Impacts of Bradyrhizobium inoculants on growth and yield of tropical soybean (*Glycine max (L.) Merr.*) cultivars, soil health and soil microbiome

by

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B.S., University for Development Studies, 2007
M.S., Kansas State University, 2015

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

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Department of Agronomy
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Abstract

Microbial inoculation of grain legumes improves crop yield and soil quality. Grain legumes such as soybean as requires host specific *Brayrhizobium japonicum* to enhance growth, nitrogen fixation, and grain yield. However, limited information exists on how commercial *Bradyrhizobium* inoculants affect symbiotic plant performance and yield of soybean, and as well as soil health in Ghana's cropping systems. A field study (2-yr) was conducted at CSIR-Savanna Agricultural Research Institute's experimental field at Nyankpala, Ghana to determine the impacts of *Bradyrhizobium* inoculants on; (1) growth, nodulation, nitrogen fixation, grain yield of soybean, and (2) soil biological and chemical properties. We also evaluated the commercial inoculants effects on the subsequent maize and soybean crops. The experiment was laid out as a split-plot design where the main plot consisted of tropical soybean (*Glycine max crosses* (TGX)) varieties; Jenguma (TGX1448-2E), Afayak (TGX1834-5E), and Songda (TGX 1445-3E). The subplot consisted of three commercial *Bradyrhizobium japonicum* inoculants with different strains, Biofix (USDA 110), NoduMax (USDA 110) and Legumefix (USDA 532c) plus an uninoculated control. Assessment was made on nodulation pattern, shoot biomass, nitrogen fixation, grain yield, and residual N balance. Bulk and rhizosphere soils were sampled and analyzed for soil pH, available soil N (NO_3-N and NH_4^+-N) and P, and soil microbial community structure by phospholipids fatty acid (PLFA) analysis. Inoculants improved nodulation, shoot biomass, nitrogen fixation and grain yield of soybean. Greater responses were associated with NoduMax and Biofix. Inoculation increased grain yield by ~30 %. Commercial inoculants also increased microbial biomass, and available P and NH_4^+-N.

Afayak outperformed the other soybean varieties for biomass dry matter, nodulation (nodule number) and grain yield. Afayak also stimulated greater microbial biomass and available

P compared to Jenguma. Furthermore, enhance microbial biomass was found in the rhizosphere compared to the bulk soil due to soil enrichment with root exudate and commercial inoculants.

In assessing, the previous year commercial inoculants effect on the subsequent soybean and maize crops, three (3) independent mineral N fertilizer rates (0, 50 and 100 kg N ha^{-1}) were added to the soybean-maize rotation phase. Biofix yielded superior maize shoot dry matter and grain yield. Maize grain yield from previous commercial inoculants was equivalent to grain yield from 50 kg N ha^{-1}mineral N fertilizer). Thus inoculating soybean with commercial inoculants reduced mineral N nutrition for the subsequent maize crop by 50%. In the soybean-soybean phase, the previous Biofix and the uninoculated control produced significant soybean grain yield than the previous NoduMax. In conclusion, TGX soybean varieties exhibited superior performance when inoculated with commercial inoculants especially Biofix and NoduMax. However yearly inoculation of soybean is needed to sustain enhanced grain yield and soil quality in Northern Ghana.

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Dedication

I dedicate this work to my wife, children
and
everyone who helped me in achieving this dream.

Chapter 1 - General Introduction

Introduction

Integration of grain legumes into cropping systems is a sustainable intensification practice for enhancing human nutrition, soil quality, and crop development. Grain legumes are important sources of protein, oil, vitamins, and minerals (Robaina et al., 1995). Therefore, the consumption of grain legumes improves nutritional security in areas where access to animal protein is limited. Abaidoo et al. (2014) documented several dishes prepared from grain legumes in Ghana. Grain legumes are also important sources of feed for poultry and ruminant livestock industry because of their nutritional value (Robaina et al., 1995). Grain legumes also contribute to soil quality by supplying biologically fixed N through a symbiotic association with a group of soil bacteria called *Rhizobiacae*. This fixed N reduces capital expenditure of purchasing of mineral N fertilizer. Grain legumes are excellent for green manuring as they improve soil structure and aggregation, soil biology, minimize erosion and leaching of nutrients.

Grain legumes commonly found in sub-Saharan Africa cropping systems include pigeon- pea (*Cajanus cajan*), Bambara-groundnut (*Vigna subterranea* (L.) Verdc.), cowpea (*Vigna unguiculata*), groundnut (*Arachis hypogaea*), common bean (*Phaseolus vulgaris*) and soybean (*Glycine max (L.) Merr*). These grain legumes are introduced into cropping systems either as a monocrop or integrated as an intercrop or in rotation with other crops. Apart from soybean, the other grain legumes (pigeon pea, Bambara-groundnut, cowpea, and groundnut) are capable of forming nodules with the cross nodulating native soil *Rhizobium* spp. due to their previous cultivation history. On the other hand, soybean is not native to Africa and requires a host-specific *Bradyrhizobium japonicum* for efficient nodulation to enhance biological fixed N. (BNF) (Abaidoo et al., 2007; Grönemeyer et al., 2014). Other bacterial symbionts that are capable of

forming effective root nodules include *Bradyrhizobium elkanii* (Kuykendall et al., 1992), *Bradyrhizobium liaoningense* (Xu et al., 1995) and *Sinorhizobium fredii* (Chen et al., 2006).

Inoculation with these *Bradyrhizobium* strains becomes necessary in areas with no previous soybean history, low population of these bacteria or ineffective bacteria population. Inoculation with commercial *Bradyrhizobium* inoculant enhances effective symbiotic association. Nonetheless, until recently, most laboratories in sub-Saharan Africa (SSA) were not well equipped or resourced to produce, store, and distribute commercial *Bradyrhizobium japonicum* inoculants (Pulver et al., 1982). The most appropriate option was to import the commercial *Bradyrhizobium* inoculants but comes with the challenges of importation duty (expense), storage and distribution. In some instances, the commercial *Bradyrhizobium* inoculants do not consistently yield the desired results due to variability associated with climatic and soil conditions (Osunde et al., 2003; Okogun and Sanginga, 2003; Chianu et al., 2011; Gyogluu et al., 2016).

Pulver et al. (1982) suggested that an alternative to commercial inoculants was to develop soybean genotypes that are capable of establishing a symbiotic association with the native rhizobia in sub-Saharan Africa soils. Most tropical soils have numerous native slow-growing rhizobia ("cowpea-type rhizobia") which are capable of forming effective symbiosis (Pulver et al., 1982). The International Institute of Tropical Agriculture (IITA), Ibadan in Nigeria developed promiscuous nodulating soybean genotypes, designated as Tropical *Glycine max* crosses (TGX) by crossing a host-specific soybean genotype from the USA with promiscuous soybean genotype from China (Pulver et al., 1982). These soybean genotypes are capable of forming effective nodules with the native *Rhizobium spp* in sub-Saharan Africa soils (Abaidoo et al., 2007; N'cho et al., 2015).

In Ghana, the improved TGX soybean varieties include Jenguma, Salintuya-2, Quarshie, Anidaso, Afayak, Songda and Soung Pungu (Denwar and Wohor, 2013), TGx1485-1E, TGx1740-2F, TGx1448-2E, TGx1440-1E and Salintuya-1(Pule-Meulenberg et al., 2011). These varieties traits include high yielding, shattering tolerance, pest and disease resistance, early maturing, drought tolerance and striga resistance. (Dwivedi et al., 2015). Nonetheless, limited information is available on their responses to commercial *Bradyrhizobium* inoculants and symbiotic N contribution. Therefore, the contribution of TGX soybean varieties to the N economy of Ghana's cropping systems is generally unknown.

The proportion of N-fixed by soybean is affected by cultivar selection, the environment, the *Rhizobium* strain and management (Dwivedi et al., 2015). It is necessary to select soybean varieties or genotypes with high N-fixing, and high-yielding capabilities on location-specific performance for the resource-poor farmers in Ghana, (Belane et al., 2011). The selection of soybean varieties based on their agroecological zone performance are crucial in promoting higher production, productivity, and soil quality.

This study therefore aimed to determine (1) the impacts of commercial *Bradyrhizobium japonicum* inoculants on plant growth, symbiotic performance and N contribution of selected TGX soybean varieties, (2) the impacts of commercial *Bradyrhizobium japonicum* inoculants on selected soil health indicators and soil microbial ecology of Northern Ghana's cropping systems, (3) the impacts of the previous inoculation on the subsequent crops and selected soil health indicators, (4) the native *Bradyrhizobium* populations and compared their symbiotic performance (nodule formation and pattern) against a known *Bradyrhizobium japonicum* strains

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Chapter 2 - Literature Review

History and Domestication of Soybean

Soybean domestication started in China around 1700-1000 BC., where it was grown for food, animal feed, medicinal and recreational purposes (Kolapo, 2011). Soybean was introduced into sub-Saharan Africa (SSA) through the east coast by Chinese traders in the nineteenth century. The first documentary evidence of soybean cultivation dated back in 1903 in South Africa (Shurtleff and Aoyagi, 2009). The first commercial cultivation of soybean was documented in 1907 and 1908 in Tanzania and Malawi, respectively (Khojely et al., 2018).

In Sub-Saharan West Africa (SSWA), soybean was believed to have also been introduced in the nineteenth century by the early missionaries. In Nigeria, soybean was first cultivated in 1908 in Benue State (Khojely et al., 2018) as an intercrop in sorghum and maize or as a mixed crop on smallholder farms. In Ghana, there is no precise date on when soybean was introduced. Oral literature or folk literature has it that soybean was introduced into Ghana by the Basel or Presbyterian missionaries around 1907. The missionaries used soybean for green manuring on their farms before its economic values were identified. However, recent work by Shurtleff and Aoyagi (2009) documented 1909, as the earliest date when soybean was seen in Ghana, with England as a possible source. Presently, a significant proportion of the soybean grown in Ghana comes from the Guinea and Sudan Savanna zones of Northern Ghana, and the Forest-Savanna Transitional zone of the Brong Ahafo and the Ashanti Regions of Ghana, respectively.

Global Soybean Production

The top five soybean producing countries in the world are the USA, Brazil, Argentina, China and India producing 108.0, 86.8, 53.4, 12.2 and 10.5 million metric ton (MMT),

respectively (Karuga, 2018). The land areas under soybean seed production were 34, 29, 20.3, and 235 million ha by the USA, Brazil, Argentina, and China respectively.

Globally, the whole continent of Africa produces about 1.26 million tons (MT) of soybean on 1.16 million ha of lands (Kolapo, 2011). In sub-Saharan Africa (SSA), soybean production had increased significantly over the past four decades, starting on a land size of 20,000 ha^{-1} with a grain yield of 13,000 tons in the early 1970s to about 1.5 million ha with a grain yield of about of 2.3 million tons (MT) in 2016 (Khojely et al., 2018). The top five soybean producers in SSA as at 2016 are South Africa, Zambia, Nigeria, Zimbabwe and Uganda (Appendix Figure A.1) (Kapo et al. 2011; Khojely et al., 2018). Other countries with sizeable commercial production and with the possibility of expansion include Malawi, Sudan and Ethiopia (Khojely et al., 2018). In sub-Saharan West Africa (SSWA) Nigeria and Ghana are the two leading producers of soybean.

Uses and Economic Benefits of Soybean in Africa

Soybean as grain legume has numerous benefits that range from economics, health, food and nutrition, livestock feeds, industrial and soil quality improvement. Soybean grain is an important source of protein (40 %), fat and oil (20%), vitamins and minerals. Soybean seeds also contain essential amino acids such as cysteine and methionine. It is usually referred to as the golden bean due to its numerous benefits especially in the area of nutrition (Kolapo, 2011). Thus soybean can be used to reduce malnutrition and food insecurity in areas where access to animal protein is limited. Soybean seeds are also used for making vegetable oil for human and animal consumption, and for industrial processing of food (Kolapo, 2011). Other refined soybean seeds products include margarine and shortening (Kolapo, 2011). In industry, soybean is used to produce lubricating oil, detergents, and toiletries.

In the livestock sector, soybean is also used in preparing feed for livestock's, especially poultry. Ground soybean is mixed with other poultry feeds to provide protein, vitamins, and minerals for the birds.

On health grounds, the consumption of soy foods will reduce malnutrition and boosted the immune systems of children, the aged, the sick and HIV/AIDS-infected patients (Kolapo, 2011). Eating soybean meal can help reduce obesity and coronary heart disease (Kolapo, 2011). Available evidence suggests that soy foods can help minimize bone loss that naturally happens after menopause in women (Kolapo, 2011).

In Ghana, soybean is used for preparing various local dishes including, soy flour, soy milk, soy ice-cream, soy-yogurts, soy-biscuits, soy-kebabs (Tofu), fermented-soy flour (dawadawa) and soy-fortified porridge, "soy-fortified banku" (maize meal), soy-fortified soup, "soy-fortified tubani" (steam cowpea flour meal) and "koose" (Soy-fortified –cowpea cake) (Abaidoo et al. 2014; Khojely et al., 2018). Adding value to soybean through soybean processing help create employment especially for women. For instance, in northern Ghana, women prepare various soy-products and sell commercially, thus generating income for the family and helping to empower women economically (Khojely et al., 2018).

Commercial Bradyrhizobium Inoculant Impact on Soybean Grain Yield

The impact of commercial *Bradyrhizobium* inoculant on soybean is well documented, although results varied geographically. In Canada, inoculation with different *Bradyrhizobium japonicum* strain improved nodulation, shoot dry matter, shoot nitrogen and grain yield than uninoculated control (Zhang et al., 2002). Among the *Bradyrhizobium japonicum*, strain USDA 30 and 31 outperformed 532c respectively (Zhang et al., 2002). In Kenya, greenhouse study revealed that inoculation with commercial inoculants such as Legumefix, Vault LvL and

1495MAR enhanced shoot yield in soybean (Thuita et al., 2012). In Ethiopia, inoculation with commercial *Bradyrhizobia japonicum* strain USDA 110 increased soybean yield with location by *Bradyrhizobia japonicum* strain specificity. (Muleta et al., 2017).

Similarly, Pulver et al. (1982) observed that inoculation of promiscuous soybean cultivars with *Bradyrhizobium japonicum* resulted in increase in nodule dry weight, nodule number, shoot growth, and seed yield compared to their host-specific counterpart from the USA in Nigeria. Nonetheless, a recent study on comparative analysis of promiscuous soybean cultivar across different locations in SSA indicates that inoculation of promiscuous soybean variety does not always improve grain yield (van Heerwaarden et al., 2018). Gyogluu et al. (2016) observed inoculation of different promiscuous soybean genotypes with *Bradyrhizobium japonicum* strain WB74 did not consistently increase grain yield in South Africa even though some varieties exhibited a variable response to the inoculation. This is an indication of genotype by *Bradyrhizobium japonicum* strain specificity. Likewise, variable grain yield was observed for promiscuous soybean cultivar inoculated with commercial *Bradyrhizobium* inoculant due to seasonal effects in Tanzania (Chowdhury et al., 1983). These results suggest that the efficacy of commercial *Bradyrhizobium* inoculant can significantly be influenced by the seasonal pattern.

In Northern Ghana, inoculation of soybean with commercial *Bradyrhizobium* inoculant is a new technology which started about 8-yrs ago. The commercial inoculants were imported mainly from the UK, Kenya and later Nigeria. Results emerging from soybean inoculation studies indicated high variability in terms of nodulation and yield (shoot and grain) across the soybean production areas in Northern Ghana (Giller, 2010). In some cases, inoculation improved grain yield. Ulzen et al. (2016), reported a significant increase in grain yield of promiscuous soybean variety (*Glycine max, var Jenguma*) when inoculated with two different commercial

inoculants (Legumefix and Biofix) in the Guinea Savanna of Northern Ghana. In other locations, inoculation did not necessarily increase grain yield. Rather, there were challenges associated with non-responsive soils (Giller, 2010). Further research is therefore needed to (1) understand the inconsistent grain yield performance due to inoculation, and (2) to evaluate the non-responsiveness of soils to inoculation.

Estimating the Qquantity of Nitrogen Contributed by Soybean

Soybean is an excellent grain legume to introduce into sustainable intensification systems due to its ability to fix atmospheric N through a symbiotic association with a soil bacteria called *Rhizobium*. Soybean requires host specific Rhizobium called *Bradyrhizobium japonicum* to enhance nodulation, N_2fixation, biomass production and grain yield. The amount of N fixed or the residual N balance by soybean is affected by genotype (cultivar and maturity grouping), the rhizobial strains, the environment and the management (Dwivedi et al., 2015). The amount of N fixed by soybean can either eliminate the need to apply mineral N fertilizer or reduce the quantity of mineral N fertilizer to apply to the subsequent crop (Sinclair et al., 2014). The amount of N-fixed or the residual N balance contributed by soybean is generally variable. While some authors reported a net positive residual N balance for soybean (Sanginga et al., 2002; Ennin et al., 2004), others documented net negative residual N balance (Ogoke et al. 2003; Osunde et al. 2003a; and Singh et al. 2003). Schipanski et al. (2010) documented about 20-30 kg N ha^{-1} as the residual N contributed by soybean for the succeeding crop in the USA. Singh et al. (2003) reported net negatives (-4 to -8 kg N ha^{-1}) residual N balance for early maturing soybean lines and net positive (+ 4.0 kg N ha^{-1}) residual N balance for medium to late maturing soybeans in the Guinea Savanna zone of Northern Nigeria. Sanginga et al. (2002) observed differences in the amount of residual N balance contributed by soybean for the subsequent crop based on three

3 different approaches in the Guinea Savanna zone of Nigeria. They document -8 to + 43 kg N ha^{-1} for whole plants based on the N difference methods, 10.6 to 24.3 kg N ha^{-1} using indirect ^{15}N labeling method and 16 to 23 kg N ha^{-1} using the N difference methods. Osunde et al. (2003) found no treatment difference in the net residual N balance contributed by soybean cultivar, inoculation, and location in the Southern Guinea Savanna of Nigeria. Nonetheless, the net residual N balance ranges from ~ -36 kg N ha^{-1} to 66 kg N ha^{-1} when residues were retained, and - ~ 63 kg N ha^{-1} to ~ 99.4 kg N ha^{-1} when residues were completely removed in the Guinea Savanna Zone of Nigeria (Osunde et al., 2003). Casky et al. (2007) also reported about 20 to 45 kg N ha^{-1} in the Guinea Savanna of Nigerian.

In Northern Ghana, scanty information is available on the amount of N fixed and the residual N balance contributed by soybean. In the northern Guinea Savanna, Kaleem (1990) documented ~ 195 kg N ha^{-1} as the amount of N fixed by soybean as a monocrop. Nonetheless, the residual N balance was net negative of -36 kg N ha^{-1}. In intercrop systems, soybean fixed ~ 118 kg N ha^{-1} and its residual N balance was net negative of -56 kg N ha^{-1}. Results suggested that soybean contributed to the depletion of soil available N. Greater N loss was associated with soybean in intercropping systems compared to the mono (sole) cropping systems. Previous work by Pule-Meulenberg et al. (2011) at Wa in the Upper West Region of Ghana also recorded about 100 kg N ha^{-1} from two uninoculated TGX soybean cultivars (TGx1445-3E and Salintuya-1) and with about 60 % of their N derived through symbiotic fixation. Nonetheless, results from this study were inconclusive.

The amount of N contributed by soybean can significantly be altered by the agro-ecological zone where the crop is cultivated. Recently, Kermah et al. (2018) reported on the residual N balance for different grain legumes grown on fertile soils in both the Sudan Savanna

Zone (SSZ) and the Guinea Savanna Zone (GSZ) of Northern Ghana. In the Sudan Savanna Zone (SSZ), the residual N balance for soybean after grain yield export was positive (+) 9 kg N ha^{-1} (Kermah et al., 2018). However in the Guinea Savanna Zone (GSZ), the residual N balance after grain yield export was + 2 kg N ha^{-1} for soybean (Kermah et al., 2018). It should also be noted, that the retention of soybean residues also improves soil structure and soil aggregation, and minimizes loss of nutrients through soil erosion.

Nonetheless, in-depth information is needed on the amount N fixed by recently released soybean varieties and their residual N balance. Additionally, information on the extent to which commercial inoculants affect nitrogen (N) fixation and residual N balance in soybean are not well documented in Northern Ghana cropping systems.

Rhizosphere Microbial Community Affected Ssoybean Cultivar Selection and Inoculation

The rhizosphere is the volume of the soil under the influence of the plant roots or an area of the soil surrounding the rhizoplane. The common group of microorganisms found in the rhizosphere consists of bacteria, fungi, and Actinomycetes (Cavaglieri et al., 2009). Microorganisms in the rhizosphere can induce positive (beneficial) or negative (harmful) effects on plant health. Microorganisms that induced beneficial (positive) interaction with plant roots are useful for enhancing sustainable agriculture. Some beneficial microbial interactions include: (1) arbuscular mycorrhizal fungi (AMF) interacting with plant roots to enhance the uptake of phosphorus and water from the soil, (2) rhizobium in symbiotic association with the roots of legumes contributing to nodulation and nitrogen fixation, and (3) plant growth promoting rhizobacteria (PGPR) such as *Pseudomonas, Enterobacter, and Arthrobacter* inhabiting the rhizosphere of plants and stimulating direct and indirect beneficial effects on roots (Burdman et

al., 2000; Cocking, 2003). The direct benefits of PGPR include promoting plant growth by providing nutrients and hormones. The indirect benefits consist of stimulating greater resistance to diseases (suppression of plant disease) and triggering induced systematic resistance (a form of defense). Despite the numerous benefits of PGPR, there is generally inadequate information on the rhizosphere PGPR ecology (Lambers et al., 2009; Singh et al., 2011; Lagos et al. 2015) of legumes like soybean

Temporal selection pressure or external stress (climatic and edaphic factors) can alter the microbial community structure. The key indicators (variables) that induce changes in the soil microbial community structure include soil structure, soil texture, soil pH, mineral nutrients, soil organic carbon, total N and management history (Marschner et al., 2001). The application of mineral N fertilizer affected the diversity of the microbial community due to stimulated change in the composition of plant and soil (Santos-González et al., 2011; Ramakrishnan et al., 2017). Previous work of Buyer et al. (2002) indicated that soil type induces greater influence on microbial community structure than plant community. Recent work by Santos-González et al. (2011) also corroborated the findings of Buyer et al. (2002) that soil types induce greater influence on the soil microbial community structure than crop cultivar selection. On the contrary, Marschner et al. (2001) conclusively stated that the influence of soil types on microbial community structure is still a difficult question, given that no general principles had been developed yet. Regarding the sources of the soil, Buyer et al. (2002) observed no distinct difference in the microbial community structure in the rhizosphere and the bulk soil because both have similar microbial community structure, mainly slow-growing heterotrophs and oligotrophs.

Crop genotype or cultivar selection affect the soil microbial community. Wang et al. (2014) observed that soybean cultivar selection significantly affected rhizosphere bacteria.

Similarly, Cavaglieri et al. (2009) reported that plant growth induced significant changes in the soil microbial community structure. Ramakrishnan et al. (2017) observed that inoculation with microbial inoculant altered the rhizosphere microbial community structure of chicken pea with the changes due to variety specificity. Trabelsi and Mhamdi (2013) also established that inoculation with microbial inoculant stimulated greater changes in the microbial population and composition of the taxonomic groups. Nonetheless, in sub-Saharan West Africa the extent to which soybean cultivar selection alter or affect the soil microbial community structure is not well investigated or documented.

