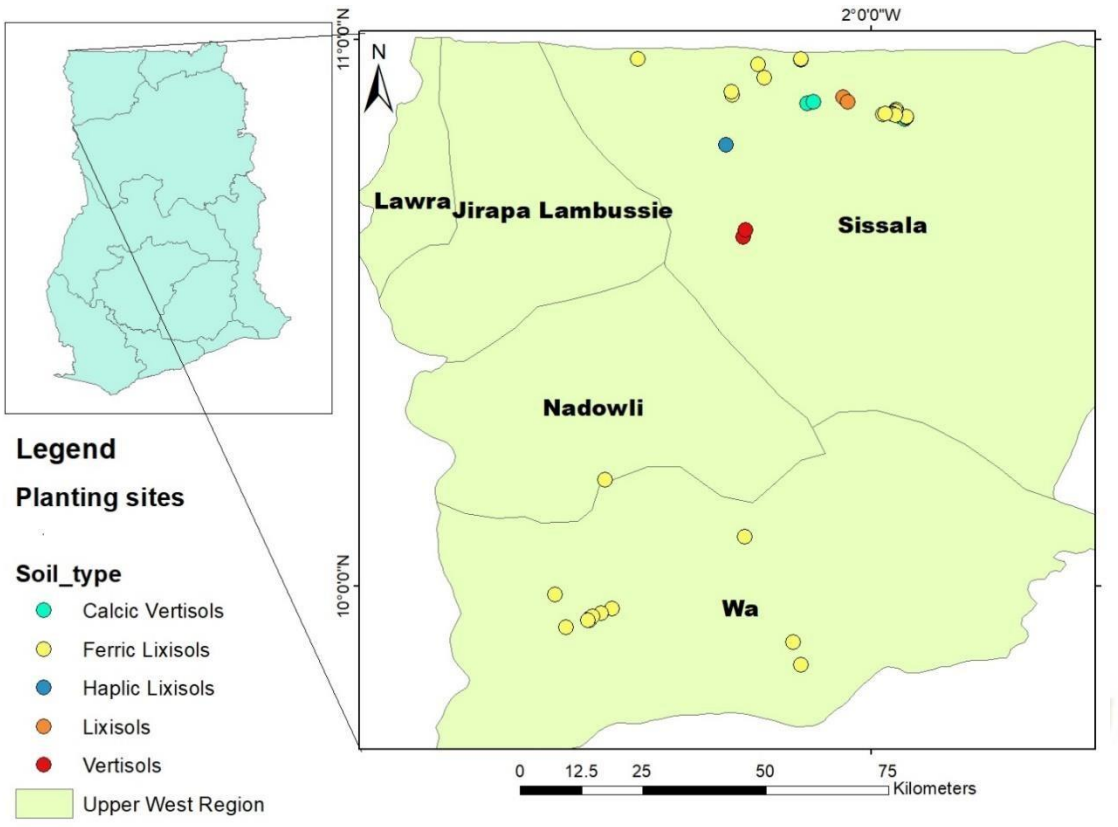


Figure 3.3. Map showing study locations with dominant soil types in the Upper West region.

3.11.2. Training of agricultural extension agents (AEAs) on protocol (treatments)

Due to the large number of demonstration sites, the experiment was conducted in partnership with AEAs and farmers. It was imperative to equip the AEAs with technical knowledge for successful implementation of the trials. The training focused on the handling, application of *Bradyrhizobium* inoculant and phosphorus fertilizer, selection of sites, good agronomic practices and data collection.

3.11.3. Mobilization of farmers

Mobilization of farmers was done through community sensitization and education about improved soybean technologies with the AEAs. Interested farmers were selected by the AEAs, organized into groups of 20 – 25 people. Within a farmer group, a lead farmer was selected. The lead farmers were trained on the handling and application of *Bradyrhizobium* inoculant and phosphorus fertilizer and good agronomic practices. Each farmer received an improved soybean variety, *Bradyrhizobium* inoculant (Nodumax) and triple super phosphate (TSP). As a requirement, farmers were asked to set up the trials at locations visible to others especially non-participating farmers.

3.11.4. Field preparation, layout, inoculation and sowing

The fields' preparation, layout, inoculation and sowing were carried out as described under section 3.8.1.

3.11.5. Treatments and experimental design

There were four (4) treatments: inoculant only (I), TSP (only) (P), no input (control) and a combination of TSP and inoculant (P + I). The treatments were tested in a simply non-replicated trial where each farm within a district was considered a replicate. The *Bradyrhizobium* inoculant (Nodumax) contained 10^9 cells g^{-1} of *Bradyrhizobium japonicum* strain USDA 532c. The TSP (46% P_20_5) was applied at a rate of 30 kg P ha^{-1}. The mode of application was band placement. About 136 and 45 demonstration trials were established at Northern and Upper West regions, respectively with the help of farmers and AEAs.

3.11.6. Data collection

Soils were sampled from a depth of 0 – 20 cm for physical and chemical analyses and enumeration of indigenous rhizobia population before planting at some sites in Northern and Upper West regions. Detailed description of the laboratory procedures are given under section 3.2.3. Rhizobia population was assessed through most probable number count. At maturity, the soybean plants were harvested, threshed and winnowed. The seeds were air dried until constant weight was attained and weighted accordingly with standard electronic scale. Grain yield was estimated on per hectare basis.

3.11.7. Proximate (active) carbon determination

The active carbon was determined following the procedure of Culman *et al.* (2012). The active carbon was prepared by dissolving 147 g of calcium chloride ($CaCl_2$) in 900 ml deionized water and made up to volume in a 1L volumetric flask with continuous stirring. A 31.06 g of potassium permanganate ($KMnO_4$) was weighed into 1000 ml beaker and dissolved with the already prepared $CaCl_2$. The resultant solution was placed on a hot plate at 60 ºC with continuous stirring using magnetic stirrer. A standard series of 0.005, 0.01, 0.015, and 0.02 M were prepared by pipetting 0.25, 0.5, 0.75 and 1.0 ml, respectively into 10 ml volumetric flask and made to the volume with deionized water. A working standard was prepared by adding 0.5 ml of each of the stock solution to 49.5 ml of deionized water in 50 ml centrifuge tubes. A 2.0 ml of $KMnO_4$ was added to 5 g soil in a 50 ml centrifuge tube and topped up with 18 ml deionized water. It was shaken for 2 minutes at 240 revolution per minute (rpm). The centrifuges were uncapped and allowed to settle for 10 minutes. A 0.5 ml of the supernatant was pipetted into 49.5 ml deionized

water and read on spectrophotometer at 550 nm wavelength. The equation, after Weil *et al.* (2003) was used to calculate the active carbon:

$$\text{Active Carbon (mg kg soil)} = [\ 0.02 \square (a + b \times abs)] \times (9000 \text{ mg C mol}^{\square 1}) \times \frac{0.02}{0.005}$$

where: 0.02 mol L^{-1} = initial solution concentration

a = intercept of the standard curve b = slope of the

standard curve

Abs = absorbance of unknown

9000 = milligrams of carbon oxidized by 1 mole of MnO4 changing from Mn^{7+} to Mn^{4+}

0.02 L = volume of stock solution reacted

0.005 kg = weight of air-dried soil sample.

3.11.8. Determination of responsive and non-responsive sites to inoculation and phosphorus application on soybean.

For the purpose of this work, responsive and non - responsive were defined by agronomic and economic means. For the agronomic means, the average of the total yields of the untreated control from the different locations were calculated. Standard deviation was calculated from this average and used as a threshold for comparison. Differences between treatment and control yields were compared to the standard deviation, differences higher than the standard deviation for a location was considered responsive and where differences were lower than the standard deviation, the location was considered nonresponsive. The rationale is that the standard deviation was a representative of all the locations under consideration. Differences less than the standard deviation was considered

as a random variation in the population. Differences higher than the standard deviation was attributed to the effect of the treatments. The computer software ArcGIS was used to draw the maps for responsive and non-responsive locations.

The economic means was through value cost ratio. For a particular location to be considered as responsive to the treatments, a farmer must break even (VCR = 1) or make profit (VCR ≥ 2), otherwise the location was considered as non-responsive to the applied treatments. The rationale is that if a particular soil is not productive, then the cost of fertilizer cannot be recovered after application due to low grain yield. The formula for VCR ratio is given under section 3.10.8. The market price of soybean was USD$ 0.43 for 1 kg of seeds (GH 1.5 exchange rate of 3.5).

3.11.9. Statistical Analysis

Cumulative probability curves were drawn to determine the distribution of soybean response to P, I and P+I and probability of obtaining a certain amount of grain yield with a particular treatment in relative to the control. Statistical analyses were performed in R version 3.3.2 (Team, 2017). The effects of the treatments were estimated with linear mixed model: treatment as fixed term and location as random term. Treatment means were separated by lsmeans with Tukey adjusted p-values. Linear mixed model regression was performed to identify the soil and environmental factors influencing yield variability.

Only locations with complete data set were used in the analysis

CHAPTER FOUR

4.0 RESULTS

4.1. Study 1. Symbiotic effectiveness and economic benefits of introduced

Bradyrhizobium

4.1.1. Physical and chemical properties of the study locations

The physical and chemical properties of soils of the experimental sites are given in Table 4.1. The soils of the study locations were predominantly sandy loam except at Nyagli, which was sandy. The pH recorded at all the locations was near neutral. Total N and organic carbon were very low at all the locations. The soybean field at Kpalga had the lowest amount of organic carbon (Table 4.1). Nyagli had the lowest total N content (Table 4.1). Available P ranged from low to very low (Table 4.1). Exchangeable Ca and Mg were adequate while DTPA-Fe was low. Exchangeable K was moderate for soybean field at Kpachi and cowpea fields at Kpalga and Nyagli. The cowpea and soybean fields at Kpachi and Kpalga had low levels of exchangeable K. These ratings were done according to Landon (2014). This demonstrates the inherent low fertility status of the study locations.

Table 4.1 Soil physicochemical properties of the study sites.

	Locations				
	Kpachi		Kpalga		Nyagli
Soil parameters	Soybean	Cowpea	Soybean	Cowpea	Cowpea
pH(1:2.5) (H_2O)	6.34±0.04†	6.46±0.05	6.75±0.16	6.90±0.082	6.69±0.045
Total N (%)	0.071±0.001	0.064±0.003	0.055±0.004	0.076±0.002	0.041±0.002
Available P (mg kg^{-1})	3.04±0.025	3.20±0.2	2.60±0.16	3.04±0.033	2.60±0.16
Exchangeable K ($cmol_{(+)}$ kg^{-1})	0.21±0.012	0.15±0.02	0.12±0.02	0.21±0.02	0.27±0.004
Organic C (%)	0.34±0.033	0.22±0.16	0.04±0.007	0.38±0.03	0.24±0.016
Exchangeable Ca ($cmol_{(+)}$ kg^{-1})	7.6±0.2	5.26±0.12	4.62±0.1	5.52±0.02	7.5±0.017
Exchangeable Mg ($cmol_{(+)}$ kg^{-1})	7.8±0.16	5.32±0.14	5.18±0.16	5.86±0.082	8.08±0.06
Fe (mg kg^{-1})	1.03±0.03	1.01±0.02	0.88±0.1	1.09±0.09	0.45±0.04
Sand (%)	57.68±0.05	57.68±0.05	57.82±0.05	57.82±0.05	89.68±0.4
Silt (%)	38.48±0.08	38.48±0.08	38.56±0.07	38.56±0.07	7.48±0.5
Clay (%)	3.84±0.06	3.84±0.06	3.62±0.025	3.62±0.025	2.84±0.16
Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam	Sand

† Represent standard deviation of the means.

4.1.2. Rainfall pattern during the cropping season at the study locations

The accumulated and daily number of days of rainfall received at the study sites are shown in Figures 4.1A and 4.1B. There were short periods of dry spells between 10 and 20 and 30 and 40 days after planting. In general, however, the rainfall pattern at Nyankpala was much better than Nyagli. There were 10 days of short dry spells in Nyagli with an average daily rainfall of 0.74 mm during the flowering stage of the plant (Figure 4.1A) whereas in Nyankpala, average rainfall was ≥ 10 mm during the same growth stage (Figure 4.1B).

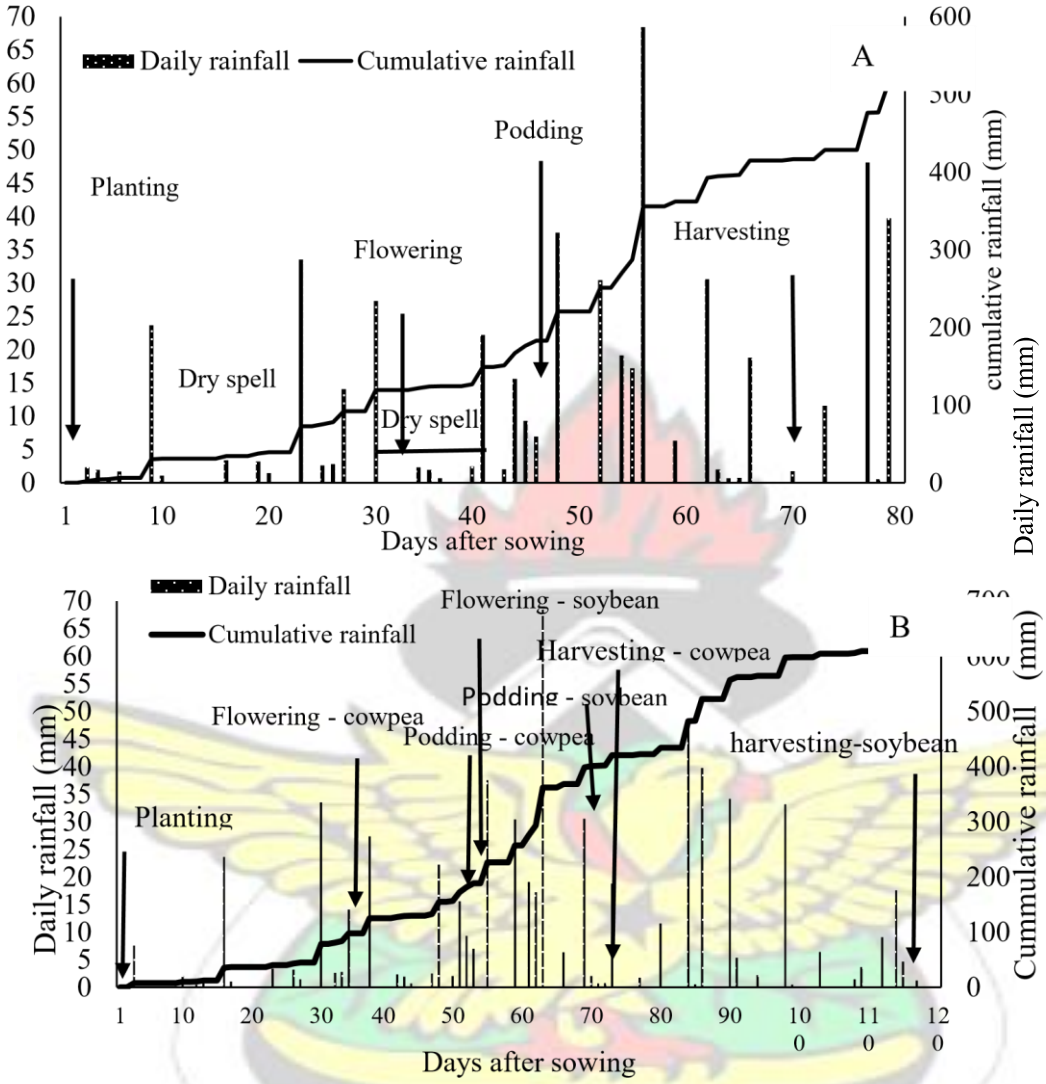


Figure 4.1. Rainfall distribution during the cropping season (A: Nyagli and B: Nyankpala).

4.1.3. Estimation of rhizobial numbers

Number of *Bradyrhizobium* cells g^{-1} inoculant and the population sizes of the indigenous rhizobia per location are presented in Table 4.2. The study sites had low numbers of indigenous rhizobia (<10 rhizobia cell g^{-1} soil). The population of *Bradyrhizobium* in the various inoculants ranged from 10^6 to 10^8 cells g^{-1} inoculant**.**

Table 4.2. Most Probable Number count of rhizobia in the inoculant and the soils at the study sites.

Inoculant	Rhizobia cells (g^{-1} peat)	Confidence Interval (P = 0.95)
Biofix	3.1×10^8	$8.1 \times 10^7 – 1{,}1 \times 10^9$
Legumefix	1.0×10^8	$2.6 \times 10^7 – 3.8 \times 10^8$
BR 3267	5.8×10^7	$1.5 \times 10^7 – 2.2 \times 10^8$
BR 3262	3.1×10^6	$8.1 \times 10^5 – 1.1 \times 10^7$

Location	Rhizobia cells (g^{-1} soil)	Confidence Interval (P = 0.95)
Kpachi	4.5	1.6 – 13.2
Kpalga	8.7	3.0 – 25.1
Nyagli	2.0	0.7 – 5.9

4.1.4. Response of soybean and cowpea to *Bradyrhizobium* inoculation

Bradyrhizobium inoculation significantly (P = 0.001) increased soybean nodulation over the uninoculated plants with or without nitrogen in Nyankpala (Table 4.3). Percentage increases in nodule number due to inoculation with USDA 110 and 532 C were 91 % and 118 %, respectively. Inoculation increased nodule weight by more than 2 and 3 folds compared to uninoculated control and N fertilized plants, respectively (Table 4.3).

In general, inoculation did not significantly (P = 0.22) increase shoot biomass of soybean (Table 4.3). However, inoculation of cowpea with BR 3262 and BR 3267 significantly (P = 0.001) increased shoot dry weight by 38 % and 37 %, respectively, when compared with yields the uninoculated control in Nyankpala (Table 4.3). Inoculation of cowpea with BR 3262 produced a higher shoot dry weight which was statistically similar to the weight

produced by plants inoculated with BR 3267 and uninoculated control which received 100 kg N ha^{-1} (Table 4.3). Both strains were equally effective in significantly (P = 0.001) increasing nodule number and weight (Table 4.3).

The effects of *Bradyrhizobium inoculation* on the grain yield of soybean and cowpea in Nyankpala are presented in Table 4.3. Inoculation of soybean with USDA 110 and 532 C significantly (P = 0.001) increased grain yield by (19 %) and (12 %), respectively, over the uninoculated control. Soybean plants that were inoculated with USDA 110 and 532 C produced grain yields that were 97 and 92 %, respectively, of the potential yield of Jenguma, which is 2.5 tons ha^{-1}. The orthogonal contrast analysis revealed that the inoculated plants performed better than the nitrogen treated plants (Table 4.3). The BR 3267 inoculated cowpea gave significantly (P = 0.001) higher grain yield than the uninoculated control. On the other hand, the effect of BR 3262 on grain yield of cowpea was not significant (Table 4.3). Cowpea plants inoculated with BR 3267 and BR 3262 produced grain yields that were 46 and 37% respectively of the potential yield of Songotra which is 2.5 tons ha^{-1}.

Table 4.3. Response of soybean and cowpea to *Bradyrhizobium inoculation* in Nyankpala.

SOYBEAN				
Treatment	Nodule Number	Nodule dry weight (mg per ten plant)	Shoot dry weight. (kg ha^{-1})	Grain yield (kg ha^{-1})
Biofix	166 b	720.0 b	695 a	2428 bc
Legumefix	190 b	752.0 b	743 a	2302 b
Nitrogen (100 kg N ha^{-1})	66 a	213.4 a	728 a	2566 c
Control	87 a	339.9 a	611 a	2047 a
CV (%)	29.2	30.9	21.4	10.2
CONTRAST				
Inoculant v Nitrogen	<0.001	<0.001	0.882	0.039
Legumefix v Control	<0.001	<0.001	0.057	0.024
Biofix v Legumefix	0.652	0.652	0.472	0.246
COWPEA				
Treatment	Nodule number	Nodule dry weight (mg per ten plants)	Dry matter yield (kg ha^{-1})	Grain yield (kg ha^{-1})
BR 3267	81.0 c	527.8 c	2696 b	1144 b
BR 3262	96.7 d	465.2 c	2708 b	917 a
Nitrogen (100 kg N ha^{-1})	39.3 a	181.2 a	2928 b	1278 b
Control	57.4 b	320.2 b	1964 a	828 a
CV (%)	22.1	30.3	10.5	18.0
CONTRAST				
Inoculant v Nitrogen	<0.001	<0.001	0.041	0.002
BR 3262 v Control	<0.001	<0.001	<0.001	0.303
BR 3267 v BR 3262	<0.001	0.009	<0.001	<0.001

Values are means of 10 plants per plot for nodule number and nodule dry weight

Bradyrhizobium inoculation significantly (P = 0.013) increased cowpea nodule number over the uninoculated plants with nitrogen at Nyagli in the Upper West region (Table 4.4). Nodule numbers produced by the inoculated plants were not significantly different from that of the uninoculated control. The BR 3267 inoculated plants produced significant (P = 0.004) increase in nodule dry weight of 62 % relative to the uninoculated control (Table

4.4**).** The nitrogen fertilized plants produced the least nodule number and nodule dry weight (Table 4.4).

Shoot biomass was not significantly (P = 0.135) affected by the treatments (Table 4.4). Nonetheless, the inoculated plants consistently recorded higher shoot biomass than the control. The grain yield of cowpea in Nyagli in the Upper West region was generally low and there was no significant (P = 0.433) difference among the treatments (Table 4.4). No significant differences were observed between the BR 3262 and BR 3267 strains in all measurements in this location (Table 4.4). The contrast analysis did not show any significant difference between any pair of treatments for nodule number, shoot dry weight and grain yield (Table 4.4).

Table 4.4. Response of cowpea to Bradyrhizobium inoculation in Nyagli.

Treatment	Nodule Number	Nodule dry weight (mg per ten plants)	Shoot dry weight. (kg ha^{-1})	Grain yield (kg ha^{-1})
BR 3267	52.4 b	368.0 c	719 a	758 a
BR 3262	48.4 b	318.2 bc	785 a	695 a
Control	44.4 b	227.6 ab	607 a	635 a
Nitrogen (100 kg N ha^{1})	33.4 a	155.4 a	810 a	649 a
CV (%)	17.4	28.1	18.5	18.2
CONTRAST				
Inoculant v Nitrogen	0.002	0.012	0.453	0.281
BR 3262 v Control	0.431	0.315	0.059	0.461
BR 3267 v BR 3262	0.431	0.081	0.456	0.441
BR 3267 v control	0.129	<.001	0.214	0.145

Values are means of 10 plants per plot for nodule number and nodule dry weight

The Wilks lambda values from MANOVA for soybean (0.067, p = 0.00) and cowpea (0.039, p = 0.00) showed that 93.3 and 96.1 % of the variations observed in soybean and cowpea grain yield, respectively, were due to inoculation (Table 4.5).

Table 4.5 MANOVA for cowpea and soybean grain yield in response to inoculant and site interaction.

Term	d.f.	Wilk's lambda	Rao F	n.d.f.	d.d.f.	F prob.
Cowpea						
Strain	3	0.0393	9.91	15	67	0.000
Site	1	0.1873	20.83	5	24	0.000
Strain. Site	3	0.4309	1.58	15	67	0.102
Soybean						
Strain	3	0.0671	7.38	15	67	0.000
Site	1	0.2193	17.09	5	24	0.000
Strain. Site	3	0.6023	0.90	15	67	0.572

n.d.f = this is the number of degrees of freedom in the model; d.d.f = this is the number of degrees of freedom associated with the model errors

4.1.5. Economic returns

Returns on investments based on VCR were positive and therefore indicated that all the applied strains were economically viable but USDA 110, 532 C were profitable for soybean while BR 3267 was profitable for cowpea. However, considering VCRs of the strains (USDA 110 (8.7), 532 C (4.1), BR 3267 (4.6) and Urea (2.0)), the use of strains would be more profitable in Nyankpala under the current environmental and climatic conditions than urea (Figure 4.2). At Nyagli in the Upper West region, the VCR for BR 3267 and B3 262 were 1.8 and 1.3, respectively while that of Urea was 0.9. Unlike, Nyankpala, the use of strains in Nyagli would not be economically viable under the current environmental and climatic conditions (Figure 4.2).