Cropping systems or crop management induced significant changes in the soil microbial communities. Soybean monoculture tends to favor the dominance of fungi in the rhizosphere microbial community structure (Liu and Herbert, 2002; Chen et al. 2006; Wang et al., 2012). This is because soybean monoculture tends to stimulate isoflavones production which is a substrate for fungi (Wang et al., 2012). Nonetheless, soybean monoculture tends to increases cyst nematode and pathogenic fungi which decrease nodulation and nitrogen fixation, and lower N mineralization compared to soybean in crop rotation (Liu and Herbert, 2002; Ruan et al., 2003; Wang et al., 2012). This partly explains the reason for reduced nitrogen availability in continuous soybean monoculture systems (Wang et al., 2012).

Crop rotation increases the diversity of bacterial in the rhizosphere (Castro-Sowinski et al., 2007). Vargas Gil et al. (2011) also observed that the adoption of crop rotation significantly increased the microbial community structure and the total microbial biomass estimated by phospholipids fatty acids (PLFAs) analysis. Phospholipids fatty acids are (PLFAs) are found in intact cell (live cells) and linked to a specific component of the cell membrane. Changes in PLFAs biomarkers or profiles are a useful indicator to monitor changes in the whole microbial

community structure (Yao and Wu, 2010). Phospholipids fatty acids indices such as bacterial: fungal ratio is an important index that is used to represent changes in microbial community structures due to changes in management or environmental stress (Yao and Wu, 2010). Higher bacterial:fungal (F:B) is associated with improving (higher) soil fertility or soil quality while lower bacterial: fungal (F:B) is associated with reducing or declining soil fertility or soil quality (Liu and Herbert, 2002; Yao and Wu, 2010).

In Northern Ghana, research on the extent to which crop genotype, soil type, crop development, and managements (including commercial *Bradyrhizobium* inoculant) affect the microbial community structure of the rhizosphere and the bulk soil is not well investigated. In-depth knowledge or understanding of the roles soil microbial communities play in the production of crops like soybean, cowpea, groundnut, sorghum, maize would help in developing sustainable crop production techniques.

Factors that Contribute to Failure of Inoculation

There are many success stories on inoculation of soybean *(Glycine max* (L.) Merrill) with commercial *Bradyrhizobium* inoculants. For instance, in sub-Saharan Africa, inoculation of soybean with *Bradyrhizobium japonicum* was reported to increase yield from 500 to 1500 kg ha^{-1} (Cummings and Andrews, 2003). Nonetheless, inoculation can sometimes fail due to lack of persistence of the *Rhizobium* strain in the inoculant. The persistence of inoculant *Bradyrhizobium japonicum strain* is an important factor that determines the success of inoculation. Therefore the frequent lack of persistence may be due to (a) poor quality of inoculant with low viability (b) low competitiveness of an inoculant *Bradyrhizobium japonicum* strain compared to the native *Rhizobia,* (c) inability of an inoculant *Bradyrhizobium japonicum* strain to withstand environmental stress (tolerate the physical and chemical conditions in the

soil) as documented by Catroux et al.(2001) and Cummings and Andrews (2003). These constraints need to be diagnosed and corrected to ensure the success of inoculation.

The success of symbiosis depends on the ability of the *Rhizobia* to show high (a) competitiveness –*the ability of the strain to compete against other strains* (b) infectiveness - *the ability to form nodules in a stressed environment*, and (c) effectiveness- *the ability to fix nitrogen* (Catroux et al., 2001; Cummings, 2005). Nonetheless, such traits are exchanged for (a) plant selectiveness or promiscuity, (b) *Rhizobium* ability to survive in soil and to outcompete with other *Rhizobium* strains (c) infection of plants and fixing nitrogen (Dwivedi et al., 2015). Therefore the future success of biological nitrogen fixation should focus on improving the host plant, the *Rhizobia* and the crop environment (Dwivedi et al., 2015). This should begin with the host plant. For instance, in the case of soybeans in sub-Saharan Africa (SSA), low soil N fertility and high cost of N fertilizers should induce plant breeders to develop and select cultivars that can (1) grow under low N conditions, and also (2) respond to inoculation with an elite *Rhizobium strains* thereby enhancing nitrogen fixation (Dwivedi et al., 2015). For the *Rhizobia*, previous studies had revealed the possibility of selecting *Rhizobia* strains with higher tolerance to environmental stresses such as higher temperature (Dwivedi et al., 2015).

Environmental stresses are also capable of altering the symbiotic performance or the interaction of the *Rhizobium* with the host plant (Shiro et al., 2012; Hungria and Kaschuk, 2014). Low pH and too low or too high temperatures affect the efficacy of an elite *Rhizobium* in an inoculant. In sub-Saharan Africa, about 70% of the soils used for crops production are acidic (Cumming and Andrews, 2003). As pH decreases, toxic metal ions, particularly Al^{3+} become soluble in soil solution. Increased Al^{3+} in soil solution reduces the availability of calcium and phosphorus resulting in the inhibition of nodulation (Cumming and Andrews, 2003; Dwivedi et

al., 2015). Furthermore, Hungria and Vargas (2000) also stated that water stress or moisture deficit, high temperature, and low pH are the principal factors for failure of nodulation and low N_2 fixation. For instance, in Brazil, it was observed that commercial inoculants with *Rhizobium leguminosarium bv phaseoli* (SEMIA 4064) tend to lose their ability to fix N under extreme environmental stress conditions (Cumming, 2005). In such a situation, a holistic approach needs to be adopted. For example, in areas where inoculation failed or where there are challenges with the production and distribution of inoculants, plants capability in establishing symbiosis with indigenous *Rhizobia* should be improved (Mpepereki et al., 2000). This strategy was employed in developing or breeding the promiscuous nodulating soybean cultivars (tropical *Glycine max* cross; TGX) in sub-Saharan Africa (Abaidoo et al., 2007; Tefera, 2011). These cultivars are capable of forming nodules with the indigenous *Rhizobium spp*. (Abaidoo et al., 2007; N'cho et al., 2015). Nonetheless, promiscuity does not ensure the appropriate combination of the plant with the most efficient *Rhizobia*, as often documented in common bean (Dwivedi et al., 2015).

The inoculum (*Rhizobium* or *Bradyrhizobium*) in commercial inoculants is regarded as ineffective if it fails to stimulate nodules formation. Thus ineffective nodulation is indicative of the inoculum failing to outcompete the indigenous rhizobia. Generally, about ten (10) ml indigenous *Rhizobia* g^{-1} of soil can effectively eliminate any response to inoculation (Thies et al., 1991; Dwivedi et al., 2015). The straightforward approach to poor nodulation in particular environments is by selecting an 'elite' indigenous *Rhizobia* isolate as an inoculum. The 'elite' indigenous *Rhizobia* must (1) be an effective symbiotic partner of the crop genotype; (2) remain viable in the inoculant carrier and (3) be genetically stable (Cumming and Andrews, 2003; Cummings, 2005). This approach was successfully employed in isolating *Rhizobium tropici* from the soils of the Brazilian Cerrados and used as an inoculum for commercial inoculants since

1998 (Dwivedi et al., 2015). The same strategy has been recommended for common bean *(Phaseolus vulgaris L.)* which failed to respond to commercial inoculant in Brazil (Cumming and Andrews, 2003; Dwivedi et al., 2015).

In Ghana, elite indigenous *Rhizobia* strain was isolated and used to inoculate cowpea (Vigna unguiculata (L.) Walp.), observed grain yield was comparable to grain yield from plants fertilized with70 kg N ha^{-1} (Cumming and Andrews, 2003). Recently, elite indigenous *Rhizobia* strains (especially KNUST 1002) which closely relates *Bradyrhizobium yuanmingense* had been identified and isolated for groundnut in Ghana (Osei et al., 2018). Nonetheless, to date, there is a dearth of information available on elite indigenous *Rhizobia* strain for TGX soybean genotype or varieties in Ghana's cropping season.

Given the underlying challenges, further research is needed to investigate the research gaps mentioned above. The present study sought to determine how commercial *Bradyrhizobium japonicum* affect plant growth, symbiotic performance and N contribution of TGX soybean cultivars, as well as soil health and soil microbiome in Northern Ghana cropping systems. The study also sought to determine the effect of previous commercial *Bradyrhizobium japonicum* on the subsequent crops and selected soil health indicators.

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Chapter 3 - Bradyrhizobium Inoculants for Soybean Cultivars in Northern Ghana Farming Systems

Abstract

Bradyrhizobium inoculants enhance N fixation, growth, and yield of grain legumes such as soybean. In Ghana, inoculation of soybean with commercial *Bradyrhizobium* inoculants is a new low-cost technology. A dearth of information exists on how commercial *Bradyrhizobium* inoculants affect growth, nodulation, and yield of soybean in Northern Ghana. A field study (2-yr) was conducted at CSIR-Savanna Agricultural Research Institute's experimental field at Nyankpala, Ghana to assess how commercial inoculants affect growth, nodulation, nitrogen fixation and grain yield of promiscuous nodulating soybean varieties. The experiment was laid as a split-plot design with the main plot factor as tropical soybean (Glycine max crosses (TGX) varieties; Jenguma (TGX1448-2E), Afayak (TGX1834-5E), and Songda (TGX 1445-3E). The subplot factor consisted of three commercial Bradyrhizobium inoculants, namely Biofix (USDA strain 110), NoduMax (USDA strain 110) and Legumefix (USDA 532c) plus uninoculated control. Nodulation pattern and shoot biomass yield were assessed at vegetative (V8, 8-leaf stage), full bloom (R2), beginning to pod (R3), full pod (R4) and full seed (R6) stages respectively, and N fixation and grain yield at maturity. Inoculation with Biofix and NoduMax increased nodulation (nodule number and dry nodule mass) on the root crown, taproot and side root compared to the uninoculated control. Nodulation on the upper (5 cm) root segments and the whole root systems were affected by the interaction of inoculants and growth stage. Nodulation varied with growth stage and peaked at the R4 stage, with pronounced effects associated with Biofix and NoduMax. In 2017, Biofix and NoduMax inoculants produced greater shoot dry matter compared to the other treatments. Similarly, enhanced grain yields up to 30% were achieved with Biofix and NoduMax compared to the uninoculated control in both 2016 and

2017. For the soybean variety effect, Jenguma and Afayak had greater number of nodules on the crown, taproot and side roots than Songda. Afayak and Songda produced greater shoot biomass than Jenguma in 2017. Averagely (2-yr), Afayak produced greater grain yields than Jenguma and Songda. Biofix and NoduMax seem to be the most promising commercial inoculants to enhance nodulation, biomass production, and grain yield. Afayak has the greatest potential for dissemination due to its superior nodulation, shoot dry matter and grain yield.

Introduction

Soybean (*Glycine max L. Merr*.) is an important grain legume with high oil (20%) and protein (40%) content making it an important food source for humans and livestock and poultry. Soybean can reduce malnutrition in areas that have limited access to animal protein. Soybean fixes N by a symbiotic association with *Bradyrhizobium japonicum* (Abaidoo et al., 2007; Grönemeyer et al., 2014). Other bacteria capable of forming effective nodules on soybean include *Bradyrhizobium elkanii* (Kuykendall et al., 1992), *Bradyrhizobium liaoningense* (Xu et al., 1995) and *Sinorhizobium fredii* (Chen et al., 1988). Inoculating soybean with these bacteria is necessary in soils where: (1) soybean is newly introduced, (2) there is no history on the availability of native rhizobia strains or the native population is low (Catroux et al., 2001; Laranjo et al., 2014); (3) environmental conditions are unfavorable or hostile to rhizobia survival (Catroux et al., 2001; Lindström et al., 2010; Laranjo et al., 2014); or (4) when an introduced *Badyrhizobium* strain losses its infectiveness or effectiveness.

Inoculants containing effective *Bradyrhizobium* strains are used to stimulate nodulation, biological N fixation, and enhance soubean yield. Inoculants are termed effective or efficient, if the introduced rhizobia strains: (1) are more competitive in nodulation, nodule occupancy, and N fixation than the native soil rhizobia population (Laranjo et al., 2014); (2) regulates the

nodulation process using the recommended rates (Somasegaran and Hoben, 1994; Laranjo et al., 2014); or (3) remains persistent in the soil over time and nodulates specific legume genotypes (host specific) (Catroux et al., 2001; Laranjo et al., 2014). Nonetheless, the ineffectiveness of introduced rhizobia strains to nodulate a host legume is due to the presence and competitiveness of native rhizobia. For instance, Laranjo et al. (2014) observed that rhizobia strains of commercial inoculants dominated nodulation 5-15 years after the first application. On the contrary, Kamicker and Brill (1987) reported that native rhizobia were responsible for 98 % of the nodulation in soybean compared to the commercial inoculant strain. Several authors have quantified the difference between infectivity of introduced rhizobia strain and the native rhizobia strain (Weaver and Frederick, 1972; Kamicker and Brill, 1987; Abaidoo et al., 2007).

Field evaluation of inoculant efficacy, *Bradyrhizobium* strain compatibility, and inoculant technology is generally based on an assessment of symbiosis parameters including N_2 fixation and nodulation (number nodule and weight, nodule pattern and distribution on root) (Cardoso et al., 2009). The symbiotic compatibility of *Rhizobium or Bradyrhizobium* strains can also be assessed via the relative effectiveness or efficiency of initiating nodules (Bhuvaneswari et al., 1988). Efficiency is defined as the number of *Rhizobium* in an inoculum to achieve a given number of the nodules in the first susceptible region of the root (Bhuvaneswari et al., 1988). The effectiveness of the *Rhizobium or Bradyrhizobium* strain in initiating nodulation indicates the compatibility of the two symbionts (Bhuvaneswari et al., 1988). The location of the nodules on root indicates when the nodule initiation began after inoculation (Bhuvaneswari et al., 1988). *Rhizobium* inoculation increased nodule formation on the crown root compared to secondary roots in peanut, soybean and common bean (Cardoso et al., 2009). Kamicker and Brill (1987) observed increased nodulation on the lower root segments (secondary root) of soybean when

Bradyrhizobium inoculation was done vertically compared to in-furrow seed inoculation. They also observed that nodules on the tap root were the first to form and to senesce while nodules on the lateral root formed latter. Thus nodules on the lateral root may tend to be younger with perhaps more N fixing ability compared to the old tap roots nodules (Kamicker and Brill, 1987). As far as we are aware, no detail work had been done on assessing commercial *Bradyrhizobium* inoculant effect on root system nodulation pattern and characterization (positioning) for soybean cultivars in sub-Saharan Africa (SSA).

The performance of an introduced *Bradyrhizobium* strain can be altered by both climatic and edaphic factors. *Bradyrhizobium* strain tends to maintain superior performance if used in similar environmental conditions from which they were isolated (Zhang et al., 2003). For instance, Hume and Shelp (1990) observed that *Bradyrhizobium* strain 532c produced greater grain yield compared to other *Bradyrhizobium* strains (USDA110, 142, and 143) when evaluated in the temperate environment of Canada. Other researchers also observed that inoculating soybean with *Bradyrhizobium* strain USDA 30 and 31 increased nodulation, shoot nitrogen content and grain yield compared to *Bradyrhizobium* strain 532c in Canada (Zhang et al., 2002, 2003). Ulzen et al. (2016) found no significant differences in nodulation, shoot biomass and grain yield of promiscuous nodulating soybean when inoculated with two different *Bradyrhizobium* strains 532c (Legumefix) and USDA 110 (Biofix) in the Guinea Savana Zone of Northern Ghana. However, Ulzen et al. (2016) suggested that inoculation was necessary for enhanced nodulation, shoot biomass and grain yield of soybean. These contrasting observations necessitate the need for further evaluation of commercial inoculant with different *Bradyrhizobium* strains on soybean, especially in the tropics.

In Ghana and other West Africa countries, soybeans are not inoculated with commercial inoculants, although soils do not contain the appropriate *Bradyrhizobium japonicum* required for soybean production. The soybean varieties grown are those designated as tropical *Glycine max* crosses (TGX crosses), and commonly referred to as promiscuous nodulating soybean. These soybean genotypes were bred by IITA (International Institute for Tropical Agriculture), to effectively nodulate with the native *Bradyrhizobium* spp (Abaidoo et al., 2007; N'cho et al., 2015). Agronomic evaluation of TGX soybean lines or varieties to commercial inoculants had yielded variable response on nodulation, grain yield and N-fixation across different locations in sub-Saharan Africa (Pulver et al., 1982; Sanginga et al., 1997a; Osunde et al., 2003; Okogun and Sanginga, 2003; Chianu et al., 2011; Gyogluu et al., 2016). These results necessitate the need to assess the performance of TGX soybean genotypes and their responses to *Bradyrhizobium* inoculation on site-specific based.

In Northern Ghana cropping systems, a paucity of information exists on how commercial inoculant affect, nodulations, shoot biomass and N-fixation in modern TGX soybean varieties (Songda, Afayak, Soung-Pungun). The present study seeks to assess how *Bradyrhizobium japonicum* inoculation impacts nodulation pattern, plant performance, nitrogen fixation and grain yield of recent TGX soybean varieties in farming systems of the Northern Guinea Savanna zone of Ghana. We hypothesized that inoculation would increase nodulation, nitrogen fixation, shoot biomass and grain yield in promiscuous soybean cultivar, and we also expected cultivar by commercial inoculant type interaction.

Materials and Methods

Study Site

A 2-yr field study was conducted at the CSIR-Savanna Agricultural (SARI) Research Institute (SARI) research field located in Nyankapala (N 09.39253º W 001.00228º 189 m and N 09.39172º W 001.00286º 188 m) in the Northern Region of Ghana during the 2016 and 2017 cropping seasons. The area has a monoidal rainfall pattern which lasts for a period of 5-6 months annually with peak rainfall occurring in July to September.The 2016 site was previously cropped to maize for three consecutive years with mineral fertilizer. The 2017 site was previously cropped to cowpea in 2015 and maize in 2016 where the mineral fertilizer was applied. After harvest, the site was left fallow, and crop residues were retained on the fields.

Baseline soil samples were collected from 0-20 cm, air dried and passed through 2 mm sieve before the establishment of the field trial. The soil was as classified as a Typic-plinthic Paleustalf according to the U.S. Soil Taxonomy. The description and soil properties at the trial sites are presented in Table 3.1.

The field was disk plowed, harrowed and manually leveled using hoes. Ridges at 50 cm part were manually constructed using hoes. Each experimental unit or plot was 4 x 4 m^2 with a total of eight ridges.

Experimental Design

The experimental design was a split-plot with a randomized complete block design (RCBD). The main plot consisted of three promiscuous soybean cultivars (Tropical *Glycine max* crosses, TGX), Jenguma (TGX1448-2E) Afayak (TGX1834-5E), and Songda (TGX 1445-3E). The subplot consisted of three different commercial inoculants: Biofix, Legumefix, and NoduMax in addition to an uninoculated control. The treatments were replicated four times.

Source of Seeds

Soybean seeds were acquired from the soybean breeding division of SARI, Nyankapala. All soybean varieties were resistant to rust disease (*Phakopsora pachyrhizi and Phakopsora meibomiae*) and with a maturity period of 110-118 days. Jenguma and Afayak were non-shattering cultivars while Songda was a shattering cultivar (can be as high as 20% if not harvested early). Afayak (TGX1834-5E) and Songda (TGX 1445-3E) were released in 2012 and are an excellent trap-crop for Striga (*Striga hermonthica*), parasitic weed (Denwar and Wohor, 2012). Jenguma (TGX1448-2E) was an existing variety released in 2003, hence will be referred to as the traditional variety. It is also an excellent trap-crop for Striga.

Source of Inoculants

The inoculants were obtained from commercial sources and were peat based. Legumefix contained *Bradyrhizobium japonicum* strain 532c (Thuita et al., 2018) and was obtained from Legume Technology Ltd., UK. Biofix was obtained from MEA fertilizer in Nairobi, Kenya and contained *Bradyrhizobium japonicum* strain USDA 110 (Ulzen et al., 2016; Thuita et al., 2018). NoduMax also contained *Bradyrhizobium japonicum* strain USDA 110 and was obtained from International Institutes for Tropical Agriculture (IITA), Ibadan, Nigeria. Both Biofix and NoduMax contained a minimum of 1×10^9 viable cells g^{-1} of inoculant while Legume fixes contained a minimum of 2×10^9 viable cells g^{-1} of inoculant according to the manufacturer.

The Bradyrhizobia population in the commercial inoculant was enumerated with yeast-mannitol agar (YMA) with congo red (CR) using ten-fold serial dilution techniques (Somasegaran and Hoben, 1994). The estimated Bradyrhizobium population was 1.8×10^8 CFU g^{-1} for Legumefix and 1.8×10^9 CFU g^{-1} for Biofix.

The native soil rhizobia population was also estimated with yeast-mannitol agar (YMA) with congo red (CR) using ten-fold serial dilution technique and the result was expressed in colony forming unit (CFU) (Somasegaran and Hoben, 1994). This was followed up with the most probable number technique (MPN) using growth pouches in a controlled environment room as reported by Somasegaran and Hoben (1994). The native rhizobia population estimated by were 5. 8 x 10^2 cells g^{-1} soil and 5. 0 x 10^2 CFU g^{-1} soil respectively.

Inoculation and Sowing of Seeds

Inoculation of soybean seed was done following the procedure of Hungria et al. (2006). Briefly, 10 g of the inoculant was added to 1 kg of seed. A 10% gum arabic (wt/vol) solution was used to increase adhesion of the peat, at 300 mL 15 kg^{-1} seed. Seed inoculation was done at sowing and comprised of applying the gum arabic (*Acacia Senegal*) solution to the seeds followed by the peat inoculant and mixing after that seeds were air-dried under shade for 15-20 mins.

Seeds were manually sown on ridges at 50 cm inter-ridge (row) distance and 10 cm inter-plant distance, and ~ 5cm deep. Sowing date was 4 July and 3 July in 2016 and 2017, respectively. To prevent contamination, non-inoculated treatments were planted first before inoculated treatments. Four (4) seeds were sown per hill but thinned to two plants at 13 days after sowing (DAS). Replanting was also done eight days after seedling emergence. Maize (*Zea mays* L.), was also planted along with the soybean as a reference crop at 50 cm inter-ridge (row) distance and 60 cm inter-plant distance and ~ 5cm deep. *Zea mays* var Abrohema, and *Zea mays* var Wang-data were the hybrid maize varieties planted in 2016 and 2017 respectively. Both have a maturity period of 100-118 days. Plant establishment was assessed 26 days after sowing (DAS). The entire plant population per plot was counted and recorded.

Agronomic Management

Fifteen days after sowing (DAS), K and P were applied at a rate of 30 kg K ha^{-1} and 30 kg P ha^{-1} as Muriate of potash (MoP) and Triple Super Phosphate (TSP), respectively. The fertilizer was banded 3-5 cm from the plants at a depth of 5 cm deep on the ridges. Pre-emergence herbicide, Basagran (with the active ingredient Sodium salt of Bentazon) was applied at a rate of 1 L ha^{-1} after sowing. Subsequent, weed control was done manually using a hoe at 4, 7, and 10 weeks after sowing (WAS). A different set of hoes were assigned to each treatment to prevent cross-contamination.

Sampling and Data Collection

Biomass Sampling

Sampling was done according to the development stages of soybean as reported by Fehr et al. (1971) and Fehr and Caviness (1977). Briefly, plant biomass was sampled at 33 (6/8/16), 50 (23/8/16), 73 (15/9/16), 87 (29/9/16), and 108 (20/10/16) days after sowing (DAS) representing V8 (8-leaf), R2 (full flower), R4 (full pod), R6 (pod-fill or seed-fill) and R8 (seed-maturity) in 2016. In 2017, plant biomass was sampled at 35 (9/8/17), 51 (23/8/17), 64 (5/9/17), 79 (20/9/17), and 88 (29/9/17) days after sowing (DAS) representing V8 (8-leaf), R2 (full flower), R3 (beginning to pod), R4 (full pod), and R6 (Pod-fill or Seed-fill). At each sampling stage, 10 plants were randomly sampled per plot, avoiding the areas marked for grain harvest (Hungria et al., 2006). For sampling, plants were uprooted carefully with a spade avoiding chopping off the roots. Soils attached to the roots were gently shaken off and roots with nodules were detached from the plants and bagged separately. Fallen nodules were also collected. In the laboratory, shoot biomass was washed with water to remove soil particles and air dried for about 20 mins in a cool place. Shoot biomass was then weighed and partitioned into leaves (leaf + petioles) and stems. Both stems and leaf biomass were weighed and dried in a forced-air oven at

70 ^{o}C for 48 hrs. Shoot biomass (leaf + stem) was expressed as shoot dry matter (g plant^{-1}). In instances, where plants had pods, the pods were dettached separately, counted, weighed, and oven-dried at 70 ^{o}C and then re-weighed. Root biomass (root with nodules) was placed on top of a 1 mm sieve and dipped into water to remove any soil particles. Fallen nodules were captured on top of the sieve. Root plus nodules were air dried for 15-20 mins in the laboratory. Nodules were detached after scoring. Root biomass was oven dried at 70 ^{o}C for 48 hrs and weighed.

Nodulation

Nodule position on the roots (nodules on the crown, taproot, lateral (side) root, and root tip) and nodule distribution pattern (nodules at the upper 5 cm root section and lower 5 cm root section), nodule scoring, and nodule dry weight (nodule mass) were assessed following the procedure reported by Kamicker and Brill (1987) and Cardoso et al. (2009). Briefly, ten roots with the nodules were partitioned into two halves. The first half (5 plant roots) was used for assessing nodule position on roots. The total number of nodules on a whole root was the sum of the nodules from the different locations or position of the roots.

The other five plant roots were used for assessment of nodule distribution pattern. The root was partitioned into two segments namely upper and lower. The nodules were counted, detached from the roots, weighed, and oven dried at 60^{o}C for 48 hrs and then re-weighed. Nodules detached during sampling were collected and referred to as dropped nodules. The sum of nodules on the two root segments constitutes the entire root nodulation pattern.

Reference plant sampling

Maize (*Zea mays* L.), the reference plant for assessing N-fixation was sampled following the procedure reported by Pule-Meulenberg et al. (2011) and Gyogluu et al. (2016). Briefly, ten maize plants per plot were sampled at the same sampling time for soybean. Roots were detached

from the plants. Both shoot and root were washed, air dried for 30 mins, weighed, and oven dried at 70 ^{o}C for 48 hrs and then weighed again.

Plant height

Plant height was randomly determined on five (5) plants tagged at harvest.

Yield and Yield Component

Grain yield was determined at full maturity using farmer practice (Osunde et al., 2003). Harvest was restricted to the four central rows of each plot leaving out 0.5 m border area at both ends of each row. Before harvest, the plant population in the harvest area was determined. Plants were then uprooted, bagged and taken to the laboratory. Seeds were threshed manually, cleaned by winnowed and then weighed. A grain sub-sample was oven dried at 70 ^{o}C for 48 hrs and weighed. Grain yield was expressed as Mg ha^{-1}.

Pod load (the number of pod per plant) was determined on 20 plants randomly collected from the harvest area. The number of pods was counted and then expressed as pod plant^{-1}. Pod dry weight was also determined on the same 20 plants sampled for pod load. Pods were detached, weighed, and oven dried at 70^{o}C for 48 hrs, and then re-weighed. Pod dry weight was expressed as Mg ha^{-1}. Haulm dry weight (dry stover) was determined from 20 plants, oven dried at 70 ^{o}C for 48 hrs, and then re-weighed. Halum dry weight was expressed as Mg ha^{-1}. A thousand seeds were randomly counted, weighed and expressed as 1000 seed weight plant^{-1}.

Harvest index which is the ratio of harvested grain to total shoot dry matter was also determined (Unkovich et al., 2010). Harvest index was used as an estimate of reproductive efficiency.

$$\text{Harvest Index } = \frac{Grain\ yield\ (\text{Mg}\ ha^{-1})}{Shoot\ dry\ matter\ (\text{Mg}\ ha^{-1})}$$

Inoculation response was estimated as;

$$\text{Inoculation responses (\%)} = \frac{(\text{Yield of inoculated plot} - \text{Yield of control plot})}{(\text{Yield of control plot})}\ x\ 100\ \%$$

Estimating N content of plant dry matter and grain yield and N-fixation

The N content of ground leaf, stem, pre-mature pod, root, grain yield, and haulm dry wt was determined by dry combustion using Carlo Erba elemental analyzer EA112 (Thermo Fisher Scientific) following the procedure reported by Zhang et al. (2003). The N content of shoot (leaf + stem), pre-mature pod, root and as well as the whole plant was determined at the R6 stage. The N content of the grain yield and haulm (stover) dry weight was assessed at final harvest. Nitrogen content was estimated by multiplying N concentrations by a dry matter of the different plant parts (Pampana et al., 2018). Grain protein content was estimated by multiplying the grain N concentration by 6.25. Total N fixed was determined by the N difference method at the R6 stage (Zhang et al., 2003; Pampana et al., 2018). Shoot N content was calculated as the leaf + stem N content. Whole plant N was estimated as the sum of shoot N, pre-mature pod N and root N. The shoot and root N content of the reference plant (maize) was also estimated. The reference plant N content was subtracted from the soybean whole plant N content to determine the amount of total N fixed in kg N ha^{-1}. The N content (N %) of shoot, root, grain, and haulm dry wt was multiplied by their respective dry matter (kg ha^{-1}) and expressed in kg N ha^{-1}

Residual Nitrogen Balance

Residual N balance was estimated using two approaches as reported by Adu-Gyamfi et al. (2007) and Zoundji et al. (2016). For Budget 1: Residual N balance was estimated as = Total N fixed - grain N uptake - Haulm N uptake. In budget 1, we assumed that both grain and haulm

(dry stover) were removed at harvest. For Budget 2: Residual N balance was estimated as = Total N fixed - grain N uptake. In budget 2, we assumed only grain was removed at harvest.

Economic Analysis

Economic return on using commercial inoculant was estimated by the value-cost ratio (VCR) because data on full production costs such as labor, inputs and machinery cost were not available (Xu et al., 2009). The VCR equation was adopted from Xu et al. (2009) and Kihara et al. (2016).

$$\text{VCR} = \frac{\text{Additional soybean yield due to inoculation (kg)} \; x \; \text{soybean price (kg}^{-1})}{\text{Amount of inoculant applied (kg)} \; x \; \text{Price of the Inoculants}}$$

Additional soybean yield due to inoculation, i.e. ((Yield of the inoculated plot – Yield of control plot) x soybean grain price (per kg)). Soybean grain price was 0.40375 USD kg^{-1} and 0.42525 USD kg^{-1} in 2016 and 2017 respectively. Amount of *Bradyrhizobium* inoculant applied (kg) x Price of the *Bradyrhizobium* Inoculant (per kg). Price of the *Bradyrhizobium* Inoculant applied (kg ha^{-1}) was 33.17 USD

A VCR greater than one would imply that commercial inoculant use was profitable if no additional cost was incurred. However, this may not be the case of commercial inoculant use in developing countries like Ghana due to handling, transaction costs, governmental bureaucracy coupled with other associated risks. Therefore, a VCR of 2.0 or greater would generally be considered profitable for farmers to use commercial inoculant as reported by Xu et al. (2009) for mineral fertilizer.

Statistical Analysis

Data were tested for normality using shapiro-wilk test in Sigmaplot 13.0. Data were then analyzed using SAS Proc Mixed Model version 9.4 . Copyright © 2014 SAS Institute Inc., Cary,

NC, USA (SAS Institute, 2014). For analysis of variance (ANOVA), inoculant, variety, and growth stage was considered as fixed effects. Block (replication), and interaction of block and variety were also considered as a random effect. Data on nodulation were fitted using compound symmetry heterogeneity (CSH). Growth stage was fitted as a repeated measure and with slice effect option. Unless otherwise stated significant difference among treatments was declared at at the $\alpha = 0.05$ probability level. Mean separation was done using Fisher's LSD. Before the analysis of variance (ANOVA), covariance structures (UN, AR(1), CS and CSH) were assessed to objectively compare the goodness of fit criteria in the PROC MIXED model. The REML log likelihood (REML logL), Akaike information criterion (AIC), and Schwarz Bayesian criterion (SBC) were all evaluated (Littell et al., 1998a). The AIC and SBC are adjusted versions of REML logL to impose a penalty according to the number of parameters estimated. The smalller the value of SBC, the better the structure. Thus, CSH was used as the covariance structure.

Results

Baseline Soil Analysis and Weather Data

The soil at the study site was inherently low in fertility (Table 3.1). Soil organic C and total N were below 0.4 % and 0.1% respectively. Available P was below the critical level of 20 mg kg^{-1} in both years. Soil available N was low (7.2 mg kg^{-1}) in 2016 and high (22 mg kg^{-1}) in 2017. Soil pH was slightly acidic but within the range required for soybean production in the tropics.

Rainfall and temperature are highly variable (Fig 3.1). Based on the mean temperature, 2016 cropping season (June – Nov) was a bit warmer than in 2017. The temperature in 2017 was similar to the 17-yr average. Rainfall was higher in 2017 than in 2016. Nonetheless, rainfall distribution was generally more uniform in the 2016 cropping season than in 2017. Rainfall distribution during the 2016 and 2017 cropping season (June – Nov) was generally higher than the 17-yr average.

Biomass Yield

Shoot biomass was affected by the interaction of soybean variety and growth stage in both 2016 and 2017 respectively (Fig. 3.2a & b). Shoot biomass increased with growth stage and varied with soybean variety peaking at the R6 stage. In 2016, Afayak and Jenguma produced greater shoot biomass than Songda with a pronounced effect at the R4 stage. At R6-stage, Afayak produced the greatest shoot biomass over the other soybean varieties (Fig. 3.2a & b). In 2017, increased shoot biomass was observed at the R6-stage with Afayak and Songda compared to Jenguma (Fig. 3.2a & b). At the R4-stage, Afayak yielded significantly more shoot biomass compared to Jenguma.

Shoot biomass was also affected by the interaction of inoculant type and growth stage in 2016 (Fig. 3.2c & d). Inoculation increased shoot biomass and varied with growth stage. Shoot biomass peaked at the R6-stage and with NoduMax compared to the other treatments (Fig. 3.2c & d). Legumefix produced the least shoot biomass at the R6 stage. At the R4-stage, Biofix yielded greater shoot biomass than the uninoculated control. In 2017, shoot biomass was not affected by the interaction effect of inoculant type and growth stage (Fig. 3.2c & d). However, Biofix and NoduMax tended to produced greater shoot biomass than the other treatment. Nonetheless, inoculation with Biofix increased shoot biomass compared to uninoculated control and Legumefix (Fig. 3.3).

Nodulation

Crown-root nodules

There were soybean varietal differences in the number of nodules found on the crown root (Table 3.2). Afayak produced a greater number of nodules on the crown root than Jenguma and Songda in both 2016 and 2017 (Table 3.2). There was also an interaction of inoculant and growth stage on the number of nodules formed on the crown root in 2016 (Fig. 3.4). Inoculation stimulated a greater number of nodules on the crown root compared to the uninoculated control and varied with growth stage (Fig. 3.4). At the R2 stage, inoculation with Biofix and NoduMax increased the number of nodules on the crown root compared to the Legumefix. The number of nodules on the crown root peaked at the R4 stage and declined at the R6 stage (Fig. 3.4). In 2017, the number of nodules on the crown root was only affected by the main treatment effects (Table 3.2). Inoculation improved the number of nodules on crown roots by 51% compared to the uninoculated control. NoduMax also produced a greater (22 %) number of nodules on crown root compared to Legumefix. Meanwhile, the number of nodules on crown roots significantly increased with growth stage before declining after the R4 stage (Table 3.2).

Taproot nodules

The number of nodules on the taproot was affected by the interaction of soybean variety and growth stage in 2016 (Table 3.2 and Appendix Fig. B.1). The number of nodules on the taproot increased with growth stage and varied with soybean varieties but declined at the R6 stage (Appendix Fig. B.1). At both the R2 and the R6 growth stage, Afayak produced greater nodules on the taproot than Jenguma and Songda. At the R4 stage, the number of nodules on the taproot was enhanced by Jenguma. In 2017, the number of nodules on the taproot was significantly affected by soybean variety (Table 3.2). Afayak produced a greater number of nodules on the taproot than Jenguma and Songda.

The commercial inoculants increased the number of nodules on taproot compared to the uninoculated control by 33% and 56 % in 2016 and 2017 respectively (Table 3.2). NoduMax also produced a greater number of taproot nodules than Legumefix in 2017. The number of taproot nodules increased with growth stages reaching a maximum at the R4 stage (Table 3.2).

Lateral root nodules

In 2016, nodules on the lateral root were affected by the 3-way interaction of soybean variety, commercial inoculant, and growth stage (Table 3.2; Fig. 3.5a, b & c). Inoculation increased the number of nodules on the lateral root and varied with soybean variety and growth stage. The number of nodules on the lateral root climaxed at the R4 stage with a significant effect associated with Jenguma and all the commercial inoculants (Biofix, Legumefix, and NoduMax) and Afayak with Biofix and Legumefix (Fig. 3.5a, b & c). In 2017, the number of nodules on the lateral roots was affected by the interaction of inoculants and growth stage (Fig. 3.6a). Inoculation with increased the number of nodules on the lateral root compared to the uninoculated control and varied with growth stage (Table 3.2 & Fig. 3.6a). At the R3 stage, NoduMax yielded a greater number of nodules on lateral root compared to Legumefix. There

was a soybean variety main effect on the number of nodules formed on the lateral root in 2017 (Table 3.2). Afayak and Jenguma produced a greater (28%) number of nodules on the lateral root than Songda (Table 3.2).

Whole root position total number of nodules

In 2016, the total number of nodules on the whole root by position was significantly affected by the 3-way interaction of soybean variety, commercial inoculant, and growth stage (Fig. 3.5d, e and f). The total number of nodules on the whole root by position was significantly increased with commercial inoculant and growth stage and varied with soybean variety. At the R4 stage, the total number of nodules on the whole root by position reached a peak with greater differences associated with Jenguma and all the commercial inoculants (Biofix, Legumefix, and NoduMax), and Afayak plus Biofix and Legumefix, and finally with Songda with Biofix and NoduMax compared to the uninoculated control. The total nodule number on the whole root system by position declined after the R4 stage.

In 2017, inoculation significantly improved the total number of nodules on the whole root by position than uninoculated control and varied with growth stage (Fig. 3.6b). At the R3 stage, the number of nodules on the whole root by position reached a maximum, with Biofix and NoduMax demonstrating greater response than Legumefix (Fig. 3.6b). Afayak had an increased number of nodules on the whole root than the other soybean varieties (Table 3.2).

Nodules on the upper 5 cm of the root segment

The number of nodules found on the upper 5 cm of the root segment was affected by soybean variety (Appendix Table B.1). In 2016, Afayak had significantly greater number of nodules on upper (top) 5 cm of the root segment compared to Songda (Appendix Table B.1). In 2017, Afayak had greater numbers of nodules on the upper 5 cm root segment than Jenguma and

Songda. Jenguma also had an increased number of nodules in the upper 5 cm root segment than Songda (Appendix Table B.1).

Further, the number of nodules on the upper 5 cm root segment was significantly affected by the interaction of commercial inoculant and growth stage in 2016 and 2017 (Appendix Table B.1 & Fig. 3.7). Inoculation increased the number of nodules on the upper 5 cm root segment and varied with growth stage compared to the uninoculated control in both years (Fig. 3.7a & b). In 2017, NoduMax had a greater number of nodules on the upper 5 cm root segment compared to the Legumefix. Meanwhile, the number of nodules on the upper 5 cm root segment significantly declined after the R4 growth stage (Fig. 3.7a & b).

Nodules on Lower 5cm root segment

The number of nodules on the lower 5 cm root segment was significantly influenced by the three-way interaction of soybean variety, commercial inoculant, and growth stage in 2016 (Appendix Table B.1 & Appendix Fig. B.2). The number of nodules in the lower 5 cm root segment increased and varied with soybean variety, commercial inoculant and growth stage. In general, Jenguma had the greatest response to commercial inoculant on the number of nodules of the lower 5 cm root compared to the other treatments. In 2017, the number of nodules on the lower 5 cm root segment was affected by the 2-way interaction of growth stage and commercial inoculant, and soybean variety and growth stage (Appendix Table B.1 & Appendix Fig. B.3). Inoculation with commercial inoculant stimulated a greater number of nodules on lower 5 cm root segment and varied with growth stage compared to uninoculated control (Appendix Fig. B.3). Inoculation effect on the number of nodules on the lower 5 cm root segment became obvious from R3 to R6. The number of nodules on the lower 5 cm root segment increased with growth stage and declined at the R6 growth stage. Afayak and Songda yielded a greater number of nodules on the lower 5 cm root segment than Jenguma, and this varied and increased with

growth stage. Soybean varietal differences became obvious between R3 and R4 growth stages in 2017 (Appendix Fig. B.3).

The entire root nodulation

The number of nodules on the entire root was affected by the soybean variety in 2016 and 2017 (Appendix Table B.1)**.** In 2016, Afayak yielded a greater number of nodules on the entire root (18%) compared to Songda. In 2017, Afayak and Jenguma had increased numbers of nodules on the entire root than Songda. The number of nodules on the entire root was affected by the interaction of commercial inoculant and growth stage in 2016 and 2017 (Fig. 3.7c & d). Inoculation increased the number of nodules on the entire root compared to the uninoculated control and varied with growth stage in 2016 and 2017 (Fig. 3.7c & d). Additionally, inoculation with NoduMax increased the number of nodules on the entire root compared to Legumefix in 2017 (Fig. 3.7c & d). The number of nodules on the entire root decreased after R4 stage.

Nodule dry mass

Crown nodule mass

Crown root nodule mass was significantly affected by the interaction of soybean variety and growth stage (Fig. 3.9a). Afayak and Songda had greater nodule mass than Jenguma at R3 and R4 stage. The peak nodule mass occurred at the R3 stage. After the V8 stage, Jenguma had the lowest crown root nodule mass compared to the other soybean varieties. The crown root nodule mass increased with growth stage and then declined after the R4 stage. Inoculation produced greater nodule mass compared to the uninoculated control (inoculation with NoduMax, Biofix, and Legumefix increased nodule mass to about 105%, 80% and 47% relative to the uninoculated control respectively) (Table 3.3). NoduMax also increased nodule mass ~ 39 % than Legumefix (Table 3.3).

Taproot nodule mass

Taproot nodule mass (nodule dry matter wt.) was affected by the main effects (Table 3. 3). The taproot nodule mass produced by Afayak and Songda was about 84% and 52% greater than Jenguma respectively (Table 3.3). Inoculation with Biofix, Legumefix, and NoduMax increased the taproot nodule mass by 113%, 60% and 30% than the uninoculated control (Table 3.3). Nodule mass significantly differed among the commercial inoculant (Table 3.3). NoduMax enhanced nodule mass of 33% and 63% more than Biofix and Legumefix respectively. Meanwhile, taproot nodule mass increased with growth stage up to R3 and after that decreased (Table 3.3).

Lateral root nodules mass

Lateral root nodules mass was not affected by soybean variety (Table 3.3). Inoculation with Biofix, Legumefix, and NoduMax improved lateral root nodule mass by 27%, 10% and 23% over the uninoculated control (Table 3.3). Within the commercial inoculants, Biofix and NoduMax increased lateral root nodules mass by 24% and 17% more than Legumefix. Lateral root nodules mass significantly increased with growth stage up to R3 stage and then decreased (Table 3.3).

Upper 5 cm root nodule mass

The upper 5 cm root nodule mass was affected by soybean variety and the interaction of growth stage and commercial inoculant (Table 3.3, Fig 3.11a). Afayak and Songda yielded significantly greater upper 5 cm root nodule mass compared to Jenguma (Fig. 3.11a). The nodule mass increased and varied with growth stage, and pronounced effect was observed at R3 and R4 stages.

Inoculation also increased nodule mass on the upper 5 cm root segment and varied with growth stage compared to the uninoculated control (Fig. 3.10a). Inoculation with NoduMax

enhanced nodule mass on the upper 5 cm root segment compared to the Legumefix and Biofix with significant effect observed at R3 and R4 stage (Fig. 3.10a). However, at the R6 stages, Biofix stimulated greater nodule mass compared to Legumefix. The overall nodule mass pattern on the upper 5 cm root segment increased with growth stage but declined after the R4 stage.

Lower 5 cm root nodule mass

There was a soybean variety by growth stage interaction effect for nodule mass on the lower 5 cm root segment (Table 3.3 & Fig. 3.11b). Nodule mass on the lower 5 cm root segment increased with the growth stage (Fig. 3.11b). At R3 and R4 growth stages, Afayak had greater nodule mass on the lower 5 cm root segment compared to Jenguma and Songda (Fig. 3.11b). Nonetheless, at the R6 stage, Songda had enhanced nodule mass on the lower 5 cm root segment compared to the other soybean varieties. Nodule mass on the lower 5 cm root segment was not affected by inoculation by commercial Inoculant (Table 3.3).

Whole root total nodule mass

The entire root segment nodule mass was affected by soybean variety in 2017 (Table 3.3). Both Afayak and Songda produced greater total nodule mass of 32% and 23% more than Jenguma respectively (Table 3.3).

Inoculation also increased total nodule mass on the entire root and varied with growth stage compared to the uninoculated control in 2017 (Fig. 3.10b). The total nodule mass on the entire root segment reached a climax at the R4 stages before declining at the R6 stage. NoduMax increased total nodule mass on the entire root segment compared to the Legumefix and Bofix with a pronounced effect observed at R3 and R4 stage respectively. The nodule mass increased varied with growth stage.

In 2016, nodule mass on the whole root system was significantly affected by the interaction of soybean variety and growth stage (Appendix Fig B.5). Nodule mass increased and

varied with growth stage, peaking at the R4 stage with Afayak and Songda producing greater nodule mass compared to Jenguma. Similarly, Legumefix and Nodumax enhanced nodule mass by more than 21% compared to the uninoculated control in 2016 respectively (Table 3.2).

Specific nodule mass

Specific nodule mass was variable with the soybean variety in 2016 and 2017 (Appendix Table B.1). In 2016, soybean variety had no significant effect on specific nodule mass. Mean specific nodule mass ranged from 7.3 with Jenguma to 8.3 with Songda. In 2017, specific nodule mass was affected by the interaction of soybean variety and growth stage (Appendix Table B.1). Specific nodule mass peaked at the V8 stage and declined at R2 stage, and after that increased partially at the R3 stage before declining again at the R4 stage and after remained stable to the R6 (Appendix Fig. B.5). Overall, Songda had greater specific nodule mass compared to the other cultivars with the growth stage progression (Appendix Fig. B.5).