NITROGEN

BR 3262

BR 3267

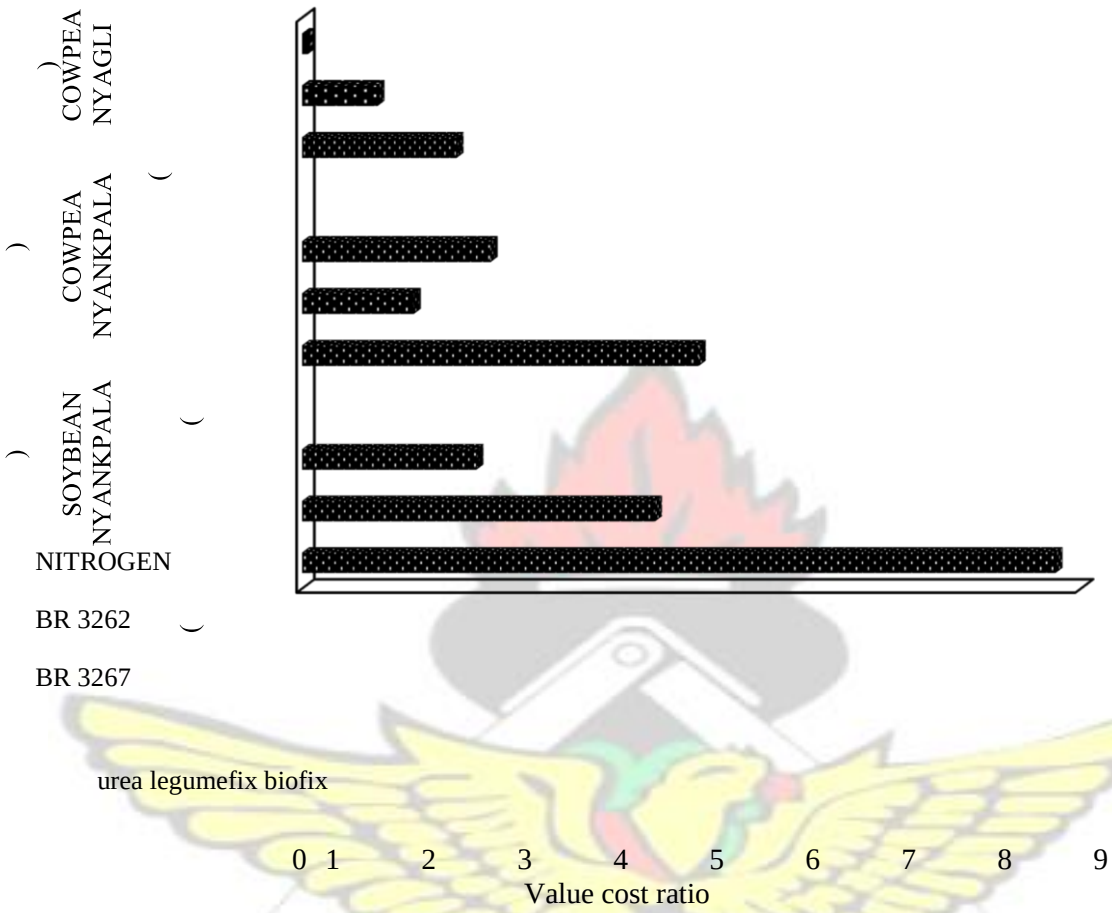


Figure 4.2. Value cost ratio of using mineral N fertilizer and *Bradyrhizobium* inoculants on soybean and cowpea.

4.2. Study 2. Persistence of *Bradyrhizobium* under field conditions

4.2.1. The physical and chemical properties of the study locations

The physical and chemical properties of the study locations are presented in Table 4.6. The pH values ranged from 6.0 to 6.3. The study locations had medium levels of nitrogen but very low levels of organic C and P according to Landon (2014). This indicates the inherent low fertility nature of the locations. The locations had relatively higher sand content than

clay. The exchangeable K was moderate whiles exchangeable Ca was low. The exchangeable Mg was high except for Kpalga according to the ratings by Landon (2014).

Table 4.6. Physical and chemical properties of the soils at the study locations

Soil parameters	locations			
	Kpalga	Tunayilli	Tanina	Busa
pH(1:2.5) (H_2O)	6.13±0.25†	6.3±0.1	6.0±0.06	6.0±0.04
Total N (%)	0.43±0.02	0.52±0.022	0.33±0.012	0.33±0.003
Available P (mg kg^{-1})	1.69±0.23	1.53±0.22	2.04±0.025	1.20±0.18
Exchangeable K ($cmol_{(+)}$ kg^{-1})	1.21±0.09	1.06±0.1	1.06±0.021	1.11±0.05
Organic C (%)	0.42±0.02	0.74±0.05	0.28±0.01	0.49±0.01
Exchangeable Ca^+ ($cmol_{(+)}$ kg^{-1})	3.15±0.11	4.41±0.65	2.66±0.01	2.93±0.09
Exchangeable Mg^+ ($cmol_{(+)}$ kg^{-1})	0.38±0.02	0.60±0.52	0.62±0.015	0.62±0.08
Sand (%)	64.42±1.50	70.08±7.04	68.92±0.02	68.52±1.60
Silt (%)	27.74±1.54	24.08±0.96	12.88±0.02	24.64±1.64
Clay (%)	7.84±1.54	5.84±6.08	18.2±0.15	6.84±0.04
Texture	Sandy loam	Sandy loam	Sandy loam	Sandy loam

† Represent standard error of the means.

A sharp numerical significant (p = 0.0001) decline of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 after 21 days of introduction into the field was observed (Table 4.7). Thereafter, the numbers declined at a slower rate for both strains. The decline rates from 42 – 142 days were not significant from each other for *B. yuanmingense* strain BR 3267. The persistence of *B. japonicum* strain USDA 110 remained constant from 21 - 142 days (Table 4.7). At the end of day 296, *B. yuanmingense* strain BR 3267 survived with $\log_{10}$ 1.9 rhizobia cell g^{-1} soil whereas *B. japonicum* strain USDA 110 survived with $\log_{10}$1.7 rhizobia cell g^{-1} soil.

Table 4.7. Persistence of *Bradyrhizobium spp.* after 296 days of introduction

Day following release	*B. yuanmingense* (BR 3267)	*B. japonicum* (USDA 110)
Rhizobial cells g soil^{-1}		
0	8.4±0.0*a†	7.4±0.0a†
21	2.5±0.03b	2.4±0.12b
42	2.3±0.05c	2.2±0.11b
81	2.2±0.05c	1.9±0.11b
142	2.1±0.07c	1.9±0.10b
296	1.9±0.06d	1.7±0.07c
P-value	*<0.0001*	*< 0.0001*

† Within column, figures followed by the same letters are not significantly different from one another at 5% probability. * represents standard error of mean.

Figures 4.3a and 4.3b shows the distribution of the *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA at the various locations (Figures. 4.3a and 4.3b). The highest point of the boxplot reflects the initial number of strains introduced and the lowest point reflect the remaining number of strains at the end of the experiment. There was slight variation between the boxes for each location but such variations were not significant (Figures. 4.3a and 4.3b). Therefore, the decline in population of the introduced strains *B. yuanmingense* strain BR3267 and *B. japonicum* strain USDA 110 revealed that in general the strains did not behave differently in the different locations (Figs. 4.3a and 4.3b).

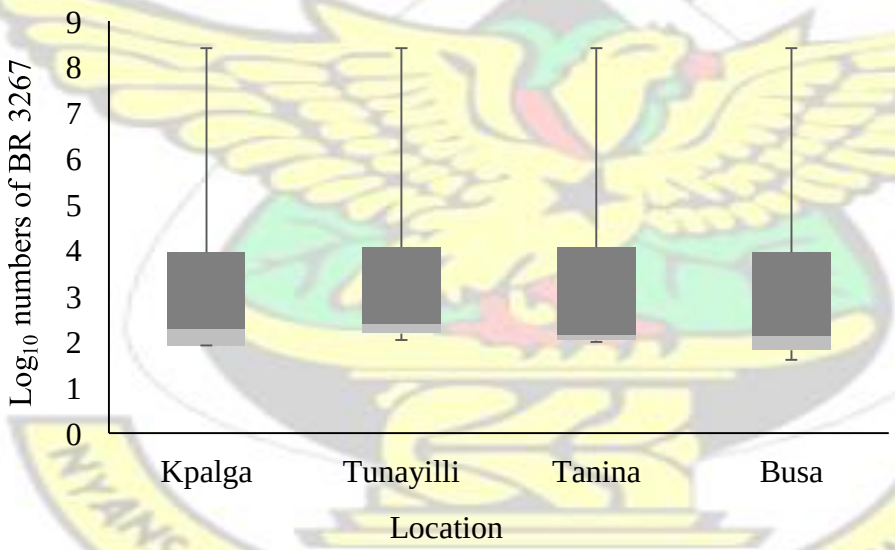


Figure 4.3a. Distribution of *B. yuanmingense* strain BR 3267 at the study locations.

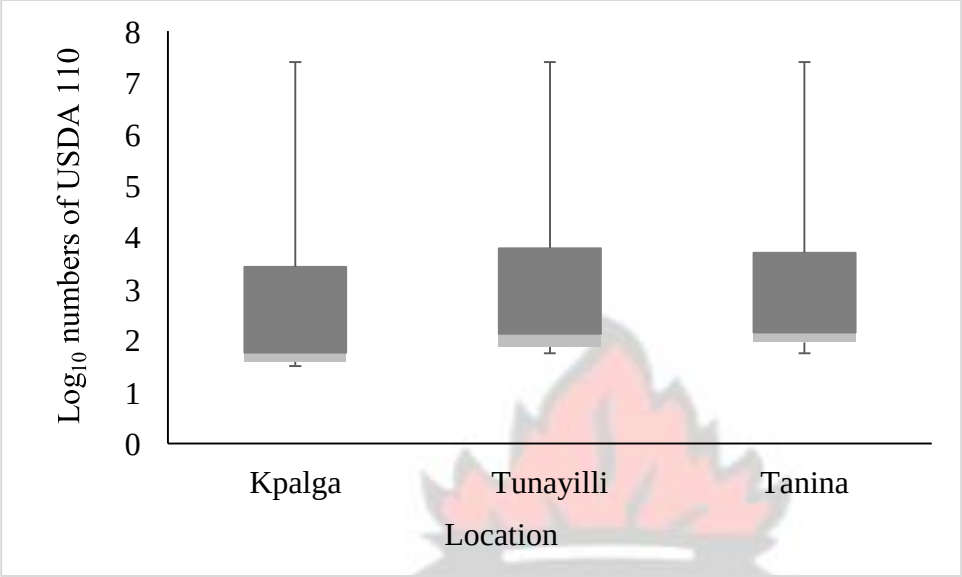


Figure 4.3b. Distribution of *B. japonicum* strain USDA 110 at the study locations.

Among the several regression functions that were applied to the decline rates of *B. yuanmingense* strain BR3267 and *B. japonicum* strain USDA 110, hyperbolic function was identified as the option that provided the best fit based on the AICC value (Table 4.8). Native rhizobia population, soil moisture and rainfall mostly influenced the persistence of *B. yuanmingense* strain BR 3267 over time (Table 4.9). Assuming that all other environmental factors remain constant, native rhizobia population, soil moisture and rainfall, accounted for 98, 96 and 45 %, respectively of the variations in the observed decline of *B. yuanmingense* strain BR 3267 (Table 4.9). Similarly, soil moisture, native rhizobia population and solar radiation were identified as the most influential factors affecting the persistence of *B. japonicum* strain USDA 110. Assuming that all other environmental factors remained constant, soil moisture, native rhizobia and solar radiation accounted for 98, 81 and 48 %, respectively of the variations in the observed decline of *B. japonicum* strain USDA 110 (Table 4.9). Holding all other environmental factors constant,

the prevailing relative humidity at the study location had no effect on the persistence of *B. yuanmingense* strain BR 3267 and *B. yuanmingense* strain BR 3267 (Table 4.9).

Table 4.8. Fitting of regression models to the survival rates of introduced *Bradyrhizobium spp*

Strain	Type of function	equation	coefficients				Standard error	r	AICC
BR3267			q_o	a	b	c			
	Hyperbolic	$y = q_o (1 + bx/a)^{-1/b}$	8.40	0.009	10.21		0.031	0.99	-38.04
	Logistics	$y = a / (1 + be^{-cx})$		2.09	-0.75	0.068	0.15	0.99	-19.36
	Exponential decline	$y = q_o \exp^{-x/a}$	7.88	30.58			1.79	0.77	7.60
	Gompertz relation	$y = a\exp^{-e^{b-cr}}$		3.23	-45.04	1.2×10^7	3.28	0.00	17.84
USDA 110	Hyperbolic	$y = q_o (1 + bx/a)^{-1/b}$	7.40	0.03	7.60		0.06	0.99	-29.06
	Logistics	$y = a / (1 + be^{-cx})$		1.86	-0.75	0.051	0.14	0.99	-19.34
	Exponential decline	$y = q_o \exp^{-x/a}$	6.82	35.62			1.56	0.77	5.89

AICC= Akaike information criterion corrected.

Gompertz relation $y = a\exp^{-e^{b-cr}}$	2.98	-39.54	1.7×10^7	2.85	0.00	16.42

Table 4.9. Fitting of hyperbolic regression to parameters that influence the survival rates of *Bradyrhizobium spp*

Strain	Parameters		coefficients		Standard error	r	AICC
BR3267		q_o	a	b			
	Soil moisture	1.72	-102.73	5.06	0.37	0.98	-8.12
	Native rhizobia population	1.58	-10.46	5.51	0.37	0.99	-8.14
	Temperature	5.56	245.32	-5.86	3.10	0.58	17.44
	Relative Humidity	3.23	3.8×10^7	2.0×10^7	0.00	0.00	18.08

	Solar energy	4.0×10^4	74.58	0.32	2.59	0.61	15.24
	Annual rainfall	5.45	0.002	10.36	2.41	0.67	14.40
AICC= Akaike information criterion corrected							
USDA 110	Soil moisture	1.85	-290.23	12.90	0.32	0.99	-9.78
	Native rhizobia population	1.56	-12.87	7.14	2.15	0.90	13.04
	Temperature	2.8×10^6	0.87	0.14	2.70	0.59	15.74
	Relative Humidity	2.92	2.1×10^7	2.1×10^7	2.85	0.00	16.42
	Solar energy	5.5×10^4	84.12	0.27	2.06	0.69	12.51
	Annual rainfall	4.0	0.0069	11.24	2.41	0.54	14.37

Temperature and soil moisture were inversely proportional to the survival rates of the introduced strains as indicated by the surface response curve (Figure. 4.4).

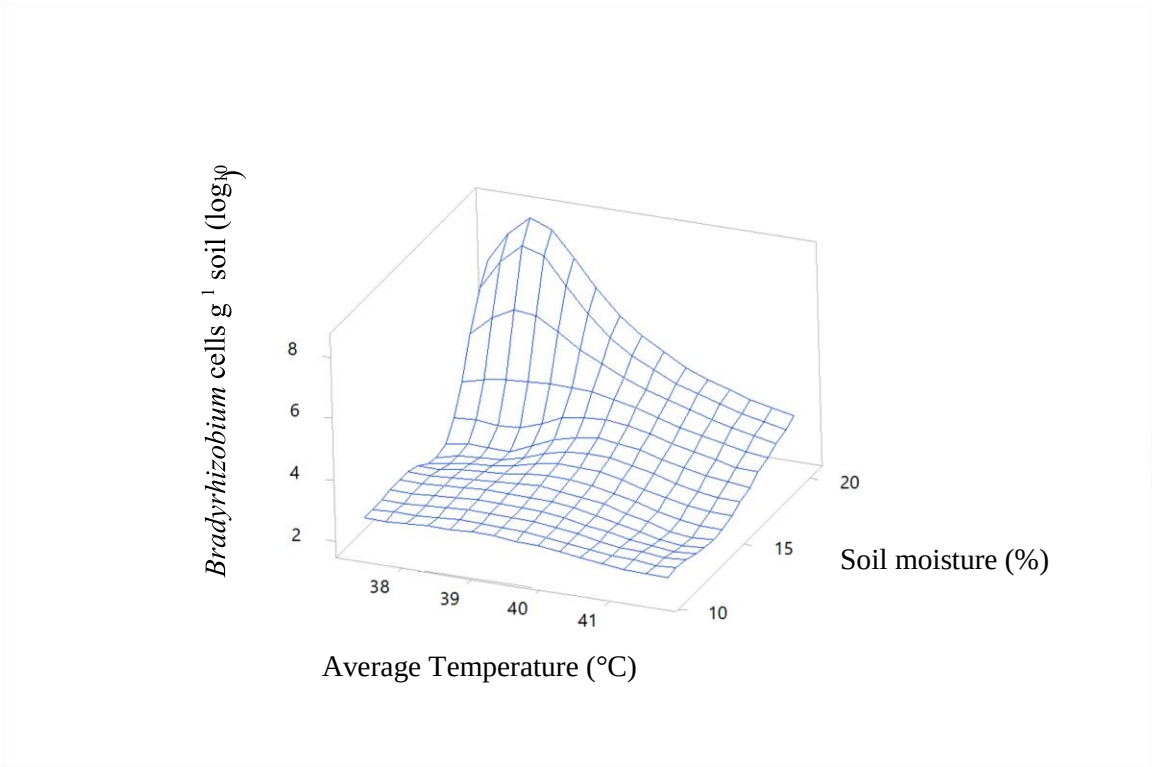


Figure 4.4. The relationship between temperature, soil moisture and introduced *Bradyrhizobium* strains

Predictions based on the hyperbolic regression function indicate that *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 can persist up to 3 years assuming the current environmental conditions remain fairly the same (Figure 4.5).

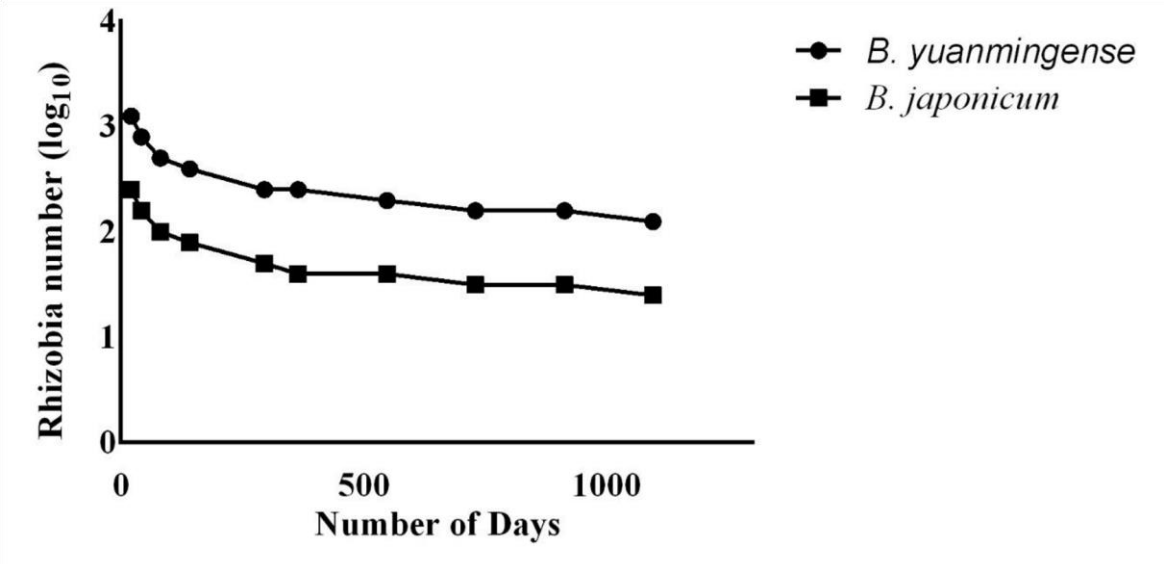


Figure 4.5. Predicted number of survived *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 for 3 years (1095 days).

To check the precision of the prediction, the predicted decline rates were regressed against the observed decline rate (Figure 4.6). The regression revealed strong relationship (r^2 = 0.98) for *B. yuanmingense* strain BR 3267 and (r^2 = 0.96) for *B. japonicum* strain USDA 110, respectively (Figure 4.6).

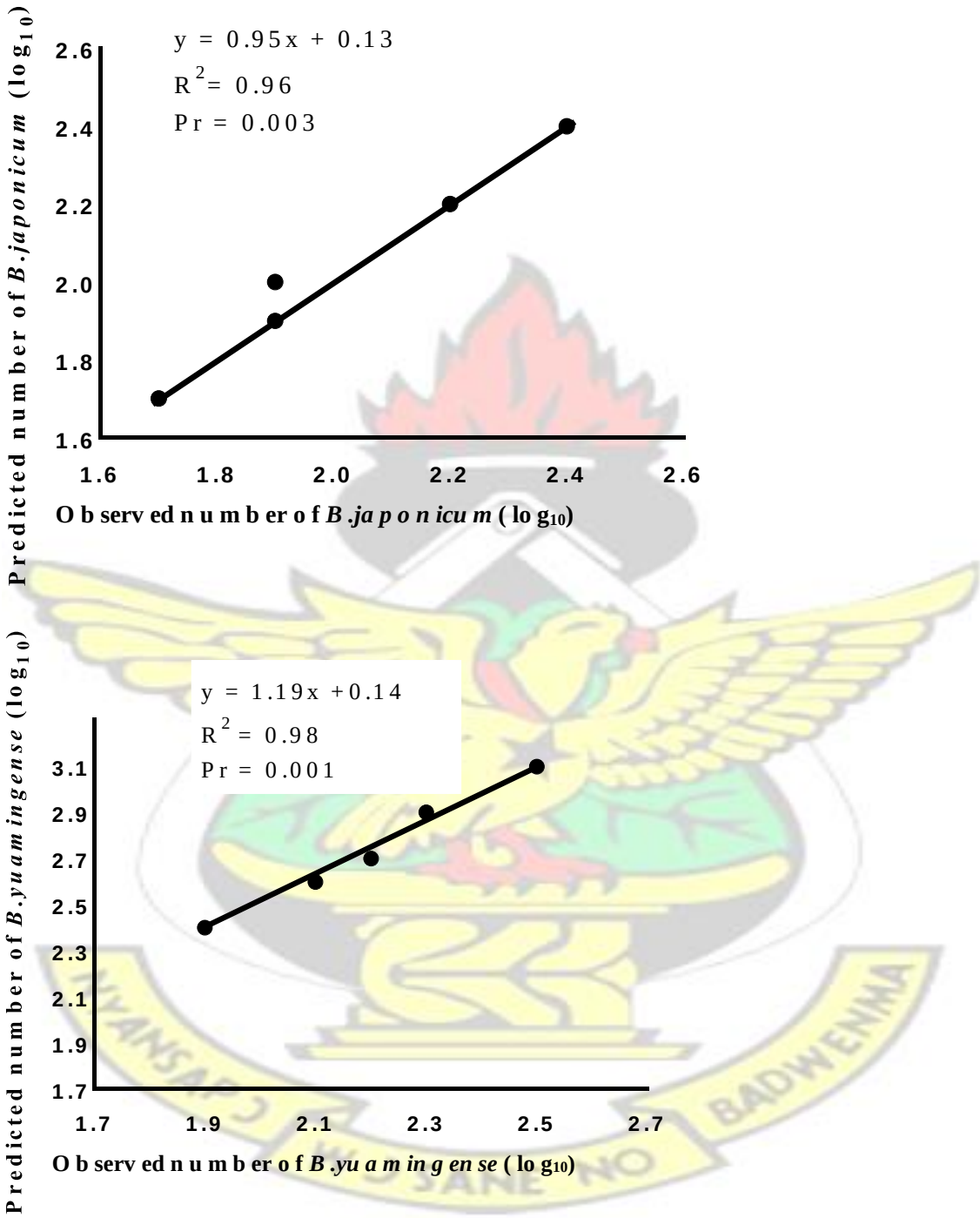


Figure 4.6. Relationship between predicted and observed decline number of *Bradyrhizobium spp.*

The multivariate non-linear regression analysis (Table 4.10) revealed the variations in magnitude and direction of the effect of environmental factors on the strains. For example, rainfall had

negative effect on persistence of the strains. Soil moisture and soil temperature effects were positive irrespective of the strain. The effect of native rhizobia population was negative on B. yuanmingense strain BR 3267 and positive on B. japonicum strain USDA 110. The effects of solar radiation and relative humidity on *B. yuanmingense* strain BR 3267 was positive but negative on *B. japonicum* strain USDA 110. Given that, the correlation coefficients were 0.98 and 0.96 for *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110, respectively, 96 and 92% of the dynamics of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110, respectively in the soil were explained by the environmental factors.

The proximate analysis of the carrier materials for the two strains are illustrated in Table 4.11. Except for the percentage fat, fibre and protein contents, there was no significant differences in the other properties of peat and filter mud.

Table 4.10. Multivariate non-linear regression of the factors affecting rhizobia survival.

Parameters / coefficient	Strain	
	*B. yuanmingense (*BR 3267)	*B. japonicum* (USDA 110)
Intercept	-16.12	-53.38
Native rhizobia population	-7.17	8.09
Soil moisture	1.42	1.05
Daily rainfall	-0.88	-0.01
Soil temperature	0.149	0.80
Relative humidity	0.024	-0.019
Solar radiation	0.000	-0.002
Correlation coefficient (r)	0.98	0.96

Table 4.11.Properties of the carrier materials for USDA 110 (filter mud) and BR 3267 (Peat)

Parameters	Strain			
	B. yuanmingense (BR 3267)	*B. japonicum* (USDA 110)	*P-value*	Confidence interval
Fat (%)	0.33 ± 0.006†	0.79 ± 0.0064	0.001	0.44 – 0.48
Fiber (%)	10.79 ± 0.58	7.43 ± 0.53	0.024	0.82 –5.83
Ash (%)	30.66 ± 0.58	29.59 ± 0.56	0.281	-1.53 – 3.68
Moisture (%)	42.00 ± 0.58	37.20 ±1.2	0.09	-1.60 – 9.87
Protein (%)	8.25 ± 0.65	5.15 ± 0.49	0.012	1.02 – 5.17
Carbohydrate (%)	9.50 ± 0.37	10.62 ± 0.42	0.12	-0.41 – 2.49

† represents standard error of the mean

4.3. Study 3. Improving cowpea response to *Bradyrhizobium* inoculation through the addition of phosphorus fertilizer and organic manure

4.3.1. Physical, chemical characteristics and most probable number count

The physical and chemical characteristics of the study locations are presented in Table 4.12. The concentrations of major nutrients (N, C and P) were very low according to the ratings of Landon (2014). The locations had moderate levels of exchangeable K. The pH of the soil at various locations was classified as medium. The soil contains high proportion of sand at the various locations and the texture classified as sandy loam (Table 4.12)

Table 4.12. Physical and chemical properties of the soils at the study locations

Soil parameters	Kpalga	Tunayilli	Busa
pH(1:2.5) (H_2O)	6.4±0.5*	6.6±0.02	6.4±0.5
Total N (%)	0.043±0.02	0.052±0.022	0.033±0.003
Available P (mg kg^{-1})	1.69±0.23	1.53±0.22	1.20±0.18
Exchangeable K ($cmol_{(+)}$ kg^{-1})	1.21±0.09	1.06±0.1	1.11±0.05
Organic C (%)	0.42±0.02	0.74±0.05	0.49±0.01
Exchangeable Ca ($cmol_{(+)}$ kg^{-1})	3.15±0.11	4.41±0.65	2.93±0.09
Exchangeable Mg ($cmol_{(+)}$ kg^{-1})	0.38±0.02	0.60±0.52	0.62±0.08
Sand (%)	64.42±1.50	70.08±7.04	68.52±1.60
Silt (%)	27.74±1.54	24.08±0.96	24.64±1.64
Clay (%)	7.84±1.54	5.84±6.08	6.84±0.04
Texture	Sandy loam	Sandy loam	Sandy loam

* Represents standard error of the means.