Inoculation affected specific nodule mass in 2016 and 2017. The control had a significant specific nodule mass compared to the other treatments in both years (Appendix Table B.1). Further, specific nodule mass was significantly greater at the R6 stage compared to the other growth stages in 2016 (Appendix Table B.1). In 2017, the V8 growth stage produced higher specific nodule mass, followed by R3 stage while R2, R4, and R6 yielded lower specific nodule mass (Appendix Table B.1)

Plant Height

Afayak produced plants with greater height compared to Jenguma and Songda (Appendix Table B.2). Inoculation with NoduMax increased plant height compared to the Legumefix and the uninoculated control (Appendix Table B.2). Likewise, Biofix inoculated plants were significantly taller than uninoculated control plants. Average increased plant height due to inoculation with Biofix and NoduMax was 11% compared to the other treatments.

Yield and Yield Components

Pod load was significantly affected by soybean variety in 2016 and 2017 respectively (Appendix Table B.2). Afayak and Jenguma had greater number of pod plant^{-1} than Songda in 2016. In 2017, Jenguma yielded greater number of pods per plant compared to Songda. The 2-yr average showed Afayak and Jenguma produced a significantly greater number of pods per plant than Songda. Inoculation did not increase the number of pods plant^{-1} in both 2016 and 2017 (Appendix Table B.2).

Pod yield was significantly affected by soybean variety in 2016 and 2017 (Appendix Table B.2). In 2016, Afayak produced significantly greater pod yield of 27% and 57% more than Jenguma and Songda respectively. Jenguma also had higher pod yield up to 25% more than Songda. In 2017, pod yield of Jenguma was 55% more than Songda. On average (2-yr) Afayak produced greater pod yield than the other varieties (Appendix Table B.2). Inoculation with Biofix and NoduMax increased pod yield compared to the uninoculated control in 2016 (Appendix Table B.2). In 2017, inoculation had no significant effect on pod yield. Average (2-year) pod yield produced by NoduMax was 25% more than the other inoculants (Appendix Table B.2)

In 2016, Afayak produced significant more grain of 29% and 33% than Jenguma and Songda, respectively (Fig. 3.12a). In 2017, grain yield was not affected by soybean variety. However, Afayak produced marginally greater grain yield of 13% and 5% than Jenguma and Songda, respectively (Fig. 3.12a). On average (2- yr), Afayak increased grain yield over Jenguma and Songda (Fig. 3.12a)

Commercial inoculant significantly affected grain yield in 2016 and 2017 (Fig. 3.16b). In 2016, Biofix and NoduMax produced superior grain yield than the uninoculated control ($P = 0.0576$). In 2017, NoduMax yielded greater grain yield compared to the Legumefix, and the

uninoculated control. Inoculation with Biofix and NoduMax increased grain by 23% and 21% in 2016, and 22% and 36% in 2017 respectively over the uninoculated control (Fig. 3.12b). Legumefix also marginally increased grain yield by 15% in 2016 and 11% in 2017 over the uninoculated control respectively although not statistically different. Averagely (2-yr) Biofix and NoduMax significantly improved grain yield by 21% and 29% over the uninoculated control. (Fig. 3.12b).

Afayak had greater 1000 seed weight compared to both Jeguma and Songda in 2016 and 2017 (Appendix Table B.3). Commercial inoculant did not increase 1000 seed weight (Appendix Table B.3).

Haulm yield (haulm dry matter) was significantly affected by the interaction of soybean variety and inoculant type in 2016 (Appendix Table B.3). Inoculation of Songda with Legumefix resulted in greater haulm yield compared with the uninoculated control soybean varieties and Songda with NoduMax (Appendix Fig. B.6). Inoculation of Afayak with NoduMax improved haulm yield compared to Songda inoculated with NoduMax, and the uninoculated control Afayak and Songda. In 2017, inoculation with Biofix, Legumefix, and NoduMax increased haulm yield by 14%, 6% and 17% over the uninoculated control although not statistically significant. Similarly, average (2-yr) haulm yield was neither affected by soybean variety nor inoculation (Appendix Fig. B.6).

Harvest index was significantly affected by soybean variety in both 2016 and 2017 (Appendix Table B.3). In both years, Afayak had a greater harvest index compared to the other soybean varieties. In 2016, inoculation did not significantly affect harvest index but mean harvest index ranged from 62% with NoduMax to 59% with Legumefix. In 2017, the harvest index was affected by the interaction of soybean variety and inoculant type. Inoculation of

Afayak with all the commercial inoculants (NoduMax, Legumefix, and Biofix) plus the uninoculated Afayak had a greater harvest index (Appendix Fig. B.7). While the uninoculated control Songda and Jenguma inoculated Legumefix and NoduMax yielded the lowest harvest index (Appendix Fig. B.7).

Economic Analysis

Based on the value to cost ratio (VCR), Afayak yielded a greater net return on investment compared to Jenguma and Songda in 2016 and 2017 (Fig. 3.14). Inoculation with a commercial inoculant produced a higher net return on investment compared to the uninoculated control in both years (Fig. 3.13). With the commercial inoculant, the net returns on investment from Biofix and NoduMax were generally higher than Legumefix in both years (Fig. 3.13). The 2-yr average, Biofix, and NoduMax provided 1.5 times more net return over Legumefix.

Biomass Dry Matter and Nitrogen Content at R6 stage

Dry matter (DM) of shoot (Leaf +Stem), pod, and root was assessed at R6 stages. Whole plant dry matter consisted of dry matter of shoot, root, and pod. In 2016, shoot DM production was not affected by soybean variety (Table 3.4). In 2017, Afayak and Songda yielded greater shoot DM than Songda (Table 3.4). In 2016, inoculation with NoduMax improved shoot DM compared to Biofix and Legumefix respectively. In 2017, inoculation did not increase shoot DM production. Nonetheless, the trend for shoot DM production was similar to those observed in 2016. Also, root DM was not significantly affected by soybean variety and with commercial inoculant in both 2016 and 2017 (Table 3.4). Afayak and Jenguma produced greater pod DM than Songda in 2016 and 2017 (Table 3.4). Inoculation with NoduMax increased pod DM compared to the other treatments in 2016 (Table 3.4). In 2017, inoculation with commercial inoculant did not significantly improve pod DM (Table 3.4).

Plant DM was not affected by soybean variety in both 2016 and 2017 (Table 3.4). In both years, Afayak produced greater mean plant DM than Songda and Jenguma but not statistically different (Table 3.4). In 2016, inoculation with NoduMax significantly improved plant DM compared to Biofix and Legumefix (Table 3.4). In 2017, inoculation with commercial inoculant did not statistically increase plant DM, however, Biofix and NoduMax had greater mean plant DM than the rest of the treatments (Table 3.4)

Shoot N content was not affected by soybean variety for both years (Table 3.5). Nonetheless, in both years Afayak had greater mean shoot nitrogen content than the other soybean varieties. In 2016, inoculation with NoduMax increased shoot nitrogen content compared to the other treatments (Table 3.5). In 2017, there was no commercial inoculant effect on shoot N content (Table 3.5). However, inoculation with Biofix and NoduMax marginally increased shoot N content compared to the other treatment.

Root N content was not affected by soybean variety and commercial inoculant for both 2016 and 2017 (Table 3.5). Root N content for soybean variety ranged from 11.0 to 11.4 kg N ha^{-1}, and 7.8 to 9.5 kg N ha^{-1} in 2016 and 2017 respectively. Root N content for commercial inoculant ranged from 9.7 to 12.2 kg N ha^{-1} in 2016 and 8.1 to 9.9 kg N ha^{-1} in 2017 respectively.

Pod N content was affected by soybean variety in 2016 and 2017 (Table 3.5). In both years, Afayak and Jenguma had enhanced pod N content compared to Songda (Table 3.5). Commercial inoculant had a variable respond on pod N content. In 2016, inoculation with NoduMax stimulated increase pod N content compared to other treatments (Table 3.5). In 2017 commercial inoculant did not affect pod N content (Table 3.5). However, the mean value for pod N content ranged from 32.9 kg N ha^{-1} with Biofix, 26.7 kg N ha^{-1} to 23.8 kg N ha^{-1} with Legumefix (Table 3.5). Whole plant N was not significantly affected by soybean variety in 2016

and 2017 respectively (Table 3.5). However, in 2016, Afayak had greater mean value for whole plant N of 263 kg N ha^{-1}, followed by Jenguma with 233 kg N ha^{-1} and then Songda with 213 kg N ha^{-1} (Table 3.5). In 2017, the trend was same where the mean value for whole plant N by Afayak was 156 kg N ha^{-1}, followed by Jenguma with 135 kg N ha^{-1} and Songda with 156 kg N ha^{-1} by (Table 3.5). Whole plant N was also affected by the commercial inoculant in both years. In 2016, inoculation with NoduMax improved whole plant N compared to other treatments. While in 2017, inoculation with Biofix induced greater whole plant N than Legumefix (Table 3.5).

Total Nitrogen Fixation

There was no significant difference in the total N fixed by the different soybean variety in both years (Table 3.5 & Fig. 3.15). Nonetheless, the total N fixed ranged from 184 to 204 kg N ha^{-1} in 2016 and 119 to 151 kg N ha^{-1} in 2017. Inoculation with NoduMax stimulated greater total N fixation compared to the other treatments in 2016 (Table 3.5 & Fig. 3.15). In 2017, inoculation with Biofix significantly increased total N fixation compared to Legumefix (Table 3.5 & Fig. 3.15).

Grain Protein Content

Grain produced by Afayak had a higher protein content of about 22% and 32% more than Jenguma and Songda respectively in 2016 (Appendix Table B.3). In 2017, protein content was not significantly affected by soybean varieties. Nonetheless, the trend for soybean grain protein content in 2017 was similar to 2016. Commercial inoculant significantly affected protein content in both 2016 and 2017. Inoculation with Biofix and NoduMax significantly increased grain protein content compared to the uninoculated control in 2016 (Appendix Table B.3). In 2017,

inoculation with NoduMax increased grain protein than Legumefix and the uninoculated control. In general, inoculation improved the quality of grain protein content.

Grain Nitrogen Uptake

Grain N uptake was variable with the soybean variety in 2016 and 2017. In 2016, Afayak had an enhanced grain N uptake compared to Jenguma and Songda (Table 3.6). In 2017, grain N uptake did not significantly differ among the soybean variety nonetheless trends were similar to 2016.

Inoculation with Biofix and NoduMax inoculants improved grain N uptake compared to the uninoculated control in 2016 (Table 3.6). In 2017, NoduMax inoculant increased grain N uptake compared to the Legumefix and the uninoculated control (Table 3.6).

Haulm Nitrogen Uptake

Afayak had increased haulm total N uptake compared to Jenguma in 2016 (Table 3. 6). In 2017, there was no statistical difference in haulm N uptake among the different soybean varieties (Table 3.6). Inoculation did not significantly improve haulm N uptake (Table 3.6).

Total Nitrogen Uptake

In 2016, Afayak had increased total N uptake which was about 22% and 29% more than Jenguma and Songda respectively (Table 3.6). In 2017, total N uptake was not significantly affected by the different soybean varieties (Table 3.6). Inoculation with Biofix and NoduMax significantly enhanced total N uptake by 21% and 19% compared to the uninoculated control in 2016 respectively (Table 3.6). In 2017, NoduMax inoculant increased total N uptake by 22% and 28% compared to the Legumefix and the uninoculated control respectively. Likewise, Biofix inoculant had greater total N uptake of about 18% more than the uninoculated control in 2017.

Legumefix marginally increased the total N uptake by 15% and 7% more than the uninoculated control in 2016 and 2017 respectively, although not statistically different.

Residual Nitrogen Balance (Residual N)

In residual N budget 1, both grain and haulm (dry stover) were exported at harvest. Residual N balance was not statistically significant among the different soybean varieties in both 2016 and 2017 respectively. Nonetheless, in 2016, Jenguma had a greater residual N (23.5 kg N ha^{-1}) than Songda (19.52 kg N ha^{-1}), and Afayak (2.7 kg N ha^{-1}) (Table 3.6). In 2017 Afayak had higher residual N (45.7 kg N ha^{-1}) than Songda (25.3 kg N ha^{-1}) and Jenguma (17.0 kg N ha^{-1}). Inoculation significantly affected residual N in both 2016 (P= 0.073) and 2017 (P= 0.086) respectively. Inoculation with NoduMax contributed to significant residual N pool of 66.8 kg N ha^{-1} compared to Biofix (-24.6 kg N ha^{-1}) and Legumefix (-6.7 kg N ha^{-1}) in 2016. In 2017, Biofix inoculant contributed greater residual N (54.7 kg N ha^{-1}) than NoduMax (6.19 kg N ha^{-1}) and Legumefix (21.9 kg N ha^{-1}). In 2016, Biofix and Legumefix had negative residual N, indicating uptake of N from the soil mineral N pool.

Residual N Budget 2

In residual N budget 2, only grain was exported, and haulm was left in the field. Residual N budget 2 was not significantly different among the soybean varieties. Nonetheless, the residual N contributed by Jenguma (45.2 kg N ha^{-1}), and Songda (45.6 kg N ha^{-1}) was higher than Afayak (32.0 kg N ha^{-1}) in 2016. While in 2017 Afayak had greater residual N (67.4 kg N ha^{-1}) than Songda (46.6 kg N ha^{-1}) and Jenguma (38.0 kg N ha^{-1}) (Table 3.6). Commercial inoculants also enhanced residual N balance in both 2016 and 2017. Inoculation with NoduMax contributed significant (P= 0.082) residual N of 92.6 kg N ha^{-1} compared to Biofix (2.9 kg N ha^{-1}) and Legumefix (20.6 kg N ha^{-1}) in 2016. In 2017, Biofix (78.5 kg N ha^{-1}) inoculant significantly (P=

0.082) the increased residual N balance than Legumefix (24.9 kg N ha^{-1}). Thus the residual N balance contributed by all the treatments in 2017 were positive compared to 2016.

Discussion

Successful soybean production depends on good soil quality and an enhanced symbiotic association with the appropriate Bradyrhizobium. In sub-Saharan West Africa (SSWA), soybean is not generally inoculated with *Bradyrhizobium japonicum* as most of the soybean genotypes belong to the promiscuous nodulating groups (Tropical *Glycine max crosses*, TGX). However, several authors had documented improved nodulation and grain yield when the promiscuous nodulating soybean (TGX-soybean) was inoculated with *Bradyrhizobium japonicum* (Abaidoo et al., 2000, 2007; Thuita et al., 2012; Ulzen et al., 2016). In this study, inoculating TGX soybean lines with commercial inoculant increased nodule number and nodule dry mass compared to the uninoculated control confirming previous research (Abaidoo et al., 2000; Zhang et al., 2003; Ulzen et al., 2016). Further, inoculation with commercial inoculant stimulated greater nodule number and nodule mass (nodule dry wt.) at different root locations. Cardoso et al. (2009) reported that *Rhizobium* or *Bradyrhizobium* inoculation increased nodule number and nodule mass on the crown root compared to other locations on the root segment of common bean, groundnut, and soybean in Brazil. They found a strong relationship between the nodulation (nodule number and nodule mass) on the crown root and the whole root, hence concluded that nodulation assessment should focus on the crown root to minimize cost and labor. Nonetheless, our results found no strong relationship between nodules on the crown root and whole root systems ($r2 = 0.429$ and $r2= 0.518$ in 2016 and 2017 respectively). Inoculation with commercial inoculant increased nodule number and nodule mass at upper 5 cm root and lower root segments compared to the uninoculated control. Cardoso et al. (2009) reported that inoculation did not

increase the number of nodules on the lower secondary root segments compared to the primary crown root. Nonetheless, results contradict earlier research by Kamicker and Brill (1987) who reported that inoculation with *Bradyrhizobium japonicum* for soybean increased the number of nodules on the lower root segments than the upper 5 cm root. They argued that the vertical movement of *Bradyrhizobium japonicum* increased nodulation on the lower root segments. The increased nodule number and nodule mass at all root positions in our study was perhaps due to seed inoculation with the commercial inoculants which allowed the introduced *Bradyrhizobium japoncium* to remain in close contact with the root after seed germination. Nodulation was generally poor or depressed on the uninoculated control. Inoculation with *Bradyrhizobium japonicum* is necessary for enhancing nodulation in TGX soybean line (Abaidoo et al., 2007; Thuita et al., 2012).

Commercial inoculant stimulated biomass production (shoot and whole plant), growth (plant height) and yield (grain yield and haulm dry matter). Inoculation with commercial inoculant also improved grain protein and N content, total N fixation, grain and haulm N uptake, total N uptake, as well as the residual N balance. Further, increased biomass production due to inoculation with commercial inoculant particularly with Biofix and NoduMax became obvious at R4 and R6 growth stage. Nonetheless, at the R6 stage, biomass production including immature pod dry wt reached a maximum with NoduMax, while Legumefix and Biofix produced the least biomass. Previous research by Ulzen et al. (2016) showed that inoculation of TGX soybean lines with commercial inoculants (Biofix and Legumefix) did not improve biomass production and pod dry wt. The non-inoculation responses by biomass production were perhaps due to sampling time (at R3 stage-beginning to the pod). The inoculation effect on biomass production became obvious after the R3 stage in the present study.

At the R6 stage, commercial inoculant improved biomass N content (shoot and whole plant) and total N fixation, with pronounced increases associated with NoduMax and Biofix. The 2-yr average N fixed by NoduMax and Biofix was 205 kg N ha^{-1} and 172 kg N ha^{-1} respectively. Legumefix fixed the least amount of N (143 kg N ha^{-1}). Zhang et al. (2003) observed that inoculation with commercial inoculant increased total N fixation although our values are slightly higher. The average (2-yr) performance of the uninoculated control (155 kg N ha^{-1}) is quite surprising, as both its biomass N content and fixed N were higher than Legumefix although not statistically significant. The total amount of N fixed was within the range of 159-227 kg shoot N ha^{-1} reported by Peoples et al. (2009). The wide variability in the quantity of N fixed in both years (2016 and 2017) can be attributed to the difference in soil fertility and weather pattern. Nonetheless, the 2017 experimental site has greater available soil N of 20 mg kg^{-1} (above the threshold level) compared to 2016 site. Perhaps, the high available N could be responsible for the depression in N_2 fixation, and nodulation. Several authors have reported that high available soil N inhibits N-fixation, nodulation and shoot biomass (Danso et al., 1990; Dakora and Keya, 1997). Additionally, inoculation with Biofix and NoduMax inoculants improved grain protein content, grain N content, and total N uptake. Several researchers have also reported that inoculation with commercial inoculants enhanced or stimulated growth, biomass production, grain yield, biomass N content and total N uptake in soybean (Zhang et al., 2003; Thuita et al., 2012; Ulzen et al., 2016; Koskey et al., 2017). Previous work by Zimmer et al. (2016) established that inoculation of soybean with commercial inoculants increased grain protein content.

Commercial inoculant increased grain yield with a greater advantage associated with NoduMax and Biofix. The 2-year grain yield response to inoculation was 14% with Legumefix,

25% with Biofix and 31% with NoduMax over the uninoculated control. Thuita et al. (2012) and Ulzen et al. (2016), also reported an increase in soybean grain yield due to inoculation with commercial inoculants. The average performance of Legumefix (14% grain yield increased over the control) in this study agrees with previous work with Legumefix inoculant (18% grain yield increase over the control) reported by Thuita et al. (2018). Generally, grain yield in 2016 was higher than in 2017. This could be due to variation in rainfall and temperature patterns and to some extent soil induced factors. The soil analysis indicated that the soil was of inherent low quality. Since soil organic C (< 0.4%), available P (< 9 ppm), and total N (< 1%) were below the critical levels required for successful soybean production. Poor soil quality can undermine crop productivity, as soils with low soil organic matter and P, and high acidity can reduce the symbiotic association, N-fixation, and grain yield.

The net returns on inoculation of TGX soybean with commercial inoculant was estimated using the value-cost ratio (VCR) with a threshold set greater than 2. Commercial inoculant increased VCR above the threshold level, surmising that the adaption of inoculant technology resulted in greater economic returns. The 2-year average, higher net return (profit) was associated with NoduMax (~\$240 ha^{-1}) and Biofix (`\$210 ha^{-1}) than Legumefix (~ \$120 ha^{-1}). The VCR obtained with Biofix and Legumefix are consistent and within the range reported by Ulzen et al. (2016). It is also apparent that commercial inoculants with the strain USDA110 (NoduMax and Biofix) yielded 2-fold net returns (profit) than the strain USDA 532c (Legumefix). They are thus suggesting that the USDA 110 to be a superior strain to use in the tropics. Nonetheless, commercial inoculant formulated with USDA 523c could be used as an alternative in areas where access to USDA 110 strain is limited. Our results seem to suggest that farmers in Northern Ghana stand to achieve greater benefits from using commercial inoculants

on their promiscuous soybean varieties. This is because inoculation is low-cost technology and can potentially increase grain yield leading to higher net returns. Inoculation of soybean should be the starting point of promoting sustainable legumes intensification practices in smallholder systems in Sub-Saharan Africa, as challenges with fundamental soil quality (such as low pH, phosphorus fixation, low SOM, and micronutrients deficiency) equally needs to be addressed.

Further, NoduMax (USDA 110) inoculant outperformed Legumefix (USDA 532c), in almost all the parameters (nodulation, shoots biomass, pod dry wt., grain yield, total N fixation, and residual N balance). This can be attributed to the difference in the *Bradyrhizobium japonicum* strains of the two inoculants. The superior performance of *Bradyrhizobium japonicum* strain and their interaction with the host (plant) can potentially be altered by the environment where they are introduced. That is, the strains ability to colonize the host root, remain motile, persist and even adapt depends on the environment. Zhang et al. (2002, 2003) observed that *Bradyrhizobium japonicum* strain USDA 31and 30 outperformed *Bradyrhizobium japonicum* strain USDA 532c in Canada when the evaluation was done on nodulation (nodule number and nodule dry wt.), shoot N and N fixation. The poor performance of USDA 532c was attributed to the slow growth rate and poor adaptability to a cold environment (cold weather). On the contrary, Hume and Shelp (1990) reported that inoculation with *Bradyrhizobium japonicum* strain USDA 532c improved grain yield, although nodulation was average compared to other *Bradyrhizobium japonicum* strains when evaluated in Canada. Similarly, Ravuri and Hume (1992) also documented that *Bradyrhizobium japonicum* strain USDA 532c fix higher N_2 g^{-1} of nodule mass. Nonetheless, the poor performance of the USDA 532c strain in the tropics suggests that climate was not a significant factor as documented by Zhang et al. (2003). The poor performance of USDA 532c may be associated with low competitiveness, genetic variation,

soils, quality control, and handling. There was also no explicit explanation for the average performance of Biofix compared to NoduMax as both commercial inoculants contain the same *Bradyrhizobium japonicum* USDA 110. The possible explanation could be quality control and handling including distribution.

Residual N balance due to inoculation with commercial inoculants was variable in both years. For Scenario one (1) N budget where both grain and haulm yield were exported from the field, inoculation could either results in positive or negative residual N balance. In 2016, residual N balance ranged between -24 and 67 kg N ha^{-1} with NoduMax yielding the greatest net positive N balance of 67 kg N ha^{-1} while Biofix and Legumefix lead to a negative N balance of -24.6 kg N ha^{-1} and - 6.7 kg N ha^{-1} respectively. The negative N balance infers that Biofix and Legumefix did not contribute N to the soil N pool by fixation but rather N uptake from the soil mineral pool. Adu-Gyamfi et al. (2007) documented negative residual N balance when both grain and stover were removed from pigeon intercropping systems. Nonetheless, in 2017, the residual N balance was a net positive ranging between 6 and 55 kg N ha^{-1}. Biofix contributed the most residual N of 55 kg N ha^{-1}. The variable residual N balance in both years is due to the difference in grain yield and harvest index. Since grain yield and harvest index in 2016 were generally higher than in 2017. The greater grain yield may have induced higher N transport into the grain. Hence the resultant low residual N balance observed in 2016.