The chemical properties of the fertisoil and cattle manure are illustrated in Table 4.13. Fertisoil had nitrogen content > 2.5 % with that of cattle manure was < 2.5%. Organic manure with nitrogen content higher than 2.5% is considered to be of good quality (Palm et al., 1997). The carbon content of cattle manure was relatively higher than that of fertisoil. However, all the two manure types had C: N ratios of less than 30 (Table 4.13). The total calcium and magnesium of the fertisoil were relatively higher than that of cattle manure

Table 4.13. Selected chemical characteristics of fertisoil and cattle manure

Parameter	Fertisoil	Cattle manure
Carbon (%)	11.7	21.15
Total Nitrogen (%)	4.5	2.43
Total Phosphorus (%)	0.37	0.24
Total Potassium (%)	0.41	0.3
Lignin (%)	10.5	5.5
Polyphenol (%)	0.08	0.02
Ash (%)	59.4	56.1
Total calcium (%)	1.3	0.65
Total magnesium (%)	0.92	0.41
C:N Ratio	1.95	6.17

The indigenous rhizobia populations of the study locations were below 50 cells g^{-1} soil (Table 4.14). However, the population at Tunayilli and Busa was relatively higher than that of Kpalga but the differences was not significant at $P \geq 0.05$.

Table 4.14. Most probable number count of indigenous rhizobia at the study locations

Locations	Rhizobia cells (g^{-1} soil)	Confidence interval
Kpalga	32.8	11.4-94.6
Tunayilli	40.4	14.0-116.5
Busa	43.6	15.1-125.6

4.3.2. Nodulation

Unlike other parameters, only the combined application of inoculant and compost were significant (p=0.001) for nodule number under the two-way interaction (Figures 4.7, 4.8 and 4.9). Adding fertisoil to *Bradyrhizobium* inoculant resulted in a significantly higher nodule number than the addition of cattle manure to inoculated and control treatments (Figure 4.8). The three way interaction involving *Bradyrhizobium*, phosphorus and organic manure was significant (p = 0.00001) for nodule number (Figure 4.10). Adding fertisoil and P to *Bradyrhizobium* inoculant caused an increase in nodule number by 32% over the combined application of *Bradyrhizobium* inoculant, phosphorus and cattle manure (Figure 4.10). The application of fertisoil and P to *Bradyrhizobium* inoculant resulted in greater increase in nodule number (165%) over the control. The effect of cattle manure and P to *Bradyrhizobium* inoculant on nodule number was 100% over the control (Figure 4.10). There was no significant (p = 0.34) difference between the locations and the treatments (Figure 4.11).

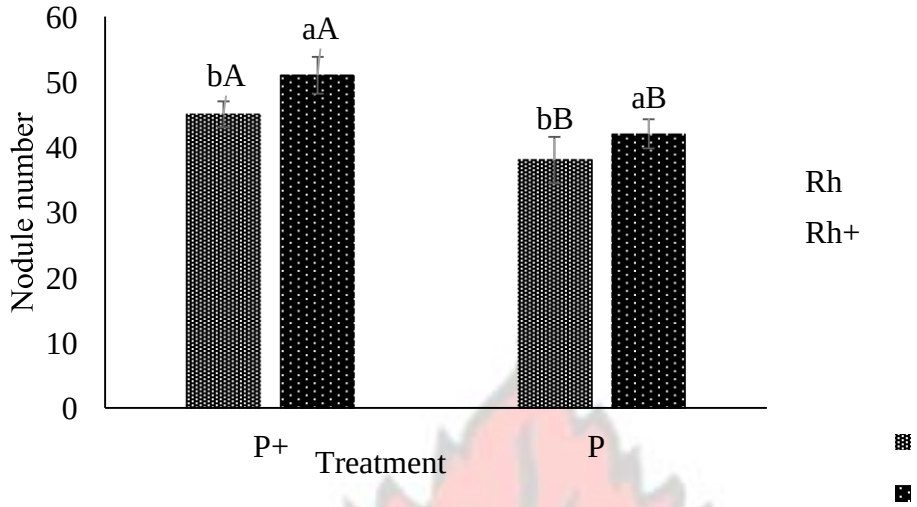


Figure 4.7. Effect of *Bradyrhizobium* inoculation and phosphorus application on nodule number of cowpea. Bars denote standard error of means. Values for nodule numbers are means of ten plants. Bars followed by different lowercase letters between (P+ and P-) and different upper case letters between (Rh+ and Rh-) are different from each other at the 5% probability level.

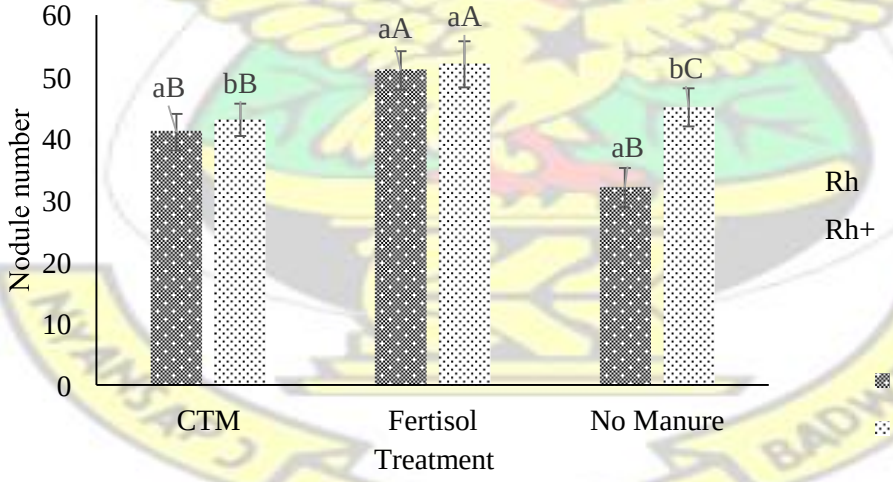


Figure 4.8. Effect of *Bradyrhizobium* inoculation and organic manure application on nodule number cowpea. Bars denote standard error of means. Values for nodule numbers are means of ten plants. Bars followed by different lowercase letters between (Rh+ and Rh-) within types of organic manure and different upper case letters between types of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

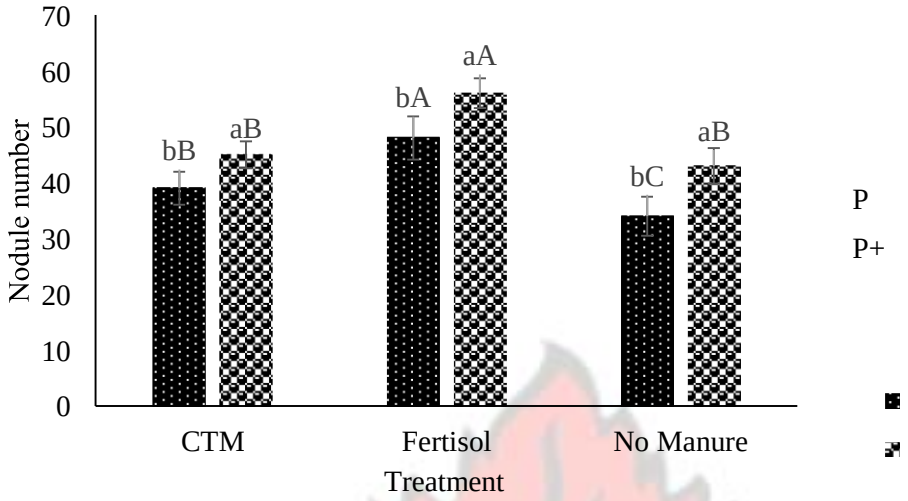


Figure 4.9. Effect of phosphorus and organic manure application on nodule number of cowpea. Bars denote standard error of means. Values for nodule numbers are means of ten plants. Bars followed by different lowercase letters between (P+ and P-) within types of organic manure and different upper case letters between types of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

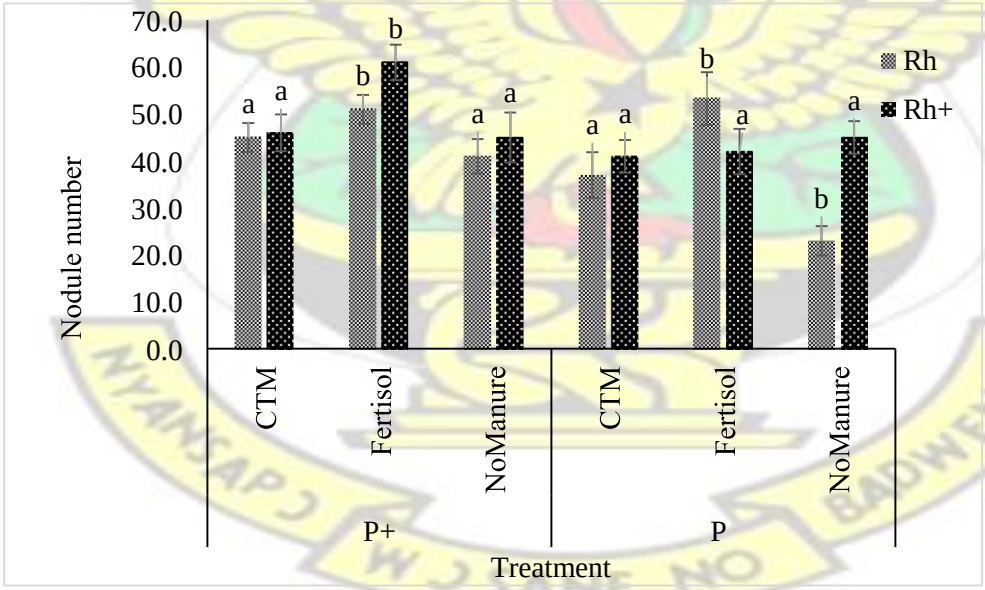


Figure 4.10. Effect of *Bradyrhizobium* inoculation, phosphorus and organic manure on nodule number of cowpea. Bars denote standard error of means. Values for nodule numbers are means of ten plants. Bars followed by different letters, comparing (Rh+ and Rh-) within each (P+ and P-) and type of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

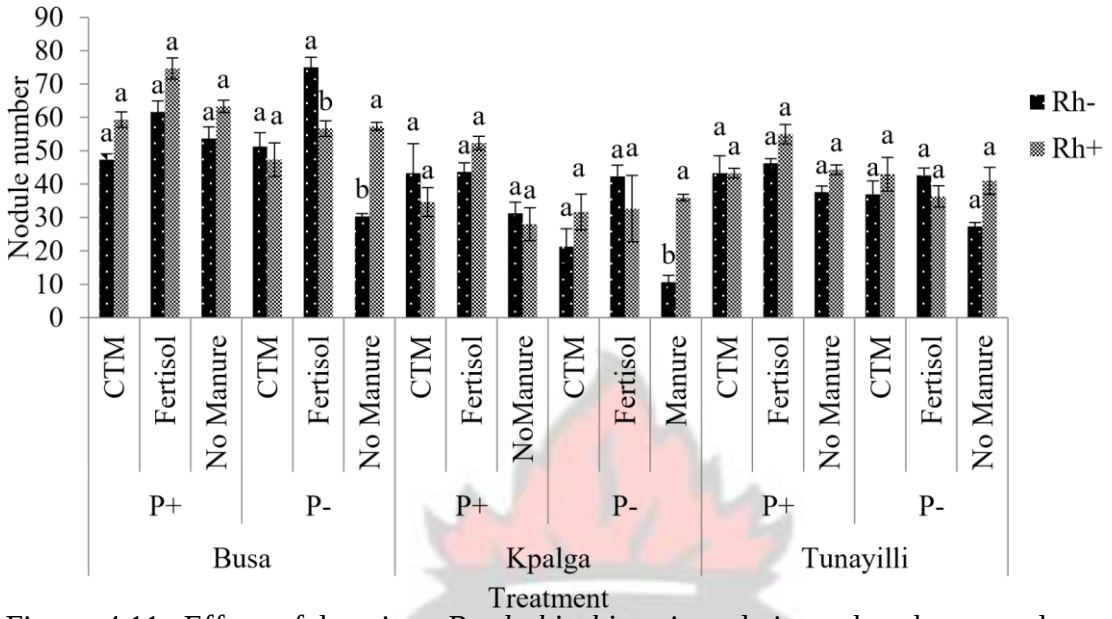


Figure 4.11. Effect of location, *Bradyrhizobium* inoculation, phosphorus and organic manure on nodule number of cowpea. Bars denote standard error of means. Values for nodule numbers are means of ten plants. Bars followed by different letters within the same location, levels of P and type of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

Except the interaction between inoculation and phosphorus, no other interaction was significant for nodule dry weight (Figures 4.12 – 4.14). Adding P to *Bradyrhizobium* inoculant increased nodule dry weight from 672 mg plant^{-1} to 1077 mg plant^{-1} (Figure 4.12). The combined application of P and *Bradyrhizobium* inoculant increased nodule dry weight 126% over the control. Comparatively, an increase of 416 mg plant^{-1} by the *Bradyrhizobium* treatments that received P over the *Bradyrhizobium* inoculant without P was recorded (Figure 4.12). The three way interaction between *Bradyrhizobium* inoculant, phosphorus and organic manure was not significant ($p = 0.23$) (Figure 4.15). Also, there was no significant ($p=0.82$) difference for nodule dry weight for the interaction between location and treatments (Figure 4.16).

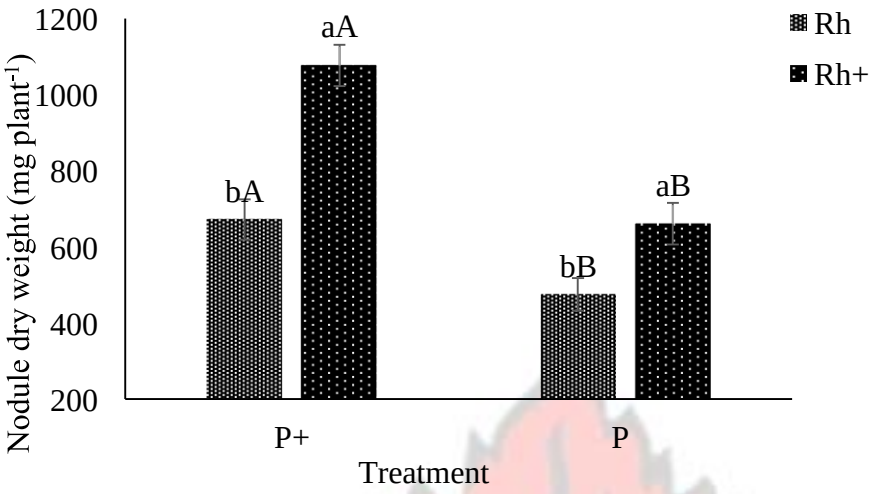


Figure 4.12. Effect of *Bradyrhizobium* inoculation and phosphorus application on nodule dry weight of cowpea. Bars denote standard error of means. Values for nodule dry weight are means of ten plants. Bars followed by different lowercase letters between (P+ and P-) and different upper case letters between (Rh+ and Rh-) are different from each other at the 5% probability level.

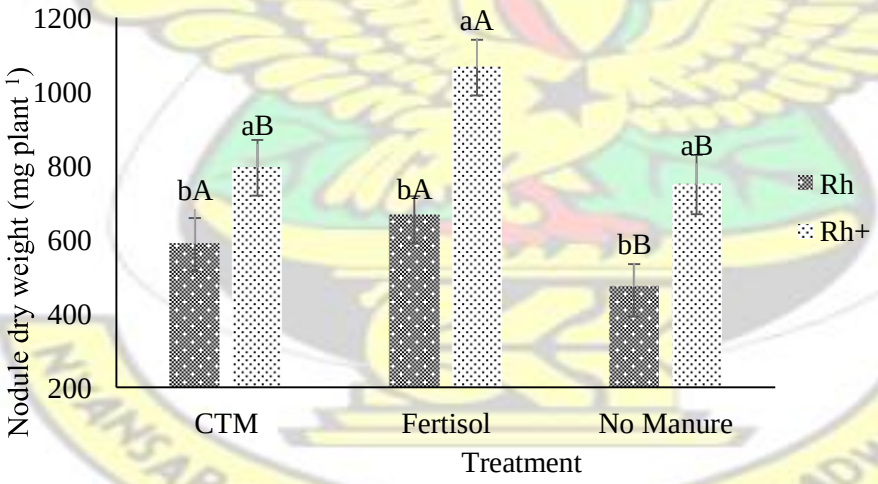


Figure 4.13. Effect of *Bradyrhizobium* inoculation and organic manure application on nodule dry weight of cowpea. Bars denote standard error of means. Values for nodule dry weight are means of ten plants. Bars followed by different lowercase letters between (Rh+ and Rh-) within types of organic manure and different upper case letters between types of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

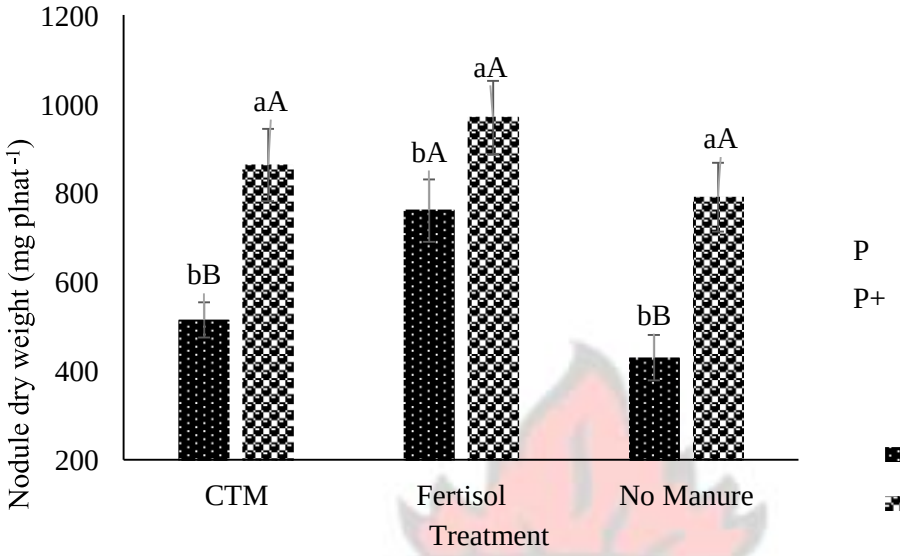


Figure 4.14. Effect of phosphorus and organic manure on nodule dry weight of cowpea. Bars denote standard error of means. Values for nodule dry weight are means of ten plants. Bars followed by different lowercase letters between (P+ and P-) within types of organic manure and different upper case letters between types of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

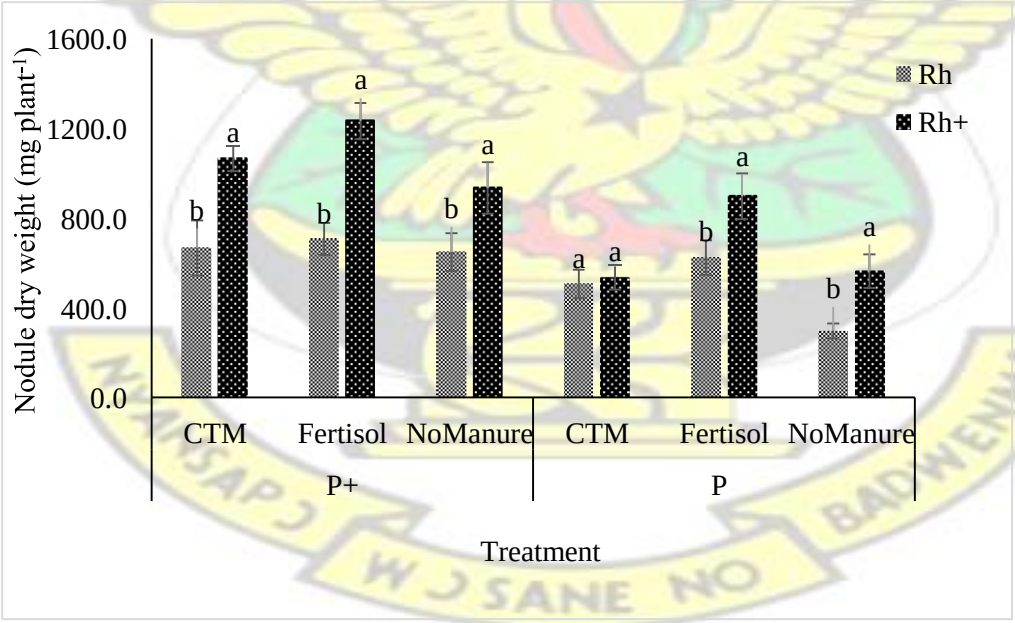


Figure 4.15. Effect of *Bradyrhizobium* inoculation, phosphorus and organic manure application on nodule dry weight of cowpea. Bars denote standard error of means. Values for nodule dry weight are means of ten plants. Bars followed by different letters, comparing (Rh+ and Rh-) within each (P+ and P-) and type of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

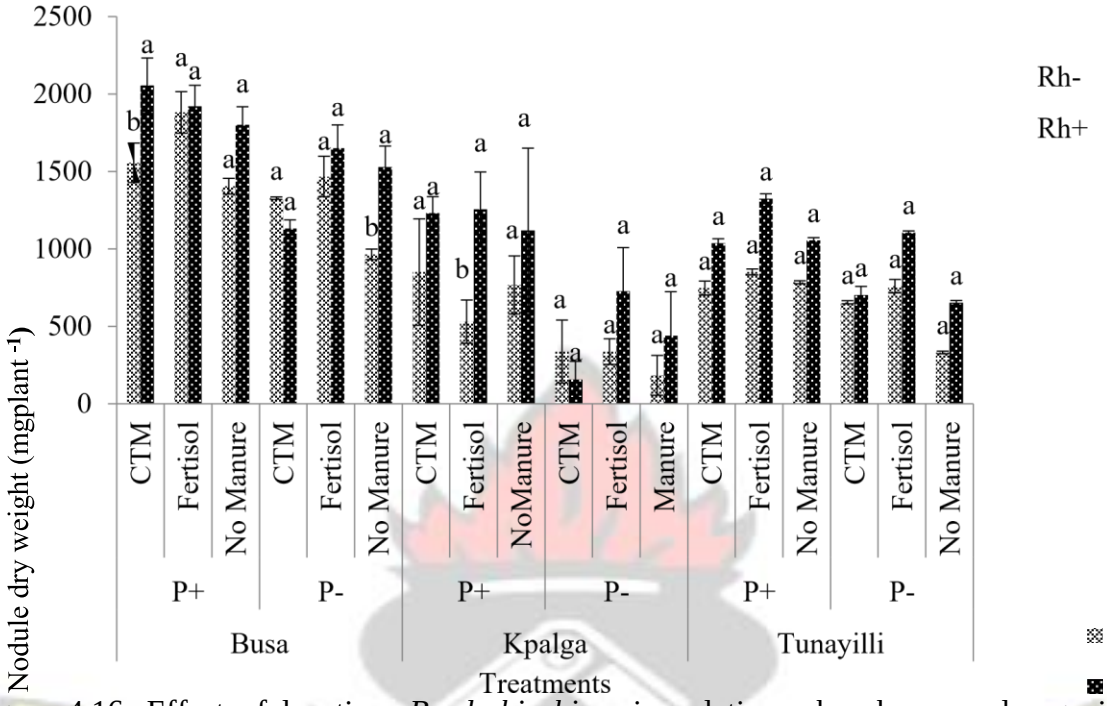


Figure 4.16. Effect of location, *Bradyrhizobium* inoculation, phosphorus and organic manure on nodule dry weight of cowpea. Bars denote standard error of means. Values for nodule dry weight are means of ten plants. Bars followed by different letters within the same location, levels of P and type of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

4.3.3. Shoot biomass yield

There were significant ($p < 0.05$) differences in grain yield for the two (Figure 4.17 – 4.19) and three (Figure 4.20) way interactions. For the addition of P to *Bradyrhizobium* inoculant treatment, increased shoot biomass yield from 1482 to 1792 kg ha^{-1} (Figure 4.17). Addition of fertisoil or cattle manure to *Bradyrhizobium* inoculation increased the dry matter yield from 1509 to 1753 kg ha^{-1} and from 1283 to 1432 kg ha^{-1} respectively (Figure 4.18). Combined application of *Bradyrhizobium* inoculant and fertisoil significantly increased shoot biomass yield over combined application of cattle manure and *Bradyrhizobium* inoculant by 320 kg ha^{-1} (Figure 4.18). Similarly, addition of fertisoil or cattle manure to P treatment increased shoot biomass yield from 1625, 1795 kg ha-1, and 1090 $kgha^{-1}$ to

1467 kg ha^{-1} respectively (Figure 4.19). The shoot biomass yield of combined application of fertisoil and P was significantly higher than the combined application of cattle manure and P (Figure 4.19). Shoot biomass increased from 856 kg ha^{-1} in the control treatment to 1057 kg ha^{-1} when fertisoil, P and *Bradyrhizobium* inoculant were applied together. Similarly, an increase in shoot biomass of 957 kg ha^{-1} was obtained when cattle manure, P and *Bradyrhizobium* inoculant were applied together (Figure 4.20). There was no significant difference in the shoot biomass yield obtained between the combined application of *Bradyrhizobium* inoculant, phosphorus and fertisoil or cattle (Figure 4.20). In almost all the cases, the treatments that received

Bradyrhizobium inoculant had better shoot biomass yield than treatments without *Bradyrhizobium* inoculant. The effect was location specific since the interaction between location and treatments were highly significant (p = 0.0046); with Busa recording low dry matter yield (Figure 4.21).