For scenario two N budget where only grain yield was exported, the residual N balance was a net positive ranging between 2-93 kg N ha^{-1} and 24-79 kg N ha^{-1} in 2016 and 2017, respectively. NoduMax and Biofix inoculants contributed the greatest positive N balance of 93 kg N ha^{-1} and 76 kg N ha^{-1} in 2016 and 2017, respectively. Zoundji et al. (2016) also reported positive residual N balance when soybean grains were exported, and stover was retained on the

field after harvest in Benin. Therefore the current practices in Northern Ghana where farmers harvest whole soybean plant (dry pod + haulm) and carry away for threshing will lead to further loss of soil nutrients (soil quality). The wide variability in the residual N balance of the two inoculants (NoduMax and Biofix) in both years is not fully understood. Nonetheless, both inoculants contained the same the *Bradyrhizobium japonicum* strain (USDA 110). Our results also suggest that the uninoculated control (2 yr average) contributed higher mean residual N balance than Biofix and Legumefix inoculant. Thus without inoculation, soybean cultivation alone will lead to positive residual N balance. Nonetheless, the grain yield may be of low nutritional quality due to low protein content resulting from low grain N uptake.

Regarding, the soybean variety, Afayak, (2-yr average) produced greater grain yield with superior protein content. The superior protein content of grain was due to increased grain N uptake. The greater grain yield by Afayak can be attributed to higher harvest index and yield components (pod load and pod dry wt.). Therefore, Afayak had greater efficiency in the partition of dry matter into grain yield. Net returns from Afayak was about 2-fold more than the other varieties. There was no varietal difference in the total N fixed, but Afayak fixed greater total N of 234 kg N ha^{-1} and 151 kg N ha^{-1} compared to 204 and 119 kg N ha^{-1} by Jenguma, and 184 and 122 kg N ha^{-1} by Songda in 2016 and 2017, respectively. A substantial proportion of the total N fixed by Afayak was translocated into the grain and the haulm thereby resulting in increased total N uptake compared to other soybean varieties. The residual N balance contributed by the different soybean variety was largely variable but not statistically different. Afayak contributed higher mean residual N balance. Residual N balance was also positive for the different soybean varieties regardless of either haulms and grains were removed. Nonetheless, greater mean residual N balance was observed when haulm was retained on the field. Results contradict the

negative N residual balance for TGX soybean lines evaluated in Benin by Zoundji et al. (2016). Nonetheless, our findings corroborate with Sanginga et al. (1997b) who documented a positive N balance for several TGX soybean lines evaluated in the southern Guinea Savanna of Nigeria.

Afayak and Songda showed superior biomass production ability over Jenguma, which was the farmer's variety. Likewise, for nodulation performance, Afayak and Songda produced nodules with greater dry matter wt. regardless of nodule location on the root (crown root, taproot, lateral root, and whole root) and root segment (upper 5 cm and lower 5cm root segment). Surprisingly, for nodule number, Afayak and Jenguma demonstrated superior ability to produce a higher number of nodules at different root location and root segment. Overall, Afayak consistently maintained superior performance over the two other varieties evaluated and seem to be potential candidates for dissemination in Northern Ghana.

Conclusion

The present study showed that promiscuous nodulation soybean responds better to inoculation with commercial inoculants. Commercial inoculants improved shoot dry matter, nodulation (nodule number and nodule mass), plant height, and grain yield, grain protein content, total N fixation, nitrogen uptake and residual N balance which was consistent with the research hypothesis. Economically, inoculation increased grain yield about ~20% over the uninoculated control. The commercial inoculants evaluated exhibited differential performance with NoduMax consistently outperforming Legumefix. This was perhaps due to strain differences as NoduMax contains *Bradyrhizobium japonicum* USDA110 and Legumefix contains *Bradyrhizobium japonicum* USDA 532c. Therefore commercial *Bradyrhizobium japonicum* inoculants with the strain USDA110 appears to be a superior candidate to use in the tropic. Inoculation is relatively a new low-cost technology in Ghana with no commercial inoculant production facility.

Nonetheless, the technology can be easily be adopted by farmers with minimal training. It will, therefore, be crucial for commercial inoculants technology to be included in a national agricultural extension program for dissemination to the farmers in Northern Ghana.

Afayak, one of the modern soybean lines showed superior performance over Songda and Jenguma. Grain yield depended on pod load and pod yield. Therefore, Afayak can be recommended be for inoculation with commercial inoculant due to improved performance. Export of both haulm and grain yield at harvest resulted in significant nutrient removal. Negative residual N balance was observed in 2016 with some of the commercial inoculant (Biofix and Legumefix) when a whole plant (haulms + grain) was exported. For the succeeding crop to benefit from residual N balance from the previous legume (soybean) cropped, residues need to be retained. Therefore the current practice in Northern Ghana where farmer harvests the whole plant does not contribute to sustainable soil intensification management.

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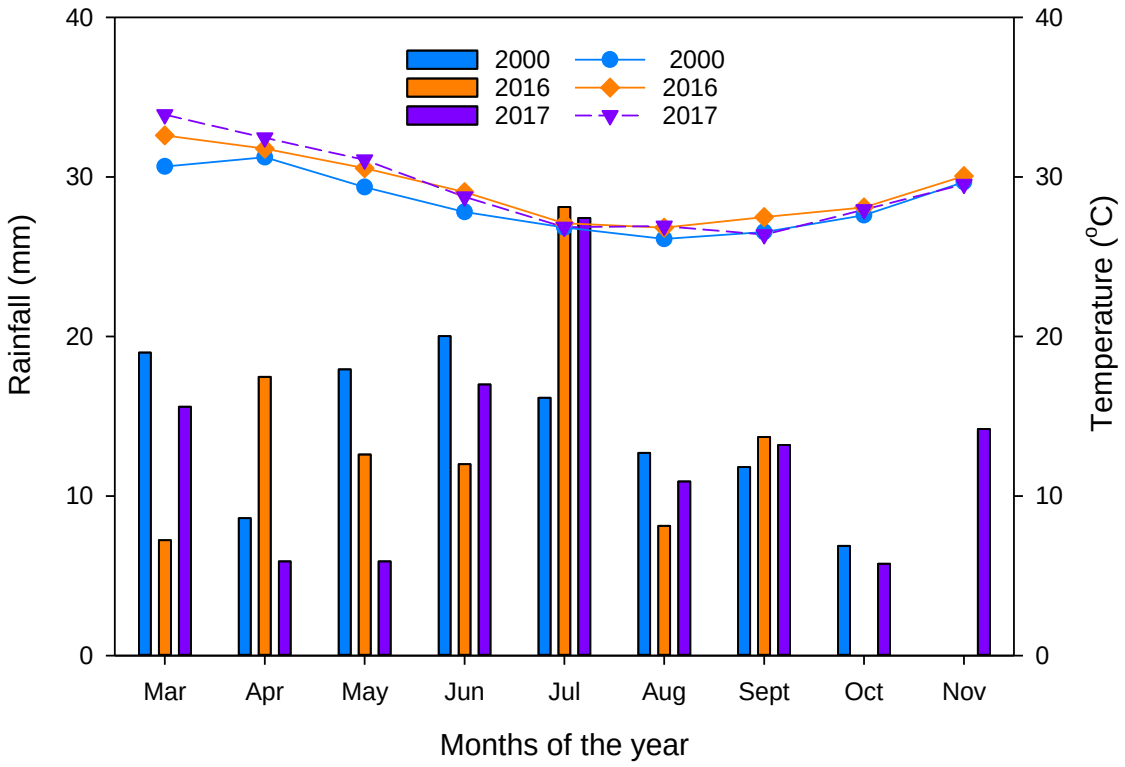

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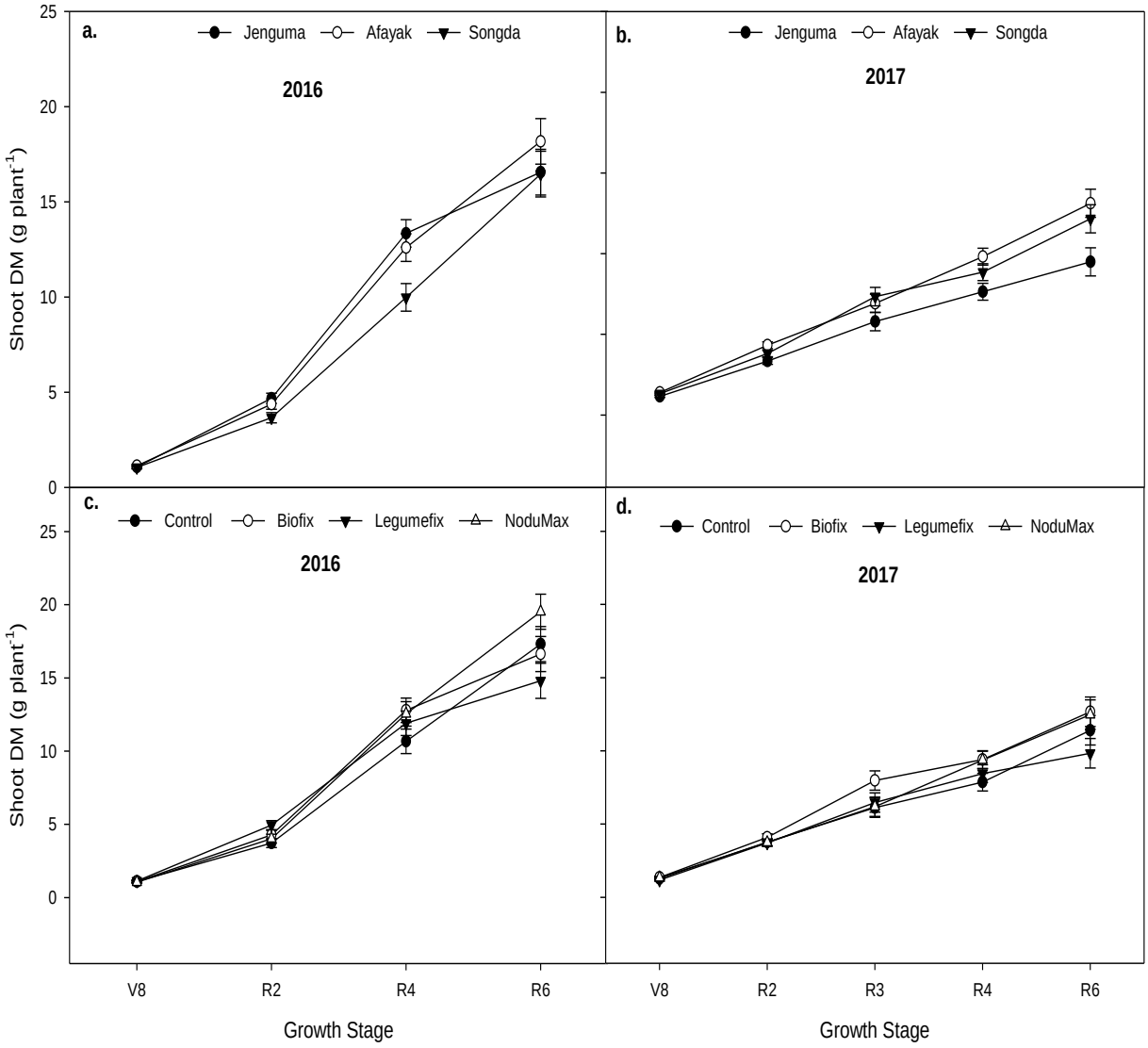

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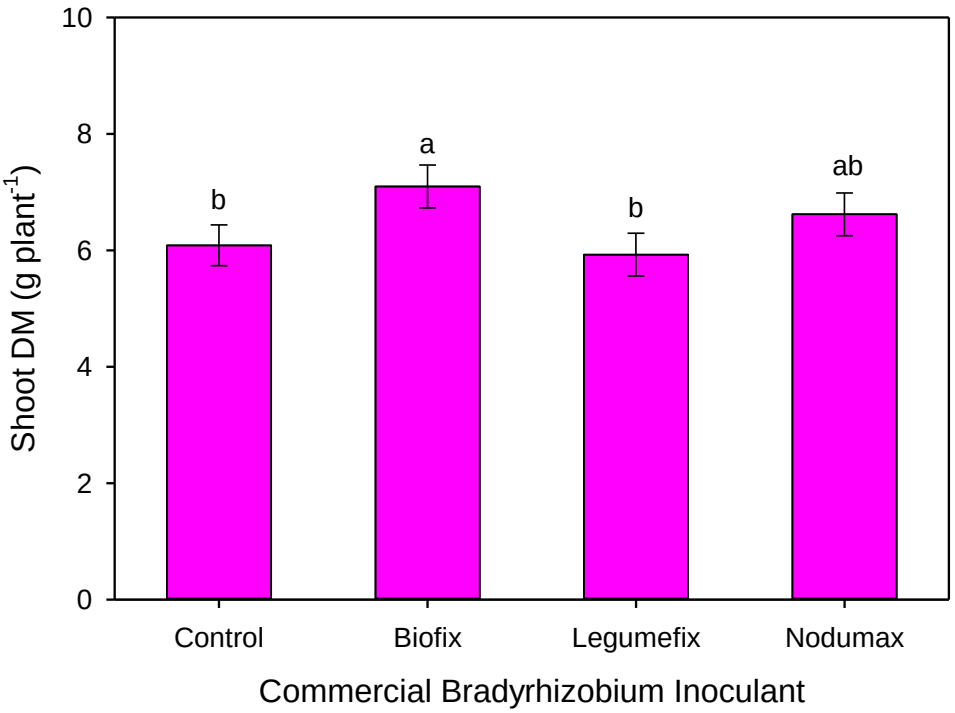

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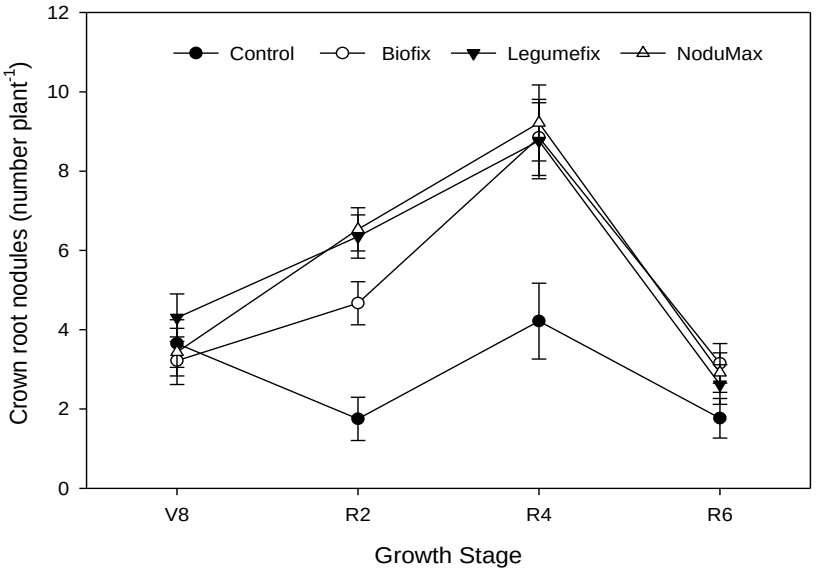

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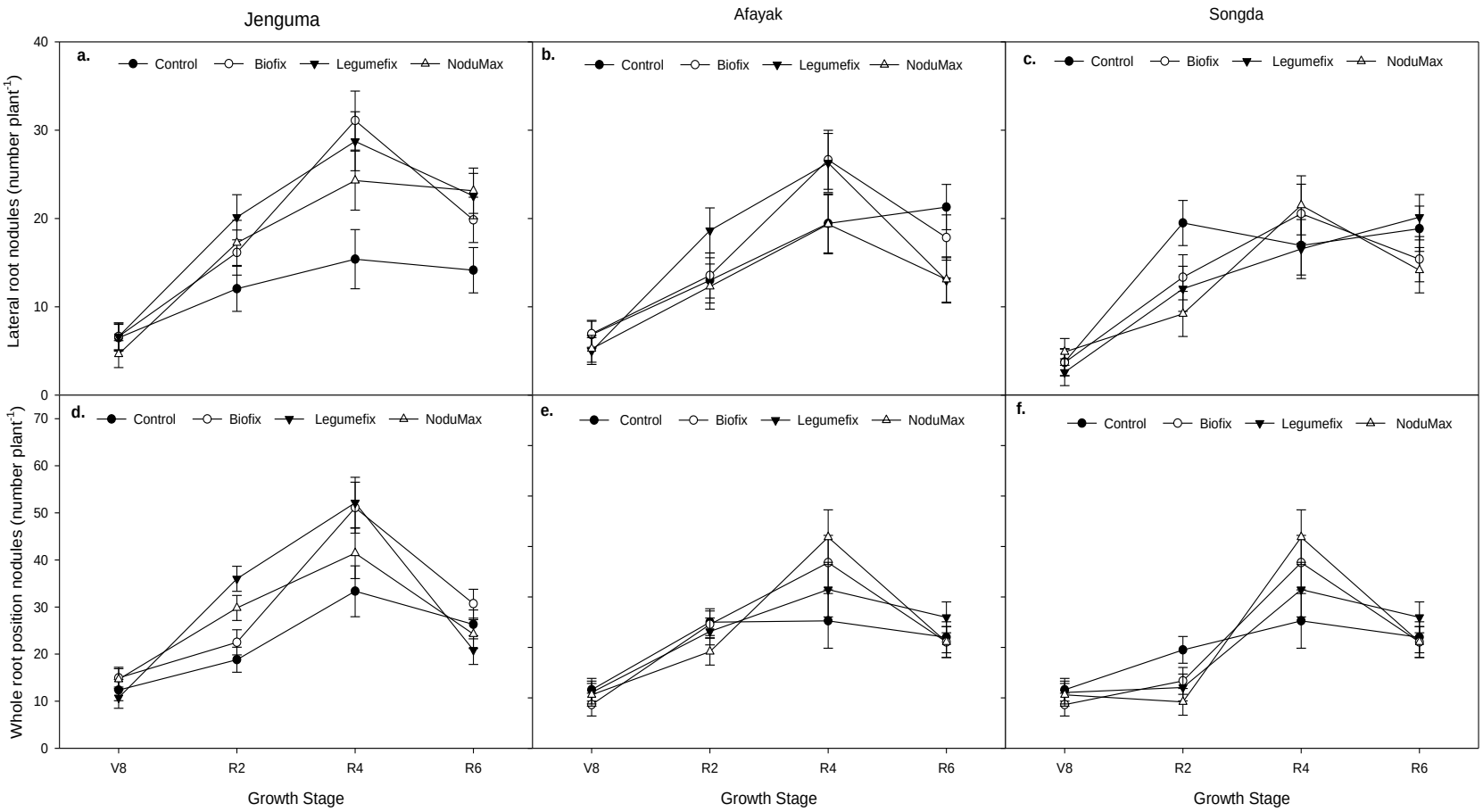

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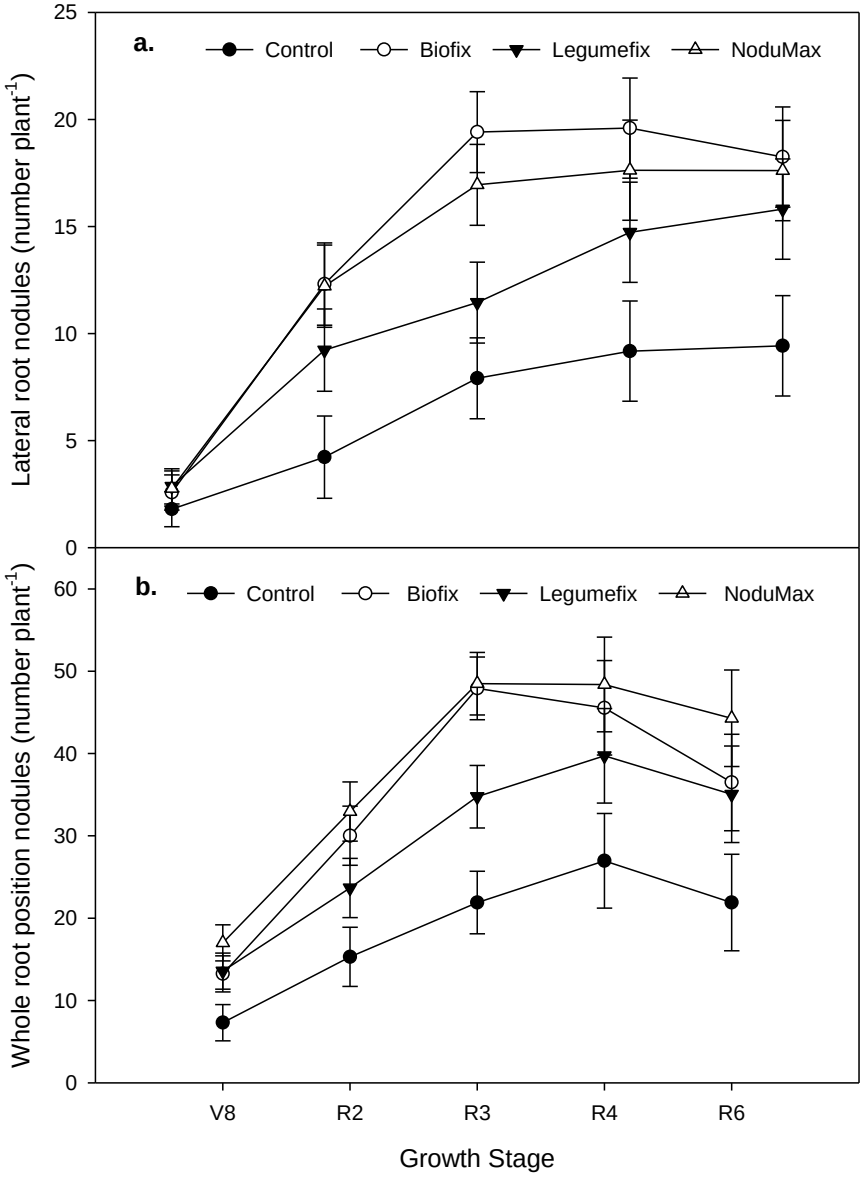