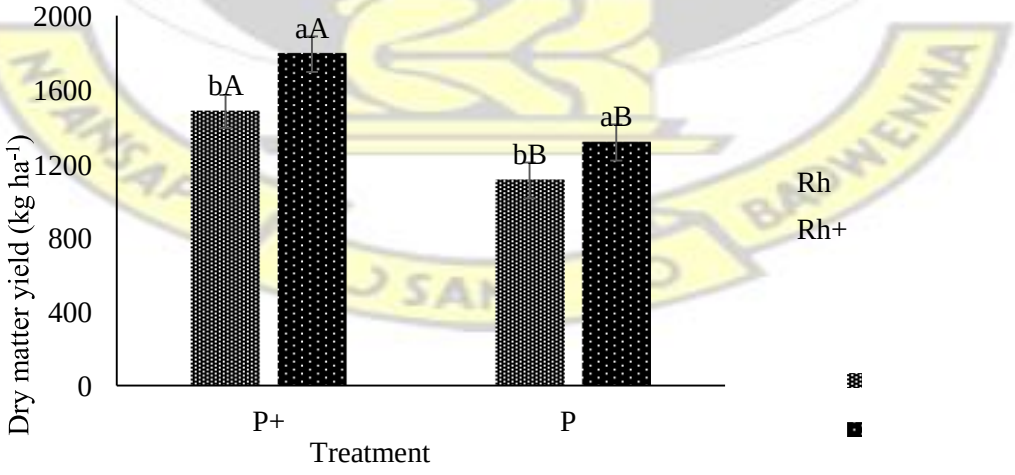


Figure 4.17. Effect of *Bradyrhizobium* inoculation and phosphorus application on shoot biomass yield of cowpea. Bars denote standard error of means. Bars followed

by different lowercase letters between (P+ and P-) and upper case between (Rh+ and Rh)- are different from each other at the 5% probability level.

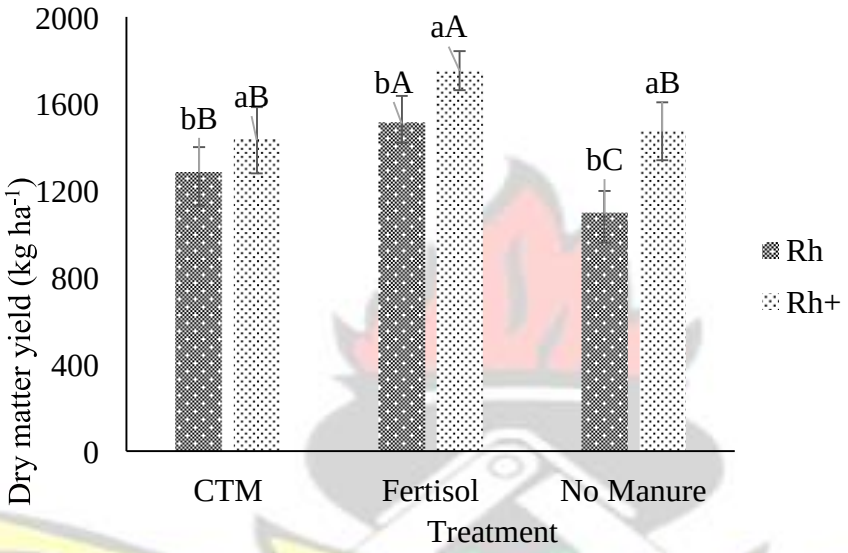


Figure 4.18. Effect of *Bradyrhizobium* inoculation and organic manure application on shoot biomass yield of cowpea. Bars denote standard error of means. Bars followed by different lowercase letters between (Rh+ and Rh-) within types of organic manure and different upper case letters between types of organic manure are different from each other at the 5% probability level. CTM=cattle manure

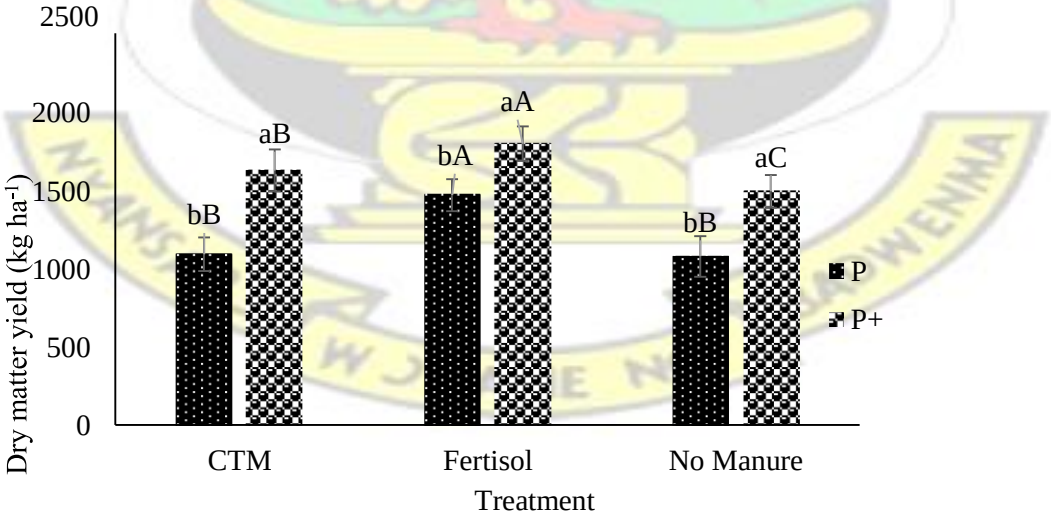


Figure 4.19. Effect of phosphorus and organic manure on shoot biomass yield of cowpea. Bars denote standard error of means. Bars followed by different lowercase letters between (P+ and P-) within types of organic manure and different upper

case letters between types of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

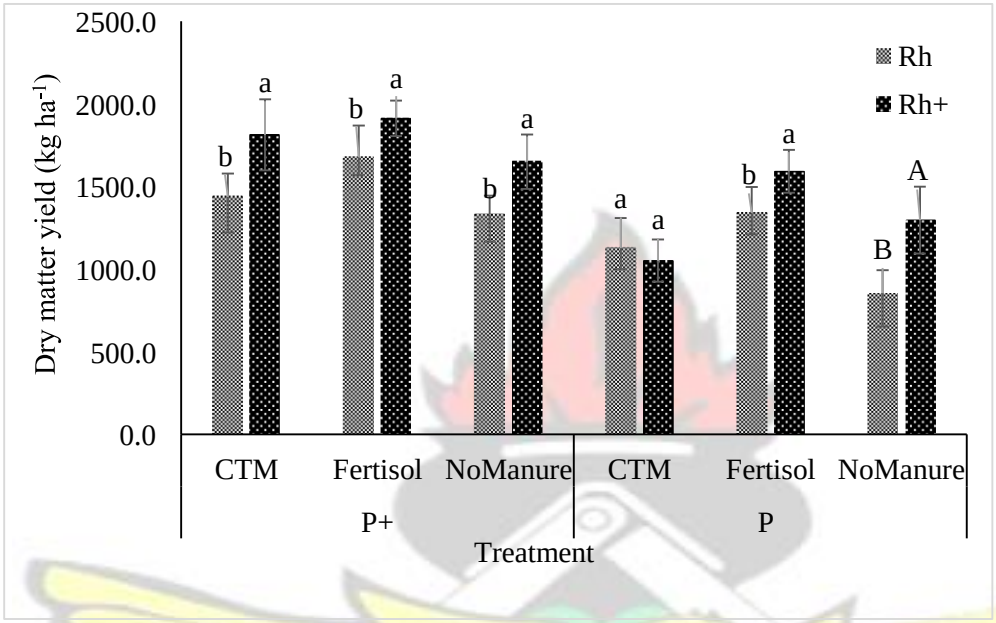


Figure 4.20. Effect of *Bradyrhizobium* inoculation, phosphorus and organic manure application on shoot biomass yield of cowpea. Bars denote standard error of means. Bars followed by different letters, comparing (Rh+ and Rh-) within each (P+ and P-) and type of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

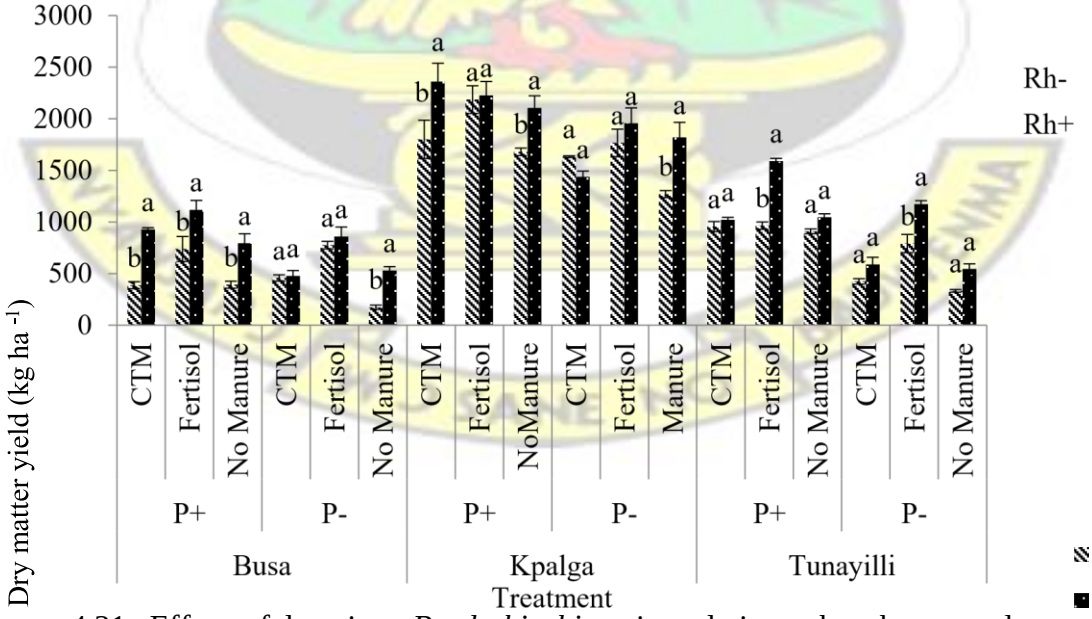


Figure 4.21. Effect of location, *Bradyrhizobium* inoculation, phosphorus and organic manure on shoot biomass of cowpea. Bars denote standard error of means. Bars followed by different letters, comparing (Rh+ and Rh-) within the same

location, levels of P and type of organic manure are different from each other at the 5% probability level. CTM=cattle manure

4.3.4. Grain yield

There were significant ($p < 0.05$) differences in grain yield for the two (Figure 4.22 – 4.24) and three (Figure 4.25) way interactions. Phosphorus and organic manure improved cowpea response to rhizobia inoculation. A grain yield of 1055 kg ha^{-1} was obtained when P was applied without *Bradyrhizobium* inoculant; however, the yield increased significantly to 1602 kg ha^{-1} with *Bradyrhizobium* inoculation (Figure 4.22). Similarly, the application of fertisoil and cattle manure without *Bradyrhizobium* inoculant, resulted in grain yields of 995 and 977 kg ha^{-1}, respectively. Addition of *Bradyrhizobium* inoculant to fertisoil and cattle manure resulted in additional grain yield increase of 495 and 295 kg ha^{-1}, respectively (Figure 4.23). The grain yield for the combined fertisoil and

Bradyrhizobium inoculant treatment was significantly higher than the grain yield of combined application of cattle manure and *Bradyrhizobium* inoculant (Figure 4.23). Combined application of phosphorus, fertisoil or and cattle manure significantly increased grain yield from 943 and 842 kg ha^{-1} to 1543 and 1480 kg ha^{-1}, respectively (Figure 4.24). Applying P to fertisoil significantly increased grain yield by 135 kg ha^{-1} over the combined application of P and cattle manure (Figure 4.24).

The combined application of fertisoil, P and *Bradyrhizobium* inoculant resulted in a significant increase of 1427 kg ha^{-1} over the control (Figure 4.25). Similarly, the combined application of cattle manure, P and *Bradyrhizobium* inoculant elicited an increase of 1278 kg ha^{-1} over the control (Figure 4.25). Applying P, fertisoil and *Bradyrhizobium* inoculant resulted in an increased grain yield of 148 kg ha^{-1}, which was higher than P, cattle manure and *Bradyrhizobium* inoculant (Figure 4.25). An increase in grain yield of 634 and 485 kg ha^{-1} resulted from applying P, fertisoil or and cattle manure with *Bradyrhizobium* inoculant

over the amendment without *Bradyrhizobium* inoculant (Figure 4.25). In almost all the cases, treatments involving *Bradyrhizobium* performed better than treatments without *Bradyrhizobium*. Unlike shoot biomass, no significant

(p=0.093) difference between location and treatments was observed for grain yield (Figure 4.26).

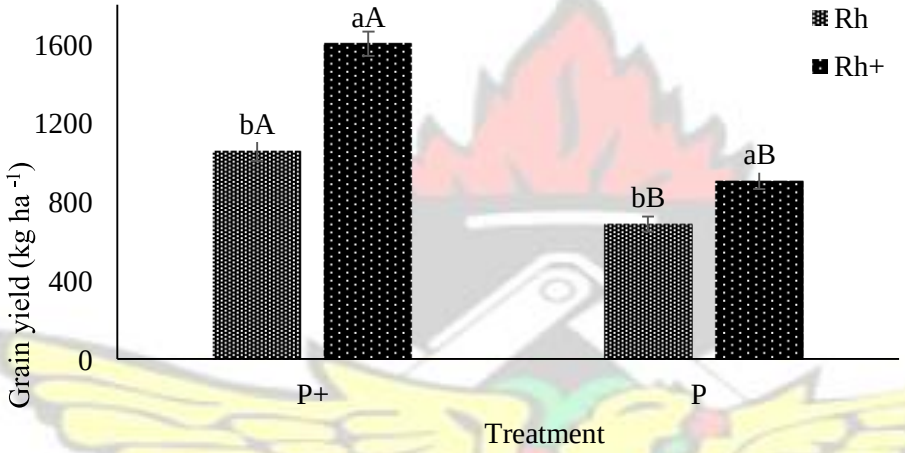
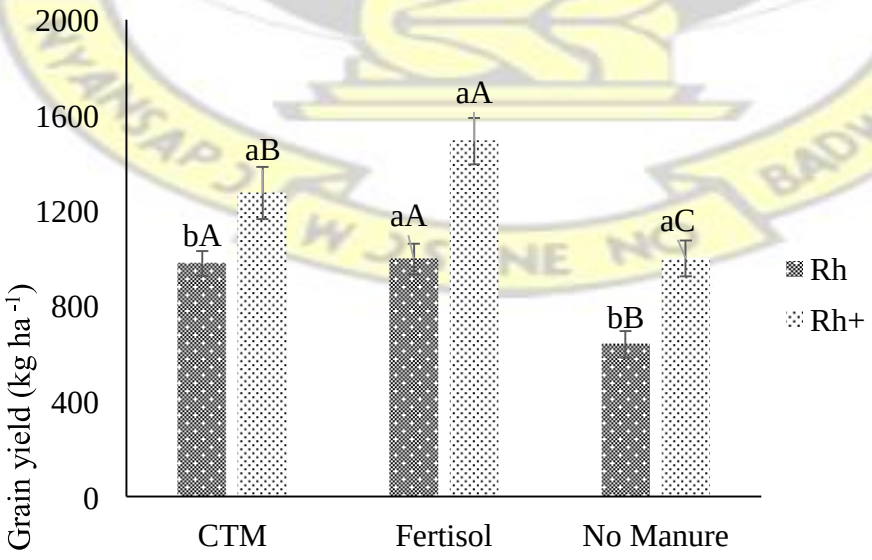


Figure 4.22. Effect of *Bradyrhizobium* inoculation and phosphorus application on grain yield of cowpea. Bars denote standard error of means. Bars followed by different lowercase letters between (P+ and P-) and different upper case letters between (Rh+ and Rh-) are different from each other at the 5% probability level.



Treatment

Figure 4.23. Effect of *Bradyrhizobium* inoculation and organic manure application on grain yield of cowpea. Bars denote standard error of means. Bars followed by different lowercase letters between (Rh+ and Rh-) within types of organic manure and upper case between type of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

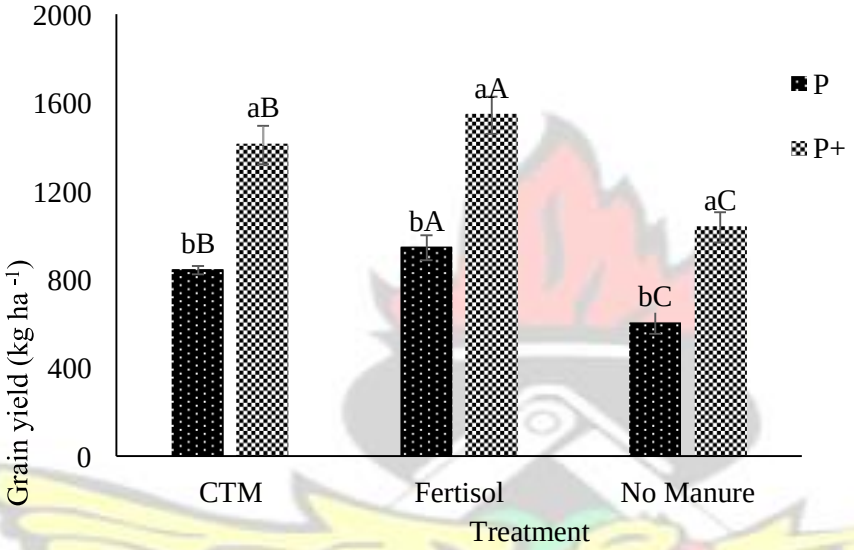


Figure 4.24. Effect of phosphorus and organic manure application on grain yield of cowpea. Bars denote standard error of means. Bars followed by different lowercase letters between (P+ and P-) within types of organic manure and upper case between type of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

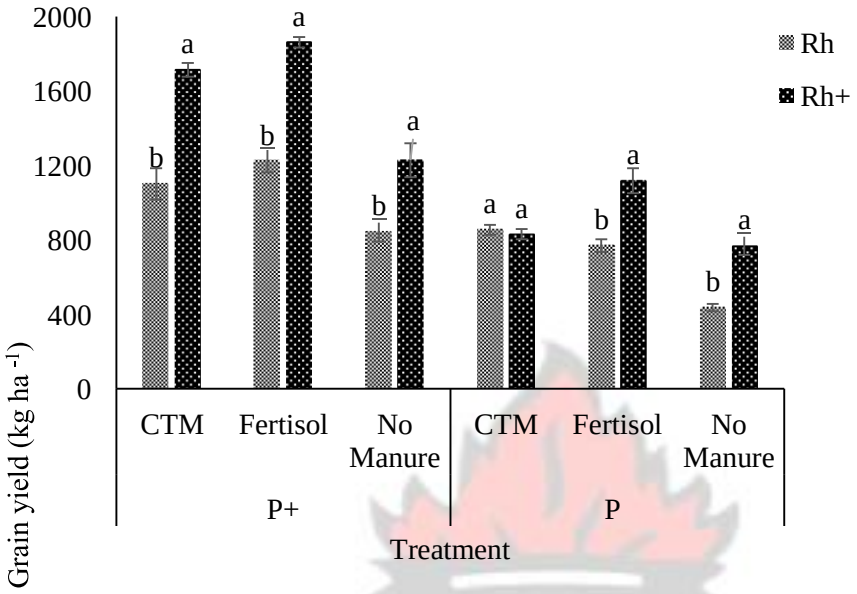


Figure 4.25. Effect of *Bradyrhizobium* inoculation, phosphorus and organic manure application on grain yield of cowpea. Bars denote standard error of means.

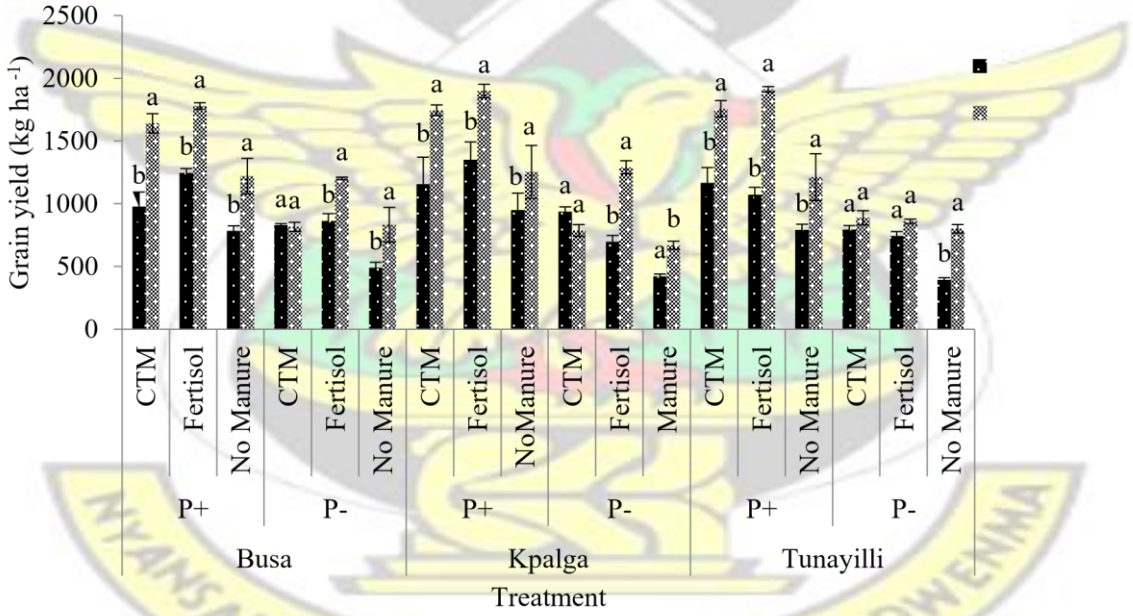


Figure 4.26. Effect of location, *Bradyrhizobium* inoculation, phosphorus and organic Bars followed by different letters, comparing (Rh+ and Rh-) within each (P+ and P-) and type of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

RhRh+

manure on grain yield of cowpea. Bars denote standard error of means. Bars followed by different letters, comparing within the same location, levels of P and type of organic manure are different from each other at the 5% probability level. CTM=cattle manure

The application of *Bradyrhizobium* inoculant, phosphorus and organic manure produced a positive added benefit in grain yield of cowpea. The combined application of *Bradyrhizobium* inoculant, phosphorus and fertisoil resulted in added benefit of 85.23 kg ha^{-1} grains of cowpea whiles the combined application of *Bradyrhizobium* inoculant, phosphorus and cattle manure had added grain yield benefit of 55.81 kg ha^{-1}.

4.3.5. Agronomic P use efficiency

Unlike the grain yield, not all the interaction levels were significant for P use efficiency. There was no significant (p = 0.0113) interaction between *Bradyrhizobium* inoculant and phosphorus (Figure 4.27); however, significant (p = 0.005) interactions between

Bradyrhizobium inoculant and organic manure (Figure 4.28), and phosphorus and organic manure (Figure 4.29) were observed. Adding fertisoil or cattle manure to *Bradyrhizobium* inoculated plots increased P use efficiency from 21 to 41 kg grain kg^{-1} P and from 32 to 33 kg grain kg^{-1} P, respectively (Figure 4.28). Application of fertisoil and *Bradyrhizobium* inoculant resulted in an increase in P use efficiency of 24% over the application of cattle manure and *Bradyrhizobium* (Figure 4.28). In contrast, a reduction in P use efficiency from 37 k to 25 kg grain kg^{-1} P and 38 to 26 kg grain kg^{-1} P was obtained when P was applied together with fertisoil or cattle manure, respectively (Figure 4.29). There was no significant interaction between P and fertisoil; and P and cattle manure. The three-way interaction between *Bradyrhizobium* inoculant, phosphorus and organic manure was significant (p = 0.0027) (Figure 4.30). The combined effect of fertisoil, P and *Bradyrhizobium* inoculant resulted in P use efficiency of 33 kg grain kg^{-1} P over the control whereas that of the combined application of cattle manure, P and *Bradyrhizobium* inoculant resulted in an increase of 32 kg grain kg^{-1} P over the control (Figure 4.30). However, when fertisoil and *Bradyrhizobium* inoculant were applied without P, the P use efficiency was 17 kg grain kg^{-1} P more than when the two were applied with P respectively (Figure 4.30). Significant (p=0.048) differences were observed for agronomic P use efficiency between locations and treatments (Figure 4.31).

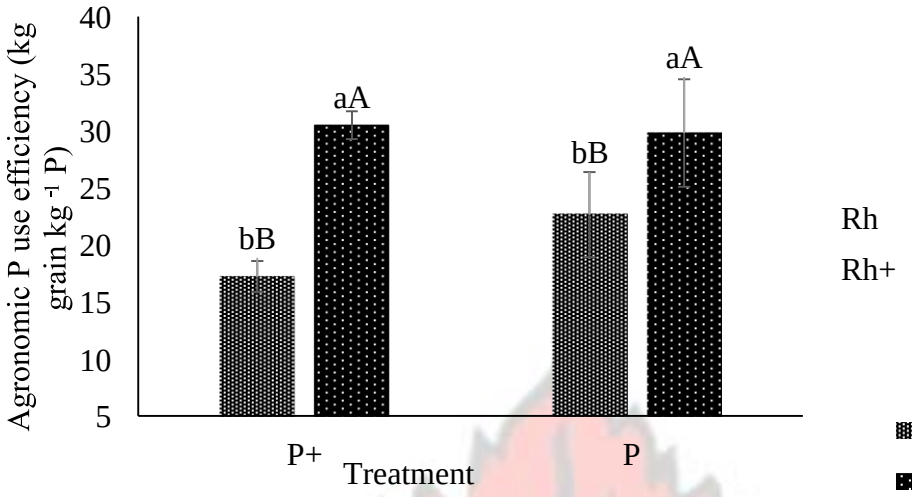


Figure 4.27. Effect of *Bradyrhizobium* inoculation and phosphorus application on agronomic P use efficiency of cowpea. Bars denote standard error of means. Bars followed by different lowercase letters between (P+ and P-) and different upper case letters between (Rh+ and Rh-) are different from each other at the 5% probability level.

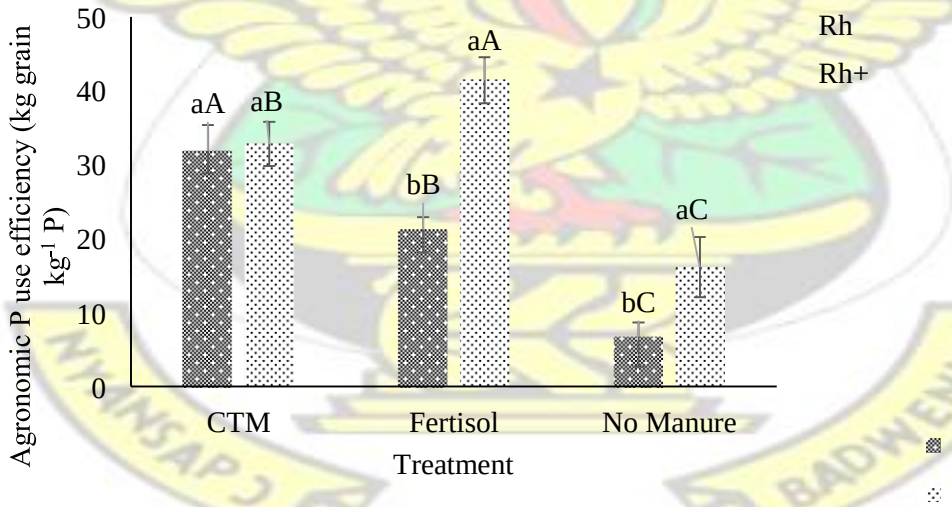


Figure 4.28. Effect of *Bradyrhizobium* inoculation and organic manure application on agronomic P use efficiency of cowpea. Bars denote standard error of means. Bars followed by different lowercase letters between (Rh+ and Rh-) within types of organic manure and upper case between type of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

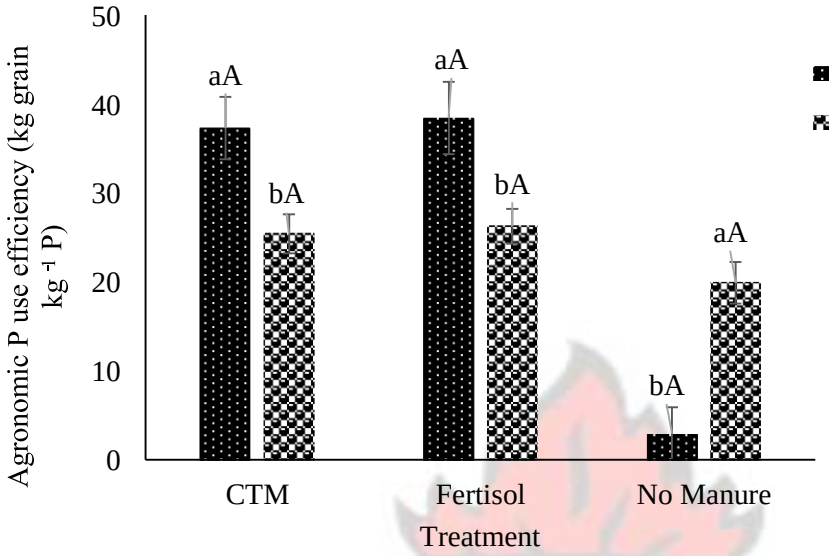


Figure 4.29. Effect of Phosphorus and organic manure application on agronomic P use efficiency of cowpea. Bars denote standard error of means. Bars followed by different lowercase letters between (P+ and P-) within types of organic manure and upper case between type of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

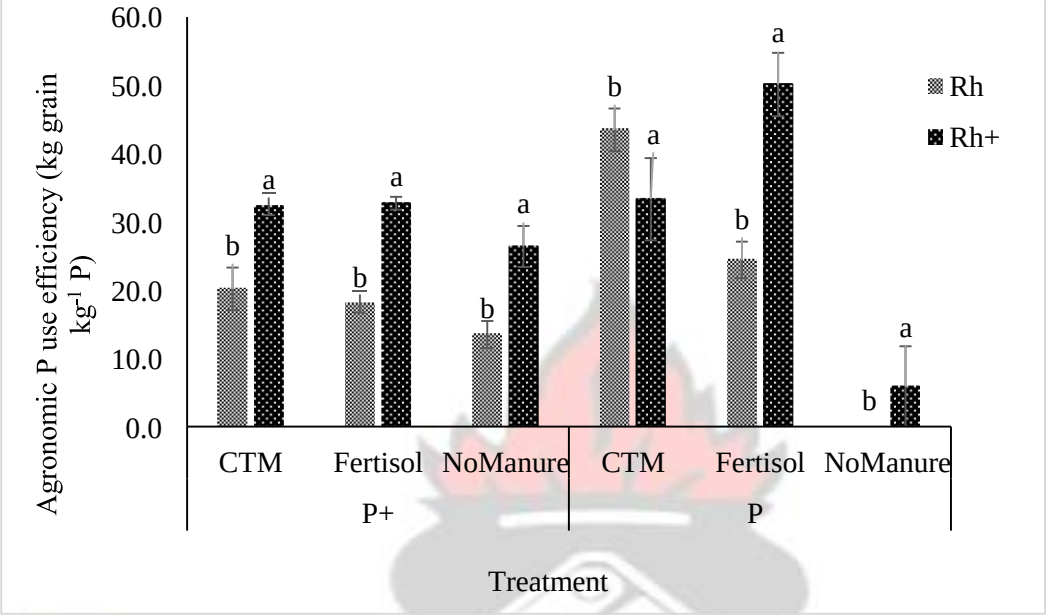


Figure 4.30. Effect of *Bradyrhizobium* inoculation, phosphorus and organic manure application on agronomic P use efficiency of cowpea. Bars denote standard error of means. Bars followed by different letters, comparing (Rh+ and Rh-) within each (P+ and P-) and type of organic manure are different from each other at the 5% probability level. CTM=cattle manure.

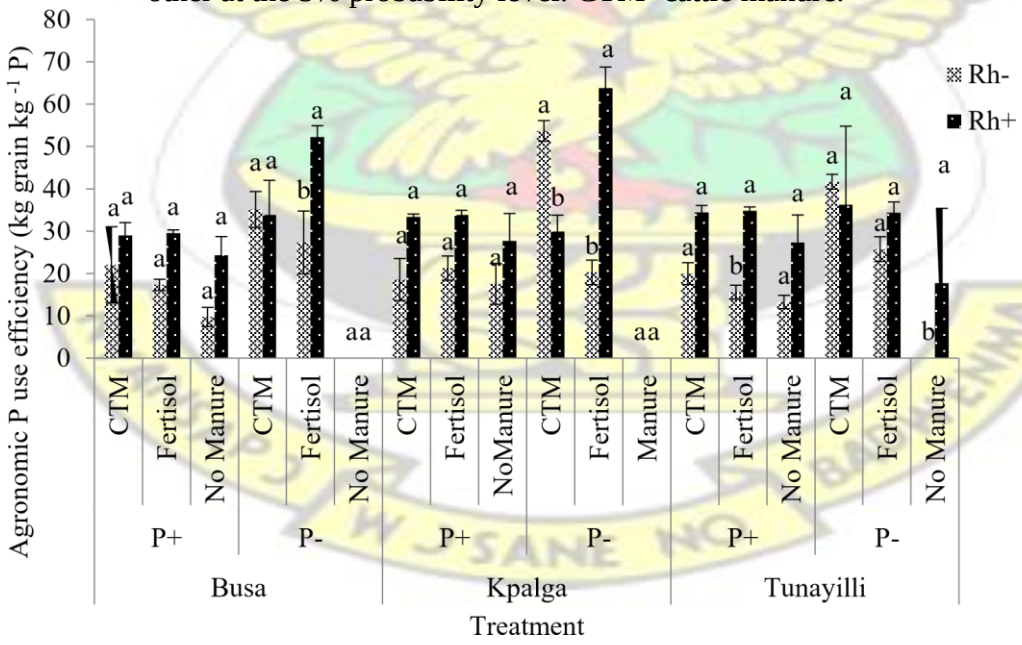


Figure 4.31. Effect of location, *Bradyrhizobium* inoculation, phosphorus and organic manure on agronomic P use of cowpea. Bars denote standard error of means. Bars followed by different letters within the same location, levels of P and type of organic manure are different from each other at the 5% probability level. CTM=cattle manure

4.3.6. Value cost ratio

The value cost ratios for the combined application of fertisoil, P and *Bradyrhizobium* inoculant was 2 while combined application of cattle manure, P and *Bradyrhizobium* inoculant resulted in a VCR of 1.7 (Figure 4.32). Except for the combined application of *Bradyrhizobium* and phosphorus, which was highly profitable, adding *Bradyrhizobium* inoculant to fertisoil or cattle manure only resulted in break-even situation (Figure 4.32). Addition of phosphorus to either fertisoil or cattle manure also resulted in VCRs of 1.5 and 1.3, respectively.

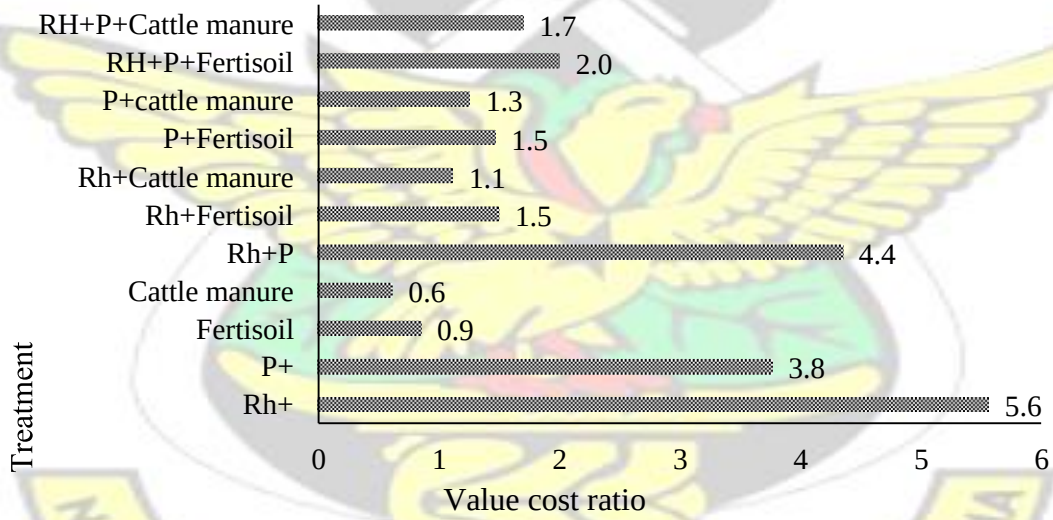


Figure 4.32. Value cost ratio of using *Bradyrhizobium* inoculant, phosphorus and organic manure in cowpea production.

4.3.7. Stepwise regression between grain yield, nodulation, P use efficiency and dry matter yield.

Stepwise regression results showed that all the growth parameters contributed significantly to grain yield (Table 4.15). Together, the growth parameters explained 67 % of the variation in grain yield in this study. The general regression equation is given as grain yield

($kgha^{-1}$) = - 40 + 0.27 dry matter yield + 6.94 P use eff. + 5.19 nodule number + 0.39 nodule dry weight. The stepwise regression showed that increase in dry matter yield, P use efficiency, nodule number and nodule dry weight would lead to significant increase in cowpea grain yield.

Table 4.15. Stepwise regression between grain yield, nodulation, P use efficiency and dry matter yield.

Term	Coefficient	Standard error of coefficient	T-value	P-Value	VIF
Constant	-40	110	-0.36	0.72	
Dry matter yield	0.27	0.062	4.43	0.000	1.21
P use efficiency	6.94	2.07	3.36	0.001	1.45
Nodule number	5.19	2.04	2.54	0.013	1.18
Nodule dry weight	0.39	0.091	4.36	0.000	1.43

VIF = Variance Inflation Factor. VIF = 1 (Not correlated). 1 < VIF < 5 (Moderately correlated. VIF >5 to 10 (Highly correlated)

4.3.8. Decomposition and nutrient release patterns of fertisoil and cattle manure

Decomposition was rapid in the first two weeks for fertisoil and cattle manure (Figure 4.33). The decomposition proceeded slowly up to the 6th week for fertisoil and 4th week for cattle manure. At the end of the tenth week, nearly 68% of the mass of fertisoil and 57% of the cattle manure had disappeared. The decomposition constants were 0.094 and 0.074 for fertisoil and cattle manure, respectively (Figure 4.33).

There was no immobilization effect for all the nutrients measured (Figures 4.34 and 4.35). For fertisoil, peak mineralization of C was attained at the 6th week. The N released increased from week 2 up to week 8 where peak mineralization was attained while phosphorus and K attained peak mineralization on the tenth week (Figure 4.34). Similar to the trend observed under fertisoil, peak mineralization of C from cattle manure was attained at week 6. All the other nutrients (N, P and K) measured attained peak mineralization at week ten (Figure 4.35).

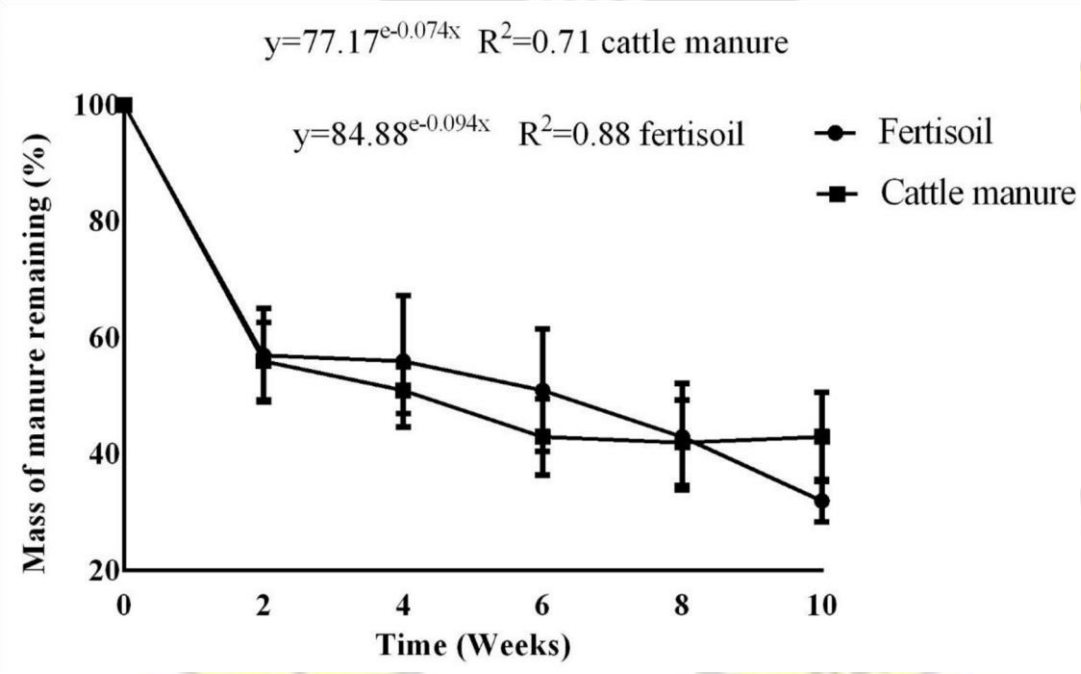


Figure 4.33. Decomposition of fertisoil and cattle manure under field conditions. Bars denote standard error of mean.

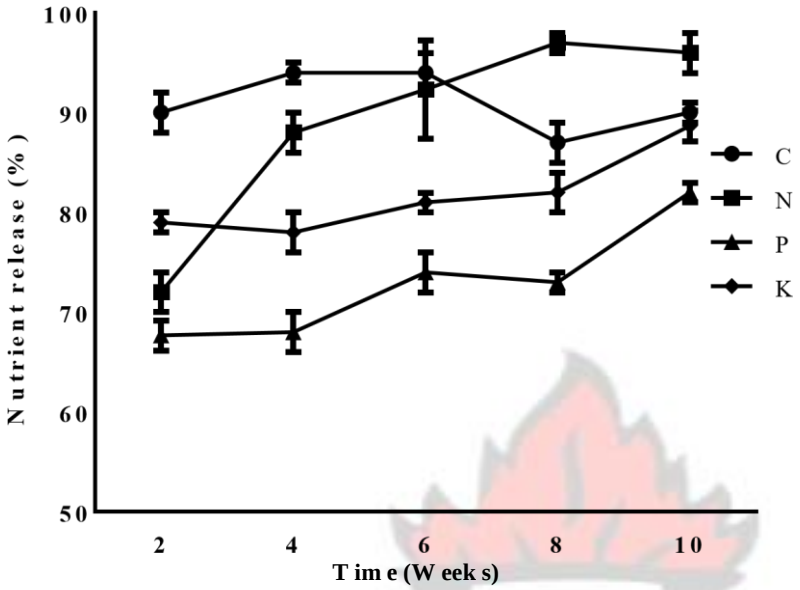


Figure 4.34**.** Carbon, nitrogen, phosphorus and potassium release patterns of fertisoil under field conditions. Bars denote standard error of mean.

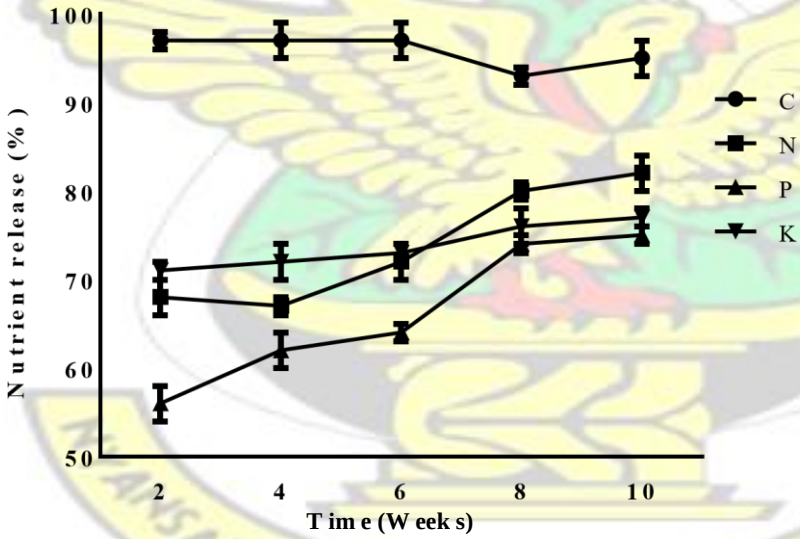


Figure 4.35**.** Carbon, nitrogen, phosphorus and potassium release patterns of cattle manure under field conditions. Bars denote standard error of mean.

Except P, there were significant differences between the other nutrients released by fertisoil and cattle manure (Appendix 2). There was significant difference between the amount of carbon released by cattle manure (Mean (M) = 95, standard deviation (SD) = 1.8) and that of fertisoil (M = 91, SD =3.0); t = 3.07, p = 0.022. Likewise, the amount of nitrogen

released by fertisoil (M =88.4, SD = 10) was significantly different from the nitrogen released by cattle manure (M = 73.8, SD = 6.87); t = 2.69, p = 0.031. Furthermore, there was significant difference between the amount of potassium released by fertisoil (M = 81, SD = 4.32) and that of cattle manure (M = 74, SD = 2.92); t = 3.34, p = 0.012.

4.4. Study 4. On – farm evaluation of soybean response to *Bradyrhizobium* inoculation and / or phosphate fertilizer.

4.4.1. Soil chemical and physical properties

The ratings for the soil chemical and physical properties were done according to the classification by Landon (2014). In the Northern region, organic carbon values recorded were very low (Table 4.16). Similarly, available P was low with little variation across the different locations. The total nitrogen contents of the study sites were largely very low. The total N concentration values ranged from 0.03 – 0.13 % across locations. Thirty three percent (33%) of the study locations in Northern region had low nitrogen content while 67% had very low nitrogen content. The exchangeable potassium was also very low. The values for exchangeable calcium ranged from low (2.8) to medium (11.44) with variations between some of the locations. The values obtained for exchangeable magnesium were between medium (0.30) and very high (4.06) with variations between locations. The soil had relatively large amounts of silt and low amounts of sand and clay. The pH ranged from medium (5.60) to high (6.99) (Table 4.16).

Table 4.16. Soil physical and chemical properties in Northern region (N=85)

Soil parameters	Median	Minimum	Maximum
pH(1:2.5) (H_2O)	6.19	5.60	6.99

Total N (%)	0.084	0.03	0.13
Available P (mg kg^{-1})	5.69	5.20	12.70
Exchangeable K ($cmol_{(+)}$ kg^{-1})	0.02	0.01	0.05
Organic C (%)	0.86	0.32	1.52
Exchangeable Ca ($cmol_{(+)}$ kg^{-1})	4.72	2.08	11.44
Exchangeable Mg ($cmol_{(+)}$ kg^{-1})	1.86	0.30	4.06
Sand (%)	21.64	5.40	50.00
Clay (%)	6.88	2.96	10.52
Silt (%)	71.96	45.04	86.08

In the Upper West Region, there was little variation between locations in respect of organic carbon (Table 4.17). The organic carbon was very low across locations with median of 0.64%. However, the available phosphorus ranged from low to medium (Table 4.17). In the Upper West region, only 5% of the 20 locations had low nitrogen content with the remaining locations having very low nitrogen. The exchangeable magnesium were generally high in half (50 %) of the locations; thirty percent (30 %) of the locations had medium exchangeable magnesium and the remaining 20 % had low amount of exchangeable magnesium. Majority of the locations had low exchangeable calcium; twenty – five percent (25%) of the locations had medium amount of exchangeable calcium. The soils had relatively high sand (76 %) and low clay content (4.7%). The exchangeable potassium recorded in the study locations were described as very low

(Table 4.17). The pH ranged from medium (5.64) to high (7.56) (Table 4.17).

Table 4.17. Soil physical and chemical properties at Upper West region (N=20)

Parameters	Median	Minimum	Maximum
pH(1:2.5) (H_2O)	6.34	5.64	7.56
Total N (%)	0.058	0.038	0.11
Available P (mg kg^{-1})	7.09	6.040	9.90
Exchangeable K($cmol_{(+)}$ kg^{-1})	0.012	0.005	0.029
Organic C (%)	0.64	0.40	1.22
Exchangeable Ca ($cmol_{(+)}$ kg^{-1})	2.51	1.62	5.66
Exchangeable Mg ($cmol_{(+)}$ kg^{-1})	0.76	0.16	2.24
Sand (%)	76.02	47.64	87.6
Silt (%)	19.48	9.28	47.28
Clay (%)	4.72	3.08	8.36

4.4.2. Indigenous rhizobia populations in the study locations

Considerable variation existed between locations in each region and between regions in indigenous rhizobia populations. The indigenous populations were relatively higher in soils in the Northern region than in Upper West region (Table 4.18).

There were significant differences among the indigenous rhizobia populations across the various locations in Northern region. The indigenous populations (varied depending on the location) ranged from as low as 11.4 to 1464 rhizobia cells g^{-1} soil. More than 50% of the locations had rhizobia numbers less than 100 cells g^{-1} soil. Within the 50%, more than half-recorded numbers less than 50 cells g^{-1} soil. The median rhizobia population in the Northern region soils was 57.1 cells g^{-1} soil (Table 4.18).

Similarly, in the Upper West region, there was significant variation among the indigenous rhizobia populations between the locations. The highest indigenous population recorded was 287 cells g^{-1} soil and the lowest was 1.1 cells g^{-1} soil. The indigenous rhizobia populations of 50% of the locations were above 100 cells g^{-1} soil and 45% had indigenous population to be less than 50 cells g^{-1} soil. The median rhizobia population in the Upper West region soils was 91.7 cells g^{-1} soil (Table 4.18).

Table 4.18. Indigenous rhizobia population (cells g^{-1} soil) of the study locations

Location		Median	Minimum	Maximum
Northern Region (N=69)	57.10	11.40	1464.90	
Upper West Region (N=20)	91.70	1.10	287.10	

4.4.3. Rainfall pattern during the cropping season at the study locations

In the Upper West region, there was rainfall after planting until day 30. Thereafter, the rainfall seldom reached 20 mm per day, culminating into dry spells just before and after flowering. In addition, there were short dry spells after flowering that continued until harvesting (Figure 4.36).