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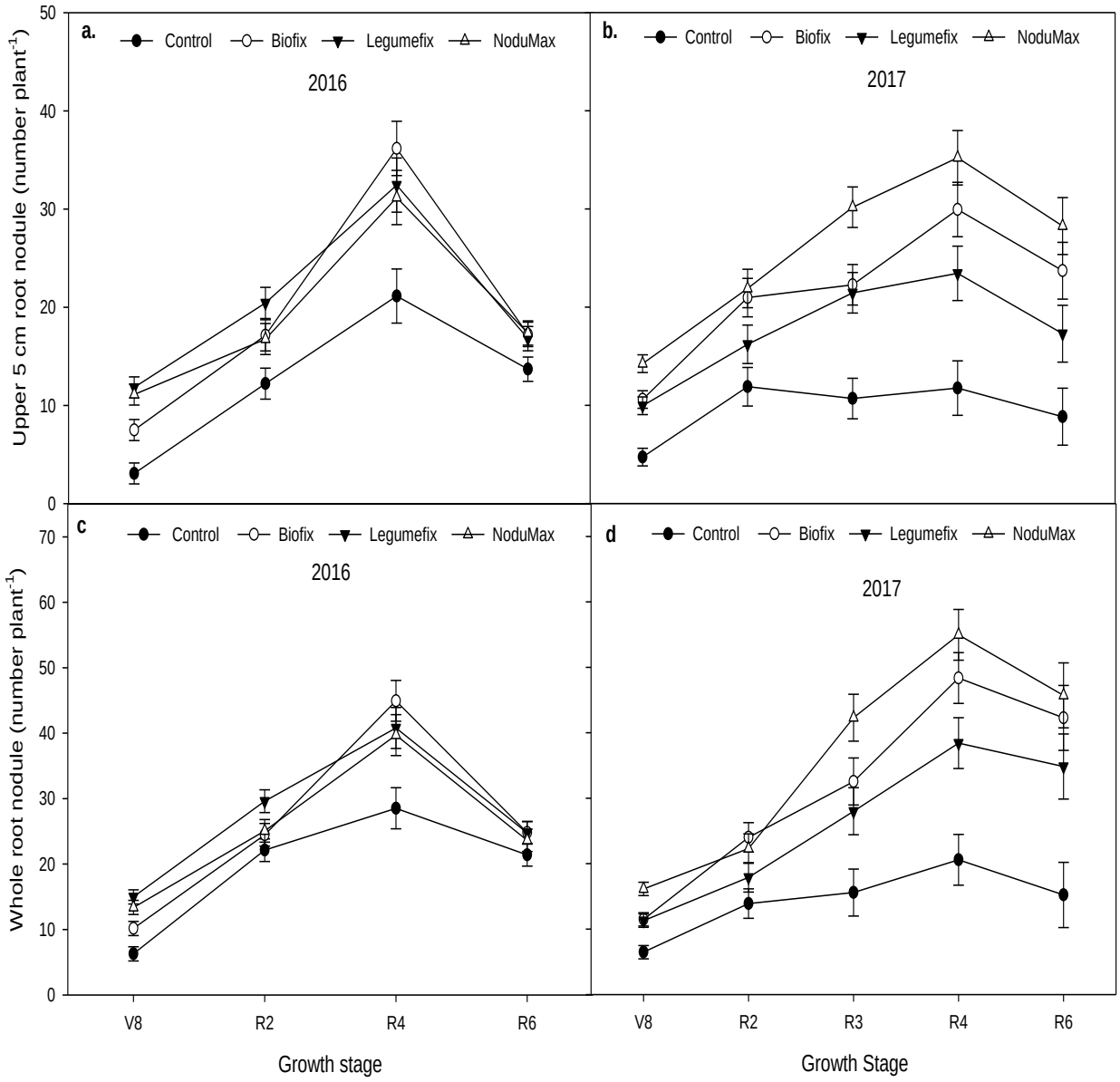

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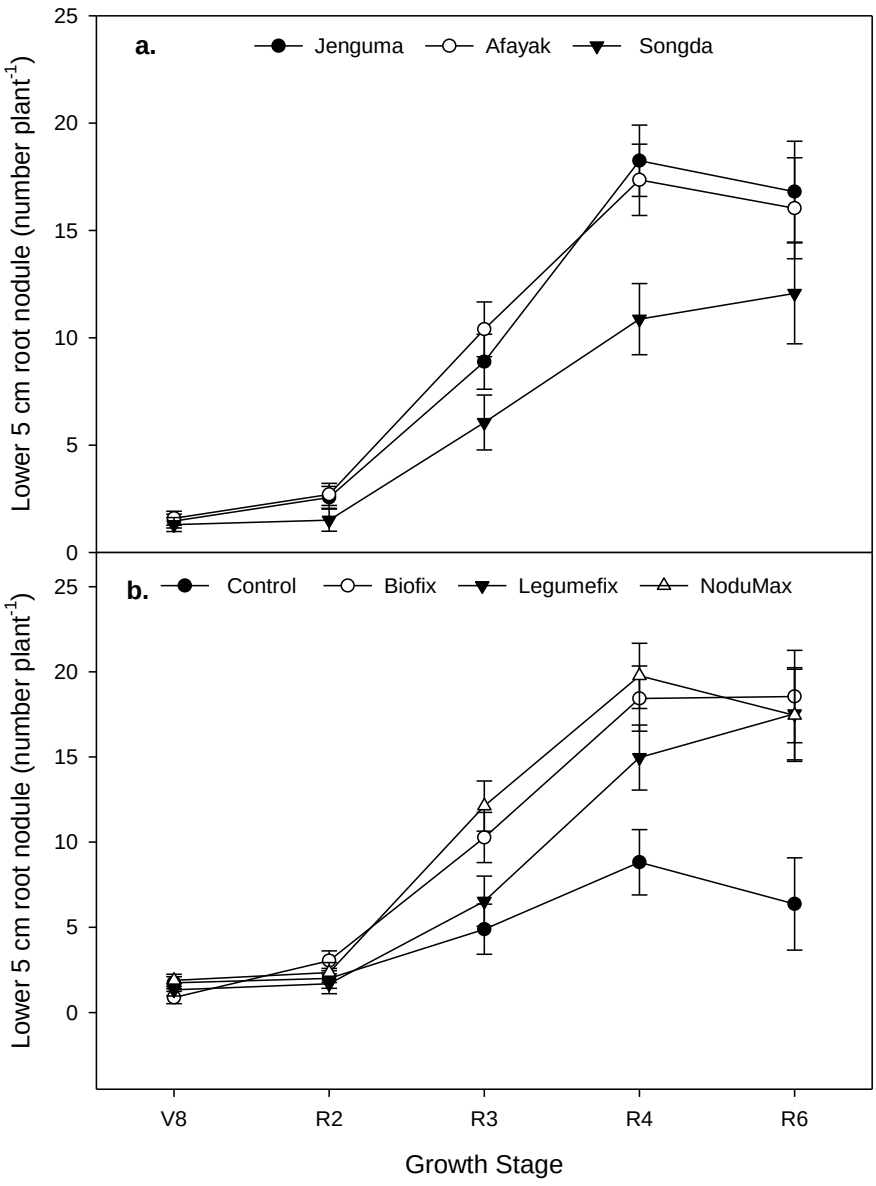

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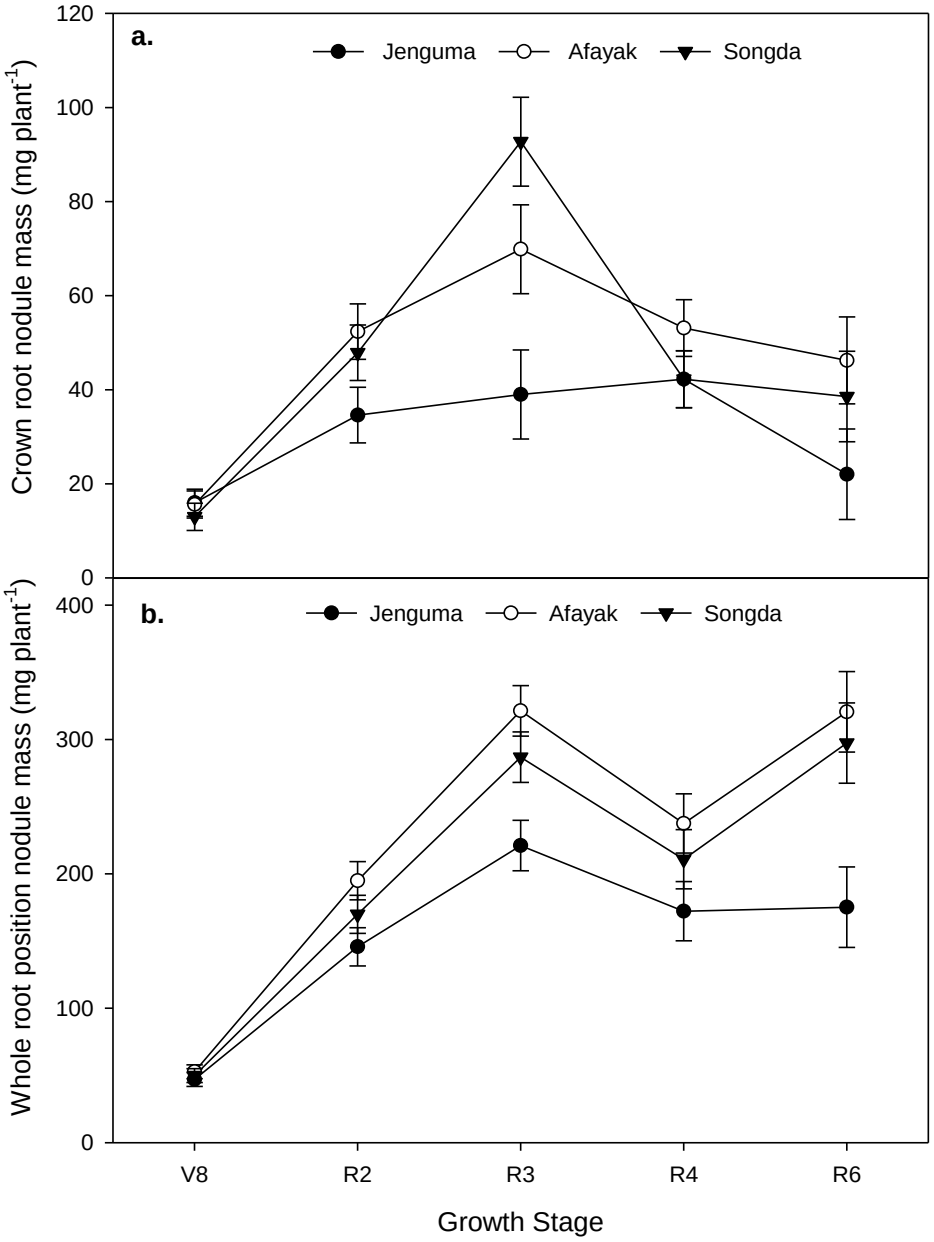

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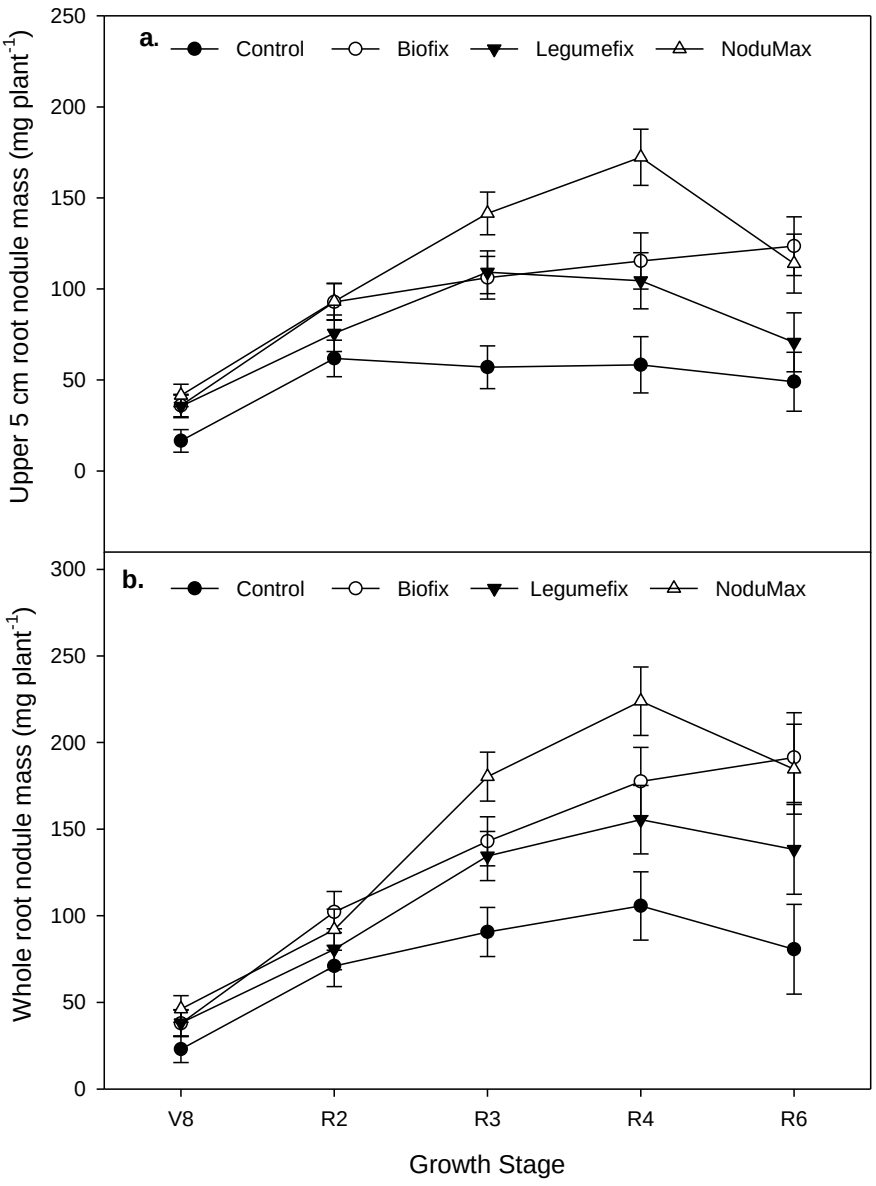

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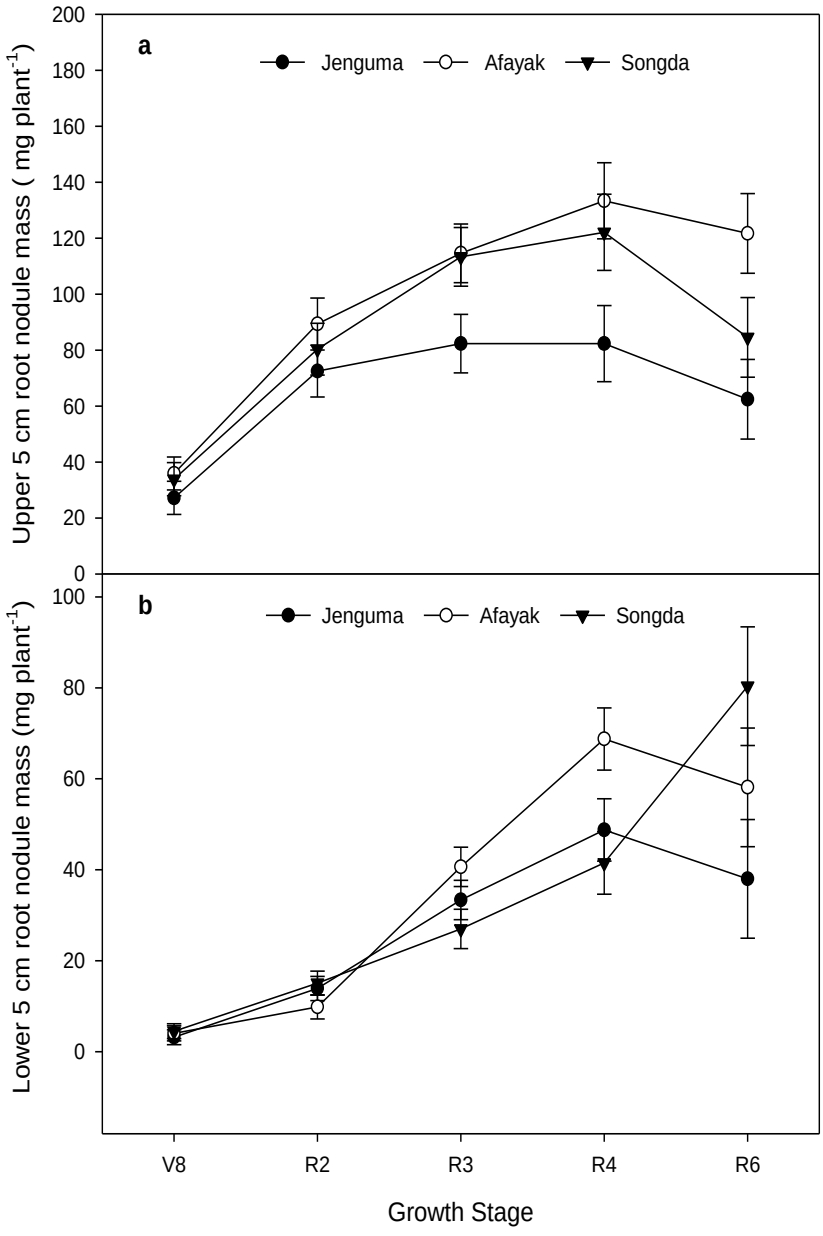

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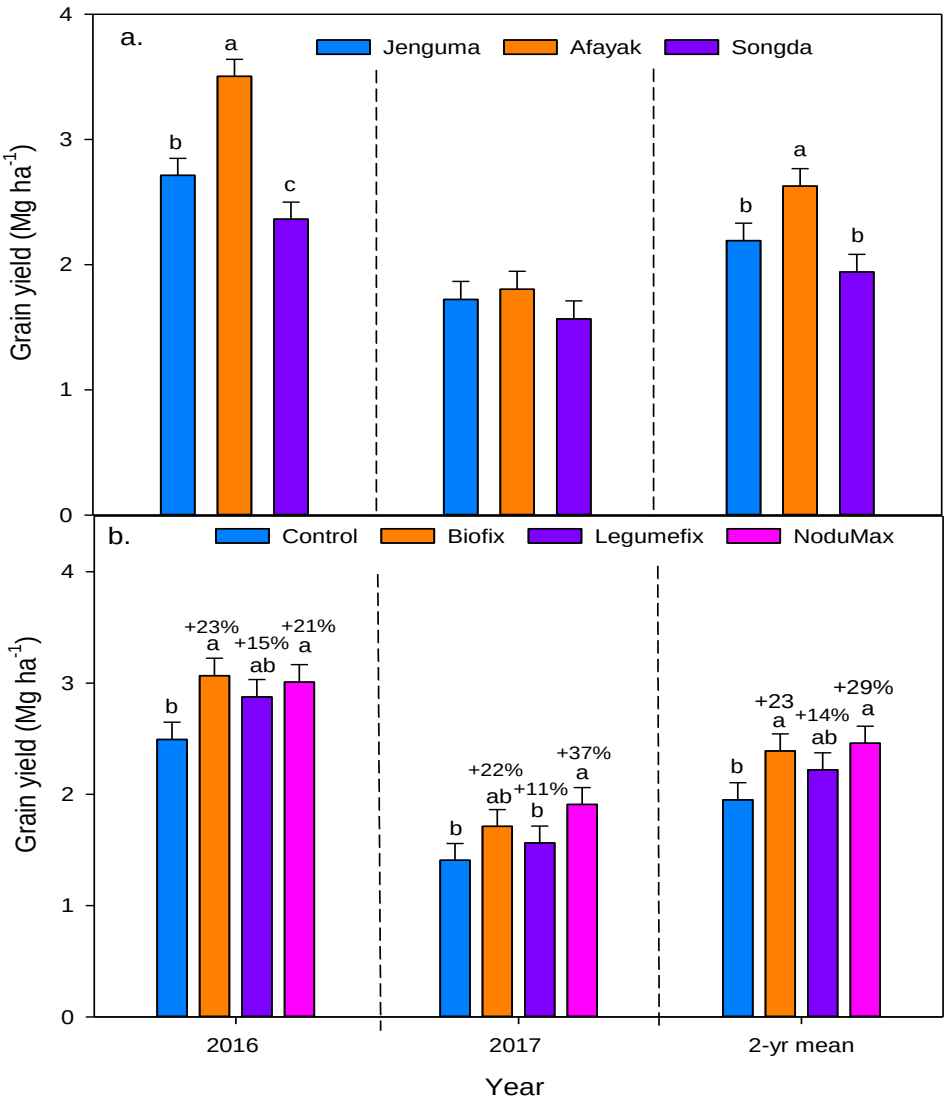
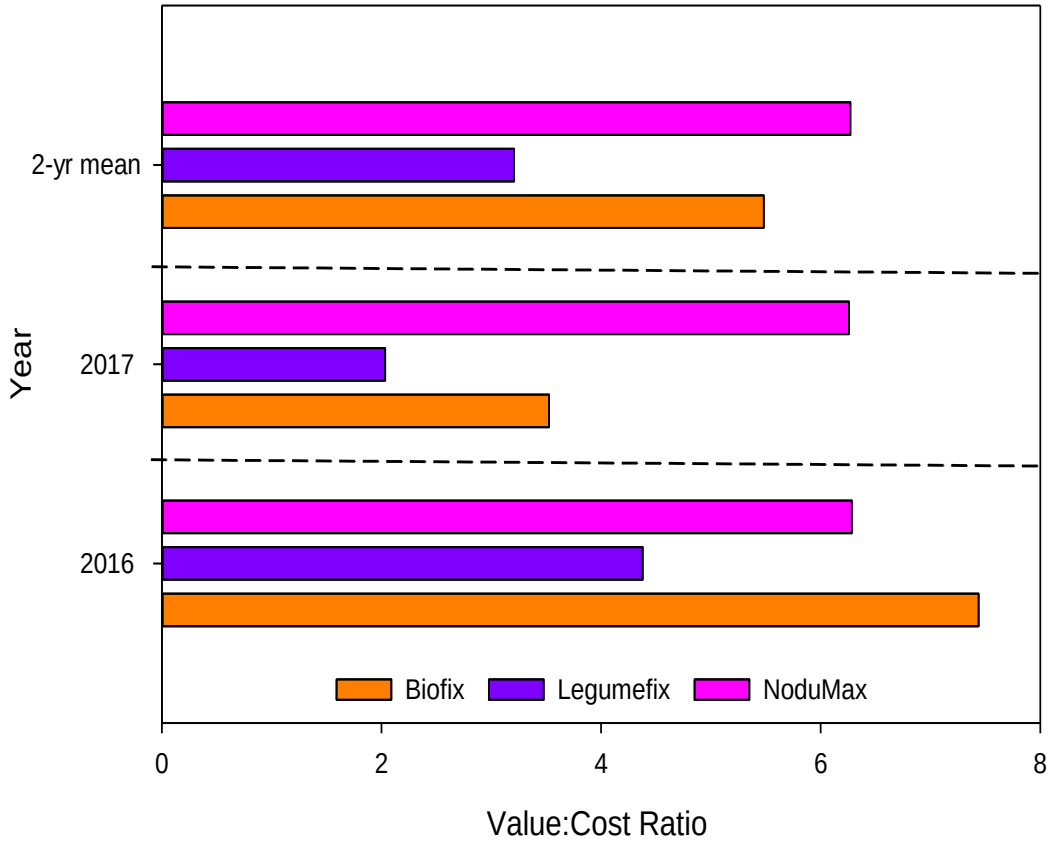


Figure 3.12. Grain yield affected by (a) soybean variety and (b) Bradyrhizobium inoculant in Nyankpala, Ghana in 2016 and 2017.

Lower case letters indicate significant differences at $p < 0.05$. Error bar is a standard error (SE).