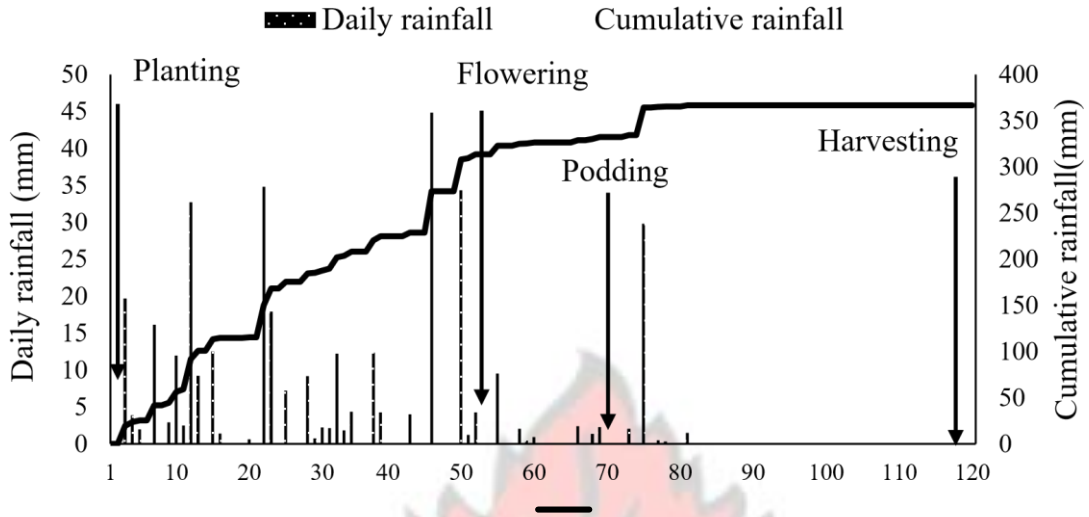


Figure 4.36. Rainfall distribution during the cropping season in the Upper West region In the Northern region, there was a dry spell from day 10 - 40 days after planting.

Thereafter, there was adequate rainfall until flowering with short dry spells up to podding.

The total rainfall received at the Northern region was higher than that of Upper West region

(Figure 4.37).

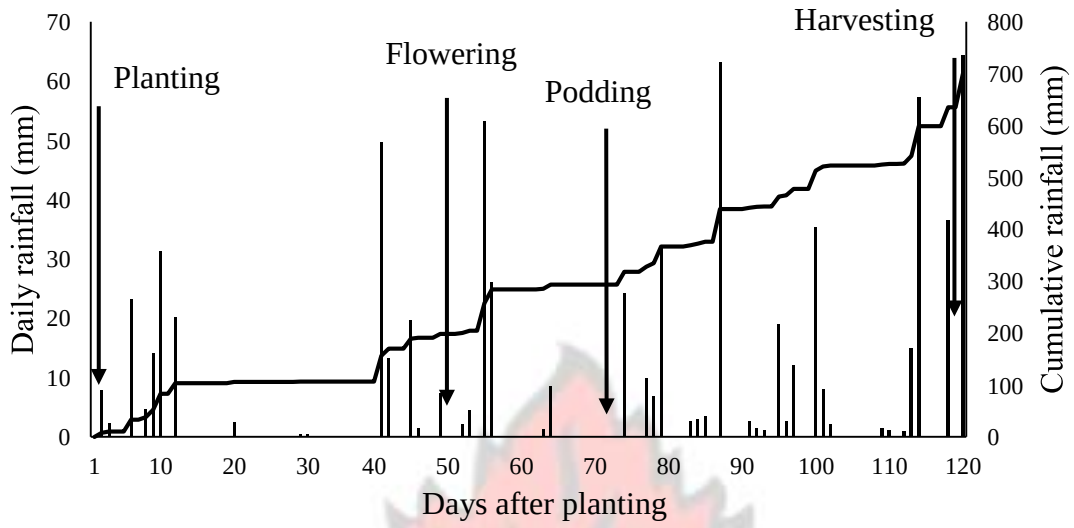


Figure 4.37. Rainfall distribution during the cropping season in the Northern region.

4.4.4. Soybean grain yields

The average grain yield from plots that received P and or inoculant (I) were significantly ($p < 0.0001$) higher than the control plots at the Northern region locations (Table 4.19). Phosphorus and inoculation effects resulted in 18 and 24% increase in grain yield over the control, respectively in the Northern region. The treatment, I+P however recorded the highest grain yield of 1371 kg ha^{-1} (Table 4.19).

Unlike, the study locations in Northern region, the grain yields recorded at Upper West region were low; but variations between treatments were also significant ($p = 0.0003$) (Table 4.19). There were significant differences between control plots and plots that received P only and P + I but not with plots that received inoculant (I) only. Plots that received I + P also produced the highest grain yield in Upper West region (Table 4.19).

Table 4.19. Average soybean grain yields in Northern and Upper West regions.

Treatment	Northern region	Upper West region
		kg ha^{-1}
Control	998.41±44.6* c†	213.04±15.8 b*†
TSP (P)	1177.00±8.3 b	263.53±9.3 a
Inoculant (I)	1237.52±2.9 ab	236.67±5.3 ab
Inoculant plus TSP (I+P)	1370.75±9.7 a	271.86±4.8 a
P-value	< 0.0001	0.0003

†Within column, means followed by same letters are not different at 0.05 probability level.
* represent standard error of the mean.

4.4.5. Distribution of soybean response to TSP and *Bradyrhizobium* inoculation in the Northern region

The absolute response (treatments yield minus control yield) and relative response (treatments yield minus control yield divided by the control yield and multiplied by 100) are presented in Figure 4.38 and 4.39. In absolute terms, 81, 83 and 81% of the locations had a positive response to P, inoculant (I) and I+P, respectively, in relation to the control in Northern region (Figure 4.38). Forty four percent of the farmers increased their grain yields in absolute terms by about 200 kg ha^{-1} or more with phosphorus only (P). In absolute terms, 56% of the farmers increased their grain yields by 200 kg ha^{-1} or more with inoculant only (I). Sixty – two percent of the farmers had absolute increase in grain yield by 220 kg ha^{-1} or more with inoculant and phosphorus combined (I + P). Gains of

1000 kg ha^{-1} grain yield or more were achieved by 2% of the locations that received I and 4% with locations that received I+P. None of the locations that received P only had yield gains of 1000 kg ha^{-1} or more (Figure 4.38). The probability of achieving a negative

response due to the application of P, inoculant (I) and / or I+P were 18, 14 and 16%, respectively (Figure 4.38).

More than half of the locations recorded a relative increase in grain yield of 20% or more with P, 20% or more with inoculant (I) and 23% or more with I+P. Seven percent of the locations achieved over 100% relative increase in grain yield with P, 8% with inoculant (I) and 15% with I+P (Figure 4.39).

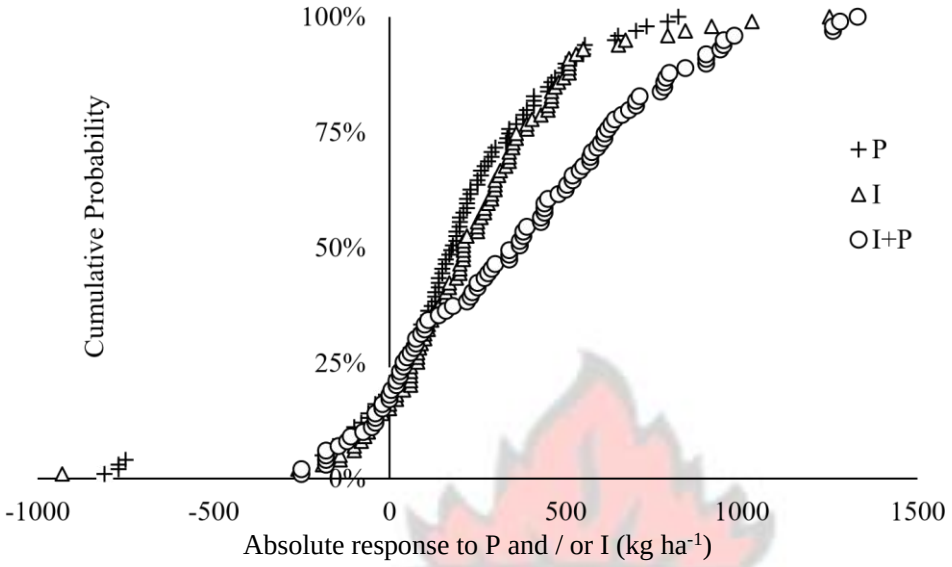


Figure 4.38. Cumulative probability of estimated absolute response of soybean grain yield in the Northern region.

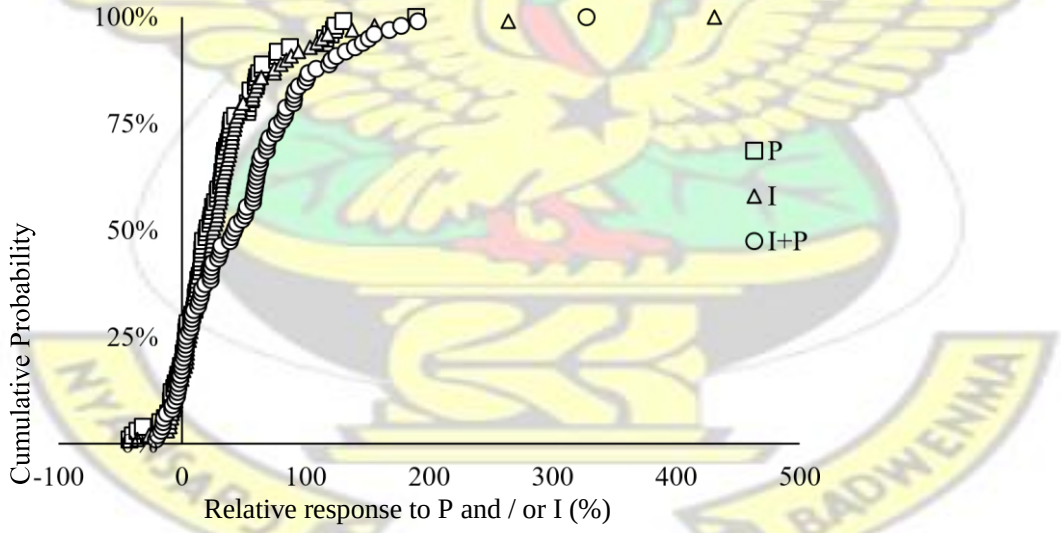


Figure 4.39. Cumulative probability of estimated relative response of soybean grain yield in the Northern region.

4.4.6. Distribution of soybean response to TSP and *Bradyrhizobium* inoculation in the Upper West region

The absolute response and relative response are presented in Figures 4.40 and 4.41. In absolute terms, 75, 76 and 86% of the locations had a positive response to P, inoculant

(I) and I+P, respectively, relative to the control in the Upper West region (Figure 4.40). Gains of 100 kg ha^{-1} or more were achieved by 22% of the locations that received P, 8% by the locations that received inoculant (I) and by 18% of the locations that received I+P. None of the locations had yield gain of 1000 kg ha^{-1} (Figure 4.40). The probability of achieving a negative response due to the application of P, inoculant (I) and / or I+P were 20%, 12% and 10%, respectively (Figure 4.40).

In terms of relative percentage increase in grain yield, half of the farmers increased their grain by 20% or more with P, 10% or more with inoculant (I) and 29% or more with I+P (Figure 4.41). On 4%, 12% and 14% of the locations, relative increase in yield of 100% or more was achieved with inoculation, P and I+P, respectively (Figure 4.41).

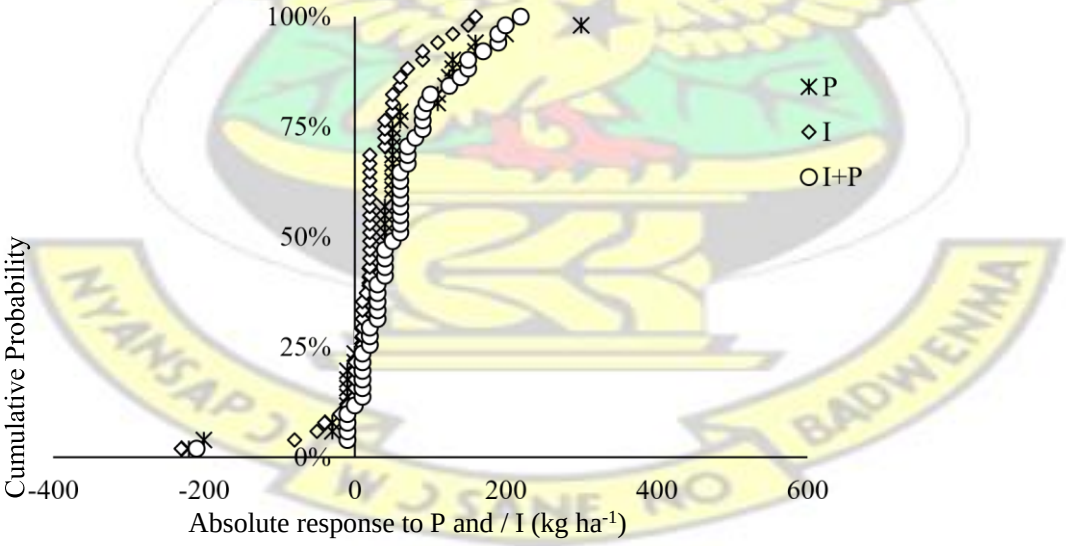


Figure 4.40. Cumulative probability of estimated absolute response of soybean grain yield in the Upper West region.

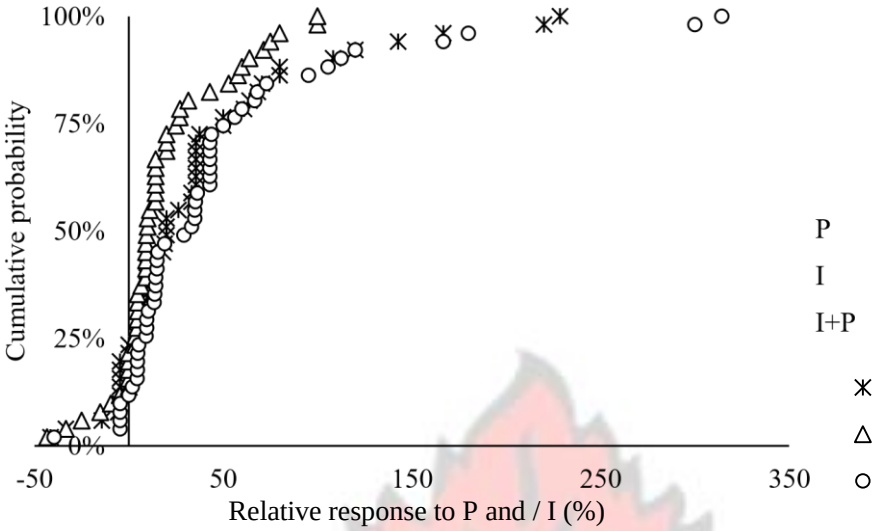


Figure 4.41. Cumulative probability of estimated relative response of soybean grain yield in the Upper West region.

4.4.7. Variability in soybean yield and response to P and / or inoculant application

Figures 4.42 and 4.43 show the performance of the treatments in the various locations. There was a wide variation in grain yield among the treatments and between locations (Figures 4.42 and 4.43). Grain yields in control plots ranged from 180 to 2560 kg ha^{-1} while that of the treated plots ranged from 250 to 3120 kg ha^{-1} in the Northern region (Figure 4.42). Except at Sheillianyilli, grain yields for all control plots at various the locations were below 2000 kg ha^{-1} (4.42).

Grain yields in control plots ranged from 50 to 600 kg ha^{-1} while those of the treatments ranged from 90 to 1000 kg ha^{-1} in the Upper West region (Figure 4.43). The lowest yield was recorded at Bawa with the control treatment whiles the highest yield was recorded at

Siriyiri with phosphorus application (Figure 4.43).

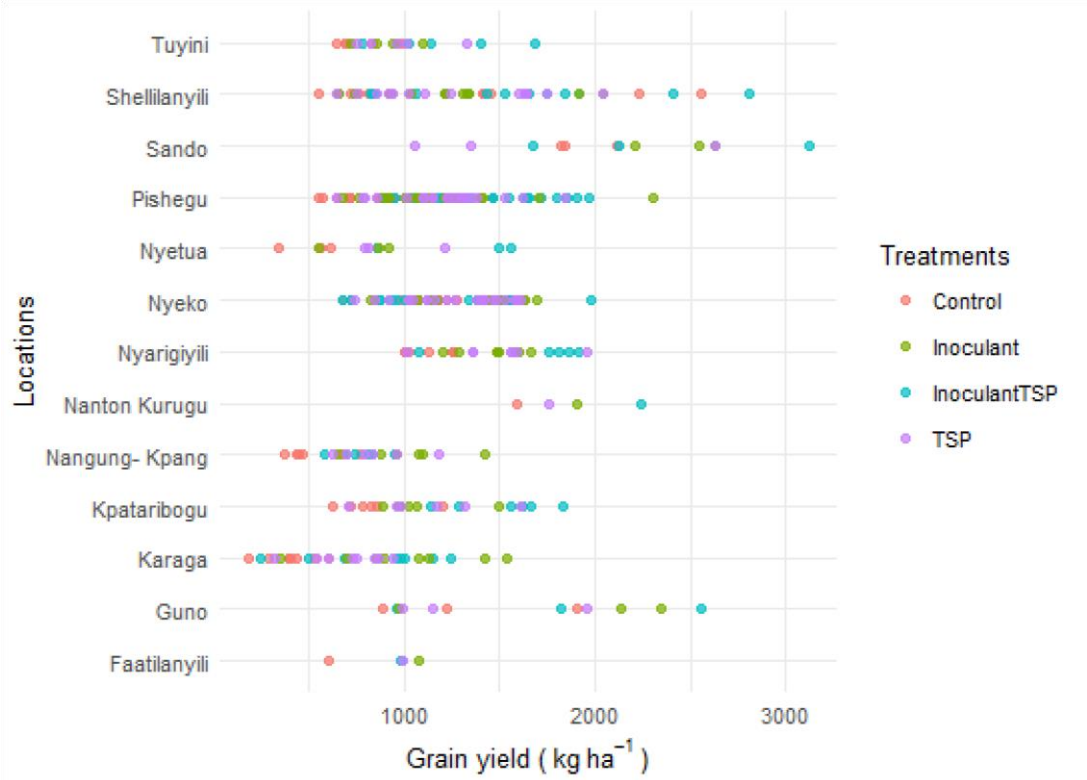


Figure 4.42. Variability in grain yield response to TSP and / or inoculants in the Northern region.

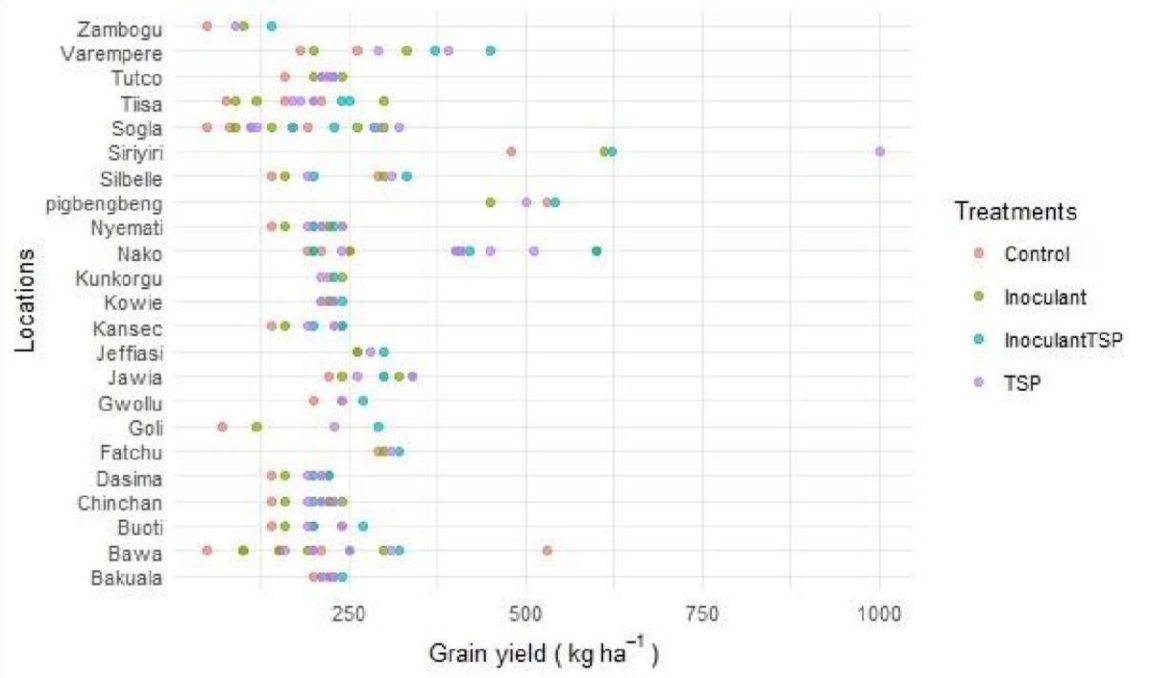


Figure 4.43. Variability in grain yield response to TSP and / or inoculant in the Upper West region.

4.4.8. Economic viability of using P and / or I in the Northern and Upper West regions

The use of inoculant and or P will only be attractive to farmers if the gross returns from the grain yield is equal to or greater than the total cost of applying inoculant and P. This procedure was also used to determine the responsive and non-responsive fields. The probability of achieving certain economic benefits which reflects the responsiveness and non-responsiveness to P and or inoculant (I) compared to the control is presented as probability distribution graph (Figures 4.44 and 4.45). In Figure 4.44, the VCR values due to the inoculant are more shifted to the far right than P and I+P indicating that inoculation would be more profitable. About 66 % of the farmers that applied P had gross returns equal to or greater than the cost of applying P. Out of the 66% farmers, 35% had

VCR of 1, 18% had VCR of 2, 9 % had VCR of 3 and 4% had VCR of 4 (Figure 4.44).

For inoculant use, 22 % of the farmers had VCR of 1, 24 % had VCR of 2, 15 % had VCR of 3 and 14 % had VCR ranging from 4 – 9 (Figure 4.44). For I+P, the ratios were much less for farmers that had VCR of 1 than that of P and I only. About 19 % of the farmers who applied I+P had VCR of I, 27 % had VCR of 2, 14 % had VCR of 3 and 4

% had VCR in the range of 4 – 5 (Figure 4.44).

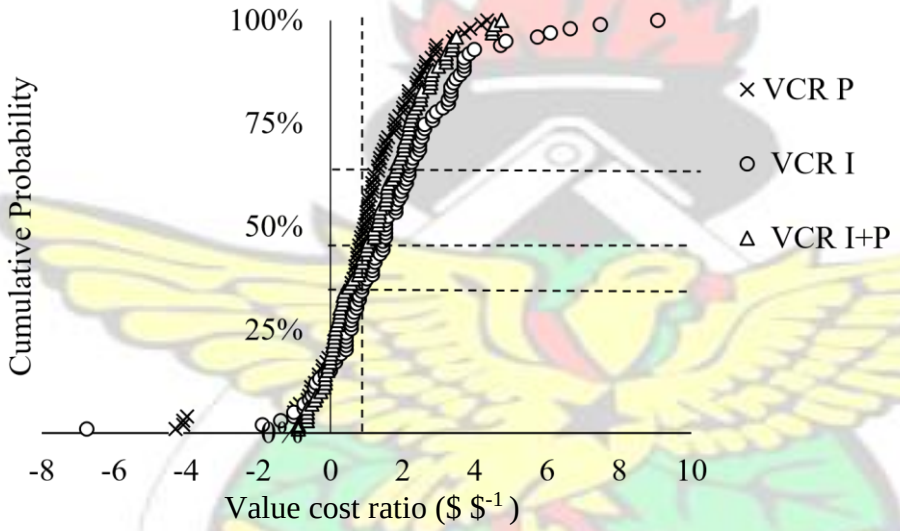


Figure 4.44. Cumulated probability of estimated value cost ratio of P and / or I in the Northern region. The cumulative probability (Y-axis) reflects the likelihood of obtaining a value larger than a given VCR (X-axis). Vertical line denotes VCR= 1 and horizontal lines intersect with the cumulative distribution curves for I, P and I+P in that order.

A large proportion of farmers in Upper West region recorded a VCR of zero (Figure 4.45). Twenty - two percent of the farmers who applied P had a VCR of one or more. Out of the 22%, only two percent had a VCR of 2 and 3 (Figure 4.45). Twenty-four percent of the farmers who used inoculant had a VCR of one or more. Out of the 24%, four percent had a VCR of two (Figure 4.45). Fourteen percent of the farmers who used inoculant with

phosphorus had a VCR of one. None of them had a VCR of two or more (Figure 4.45). The VCRs for inoculant and phosphorus were more shifted to the right than I+P indicating that inoculation and phosphorus were more profitable in Upper West region.

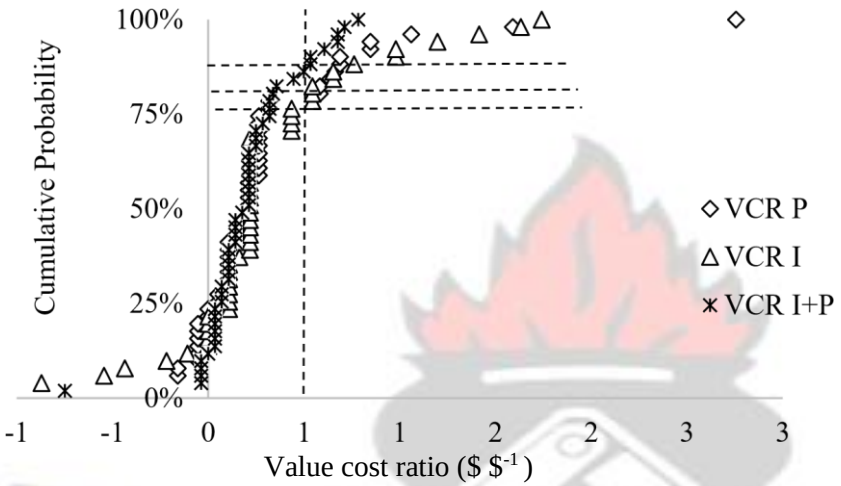


Figure 4.45. Cumulated probability of estimated value cost ratio of P and / or I the Upper West region. The cumulative probability (Y-axis) reflects the likelihood of obtaining a value larger than a given VCR (X-axis). Vertical line denotes VCR= 1 and horizontal lines intersect with the cumulative distribution curves for I, P and I+P in that order.

4.4.9. Responsive and non – responsive sites to P and / or inoculant application to soybean

There was wide variation in soybean response to P and / or I. Based on the agronomic approach described under section 3.11.8, seventeen percent (17%) of the locations within Northern Region were responsive to P, 21% responsive to inoculant (I) and 40% responsive to I+P (Figure 4.46 A). Majority of the trial locations were either non – responsive to P and or inoculation (I) (Figures 4.47 – 4.49). Seventeen (17%) percent of the locations within Upper West region were responsive to P and I+P (Figure 4.6 B). Only 6% the locations were responsive to inoculation (Figure 4.6 B).

If we consider, the economic approach, the picture changes as many sites become responsive. About 66% of the sites within the Northern region were responsive to P whiles 75 and 64% were responsive to inoculant and I+P, respectively (Figure 4.47A).

Within the Upper West region, only 22% of the locations were responsive to P, 24 and 14% were responsive to inoculant and I+P, respectively (Figure 4.47B).

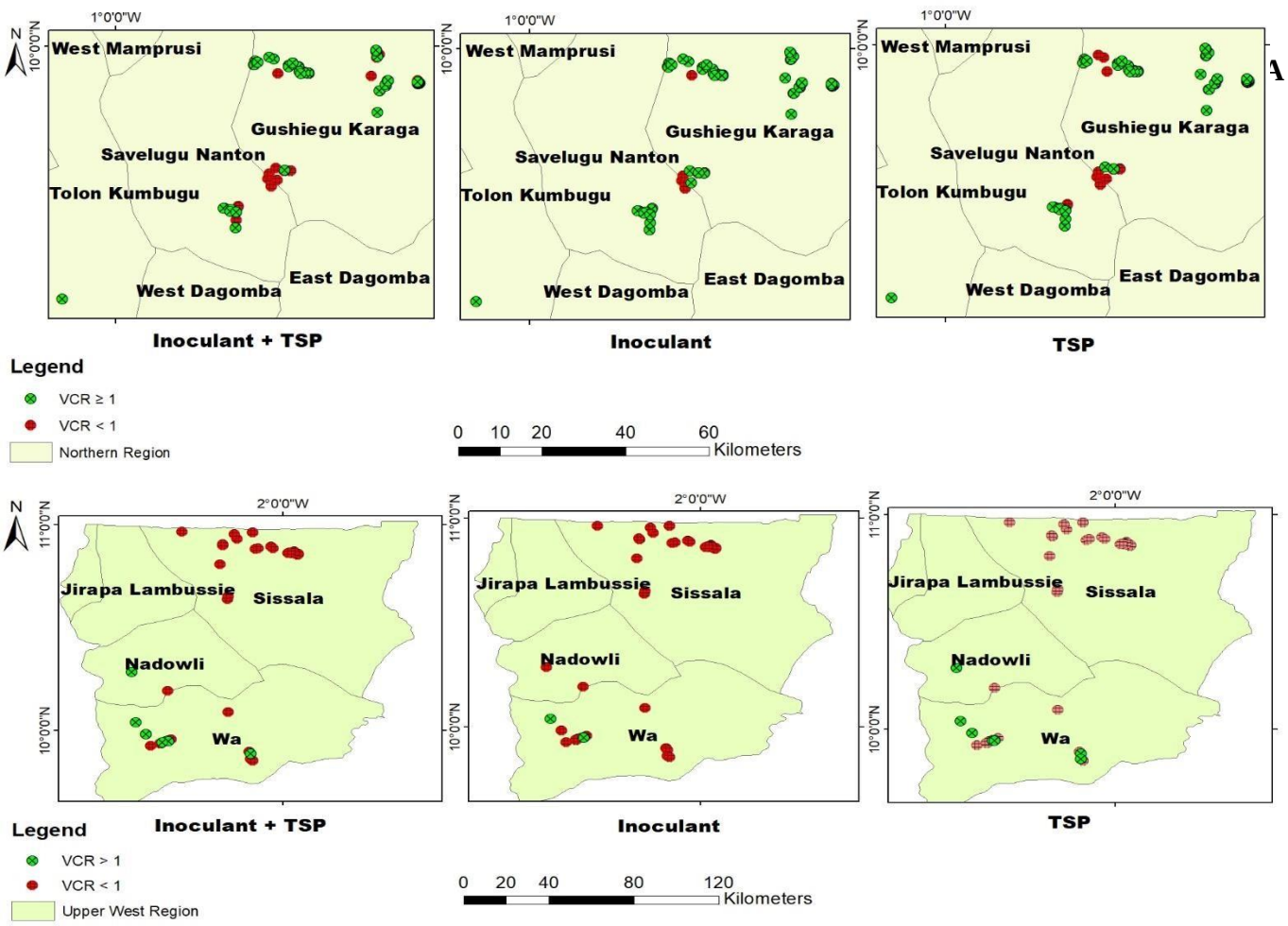


Figure 4.46. Soybean response to P and / or I in the Northern (A) and Upper West (B) regions

Figure 4.47. Value cost ratio for application of phosphorus and / or inoculant in the Northern (A) and Upper West (B) regions.

A

B

4.4.10. Understanding the variability in soybean yield and response to P and / or inoculant

It was observed under section 4.4.7 that there was a wide variation in the grain yield between the locations. This section attempts to explain such variations using the soil and environmental factors measured at the locations using linear model. Overall, the linear model explained 42% of the total variances in grain yield in the Northern region (Table 4.20). Soil factors such as nitrogen ($p = 0.004$) and phosphorus ($p = 0.045$) had positive significant effect on the grain yield. Cumulative rainfall ($p = 0.0056$) and soil types Dystric Plinthosols ($p = 5.04$ e-09) and Plinthic Lixisols ($p = 1.00$ e-05), however, had significant negative effect on grain yield. Furthermore, native rhizobia population ($p = 0.68$) had negative effect on grain yield, though it was not significant (Table 4.20).

The linear model explained 79% of the variance in grain yield observed in the Upper West region (Table 4.21). Soil nitrogen ($p = 0.0177$) and organic carbon ($p = 0.015$) had negative effect on grain yield. The effect of phosphorus ($p = 0.021$) and pH ($p = 0.011$) were significantly positive. Unlike the Northern Region, soil types (Ferric Lixisols ($p = 0.06$) and Leptosols ($p = 0.026$) had positive significant effect on grain yield. Again, native rhizobia had negative effect.

Table 4.20. Explanatory variables for variability in grain yield in selected locations of Northern region

Coefficients				
	Estimate	standard error	t value	Pr (>|t|)
(Intercept)	1.94E+05	6.93E+04	2.796	0.0059 **
Nitrogen	1.04E+03	2.86E+02	3.627	0.0004 ***
Organic carbon	3.50E+02	1.80E+02	1.95	0.05
Phosphorus	2.21E+02	1.09E+02	2.024	0.045 *
Potassium	-2.19E+04	6.78E+03	-3.226	0.0015 **
Calcium	-2.68E+00	2.13E+01	-0.126	0.90
Magnesium	-1.02E+02	5.63E+01	-1.81	0.07
Cumulative rainfall	-3.19E+02	1.13E+02	-2.809	0.0056 **
Native rhizobia	-1.18E-01	2.87E-01	-0.412	0.68
Active carbon	2.60E-01	2.02E-01	1.287	0.20
pH	1.97E+02	1.42E+02	1.391	0.17
% Sand	-2.62E+00	5.83E+00	-0.448	0.65
% Clay	1.11E+01	2.49E+01	0.444	0.66
% Silt	-1.20E+02	3.09E+02	-0.388	0.70
Texture_silt loam	1.10E+02	2.61E+02	0.42	0.68
Soil type_Dystric Plinthosols	-5.68E+02	9.13E+01	-6.22	5.04e-09 ***
Soil type_Ferric Lixisols	-6.26E+01	1.52E+02	-0.413	0.68
Soil type_Planosols	2.22E+02	1.48E+02	1.497	0.14
Soil type_Plinthic Lixisols	- 8.80E+02	1.92E+02	- 4.578	1.00 e-05 ***
Adjusted R-squared : 0.42 F-statistic: 7.461 on 18 and 145 DF, P-value: <0.0001				

Significant levels: *p < 0.05, **p < 0.01, and ***p < 0.001

Table 4.21. Explanatory variables for variability in grain yield in selected locations of Upper West Region.

Coefficients				
	Estimate	standard error	t value	Pr (>|t|)
(Intercept)	2.78E+03	9.99E+02	2.79	0.008 **
Nitrogen	-3.16E+03	1.28E+03	-2.463	0.0177 *
Organic carbon	-6.77E+02	2.68E+02	-2.521	0.015 *
Phosphorus	1.23E+02	5.17E+01	2.389	0.021 *
Potassium	5.75E+03	3.51E+03	1.639	1.08E-01
Calcium	3.28E+01	3.72E+01	0.881	3.83E-01
Magnesium	-1.49E+02	9.42E+01	-1.578	1.21E-01
Native rhizobia	-5.53E-01	4.26E-01	-1.298	2.01E-01
Active carbon	-0.06916	1.39E-01	-0.498	0.62
pH	1.17E+02	4.40E+01	2.66E+00	0.011 *
% Sand	-5.77E+01	1.86E+01	-3.099	0.003 **
% Clay	5.38E+01	2.54E+01	2.118	0.040 *
Texture_sandy loam	-1.03E+03	3.50E+02	-2.948	0.0051 **
Soil type_Ferric Lixisols	7.61E+02	3.93E+02	1.936	0.06
Soil type_Leptosols	7.71E+02	3.36E+02	2.296	0.026 *
Adjusted R-squared : 0.79 F-statistic: 17.68 on 14 and 45DF, *P-value:* <0.0001				

Significant levels: *p < 0.05, **p < 0.01, and ***p < 0.001

CHAPTER FIVE

5.0 DISCUSSION

5.1. Study 1. Symbiotic effectiveness and economic benefit of introduced *Bradyrhizobium* strains

The success of *Bradyrhizobium* inoculation primarily depends on the rhizobial strain, the legume genotype, the environmental conditions and the crop management (Woomer *et al.*, 2014). Although, inoculation response is site specific, there are two main situations where there is likely to be a response to *Bradyrhizobium* inoculation. These are where

compatible rhizobia of the host legume are absent and where native rhizobia population is low. In this study, counts of native *Bradyrhizobium* population were very low (< 10 cells g^{-1} soil) and probably ineffective. Sanginga *et al.* (1996) and Houngnandan *et al.* (2000) indicated that response to inoculation is likely to occur when the indigenous rhizobia population is less than 5 or 10 rhizobia cells g^{-1} soil. Similar results of significant nodulation in soybean due to *Bradyrhizobium* inoculation have also been reported by several authors (Albareda *et al.*, 2009; Kumaga and Ofori, 2004; Osunde *et al.*, 2003). Okogun and Sanginga (2003) reported no increase in shoot biomass after inoculation with rhizobia on soybean. In this study, similar results of no significant difference in shoot biomass in soybean was obtained which could be due to inadequate amount of nitrogen fixed by the introduced strains at that time of sampling.

Martins *et al.* (2003) observed a significant increase in nodule number of cowpea after inoculation with *Bradyrhizobium* inoculant. Nodule dry weight is very important in strain evaluation as it serves as an indicator for symbiotic efficiency (Graham *et al.*, 2004). Plants that received mineral nitrogen at a rate of 100 kg N ha^{-1} recorded the least nodulation. Such results were expected because high levels of nitrogen have been reported to affect rhizobia activity in the soil by inhibiting legume host production of lectin, which attracts the rhizobia to infect the roots. The treatment, 100 kg N ha^{-1}, was used as positive control to depict an ideal situation where nitrogen is not limiting (Thies *et al.*, 1991).

Nitrogen supplied as urea at a rate of 100 kg N ha^{-1} increased grain yield significantly. This implies that N was limiting in soils of the study sites. Yield increases may not be observed in soils, which receive quality inoculant if nitrogen is not a limiting factor

(Catroux *et al.*, 2001). Wilk's lambda values from these studies indicated that more than 93% of the variations observed in soybean and cowpea grain yields were due to the applied inoculants confirming the earlier assertion that the strains used in this study were highly effective. Martins *et al.* (2003) used BR 3267 and found a significant increase in grain yield of cowpea compared to the control. They observed no significant difference in grain yield when compared to N–fertilized plants in Brazil. The results obtained in this study is consistent with that of Boddey *et al.* (2016) who reported a significant increase in cowpea grain yield in northern Ghana after inoculating with the strain BR 3267. Fening *et al.* (2001) reported that at least 60 % of the soils in Ghana contain 1.3×10^3 cells of rhizobia capable of nodulating cowpea. To nodulate and to furnish plants with their N requirements are two different things. The data from Nyankpala suggests that the indigenous rhizobia were infective but not effective enough to supply the desired N requirement for grain yield to outweigh the control. Some reports from the past suggest that cowpea yields are not improved by rhizobia inoculation (Awonaike *et al.*, 1990;

Mathu *et al.*, 2012) but results from this study showed otherwise. The above authors made such conclusions because either the study locations had large numbers of indigenous rhizobia or the strains used were not effective enough to elicit significant responses. Inoculation with rhizobia does not always elicit significant response and its effect is site specific (Date, 2000). The results from the various locations in this research attested to that. MANOVA revealed that the study locations did not influence the performance of the strains in Nyankpala. This is a good attribute of the strains especially if they are to be used widely by farmers. The native rhizobia populations in Nyagli were too low to obviate significant response to inoculation. Therefore, other factors aside the native rhizobia population may have reduced the symbiotic performance of the introduced strains. The first step towards realisation of the benefit of inoculation is the survival of the strain in the soil and its subsequent ability to nodulate the host plant (Vachot-Griffin and Thies, 2005). The soil at Nyagli was sandy and could have influenced the survival of the strains since survival of rhizobia in sandy soils is very difficult (Zengeni *et al.*, 2006). Short dry spells during flowering probably resulted in poor flowering and consequently pod number and pod filling, thus reducing grain yield at Nyagli. Drought stress affects all physiological process including flowering and podding in plants (Serraj *et al.*, 2001). The effect of drought has a direct bearing on the host legume and indirect

bearing on the introduced strains that occupy root nodules of host plants. During such periods, the host legume closes its stomata, which is responsible for gas exchange with the atmosphere to prevent further water deficit thus reducing the amount of photosynthate produced and consequently the amount of energy supplied to the rhizobia. The end result is a reduction in the effectiveness and symbiotic performance of the introduced strain

(Sinclair *et al.*, 2007). Nutrient uptake, especially mineral nitrogen is essentially dependent on availability of water. This explains why plants fertilized with 100 kg N ha^1 recorded lower yields than inoculated plants at Nyagli. Although, there were rains after flowering to podding till harvest, photosynthetic activity barely returns to normal after moisture stress.

Gross returns of using rhizobia inoculant on cowpea and soybean was estimated using VCR. Dittoh *et al.* (2012) set a VCR threshold of 3 - 4 for an introduced technology to be considered attractive to farmers. Per such a threshold, three out of the four *Bradyrhizobium* inoculants were profitable at Nyankpala with an estimated gain of USD$ 169, USD$ 113 and USD$ 176 per hectare for Biofix, Legumefix and BR 3267, respectively. Asei *et al.* (2015) obtained a VCR of less than two when Legumefix was used to inoculate soybean in the Northern region. The discrepancy between the VCR results of this study and that of Asei *et al.* (2015) could be attributed to variability in environmental conditions such as seasonal rainfall and spatial soil fertility under which Asei *et al.* (2015) carried out their research. The VCR of the mineral N fertilizer was far below the threshold of 3 – 4 as compared to the *Bradyrhizobium* inoculants due to its high cost. This finding should serve as a basis for policy makers, government and non – governmental organisations to consider subsidizing *Bradyrhizobium* inoculants for soybean and cowpea, as it is less expensive, environmentally friendly and more likely to benefit smallholder legume farmers in Nyankpala.

5.2. Study 2. Persistence of *Bradyrhizobium* under field conditions

This study determined the persistence of introduced strains under smallholder farmers' condition and the predominant factors that affect their performance. The results showed that the *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 survived with considerable number of cells ranging from $\log_{10}1.7$ – 1.9. Native rhizobia population, soil moisture, annual rainfall and solar radiation were the predominant factors that influenced the strains persistence. Predictions based on the hyperbolic function suggested that the strains could persist for 3 years with the assumptions that little changes would occur with the prevailing environmental factors. Assuming the background indigenous populations at the various locations behaved similarly, their effect on the individual introduced strains will not be different (Crozat *et al.*, 1987) and therefore the counts should represent a build-up of total rhizobia in the soil at each sampling time.

A study on saprophytic competence of *Bradyrhizobium* is of great biological importance as it helps in predicting the nitrogen fixing ability of the legume-*Bradyrhizobium* symbiosis (Woomer, 1990). In addition, for a strain to be selected for inoculant production, it must be able to colonize the soil rapidly and adapt to the prevailing environmental conditions (Brockwell *et al.*, 1995). Mostly, the poor establishment of introduced *Bradyrhizobium* strain is due to the inability of the strain to persist between growing seasons. Our results showed that there was an initial rapid decline in the survival rates of the two strains during the first 21 days of introduction but the decline rate remained constant thereafter. There are few studies on rhizobia survival; however, the reports from such studies suggest that survival rates remain constant after initial decline in numbers due to predation by protozoa (Heijnen and Van Veen, 1991; Hirsch and Spokes, 1994; Pitkajarvi *et al.*, 2003; Woomer *et al.*, 1992). Similar trends have been

reported for other bacterial such as *Escherichia coli*, *Listeria monocytogenes*, *Salmonella dublin* after predation and parasitism by other organisms (Brennan *et al.*, 2014).

The successful establishment of introduced strains depends largely on the population size of rhizobia in the soil (Slattery *et al.*, 2004). The likelihood of survival of introduced strains is very low in the absence of host legume when native rhizobia population is high. This is probably due to the disadvantage placed on introduced strains by the native rhizobia for carbon source. Although, Woomer (1990) indicated that indigenous rhizobia by definition are adapted to the stresses in their environment, observations from the most probable number counts from this study suggest that both indigenous and introduced strains suffer the same fate in terms of reduction in numbers. Therefore, the ability of introduced strains to withstand stress and persist with higher population than the indigenous strains makes it more desirable for selection for inoculant production.

Different environmental and climatic conditions are said to influence the saprophytic competence and population dynamics of introduced strains (Pitkajarvi *et al.*, 2003). Results from this study suggests that soil moisture, temperature, solar energy and annual rainfall influence the dynamics of the introduced strains with varying significance. The most prominent factor was found to be soil moisture and this is probably due to the significant effect soil water content can have on cell viability (Rattray *et al.*, 1992). Beringer and Kay (1993) also reported that soil moisture is the obvious factor that affects rhizobia population levels in the soil. Hungria and Vargas (2000) highlighted the effect of soil moisture on rhizobia survival and growth in the soil. Our results therefore support these earlier findings, as soil moisture seemed to have directly influenced the survival of the introduced strains (Tables 4.9 and 4.10).

The results also showed that the population sizes of the introduced strains decreased as the average temperature increased and the soil moisture decreased. This can be explained by the fact that as the temperature increases the available moisture evaporates and the soil atmosphere dries up and becomes detrimental to the survival of the introduced *Bradyrhizobium*. The temperature effect accounted for less than or equal to 35% of the variations observed for decline rates of *B. yuanmingense* and *B. japonicum* at all the locations. In the tropics, high temperatures are likely to reduce the survival rate of rhizobia because it does not form heat-resistant spores and cannot survive extreme soil temperature (Hirsch, 1996; Hungria and Vargas, 2000). Tolerance to temperature is strain dependent; however, some rhizobia may survive at a temperature of 47 °C (Mpepereki *et al.*, 1996). In this study, the highest temperature recorded was 41 °C but for the most times; it was either below or up to 37 °C.

Many researchers have predicted the survival of introduced strains using Mistcherlich equation (Woomer *et al.*, 1992), logistic function (Crozat *et al.*, 1982) and Gompertz function (Corman *et al.*, 1987) but the data of this study was best described largely by hyperbolic functions. It is worth noting that the above researchers studied the persistence of introduced strains for more than a year. However, after the decline rate reached equilibrium, there were less mortalities and therefore depending on when equilibrium is attained, the subsequent years may not have so much effect on the surviving strains. Therefore, in general, the length of this study did not affect the model selection. However, the prevailing environmental variables might have had a stronger influence on the kind of model, hence the differences in the models derived for this and their work.

The purpose of this study was not to compare the survival rates of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110; however, observed differences could be explained by the differential sensitivity among strains to environmental conditions. In addition, the behavior of bacteria within soil is species and strain specific (Brennan *et al.*, 2014). McLoughlin *et al.* (1990) observed variation in the saprophytic competence of two *B. japonicum* strains CPAC 15 and CPAC 7. The observed variation was attributed to the intrinsic abilities of the strains to survive. Barthelemy-Delaux *et al.* (2014) affirmed that variation exists among the same species of rhizobia in their response to stressful conditions. The variation among the properties of the carrier materials for the two strains (Table 4.11) could not have significantly accounted for the variation in the survival of *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110. It could therefore be suggested that the inherent ability of the strains to persist cannot be overlooked. Although, we could not confirm the identity of the strains used in this work using molecular tools due to challenges in equipment, we have quantitatively determined the persistence of the introduced strains in reference to the indigenous rhizobia in the uninoculated plots using the MPN technique. Several authors have used MPN to measure the population of rhizobia over time (Barthelemy-Delaux *et al.*, 2014; Woomer *et al.*, 1992).

The multivariate non-linear regression revealed that soil moisture had positive effect with a higher magnitude on the survival of the strains in this study. It implies that soil moisture up to 27% as observed in the study favor persistence of rhizobia. Daily rainfall had negative effect implying that very low rainfall such as 0.7 mm per day observed in this study could be detrimental to the survival of rhizobia. The highest daily rainfall recorded in this study was 12.7 mm. The indigenous rhizobia populations at the introduction sites

of *B. yuanmingense* strain BR 3267 were relatively higher than that of *B. japonicum* strain USDA 110 which could have increased competition. This may explain negative effect on

B. yuanmingense strain BR 3267. The higher R-value obtained is probably due to the higher number of independent variables. Woomer *et al.* (1992) reported an R-value of 0.74 using Mistcherlich's decline function with mean annual rainfall and water holding capacity as predictors. This may indicate that the higher the number of environmental factors evaluated the better prediction of the persistence of introduced strains in soils.

Based on the results of the study, cowpea and soybean may not require re-inoculation a year after inoculation based on the number of the inoculum strain that survived, and on an assumption that little variation in native rhizobia population and soil moisture will occur during the period. The numbers will certainly increase in the presence of host and favorable conditions such as adequate soil moisture and availability of carbon source during the planting season. Hirsch (1996) reported that *R. legumnosarium* and *Sinorhizobium meliloti* populations can increase up to four-folds in the presence of their host. Although, effectiveness test was not carried out, during the MPN assay in N-free plant culture medium, the leaves of the cowpea and soybean plants were dark green compared to the control, which was yellow indicating that the strains had sustained their effectiveness.

The selection of rhizobia depends on its effectiveness and saprophytic competence. The persistence of *B. japonicum* strain USDA 110 is well known (Narożna *et al.*, 2015; Woomer *et al.*, 1992) but not that of *B. yuanmingense* strain BR 3267. Boddey *et al.* (2016) and Ulzen *et al.* (2016) have reported on the effectiveness of the strains used in this work. The results suggest that the potential for high saprophytic competence of *B. yuanmingense* strain BR 3267 has been confirmed by this work since the number of

surviving cells did not differ significantly from one location to the other. This also suggests that its introduction into wider areas with similar conditions is not likely to be affected dramatically by the varying environmental and climatic conditions and can therefore be recommended for legume-*Bradyrhizobium* dissemination programs.

5.3. Study 3. Improving cowpea response to *Bradyrhizobium* inoculation through the addition of phosphorus fertilizer and organic manure

5.3.1. Nodulation and dry matter yield

Bradyrhizobium, phosphorus and organic manure interacted to increase nodule number but not nodule dry weight. Compared to threshold of 50 cells of rhizobia g^{-1} soil (Slattery *et al.*, 2004) that can obviate significant response, the study locations had fewer number of rhizobia cells. Ulzen *et al.* (2016) observed a significant response in nodule number under similar agro-ecological conditions when native *Bradyrhizobium* population were less than 10 cells g^{-1} soil. Only the *Bradyrhizobium* and phosphorus combinations had significant effect on nodule dry weight. Kyei-Boahen *et al.* (2017) made similar observations on cowpea after combined application of *Bradyrhizobium* inoculant and P in Mozambique. Phosphorus plays an important role in *Bradyrhizobium* nutrition by supplying energy required for nitrogen fixation (O'Hara, 2001b). Response to P was expected because the study locations had low inherent P concentrations. The amount of nitrogen released by the organic manures during the 2 – 4 weeks were high enough (Figures 4.34 and 4.35) to support good growth of plants when nitrogen fixation was not optimum yet. Healthy plants are likely to benefit from enhanced symbiosis and consequently increased nodulation. This possibly accounted for the higher nodulation observed in this study. The organic carbon released was also high (Figures 4.34 and 4.35)

and probably served as substrate for the *Bradyrhizobium* and thus enhancing its survival before the optimal expression of symbiotic functions. Zengeni *et al.* (2006) had earlier reported that organic manure enhanced the survival of rhizobia. The high nodule biomass observed in this study is an indicator for high symbiotic efficiency (Graham *et al.*, 2004). It is therefore not surprising that this efficiency coupled with support of phosphorus and organic manure led to high dry matter yield and consequently higher grain yield. In contrast, Zengeni *et al.* (2006) observed no significant increase in dry matter when cattle manure was applied. The manure used in this study had > 2.5 % N and C: N ratio of < 20 compared to theirs which had < 1 % N and C: N ratio > 30. Fertisoil and phosphorus had also been used to increase nodule dry weight and dry matter yield (Ezekiel-Adewoyin, 2015).