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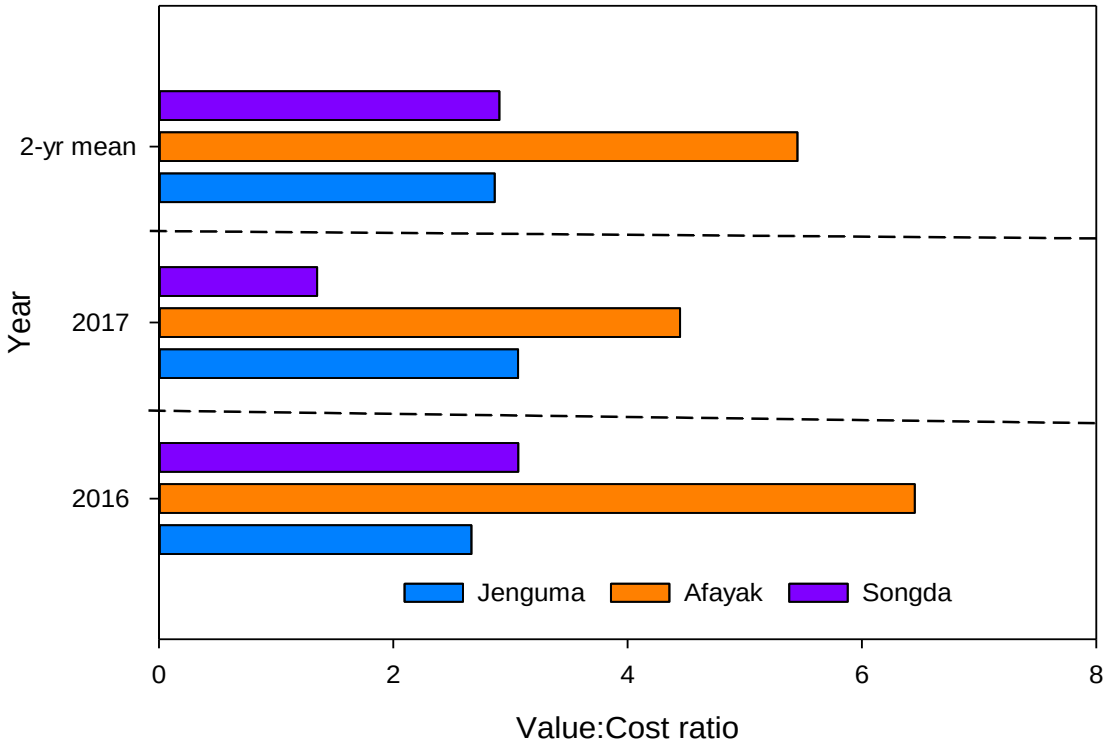

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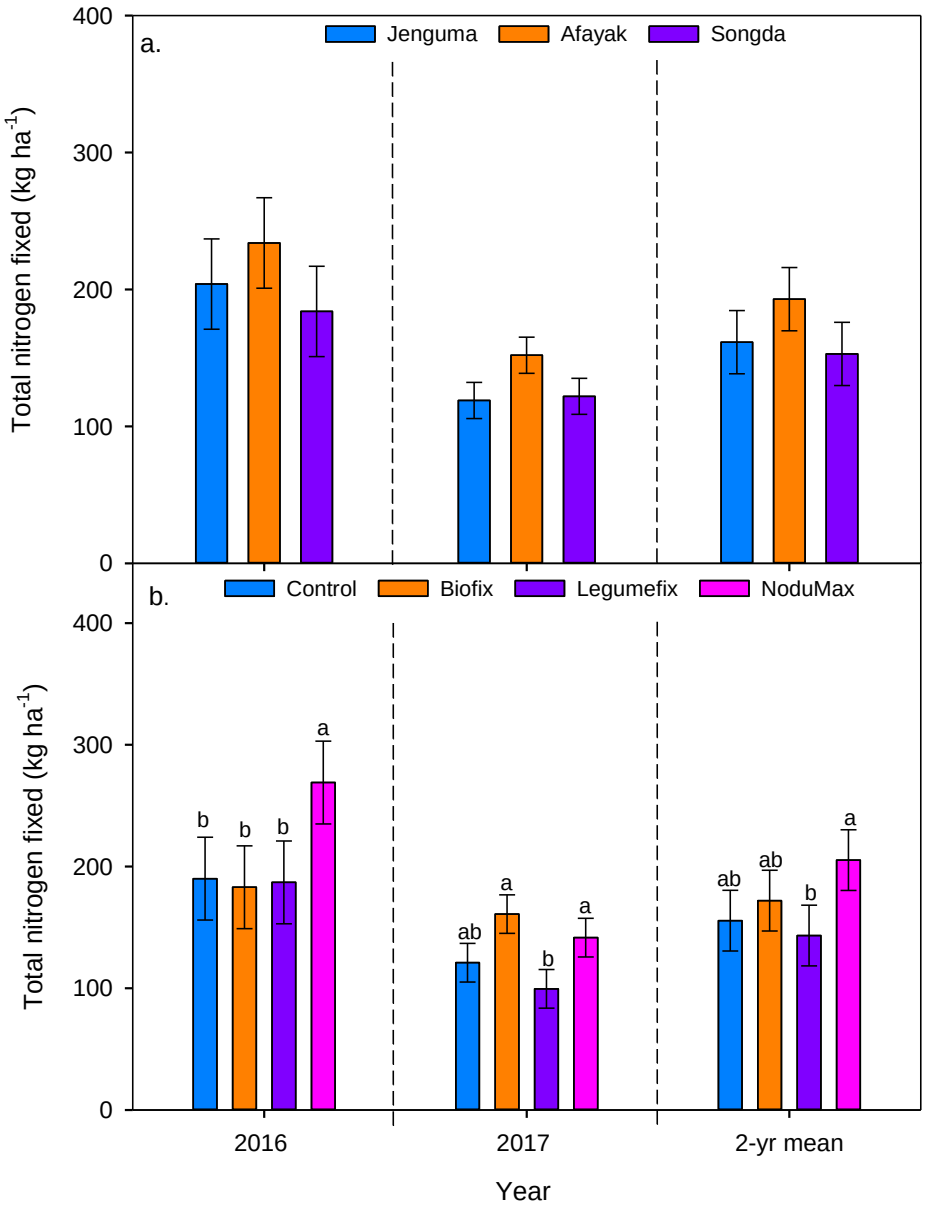

Figure 3.15. Total nitrogen fixation affected by (a) soybean variety and (b) Bradyrhizobium inoculant in Nyankpala, Ghana in 2016 and 2017. Lower case letters indicate significant differences at $p < 0.05$. Error bar is a standard error (SE).

Table 3.1. Soil physicochemical properties at an experimental site in Nyankpala, Ghana in 2016 and 2017.

Depth : 0-15 cm	**Site - 2016**	**Site - 2017**
Soil class (FAO)	Ferric Luvisol	Ferric Luvisol
Soil pH (Soil:H_2O;1: 5)	6.5	6.2
SOC (g C kg^{-1})	3.2	3.8
Total N (g N kg-1)	0.37	0.92
NH_4^+- N (mg N kg^{-1})	5.4	20.7
NO_3-N (mg N kg^{-1})	1.8	1.4
Available P (Bray-1 P) (mg kg^{-1})	6.3	8.2
Exchangeable cations:		
K (mg kg^{-1})	54.0	77.3
Ca (mg kg^{-1})	227	280
Mg (mg kg^{-1})	49.1	71.4
CEC ($cmol^+$ kg^{-1})	13.7	11.4
Sand (%)	69.0	67.4
Silt (%)	29.0	25.9
Clay (%)	2.0	6.7
Texture class	Sandy loam	Sandy loam

Table 3.2. Main effects of soybean variety, commercial Bradyrhizobium inoculant, and growth stage on root nodulation position (number of nodules on crown, tap root, lateral root per plant) and whole root position total nodule dry matter (mg plant) in Nyankpala, Ghana in 2016 and 2017.

Main Effects	Crown root		Tap root		Lateral root		Whole root position		Whole root position nodule	
	number of nodules plant^{-1}								dry wt.(mg plant^{-1})	
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Variety										
Jenguma	4.0 ^{b}	6.2 ^{b}	5.0 ^{b}	4.1 ^{b}	16.8 ^{a}	12.3 ^{a}	26.0 ^{a}	22.7 ^{a}	154 ^{b}	131 ^{b}
Afayak	5.7 ^{a}	8.6 ^{a}	6.6 ^{a}	6.5 ^{a}	14.9 ^{b}	12.7 ^{a}	27.5 ^{a}	27.8 ^{a}	207 ^{a}	185 ^{a}
Songda	4.4 ^{b}	5.4 ^{b}	4.5 ^{b}	3.4 ^{b}	13.3 ^{b}	8.9 ^{b}	22.4 ^{b}	17.6 ^{b}	205 ^{a}	171 ^{a}
Inoculant										
Control	2.8 ^{b}	3.8 ^{c}	3.9 ^{b}	2.5 ^{b}	14	6.5 ^{c}	20.9 ^{b}	12.8 ^{c}	163 ^{b}	113 ^{b}
Biofix	5.0 ^{a}	7.6 ^{ab}	5.5 ^{ab}	5.4 ^{ab}	16	14.4 ^{a}	26.6 ^{a}	27.4 ^{ab}	173 ^{ab}	179 ^{a}
Legumefix	5.5 ^{a}	6.8 ^{b}	6.2 ^{a}	4.3 ^{b}	16.0	10.8 ^{b}	27.9 ^{a}	21.9 ^{b}	208 ^{a}	154 ^{ab}
NoduMax	5.5 ^{a}	8.7 ^{a}	5.9 ^{a}	6.5 ^{a}	14.1	13.4 ^{ab}	25.8 ^{a}	28.7 ^{a}	211 ^{a}	202 ^{a}
Growth Stage										
V8	3.7 ^{c}	4.8 ^{c}	3.3 ^{d}	3.4 ^{c}	5.3 ^{d}	2.5 ^{c}	12.3 ^{d}	10.7 ^{c}	67 ^{c}	120 ^{d}
R2	4.8 ^{b}	6.4 ^{b}	5.1 ^{b}	4.6 ^{b}	14.8 ^{c}	9.5 ^{b}	24.8 ^{b}	20.4 ^{b}	193 ^{b}	97 ^{c}
R3	-	9.2 ^{a}	-	7.2 ^{a}	-	13.9 ^{a}	-	30.3 ^{a}	-	204 ^{a}
R4	7.8 ^{a}	7.6 ^{b}	9.1 ^{a}	4.7 ^{b}	22.2 ^{a}	15.3 ^{a}	39.9 ^{a}	27.6 ^{a}	201 ^{b}	186 ^{b}
R6	2.6 ^{d}	5.8 ^{bc}	3.9 ^{c}	3.4 ^{c}	17.8 ^{b}	15.3 ^{a}	24.3 ^{b}	24.4 ^{a}	294 ^{a}	205 ^{a}
Year	Pr. > F (P-value)									
Variety	0.009	0.001	0.000	0.005	0.020	0.013	0.031	0.002	0.005	0.004
Inoculant	<.0001	<.0001	0.002	<.0001	0.095	<.0001	0.001	<.0001	0.032	<.0001
Variety*Inoculant	0.197	0.648	0.696	0.998	0.013	0.820	0.361	0.991	0.717	0.226
Stage	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Variety*Stage	0.283	0.288	0.031	0.361	0.382	0.196	0.545	0.164	0.012	0.062*
Inoculant*Stage	0.000	0.690	0.233	0.690	0.092*	0.001	0.003	0.014	0.158	0.286
Variet*Inocula*Stage	0.286	0.972	0.091*	0.891	0.026	0.848	0.005	0.946	0.789	0.685

Values within a column followed by the same (letter) are not significantly different at p < 0.05 and p < 0.1 five plants mean nodules*

Table 3.3. Main effects of soybean variety, commercial Bradyrhizobium inoculant, and growth stage on nodule mass at crown root, tap root, side root, upper 5cm root and Lower 5 cm root segment in Nyankpala, Ghana in 2017.

Main Effects	Crown root	Tap root	Lateral root	Total	Upper 5 cm root	Lower 5 cm root	Total
	Nodule mass (mg plant^{-1})						
Variety							
Jenguma	31 ^{b}	18 ^{b}	46 ^{b}	152 ^{b}	65 ^{b}	27 ^{b}	91 ^{b}
Afayak	47 ^{a}	34 ^{a}	55 ^{a}	225 ^{a}	99 ^{a}	36 ^{a}	134 ^{a}
Songda	47 ^{a}	28 ^{a}	44 ^{b}	203 ^{a}	87 ^{a}	34 ^{a}	119 ^{a}
Inoculant							
Control	26 ^{c}	18 ^{c}	41 ^{c}	141 ^{c}	49 ^{c}	27	74 ^{b}
Biofix	47 ^{ab}	28 ^{ab}	56 ^{b}	206 ^{ab}	95 ^{ab}	36	130 ^{ab}
Legumefix	39 ^{b}	23 ^{b}	45 ^{b}	183 ^{b}	79 ^{b}	31	109 ^{b}
NoduMax	54 ^{a}	37 ^{a}	53 ^{a}	244 ^{a}	113 ^{a}	35	145 ^{a}
						NS	
Growth Stage							
V8	15 ^{c}	9 ^{c}	13 ^{d}	50 ^{d}	32 ^{c}	4 ^{d}	36 ^{d}
R2	45 ^{b}	29 b	38 ^{c}	170 ^{c}	81 ^{b}	13 ^{c}	86 ^{c}
R3	67 ^{a}	46 ^{a}	55 ^{b}	276 ^{a}	103 ^{a}	34 ^{b}	137 ^{b}
R4	46 ^{b}	27 ^{b}	62 ^{b}	207 ^{ab}	113 ^{ab}	53 ^{a}	166 ^{a}
R6	36 ^{b}	21 ^{b}	74 ^{a}	264 ^{a}	90 ^{ab}	59 ^{a}	149 ^{ab}
	Pr. > F (P-value)						
Variety	0.002	0.001	0.105	0.001	0.002	0.119	0.001
Inoculant	0.0002	0.0003	0.058*	0.0001	<.0001	0.234	<.0001
Variety*Inoculant	0.413	0.594	0.817	0.436	0.709	0.190	0.546
Stage	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Variety*Stage	0.007	0.158	0.682	0.005	0.082*	0.019	0.119
Inoculant*Stage	0.972	0.738	0.574	0.150	0.0006	0.428	0.003
Variety*Inoculant*Stage	0.821	0.942	0.789	0.739	0.434	0.387	0.401

Values within a column followed by the same (letter) are not significantly different at p < 0.05 and p < 0.1 five plants mean nodules*

T**able 3.4. Main effects of soybean variety, commercial Bradyrhizobium inoculant on dry matter content of shoot, root, pod, and plant at R6 growth stage in Nyankpala, Ghana in 2016 and 2017.**

	Shoot		Root		Pod		Plant	
	Mg ha^{-1} dry matter							
	2016	2017	2016	2017	2016	2017	2016	2017
Variety								
Jenguma	4.9	3.8 [b]	0.54	0.55	1.5 [a]	1.1 [a]	7.1	5.4
Afayak	5.3	5.0 [a]	0.55	0.60	2.1 [a]	1.0 [a]	8.0	6.5
Songda	5.0	4.6 [a]	0.55	0.62	0.9 [b]	0.6 [b]	6.6	5.9
LS Mean	NS		NS	NS			NS	NS
Inoculant								
Control	5.1 [ab]	4.3	0.56	0.56	1.4 [b]	0.9	7.2 [ab]	5.8
Biofix	4.8 [b]	4.8	0.53	0.62	1.4 [b]	1.0	6.8 [b]	6.4
Legumefix	4.6 [b]	3.8	0.49	0.54	1.3 [b]	0.7	6.4 [b]	5.1
NoduMax	5.9 [a]	4.9	0.61	0.64	1.9 a	1.0	8.5 [a]	6.5
LS Mean		NS	NS	NS		NS		NS
	Pr. > F (P-value)							
Variety	0.847	0.087*	0.963	0.379	0.008	0.003	0.280	0.194
Inoculant	0.076*	0.239	0.194	0.282	0.036	0.254	0.048	0.157
Variety*Inoculant	0.638	0.970	0.549	0.959	0.311	0.997	0.550	0.971

Shoot = sum (leaf + Stem)
Plant = sum (leaf + Stem + Pod + Root)
Pod= immature pod with seeds
Mean of 10 plants expressed in Mgha^{-1}based on plant population establishment
Values within a column followed by the same alphabet (letter) are not significantly different at p < 0.05 and p < 0.1, NS = Not significantly different*

Table 3.5. Main effects of soybean variety and commercial Bradyrhizobium inoculant on nitrogen (N) content of shoot, root, pod and plant dry matter and total N fixed at R6 growth stage in Nyankpala, Ghana in 2016 and 2017.

Main Effects	Shoot N content		Root N content		Pod N content		Plant N content		Total N Fixed	
	kg N ha^{-1}									
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Variety										
Jenguma	161	88	11.2	7.8	55.1 [a]	35.8 [a]	233	131	204	119
Afayak	173	123	11.0	9.1	51.3 [a]	30.5 [a]	263	164	234	151
Songda	168	105	11.4	9.5	47.0 [b]	20.1 [b]	213	135	184	122
	NS	NS	NS	NS			NS	NS	NS	NS
Inoculant										
Control	152 [b]	98 [ab]	11.9	8.7	55.1 [b]	26.7	219 [b]	134 [ab]	190 [b]	121[ab]
Biofix	150 [b]	130 [a]	11.0	8.6	51.3 [b]	32.9	212 [b]	174 [a]	183 [b]	161[a]
Legumefix	159 [b]	80 [b]	9.7	8.1	47.0 [b]	23.8	216 [b]	112 [b]	187 [b]	100[b]
NoduMax	207 [a]	112 [a]	12.2	9.9	79.1 [a]	31.9	299 [a]	154 [ab]	269 [a]	142[ab]
			NS	NS		NS				
	Pr. > F (P-value)									
Variety	0.833	0.232	0.932	0.143	0.011	0.001	0.267	0.204	0.267	0.164
Inoculant	0.020	0.085*	0.147	0.283	0.005	0.161	0.011	0.054*	0.011	0.033
Variety*Inoculant	0.712	0.939	0.427	0.390	0.346	0.986	0.649	0.969	0.649	0.794

Shoot = sum (leaf + Stem)*
Plant = sum (leaf + Stem + Pod + Root)*
Pod= immature pod with seeds
Means of 10 plants expressed in kgha^{-1}based on plant population establishment
Values within a column followed by the same alphabet (letter) are not significantly different at p < 0.05 and p < 0.1, NS = Not significantly different*

Table 3.6. Main effects of soybean variety and commercial Bradyrhizobium inoculant on grain nitrogen (N) uptake, haulms nitrogen (N) uptake, total N uptake and Residual N budget (Res. N budget) in Nyankpala, Ghana in 2016 and in 2017.

Main Effects	Grain N uptake		Haulm N uptake		Total N uptake		Res. N budget 1		Res. N budget 2	
	kg N ha^{-1}									
	2016	2017	2016	2017	2016	2017	2016	2017	2016	2017
Variety										
Jenguma	159 ^{b}	81.4	21.7 ^{b}	20.9	180 ^{b}	102	23.5	17.0	45.2	38.0
Afayak	202 ^{a}	83.4	29.3 ^{a}	21.9	232 ^{a}	105	2.7	45.7	32.0	67.6
Songda	138 ^{b}	75.5	26.1^{ab}	21.3	164 ^{b}	96.9	19.5	25.3	45.6	46.6
LSD (P < 0.05)		NS	NS	NS		NS	NS	NS	NS	NS
Inoculant										
Control	165 ^{b}	67.9 ^{b}	22.1	18.8	165 ^{b}	86.7 ^{c}	25.5 ^{ab}	34.6 ^{ab}	47.6 ^{ab}	53.4 ^{ab}
Biofix	208 ^{a}	82.4 ^{ab}	27.5	23.8	208 ^{a}	106 ^{ab}	-24.6 ^{b}	54.7 ^{a}	2.9 ^{b}	78.5 ^{a}
Legumefix	193 ^{ab}	74.7 ^{b}	27.3	18.7	193 ^{ab}	93.4 ^{bc}	-6.7 ^{b}	6.19 ^{b}	20.6 ^{b}	24.9 ^{b}
NoduMax	203 ^{a}	95.5 ^{a}	25.8	24.1	203 ^{a}	120 ^{a}	66.8 ^{a}	21.9 ^{b}	92.6 ^{a}	46.1 ^{ab}
LSD (P < 0.05)			NS	NS						
	Pr. > F (P-value)									
Variety	0.004	0.611	0.007	0.978	0.003	0.756	0.824	0.208	0.908	0.229
Inoculant	0.050	0.005	0.156	0.257	0.048	0.001	0.073*	0.086*	0.082*	0.081*
Variety*Inoculant	0.938	0.104	0.114	0.723	0.837	0.102	0.832	0.545	0.831	0.531

Total N uptake = (grain N+ haulm N) uptake,
Haulm N = Haulm dry matter N (dry stems without leaves)
Residual N budget 1= Total N fixed –Total N uptake,
Residual N budget 2= Total N fixed –Grain N uptake
Mean of 10 plants expressed in kgha^{-1}based on plant population establishment for haulms
Values within a column followed by the same alphabet (letter) are not significantly different at p < 0.05 and p < 0.1, NS = Not significantly different*

Chapter 4 - Commercial Bradyrhizobium Inoculants Impact on Soil Microbiome and Soil health

Abstract

Commercial Bradyrhizobium inoculants allow the colonization of the plant root zone by exogenous microorganisms, thus altering the soil microbial community. We conducted a 2-yrs field experiment to determine how commercial Bradyrhizobium inoculants and soybean varietal selection affects the soil microbial community structure and selected chemical soil properties. The experiment was a split-plot design where the main plot consisted of three soybean varieties: Jenguma (TGX1448-2E), Afayak (TGX1834-5E) and Songda (TGX 1445-3E). The sub-plot consisted of three commercial Bradyrhizobium inoculant; NoduMax, Biofix, and Legumefix plus an uninoculated control. Both bulk (non-rhizosphere) and rhizosphere soils were collected at growth (phenological) stages; V8 (vegetative), R2 (full flowering), R3 (beginning to pods), R4 (full pod) and R6 (pod-fill). Analyses included microbial community structure by phospholipid fatty acid analysis (PLFA), soil pH, soil available N (NH_4^+-N and NO_3-N) and available phosphorus. In the rhizosphere, Biofix and Legumefix inoculants improved microbial biomass and actinomycete abundance compared to the uninoculated control. Afayak stimulated an increase in microbial biomass and actinomycete abundance relative to Jenguma. Gram-negative bacteria and arbuscular mycorrhizae fungi abundance were also affected by a 3-way interaction of commercial inoculant, soybean variety, and growth stage. In the bulk soil, microbial biomass, actinomycete, arbuscular mycorrhizal fungi, and gram-positive abundance were affected by a 2-way interaction of commercial inoculant and soybean variety. NoduMax inoculated Afayak enhanced microbial biomass and gram+ve bacteria. Legumefix inoculated Afayak and uninoculated control Jenguma enhanced greater actinomycete and arbuscular mycorrhizal fungi (AMF) respectively. Biofix and Legumefix inoculants improved soil NH_4^+-N availability in the

rhizosphere compared to the uninoculated control. Biofix stimulated greater phosphorus availability in the rhizosphere than uninoculated control. Afayak induced higher phosphorus availability in the rhizosphere than Jenguma. Conclusively, commercial inoculants and soybean variety selection are crucial to enhancing soil microbial community structure and soil health.