5.3.2. Grain yield, agronomic efficiency and added benefits

The combined application of organic fertilizers and mineral fertilizer have gained extreme research interest and have been used to greatly enhance grain yield of maize in low fertility smallholder fields in SSA (Chivenge *et al.*, 2011; Vanlauwe *et al.*, 2001). The present study was designed to test the effect of organic and inorganic fertilizer use in combination with *Bradyrhizobium* inoculant on cowpea. These three factors combined to increased grain yield up to 327%. Yield increases could be from the complementary role played by each factor. For instance, the organic manure could have provided favorable carbon and other extra nutrients to enhance the survival of the applied *Bradyrhizobium* strain and support general plant growth. Phosphorus (P) could have supplied the needed energy for nitrogen fixation and supported the overall growth of the host plant (Crews,

1993; Keyser and Li, 1992; O'Hara, 2001a). Since biological nitrogen fixation is a symbiotic association, it is optimized when the growth of the host plants is improved. Greater yield response observed in this study could also be attributed to the addition of extra nutrients made available to the plants from the combined treatments as also reported by Palm *et al.* (2001).

Zengeni *et al.* (2006) reported an increase in yield of soybean due to application of *Bradyrhizobium* inoculant and manure. Enhanced survival of rhizobia due to the provision of carbon by manure and release of micronutrients to ameliorate limitations to response to the inoculant could explain the observed yield increases in cowpea. Palm *et al.* (1997) and Zingore *et al.* (2008) attributed the increase in yield due to organic and inorganic fertilizer application to the release of micronutrients by the organic manure. Ezekiel-Adewoyin (2015) observed greater yields in soybean using combined application of fertisoil and phosphorus. The difference in yield of treatments with fertisoil and cattle manure is due to the higher quality of the fertisoil (N = 4.5%) relative to the cattle manure (N = 2.53%). In addition, nutrients were rapidly released in high quantities from the fertisoil relative to the cattle manure. The difference in grain yield between the combination with fertisoil and combination with cattle manure could also be attributed to the added benefits of 300 kg ha^{-1} shoot biomass produced by the former as compared to added benefit of 74 kg ha^{-1} shoot biomass produced by the latter. Higher dry matter yield tends to result in higher grain yield. In general, the treatments achieved 75% (fertisoil) and 69% (cattle manure) of the potential yield of the Songotra cultivar used in this study. Even though the supplied quantities of the organic manures contributed to achieving more

than 50 % of the potential yield of cowpea, there is a high tendency of obtaining grain yields closer to the potential yield if the quantities of organic manure are increased.

Chivenge *et al.* (2011) observed that grain yields of maize increased with increasing amount of organic resources. The significant difference in grain yield between combined application of fertisoil, P and *Bradyrhizobium* inoculant and cattle manure, P and *Bradyrhizobium* inoculant suggests that addition of fertisoil could better improve cowpea response to *Bradyrhizobium* inoculant than cattle manure. Grain legumes such as cowpea require high amount of nitrogen during flowering, podding and seed filling stages for enhanced yield. The applied organic manures released up to 97% of its nitrogen to supplement that of the applied *Bradyrhizobium* during the crop cycle hence the observable yield increases in all the study locations. Addition of quality organic manure (> 2.5 % N) in the form of fertisoil resulted in fast release of nutrients which may be assimilated by plants if they are in synchrony with crop demand (Palm *et al.*, 2001; Vanlauwe *et al.*, 2001). The major nutrients required for plant growth were limiting at the study locations (Table 4.17). Therefore, responses to the treatments were expected. The addition of manure could have improved moisture retention and soil structure to create conducive environment for enhanced grain yield.

The purpose of combining *Bradyrhizobium* inoculant, P and organic manure was to maximize agronomic P use efficiency. It was generally observed that addition of P or fertisoil to *Bradyrhizobium* inoculant increased agronomic P use efficiency due to enhanced grain yield production. Adding either fertisoil or cattle manure resulted in higher grain yield but P use efficiencies were lower than those treatments without P. This indicates that yield increases were not solely due to efficient utilization of P but due to the provision of excess nutrients supplied by the additional treatments. This could also be due to luxury consumption by the plant due to excess P. Therefore, not all P captured by plant was utilized for grain yield production. Cassman *et al.* (2002) and EzekielAdewoyin (2015) reported lower agronomic efficiencies when higher P fertilizer quantities were applied. Agronomic P use efficiency is directly proportional to grain yield and inversely proportional to the amount of fertilizer applied. The importance of phosphorus nutrition and *Bradyrhizobium* in legumes have been discussed extensively by O'Hara (2001b).

Chivenge *et al.* (2011) observed high grain yields from maize but lower agronomic N efficiency from meta analyses of several works on combined application of organic manure and mineral fertilizer and attributed it to extra nitrogen from the treatments.

Chivenge *et al.* (2011) also reported both positive and negative interaction between organic manure and mineral fertilizer. Badu (2015) observed positive interaction between organic manure and mineral fertilizer on maize in Ghana. In this study, positive interactions were observed between all the three factors. The positive interactions are an indication that the three factors have synergistic effect. The stepwise regression output revealed that all the measured parameters contributed significantly towards the grain yield. These three factors could be used by smallholder farmers to increase their grain yields and close the gap between the existing farm yields of 0.6 tha^{-1} and the potential yield of 2.5 t ha^{-1} with subsequent increase in their income levels and livelihoods as demonstrated by the value cost ratios. It is worth noting that, not all the benefits of organic manure can be realized in the first year of application because of potential residual effects. Therefore, obtaining a 200 percent returns (VCR of 2) above the cost of fertisoil in the first year indicates that such a treatment combination have the potential to increase the income of farmers in the medium to long term. Many smallholder farmers cannot afford to supply mineral fertilizers at required quantities and in addition obtain organic manures in high quantities to meet the nutrient requirement of targeted plants. Therefore, the government and other non-governmental organizations in Ghana may consider disseminating integrated soil fertility management packages (such as the factors adopted in this study) that increase grain yields and improve the livelihood of the smallholder farmers. The fact that there was no significant difference between the locations and

treatments for grain yield is an indication that the performance of the treatments were not affected by locations and therefore can be introduced into wider areas with minimal uncertainties.

5.4. Study 4. On-farm evaluation of soybean response to *Bradyrhizobium* inoculation and/ or phosphorus fertilizer

5.4.1. Soybean response to TSP fertilizer and *Bradyrhizobium* inoculation

Soybean responded significantly to *Bradyrhizobium* inoculation and phosphate fertilizer in the Northern region. The average yields obtained in this study were within the range reported by Masso *et al.* (2016) who undertook similar research in 2014 at Savelugu – Nanton and Karaga district and Ronner *et al.* (2016) in Nigeria. However, it was in contrast with the findings of Falconnier *et al.* (2016) who did not observe a significant effect in soybean grain yield after applying *Bradyrhizobium* inoculant. The difference in the two results could be attributed to a number of factors including the quality of the inoculant, the initial soil nitrogen and native rhizobia population. They reported a range of 0.28 – 0.33% of soil N which was 3 – 9 times higher than the range 0.03 – 013 obtained in this work. Higher nitrogen tend to limit the activities of introduced rhizobia. KyeiBoahen *et al.* (2017), Masso *et al.* (2016), Ronner *et al.* (2016) and Aziz *et al.* (2016) have also demonstrated the beneficial effect of P on legumes and this has been confirmed in this work. Given that the soils in Northern region had very low N and P, it was not surprising that external inputs like *Bradyrhizobium* inoculant and P fertilization significantly increased grain yield. The *Bradyrhizobium* inoculants enhanced the plants access to nitrogen through biological nitrogen fixation (Masso *et al.*, 2016). Likewise, the phosphate fertilizer enhanced access to P. When P and inoculant were applied together,

a greater response was obtained which confirms the significance of P nutrition to inoculation (O'Hara, 2001a). The inoculation results obtained in the Upper West region, however, were not significant. This result is in tandem with the reports by Okogun and Sanginga (2003) and Falconnier *et al.* (2016). The median native rhizobia population for the Upper West was 91 cells g^{-1} soil, which could have obviated significant response to inoculation. Response to rhizobia inoculation is not likely when native rhizobia population is above 10 cells g^{-1} soil (Houngnandan *et al.*, 2000; Sanginga *et al.*, 1996) and up to 50 cells g^{-1} soil. In general, the yields obtained in the Upper West region were very low which may be attributed to delayed planting and poor rainfall received during the cropping season (Figure 4.36)

5.4.2. Variability in soybean grain yield

There was a wide variability in grain yield between locations and among treatments due to the spatial variability in soil nutrients and environmental factors. This seems to be a common characteristics of on – farm trials in smallholder settings in SSA as reported by several other researchers (Bielders and Gérard, 2015; Diarisso *et al.*, 2016; Falconnier *et al.*, 2016; Fermont *et al.*, 2009; Kihara *et al.*, 2016; Masso *et al.*, 2016; Ronner *et al.*, 2016; Zingore *et al.*, 2007a). The variables measured in this experiment could explain 42 -79% of the variances in grain yield in the study locations in the Northern and Upper West region locations, respectively. This finding is comparable to that of Ronner *et al.* (2016) who found out that soil, environmental and management factors explained 16 – 61% of the variability in soybean grain yields under similar experimental conditions in Nigeria. Fermont *et al.* (2009), Bielders and Gérard (2015) and Falconnier *et al.* (2016)

also reported that environmental, management and soil factors explained 20, 58 and 49%, respectively, of the variability in cassava, millet and sorghum – cowpea - soybean yields under smallholder farmers conditions. Since the soil and environmental factors measured could explain only 42% of the variability in Northern region, it means there are other factors that contributed to the yield variability that we were not able to identify. For instance, distance from home to farm and other proxies for soil fertility. It is typical of on-farm trials that large proportions of the variability remain unexplained (Bielders and Gérard, 2015; Falconnier *et al.*, 2016) but treatment contributions to the yield variability cannot also be over looked. Bielders and Gérard (2015) reported that the applied treatments contributed to 27% variation in the millet grain yield. Soil constraints are not the only driving forces for productivity; management decisions by farmers do affect productivity too (Dang and Moody, 2016). The study did not consider management as a variable factor as was done in other studies. This is because we trained and employed Agricultural Extension Agents (AEAs) who ensured that farm activities were

standardized across and therefore could contribute little to the variation in grain yield. This probably underpins the importance of AEAs in disseminating successful legume technology to smallholder farmers.

Soil nitrogen, active and organic carbon had positive effect on yield in the Northern region. Though the current level of these nutrients are low, having positive effect indicates that plant growth were not limited by these nutrients and support inoculation. In contrast, these factors had negative effect on yield in the Upper West region indicating that the current levels of these nutrients are too low to support plant growth. Sorption of P is likely to be the major reason for non-responsiveness to P in many of the locations despite the

initial low levels of P in the soil. Potassium had negative effect on grain yield indicating the low levels of potassium at the study locations were limiting the effect of the treatments. In nutrient omission trials, potassium omission resulted in yield reduction in maize and soybean (Kihara *et al.*, 2016; Seitz, 2014). Magnesium contents were high and had negative effect indicating that such levels were not desirable for the applied treatments. Considering rhizobia inoculation, it can be argued that soil with good fertility can provide nutrients for plant and rhizobia to ensure effective symbiosis. On the contrary, soils with poor fertility do hinder effective symbiosis due to poor crop nutrition. Percent sand had negative effect on yield whereas clay percent had positive effect on yield. Sand is known to have poor water holding capacity and does not support rhizobia survival (Zengeni *et al.,* 2006). Leaching of nutrients is high with sandy soils. Therefore, its negative effect on yield was not surprising. Soil texture, soil type, Mg, pH, N and rainfall have all been reported to affect yield variability in smallholders' farm (Falconnier *et al.*, 2016; Fermont *et al.*, 2009; Ronner *et al.*, 2016). Native rhizobia population had negative effect on grain yield. This confirms the assertion by Thies *et al.* (1991) that grain yield of legumes are inversely related to native rhizobia population. At the locations (e.g. Nyeko and Sheillianyilli) where native rhizobia population were relatively high, responses to inoculation were low. Depending on soil type, the effect on grain yield were either positive or negative. Falconnier *et al.* (2016) reported similar observations under smallholder farm conditions in Mali. Soil type influences soil nutrients which determines crop response to amendments (Falconnier *et al.,* 2016). Coincidently, locations such as Nyeko and Sheillianyilli in the Northern region with Dystric Plinthosols and Plinthic Lixisols as soil types were largely non-responsive to the applied treatments. Plinthosols are inherently poor in fertility due to strong weathering with underlying hardpan, which limits rooting volume and penetration (IUSS, 2014). This affects nutrient uptake and

distribution. Naturally, Lixisols also have low plant nutrients, low clay activity and high base saturation (IUSS, 2014).

Cumulative rainfall had negative effect on yield, which is comparable to the observations by Ronner *et al.* (2016) in Nigeria. Diarisso *et al.* (2016) attributed yield variability in crops in Burkina Faso to rainfall. The negative effect of cumulative rainfall on grain yield in Northern region is difficult to explain. However, these possible scenarios may be considered; excessive rainfall, which is likely to cause leaching, waterlogging, or increase in the incidence of fungal disease, which eventually, affects yield. The other scenario is the shortage of rainfall, which affects nutrient uptake and limit the ability of rhizobia to fix nitrogen. The latter may partly explain the observation of this work because there were short dry spell after flowering. Rainfall was expected to be the dominant factor explaining the variability in yield at locations in the Upper West region because of the low rains received especially during and after flowering but this was not the case. Late planting due to late rains could be a major contributory factor for the very low yields recorded in the Upper West region. Many researchers notably Bielders and Gérard (2015) and Fermont *et al.* (2009) have also attributed low yields to late planting.

The agronomic approach adopted for determining responsive and non-responsive sites indicated that a large majority of the fields were non-responsive to P and/ or I. Only 17 – 40% of the study fields in the Northern region were responsive and 6 -17 % of the sites were responsive in the Upper West region. The agronomic approach has less sites being responsive in comparison to the economic approach; this shows the robustness and conservative nature of the agronomic approach. This finding is comparable to Kihara *et al.* (2016) who reported that 11 and 25% of fields sown with maize were responsive and

non-responsive to fertilizer respectively. Kihara *et al.* (2016) used K-means clustering to determine maize response to fertilizer in their nutrient omission trial setting a yield threshold of 3 t ha^{-1}. The idea of setting threshold including setting percentage yield increase to determine responsiveness and non-responsiveness is very subjective and can lead to either over estimation or under estimation. If non-responsiveness is caused by other factors such as seasonal rainfall or management practices, other than inherent properties of the soil, it could easily be addressed. It is worth noting that though farmers do not benefit from substantial yield increases, they however, benefit from improvement in soil fertility when they incorporate the crop residues for subsequent cropping. The residues have been reported to contribute to soil organic matter pool (Nezomba *et al.*, 2015). The mean grain yields show significant responses to the applied treatments in general but it does not provide clearer information on the treatment performance on individual farms. The cumulative probability curves shows the performance of the treatments on individual farms and therefore indicates what will happen should farmers forgo their practices and accept the treatments (Vanlauwe *et al.*, 2016). Therefore, it will be misleading to make general recommendations for all farmers based on the averages (Bielders and Gérard, 2015; Ronner *et al.*, 2016). In addition to making recommendations for individual farmers, risks associated with the adoption in terms of economic benefits must be spelt out to farmers.

5.4.3. Economic viability of P and/ or inoculant application

Value cost ratio (VCR) is a simple economic tool used to verify whether it is worth investing in a given technology based on a cost recovery and potential profit (Masso *et al.*, 2016). The application of P and / or inoculant (I) were profitable for about 64 – 75%

of the farmers in Northern region. This is comparable to the results of Ronner *et al.* (2016) who reported that about 60 – 95% of farmers who used P and / or inoculant (I) in a similar trial in Nigeria achieved economic benefit. Masso *et al.* (2016) and Banka (2016) reported that the application of P and / or inoculant (I) were financially rewarding for farmers in northern Ghana. In Niger, Bielders and Gérard (2015) reported that 36% of farmers who applied Diammonium phosphate (DAP) and / or urea to their millet had

VCR greater than 1. Although, the grain yields were low in Upper West region, about 14 – 24 % of the farmers achieved economic benefits. For farmers to adopt either P and / or inoculant (I), a 100% return to investment (break-even) is often not attractive (Bielders and Gérard, 2015; Ronner *et al.*, 2016). This is the case of SSA smallholder farmers who are generally risk averse (Kisaka-Lwayo *et al.*, 2005) cited by Masso *et al.* (2016), therefore return to investments should be at least 200% as indicated by Roy *et al.* (2006) for farmers to adopt new technologies. Using a VCR threshold of 2 or more, 31% of the farmers in Northern region who applied P achieved economic benefit, 53% who applied inoculant (I) achieved economic benefit and 45% who applied P in combination with inoculant (I) achieved economic benefit. Only 2% of the farmers in the Upper West region could achieve economic benefit for applying P and inoculant (I). None benefited economically from combined application of P and inoculant (I). This was expected due to low yields and relative higher prices of the inputs. It was observed that achieving higher economic returns depended on the performance of the control plots. Buerkert *et al.* (2001) and Bielders and Gérard (2015) made similar observations.

CHAPTER SIX

6.0 SUMMARY, CONCLUSION AND RECOMMENDATIONS

6.1. Summary

The study has contributed to the development of knowledge-based approaches for improving grain legume productivity with *Bradyrhizobium* technology by:

i. evaluating the symbiotic effectiveness of introduced *Bradyrhizobium* strains; ii. determining the persistence of introduced *Bradyrhizobium* strains and the major factors that affects their survival under smallholder farmer conditions; iii. determining the response of cowpea to *Bradyrhizobium* inoculant when phosphorus fertilizer and organic manure are co-applied; iv. evaluating on-farm response of soybean to *Bradyrhizobium* inoculation and phosphate fertilizer and the factors that influence their response

The study has shown the significant effect of *Bradyrhizobium* inoculation on soybean and cowpea grain yield in relation to untreated control and N fertilization. It further pinpoints the advantage of the returns on investment in legume inoculation compared to N fertilization. Thus, the application of effective and less expensive inoculants are crucial for the success of inoculation. This study has contributed to knowledge by identifying effective strains that are economically viable for soybean and cowpea production under smallholder farm conditions in northern Ghana. The findings on cowpea however, have bridged the knowledge gap on the need to inoculate cowpea in northern Ghana where its productivity is highest.

The introduced strains *B. yuanmingense* (BR 3267) and *B. japonicum* (USDA 110) persisted on smallholder farms after 296 days of introduction. Predictions based on the

observed numbers of surviving cells indicated that the strains could persist in the field for three years. In general, persistence over time was best described by a hyperbolic function. It was found that native rhizobia population and soil moisture were predominant factors that affected the persistence of the introduced strains. Multivariate non-linear regression revealed that the effects of the environmental factors on the strains varied in magnitude and direction. This observation has to some extent improved our understanding of persistence of introduced strains in farmers' field with native rhizobia population. In addition, coefficients of the hyperbolic model have been identified for *B. yuanmingense* (BR 3267) and *B. japonicum* (USDA 110) which can be used for predicting the

persistence of these strains in similar environmental conditions like the study location. The study has shown the beneficial effect of the combined application of phosphorus, organic manure and *Bradyrhizobium* inoculant on cowpea grain yield. It further indicates that yield increases of about four-folds from the addition of phosphorus and fertisoil to *Bradyrhizobium* inoculant, in relation to the untreated control, resulting in a 200% return on investments. The study have added to the existing knowledge on the contributions of P and organic manure to *Bradyrhizobium* inoculation. It has also proposed a potential soil fertility management (SFM) technology for increasing cowpea grain yield for smallholder farmers.

The results of this study have shown that soybean response to application of phosphorus (P) and inoculant (I) alone but greater response was achieved through the combined application. It however, indicates that not all fields responded to P and / or I and that there were variations in grain yield between treatments and locations. This led to the classification of some fields as responsive and non-responsive. Soil and environmental

factors were found to be the major driving forces for the variations in yields observed. It was also shown that application of inoculant was economically profitable. These results have contributed to the existing knowledge on the importance of P and I on soybean growth. This study has identified the major soybean growing areas that respond to *Bradyrhizobium* inoculation and / or phosphorus fertilizer and the factors driving variability in smallholder farms in northern Ghana. Furthermore, this research has contributed towards understanding the responsiveness and non-responsiveness of soybean to P and *Bradyrhizobium* inoculation due to the approaches proposed in the study.

6.2. Conclusions

Based on the objectives and the results obtained in the current study, the following conclusions can be drawn:

i. There is sufficient evidence ($P < 0.05$) that Biofix (USDA 110), Legumefix (532C) and BR 3267 can be used to increase grain yields of soybean and cowpea respectively at Nyankpala in an economically viable manner. The use of these strains proved to be economically profitable. This outcome at Nyankpala confirms the null hypothesis that introduced strains are more effective than the indigenous rhizobia populations. This implies that smallholder farms can depend on effective rhizobia inoculants to bridge the yield gap and improve on their livelihoods. At Nyagli, there was no sufficient evidence ($P > 0.05$) that strains BR 3267 and BR

3262 could be used to increase grain yield of cowpea. Furthermore, the strains were not economically profitable at Nyagli. This however, contravenes the null hypothesis stated earlier.

ii. The introduced strains *B. yuanmingense* strain BR 3267 and *B. japonicum* strain USDA 110 can persist under smallholder farmer conditions with sufficient numbers for a year confirming the null hypothesis that introduced strains have the same as or better saprophytic competence than indigenous rhizobia. Under smallholder farms conditions, soil moisture and native rhizobia populations were the major determinants of the survival rates of the introduced strains in the study locations. This implies that in areas where rhizobia inoculants are scarce due to varied reasons, farmers may not worry about re-inoculation for at least a year.

iii. The present study has demonstrated that adding high quality organic manure and phosphorus to effective rhizobia strain improves grain yield of cowpea up to four folds. The increase in cowpea yield was considerably higher and significant for fertisoil with *Bradyrhizobium* inoculant and phosphorus combination than its combination with cattle manure. Thus, fertisoil with *Bradyrhizobium* and phosphorus fertilizer is the better option for improving grain yield of cowpea for smallholder farmers in northern Ghana. The findings support the hypothesis that adding organic manure and P to *Bradyrhizobium* inoculant could improve the yield of cowpea. The findings also highlight the importance of improving organic

matter content of the soils to improve general nutrition of *Bradyrhizobium* for enhanced symbiosis.

iv. Combined application of P and I is an effective means of increasing soybean grain yields on smallholder farms. Addition of *Bradyrhizobium* inoculant to P makes it economically attractive for most farmers. However, wide variability in grain yields might occur due to varying soil and environmental factors. This implies that legume-*Bradyrhizobium* technologies could be better targeted to farmers who would benefit most. The findings also confirm the null hypothesis that, response to *Bradyrhizobium* inoculation and phosphate fertilizer is highly variable but can be economically viable.

v. From the various studies conducted so far, the application of *Bradyrhizobium* inoculant or strains and P either alone or in combination were economically viable and profitable.

6.3. Recommendations

In order to increase and sustain soybean and cowpea production and increase the livelihood of smallholder farmers, it is recommended that farmers be encouraged to apply effective *Bradyrhizobium* inoculant. For greater yield response, farmers should aim at integrating phosphorus fertilizer and fertisoil with inoculation technologies.

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APPENDICES

Appendix 1. Mean number of declining *Bradyrhizobium spp* at the study locations.

Location	*B. yuanmingense* (BR 3267)	*B. japonicum* (USDA 110)
	Rhizobial cells g^{-1}soil	
Tunayilli	3.3±0.93a†	3.0±0.81a†
Tanina	3.2±0.95a	3.0±0.81a
Kpalga	3.2±0.95a	2.7±0.86a
Busa	3.1±0.97a	NA
P-value	*0.99*	*0.97*

† Within column, figures followed by the same letters are not significantly different from one another at 5% probability

Appendix 2. T-test between selected nutrients of fertisoil and cattle manure

Organic manure	Parameter	Mean	SD	*t-value*	*P-value*	95% CI
Fertisoil	Carbon (%)	91.0	3.0	3.07	0.02	0.98, 8.62
Cattle manure		95.0	1.8			
Fertisoil	Nitrogen (%)	88.4	10.0	2.69	0.03	1.76, 27.40
Cattle manure		73.8	6.9			
Fertisoil	Phosphorus (%)	73.0	5.7	1.53	0.17	-3.73, 17.33
Cattle manure		62.2	8.1			
Fertisoil	Potassium (%)	81.0	4.3	3.34	0.01	2.28, 13.2
Cattle manure		74.0	2.9			

SD= Standard Deviation. CI = Confidence Interval. Degree of Freedom = 7

Appendix 3. Cost of inoculant and / or phosphorus at the study locations.

	Cost	
Treatments	GH¢ ha^{-1}	USD ha^{-1}
P	283	79
I	138	38
P + I	421	117