Introduction

The rhizosphere is the zone of the soil adjacent to and directly under the influence of plant roots (Koranda et al., 2011). The rhizosphere is dynamic for different types of soil flora and fauna which are closely associated with soil quality and efficient crop production (Ramakrishnan et al., 2017). The rhizosphere is a hotspot of high microbial activity due to rhizodeposition, and distinctively different from bulk soil regarding mineral nutrients, organic matter, water, oxygen, pH, and redox potential (Berg and Smalla, 2009; Koranda et al., 2011). Microbial community composition, diversity, and function in the rhizosphere and bulk soil are affected by crop and soil management, and as well as crop species or crop genotype. Crop management affects the soil microbial community structure and function, and subsequently the health and productivity of the soil (Meriles et al., 2009). Management impacts the soil microbial community structure through the influence of agrochemicals and fumigants (Ibekwe et al., 2001; Meriles et al., 2006, 2009), mineral and organic amendments (Wood et al., 2015; Ridl et al., 2016; Francioli et al., 2016), biological inoculants such as AMF fungi (Trabelsi and Mhamdi, 2013; Rodriguez and Sanders, 2014), cyanobacteria and bacteria (Ramakrishnan et al., 2017). Nonetheless, little documentation exists on the impacts of human-induced management on the microbial community structure and function in sub-Saharan West Africa (SSWA).

Crops can exert species-specific influences on soil microbial community structure (Alvey et al., 2003). The impact of crop species or crop genotype selection on the soil microbial

community structure of both the rhizosphere and the bulk soil have been well documented (Marschner et al., 2004; Rengel and Marschner, 2005; Meriles et al., 2009; Bakker et al., 2015). Yao and Wu (2010) observed that cucumber cultivars altered the rhizosphere microbial community structure. Soybean genotypes changed the rhizosphere bacterial communities in both fields and controlled environment trials (Xu et al., 2009).

Crop growth and development also induce greater changes in the rhizosphere bacterial communities compared to soybean genotype due to root exudation and environmental conditions (Xu et al., 2009; Sugiyama et al., 2014). Root exudation increases qualitatively and quantitatively with crop growth and development (Maloney et al., 1997; Xu et al., 2009). Root provide substrate for microbial growth in soil. In general microbial community composition and functional processes are higher in the rhizosphere than the bulk soil due to higher exudation or rhizodeposition. Environmental conditions such as soil temperature and soil moisture vary with crop growth and development (Xu et al., 2009). Likewise, soil structure development, texture, pH, mineral nutrients, soil organic carbon, and total N induce significant shifts in microbial community structure in the rhizosphere (Marschner et al., 2001).

Soybean is an ideal crop to investigate plant-microbial interaction in the rhizosphere due to its agricultural importance and ability to form symbiotic association with arbuscular mycorrhizal fungi (AMF) and *Bradyrhizobium japonicum* (Xu et al., 2009; Sugiyama et al., 2014; Li et al., 2016). *Bradyrhizobium japonicum* stimulate nitrogen fixation (Carbonetto et al., 2016). Arbuscular mycorrhizal fungi (AMF) enhances mineral nutrient availability (especially phosphorus) and water uptake by plants. Plant-growth-promoting rhizobacteria (PGPR) interaction in soybean induce either beneficial or harmful effects to the soybean plants (Vivanco and Baluska, 2012). The beneficial effect of PGPR includes disease suppression (Mendes et al.

2011; Huang et al., 2014), increased immunity to abiotic (Selvakumar et al., 2012; Zolla et al., 2013) and biotic stresses (Badri et al., 2013b; Zamioudis and Pieterse, 2012).

Commercial microbial inoculants such as arbuscular mycorrhizal fungi, actinomycete, and rhizobium inoculants are sometimes needed to enhance efficient microbial association with soybean. Microbial inoculation promotes rapid colonization of the rhizosphere thereby altering the microbial community structure and diversity (Ramakrishnan et al., 2017). The introduction of external microorganisms into the native soil microbial pool by either seed or soil inoculation can alter the soil microbial community structure. Inoculation of chicken pea with microbial inoculant increased both the abundances of gram-negative bacteria and total PLFA microbial biomass in the rhizosphere (Ramakrishnan et al., 2017) inducing a shift in the microbial community structure from gram-positive bacteria to gram-negative bacteria. Recent work by Nyoki and Ndakidemi (2018) revealed that inoculating soybean with commercial inoculants enhanced some selected soil chemical properties; such as pH, SOC, electrical conductivity, macro (N, P, K, Ca, and Mg) and micro (Na, Fe, Cu, Mn, and Zn) nutrients in the rhizosphere.

In Ghana, research on how crop cultivar and commercial microbial inoculants affect the soil microbial community composition and diversity, and the soil physico-chemical properties are not well documented due to logistic and skilled personnel constraints. In-depth knowledge is needed to understand the extent to which commercial *Bradyrhizobium* inoculant and promiscuous nodulating soybean cultivars affect the microbial community structure and physico-chemical properties in the rhizosphere and the bulk soil.

The objective of this study was to determine how (1) commercial Bradyrhizobium inoculants affect the soil microbial community structure and selected soil chemical properties (2) TGX soybean cultivar affects the soil microbial community structure and selected soil chemical

properties. We hypothesized that inoculation would increase that gram-negative (gram-ve) bacteria abundance in the rhizosphere than the bulk soil.

Materials and Methods

Study Site

The study was conducted for two consecutive years (2016 and 2017) at the Savanna Agricultural Research Institute (SARI) research fields located in Nyankpala (N 09.39253^{o} W 001.00228^{o} 189 m, and N 09.39172^{o} W 001.00286^{o} 188 m) in the Northern Ghana Guinea Savanna Zone of West Africa.

The climate was characterized by 5-6 humid months with annual mean precipitation of 1095 mm and classified as a summer-humid dry climate (Horst and Härdter, 1994). The soil at the 2016 and 2017 experimental site was well-drained sandy loam classified as a Typic-plinthic Paleustalf according to the USDA Soil Taxonomy. The baseline soil properties are documented in Table 4.1.

The 2016 study site was previously cropped to maize for three consecutive years where mineral fertilizer was applied. The 2017 site was cropped to cowpea in 2015, and maize in 2016 with mineral fertilizer applied. At the end of the cropping season (after harvest), the area was allowed to fallow and the crop residues (cowpea and maize stover) were left on the fields.

Experimental Design and Treatments

The experiment was designed as a split-plot with a randomized complete block design (RCBD). The main plot consisted of three promiscuous nodulating soybean cultivars: Jeguma (TGX1448-2E), Afayak (TGX1834-5E) and Songda (TGX 1445-3E). The subplot consisted of three commercial *Bradyrhizobium japonicum* inoculants; Biofix, Legumefix and NoduMax in

addition to an uninoculated control. The treatments were replicated four times. The field was disc plowed and harrowed before establishment and afterward manually leveled by hoes. Ridges were manually constructed using hoes at 50 cm part. Each experimental plot measured 16 m^2 (4 x 4 m^2) with eight (8) hand-made ridges at 50 cm part.

The soybean seeds were obtained from the soybean breeding division of SARI, Nyankapala. All soybean cultivars had a maturity period of 110-118 days. These soybean varieties are excellent trap-crop for Striga (*Striga hermonthica*) and rust disease resistant (*Phakopsora pachyrhizi and Phakopsora meibomiae*) (Denwar and Wohor, 2012). Jenguma (TGX1448-2E) and Afayak (TGX1834-5E) were non-shattering cultivars while Songda (TGX 1445-3E) shatters (up to 20 %) if not harvested early. Both Afayak (TGX1834-5E) and Songda (TGX 1445-3E) were released in 2012. Jenguma (TGX1448-2E) was released in 2003 and was popular with local farmers. Maize (*Zea mays* L.) was planted along with the soybean as the reference crop.

The commercial inoculants were peat based and sourced from different vendors. Legumefix contained *Bradyrhizobium japonicum* strains *USDA 532c*, and was obtained from Legume Technology Ltd., UK. Biofix was obtained from MEA fertilizer in Nairobi, Kenya and has *Bradyrhizobium japonicum* strain *USDA 110*. NoduMax also contained *Bradyrhizobium japonicum* strain *USDA 110* and was sourced from the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria. Both Biofix and NoduMax contained a minimum of 1 x 10^9 viable cells g^{-1} of inoculant while Legumefix contained a minimum of 2 x 10^9 viable cells g^{-1} of inoculant according to the manufacturers. However, prior to inoculation, the *Bradyrhizobium* population in the commercial inoculants and the baseline soil was enumerated using ten-fold serial dilution techniques on yeast-mannitol agar (YMA) with congo red (CR) (Somasegaran and

Hoben, 1994). The Bradyrhizobium population was expressed as colony forming unit (CFU) . The estimated Bradyrhizobium population was 5.8 x 10^{2} CFU g^{-1} soil for the baseline soil, 1.8 x 10^{8} CFU g^{-1} inoculant for Legumefix and 1.8 x 10^{9} CFU g^{-1} inoculant for Biofix.

Inoculation of soybean seed was done following the procedure of Hungria et al. (2006). Briefly, 10 g of the inoculant was added to 1 kg of seed. A 10 % gum arabic (*Acacia Senegal*) (wt/vol) solution was used to increase adhesion of the peat, at 300 mL 15 kg^{-1} seed. The seeds were air-dried under shade for 15-20 mins before sowing

Seeds were manually sown on ridges at 50 cm inter-ridge (row) distance and 10 cm inter-plant distance to a depth of 5cm. Sowing was done on July 4 and July 3 in 2016 and 2017 respectively. To avoid contamination, non-inoculated treatments were planted first before inoculated treatments. Four seeds were sown per hill but later thinned to two plants at 13 days after of sowing (DAS). Replanting was done at eight days after seedling emergence. Maize (*Zea mays* L.), was also planted along with the soybean as a reference crop at 50 cm inter-ridge (row) distance and 60 cm inter-plant distance to a depth of 5cm. *Zea mays var* Abrohema, and *Zea mays var* Wang-data were the maize cultivars sowed in 2016 and 2017, respectively. Both have a maturity period of 100-118 days.

Fifteen days after sowing (DAS), 30 kg K ha^{-1} and 30 kg P ha^{-1} were applied from Muriate of potash (MoP) and Triple Super Phosphate (TSP), respectively. The fertilizer was banded 3-5 cm from the plants at 5 cm depth.

Basagran, pre-emergence herbicide (with the active ingredient (ai): Sodium salt of Bentazon) was applied at the rate 1 L ha^{-1} after sowing. Afterward, weeds were controlled manually by hoeing at 3, 6, and 9 weeks after sowing (WAS). A different set of hoes were assigned to each treatment to prevent cross-contamination.

Sampling and Data Collection

Soil Sampling

Sampling was done following the stages of soybean development as reported by Fehr et al. (1971) and Fehr and Caviness (1977). Briefly, soil was sampled 33 (6/8/16), 50 (23/8/16), 73 (15/9/16), 87 (29/9/16), and 108 (20/10/16) days after sowing (DAS) representing V8 (8-leaf), R2 (full flower), R4 (full pod), R6 (pod-fill or seed-fill) and R8 (seed-maturity) stages respectively in 2016. In 2017, soil sampling was done at 35 (9/8/17), 51 (23/8/17), 64 (5/9/17), 79 (20/9/17), and 88(29/9/17) days after sowing (DAS) representing V8 (8-leaf), R2 (full flower), R3 (beginning to pod), R4 (full pod), and R6 (Pod-fill or Seed-fill) stage respectively. At each sampling time, 10 plants were randomly sampled from the 2^{nd} and the 7^{th} ridges per plot, avoiding the areas marked for harvesting of grain yield (Hungria et al., 2006). Plants were uprooted with spade avoiding the chopping off of the roots. Soils attached to the roots were gently shaken. Soil particles tightly attached to the root surface after the gentle shake was referred to as rhizosphere soil (Alvey et al., 2003). The soil from the rhizosphere of 10 soybean plants was composited to form a single pooled sample per plot. The bulk soil (non-rhizosphere soil) was collected randomly from 10 locations per plot using an ethanol-sterilized soil probe at a depth of 0–15 cm and composited as a single pooled sample (Liu et al., 2017). Afterward, both composited rhizosphere and bulk soil were partitioned into two halves. The first half meant for phospholipid fatty acid analysis was immediately transported to the laboratory on an ice pack to prevent deterioration. At the laboratory, both the rhizosphere and the bulk soil were stored in a −40 °C freezer. Later, samples were freeze-dried for two days at -50ºC and at a pressure of 0.009 torrs. The freeze-dried samples were sieve with 1 mm mesh size to remove debris and stones before phospholipid fatty acid analysis (PLFA). The second half was air-dried immediately after

sampling. After 3-4 days samples were passed through 2 mm mesh sieve to remove debris, roots, and stones (Liu et al., 2017).

Soil Chemical Analyses

Soil available N (NH_4-N and NO_3-N) was assessed on both rhizosphere and bulk soil. Briefly, 5g of soil was extracted with 20 mL of 1M KCl solution and shaken on a digital shaker (VWR) for 1 hr. The slurry was filtered using Whatman filter paper size 42 (110 mm diameter size). The filtrate was frozen at -20^o C, and later analyzed colorimetrically for NH_4^+– N and NO_3-N concentrations by flow injection on a Lachat Quik Chem 8000 Flow Injection Analyzer (Lachat Instruments, Loveland, CO, USA) (Maul and Drinkwater, 2010). Available soil P was assessed on both rhizosphere and bulk soil using Bray-1 P method (Gil et al., 2009). Soil pH (1:5, soil: H_2O) was also assessed on both rhizosphere and bulk soil following the procedure reported by Meriles et al. (2009) with little modification. Briefly, 2g of soil was added to 10 mL of nanopure water and shaken. The suspension was allowed to stand for 15 mins, then shaken again, and then allowed to settle before the final reading was taken with Orion pH Meters (Thermo Scientific™ Orion Star™ A111 pH Benchtop Meter)

Soil Biological Analyses

Phospholipid Fatty Acid Analysis

Phospholipid fatty acid (PLFA) extraction was conducted following the procedure of Bligh and Dyer (1959) as described by Bossio and Scow (1998). Briefly, lipids were extracted from 5g of freeze-dried soil in a single-phase chloroform-methanol-phosphate buffer system. Phospholipids were separated from neutral lipids and glycolipids on solid phase extraction columns (Supelco, Inc., Bellefonte, PA, The USA). After methylation of the polar lipids, PLFA methyl esters were analyzed on an Agilent 6850 N gas chromatography (GC, Agilent Tech. Co.,

USA) equipped with an HP-5 capillary column (30 m×0.32 mm×0.25 μm) and a flame ionization detector (FID). The Sherlock Microbial Identification System (MIDI) (Microbial ID Inc., Newark, NJ, USA) was used to identify fatty acids. Nonadecanoic acid methyl ester (19:0, Sigma) was added as internal standard and used to convert fatty acid peak areas to absolute abundance. About forty-one individual PLFAs consistently present in the samples were used for data analysis. The sum of i14:0, a15:0, i15:0, i16:0, a17:0, and i17:0 was used to indicate gram-positive bacteria (gram +ve) and the sum of 16:1 2OH, 16:1ω7c, 16:1ω9c, cy17:0, 17:1ω8c, 18:1ω7c, and cy19:0 was used to indicate gram-negative bacteria (gram -ve) (Zogg et al., 1997; Liang et al., 2014). The sum of 10Me16:0, 10Me17:0, and 10Me18:0 was used to indicate actinomycetes. The biomarker 16:1ω5c to was used indicate arbuscular mycorrhizal fungi (AMF) (Olsson, 1999; Wang et al., 2012; Cobb et al., 2017), the sum of 18:1ω9c and 18:2ω6c to indicate saprotrophic fungi (SF) (Cobb et al., 2017). The sum of all the functional group's biomarkers and non-specific biomarkers (14:0, 15:0, 16:0, 17:0, 18:0, and 20:0) were added to represent total PLFA microbial biomass (Cobb et al., 2017). The PLFA based fungi (F): bacteria (B) ratios was estimated as the proportion of fungi relative to bacteria and expressed in percent:

$$\%\ proportion\ F{:}B = \frac{Fungal\ index}{Bacterial\ index}\ x\ 100$$

Statistical Analysis

Phospholipid fatty acid analysis (PLFA) profiles and the soil chemical properties (soil pH, available soil N (NH_4^+-N and NO_3-N) and available P) were analyzed using PROC MIXED model in SAS® version 9.4. Copyright © 2014 SAS Institute Inc., Cary, NC, USA (SAS Institute, 2014). Soybean variety, commercial inoculants, growth stage and their interaction were the fixed effects. Block and the interaction of Block and Soybean variety were the random effects. Growth stage was fitted as the repeated measure and with slice effect option.

Significance level among treatments was declared at $\alpha = 0.1$ probability level, as we envisaged high heterogeneity to occur on the field. Means were separated using Fisher's least significant difference (Fisher's LSD).

In 2016, samples for phospholipid fatty acid analysis (PLFA) were held in transient for 2-3 weeks before arrival at the laboratory. The only exception was V8 growth stage samples. Since phospholipid fatty acid (PLFA) is found in live microbial cells, and do not store in the soil (Zelles, 1992). It is highly likely the PLFA-soil samples held in transit somewhat deteriorated. Further, soil samples for chemical properties analyses (soil pH, available soil N (NH_4+-N and NO_3-N), and available P) were not air dried immediately after sampling but stored for a week before drying. There was also high probability that the prolong storage affected soil available N and pH results. Therefore, we restricted our results and discussion on solely 2017 data. The 2016 results are found in the appendix.

Results

Baseline Soil Analysis and Precipitation Pattern

The soil analysis (Table 4.1) revealed that the soil was of low soil quality and fertility. Soil pH was slightly acidic (6.2-6.5). Available P was low and below the critical level of 20-30 mg P kg^{-1} soil as recommended for crop production in the tropical Guinea Savanna Zone of West Africa. Soil organic C and total N were less than 0.1% and 0.07% respectively, with low CEC. Available N (NH_4^+-N + NO_3-N) was highly variable, the 2017 site had greater available N (20.7 mg kg^{-1}) than 2016 site (7.1 mg kg^{-1}). The sandy loam texture allows for high leaching potential of nutrients during excessive precipitation. Precipitation and daily temperature pattern for both 2016 and 2017 were similar (Fig. 4 1). Nonetheless, higher precipitation and cooler daily temperature were observed in 2017 relative to 2016 respectively (Fig. 4.1).

Commercial Inoculants Impact on Soil Microbial Community Structure

Gram-negative bacteria

Rhizosphere gram-negative **(**gram-ve) bacteria was affected by the three-way interaction of soybean variety, commercial *Bradyrhizobium* inoculant, and growth stage (Fig. 4.2a, b & c). The rhizosphere gram-ve bacteria under each soybean variety showed variable response to the commercial inoculants at the different growth stages. Commercial inoculant influenced on rhizosphere gram-ve bacteria was evident at the R2 growth stage, and after that declined. For Afayak, inoculation with Biofix had greater rhizosphere gram-ve bacteria compared to the other treatments at the R2 stage. For Jenguma, inoculation with Biofix enhanced rhizosphere gram-ve bacteria compared to the other treatments at the R2 stage. For Songda, the uninoculated control, and Legumefix increased rhizosphere gram-ve bacteria relative to the other treatments at the R2 stage. Across all soybean cultivars, Biofix inoculated Afayak enhanced rhizosphere gram-negative bacteria abundance. In the bulk soil (non-rhizosphere soil) gram–ve bacteria was not affected by soybean variety, commercial inoculants, and their interaction effects.

Gram-positive (gram+ve) bacteria

In the rhizosphere, gram-positive (gram+ve bacteria) abundance was not significantly affected by soybean variety, commercial inoculant, and their interaction. Average, gram+ve bacteria abundance ranged from 3.9 nmol g^{-1}soil with Songda to 4.1 nmol g^{-1}soil with Afayak, and 3.8 nmol g^{-1}soil with the uninoculated control to 4.1 nmol g^{-1}soil with Biofix.

In the bulk soil, gram-positive (gram+ve) bacteria abundance was affected by the interaction of soybean variety and commercial inoculants (Fig. 4.6a). Across all soybean varieties, greater gram+ve bacteria abundance was associated with Afayak inoculated with Biofix and Legumefix. While lower gram+ve bacteria abundance was associated with the uninoculated control Afayak and Songda, and as well as Jenguma inoculated with Biofix and

Legumefix, respectively. Regarding the soybean varieties, commercial inoculant effects on bulk soil gram+ve bacteria abundance was variable. Inoculating Jenguma and Songda with commercial inoculants did not significantly alter the bulk soil gram+ve bacteria abundance. For Afayak, Bioifx and Legumefix increased gram+ve bacteria abundance in the bulk soil compared to the uninoculated control.

Arbuscular mycorrhizal fungi (AMF)

Rhizosphere arbuscular mycorrhizal fungi (AMF) abundance was also affected by the three-way interaction of soybean variety, commercial inoculant and growth stage (Fig. 4.2d, e & f). Rhizosphere arbuscular mycorrhizal fungi (AMF) abundance varied with growth stage and commercial inoculant among the different soybean varieties. The interaction of commercial inoculant and soybean variety on rhizosphere AMF was profound at the R2 growth stage, and after that, rhizosphere AMF abundance declined. For Jenguma, the uninoculated control, NoduMax, and Legumefix had greater rhizosphere AMF than Biofix. In Afayak, inoculation with commercial inoculants had higher rhizosphere AMF abundance than uninoculated control. The uninoculated control Afayak also experienced a linear increased in rhizosphere AMF abundance with growth stage progression. For Songda, inoculation with commercial inoculants did not induce any observable differences in AMF abundance in the rhizosphere. Across all varieties, Biofix inoculated Afayak had a greater rhizosphere AMF abundance at the R2 growth stage.

Bulk soil AMF abundance was affected by the interaction of soybean variety and commercial inoculant (Fig. 4.5b). Across all soybean varieties, the uninoculated control Jenguma and Biofix inoculated Afayak had the greatest bulk soil AMF, respectively. The uninoculated control Afayak and Songda had the least bulk soil AMF respectively. For the soybean varieties,

with Jenguma, the uninoculated control had a greater abundance of bulk soil AMF than Legumefix and Biofix,. In Afayak, inoculation with Biofix facilitated greater bulk soil AMF than uninoculated control. In Songda, bulk soil AMF was not significantly affected by commercial inoculants.

Actinomycete Abundance

Rhizosphere actinomycete was significantly affected by main effects of commercial inoculants and soybean variety (Fig. 4.3a & b). Legumefix increased actinomycete abundance in the rhizosphere compared to NoduMax and the uninoculated control (Fig. 4.3b). Likewise, Boifix induced actinomycete abundance in the rhizosphere than the uninoculated control (Fig. 4.3b). Afayak had increased rhizosphere actinomycete abundance than the other varieties (Fig. 4.3a). Bulk soil actinomycete abundance was affected by the interaction of soybean variety and commercial inoculant (Fig. 4.5a). Bulk soil actinomycete abundance varied with soybean variety and commercial inoculant. Legumefix inoculated Afayak had greater actinomycete abundance in the bulk soil whereas Biofix inoculated Jenguma had lower actinomycete abundance (Fig. 4.5a). For each soybean variety, commercial inoculants induced significant variability in bulk soil actinomycete abundance (Fig. 4.5a). For Jenguma, inoculation with NoduMax enhanced bulk soil actinomycete abundance than Biofix (Fig. 4.5a). In Afayak, Legumefix increased bulk soil actinomycete abundance compared to the uninoculated control (Fig. 4.5a). Finally, in Songda, commercial inoculants had no significant influence on bulk soil actinomycete abundance (Fig. 4.5a).

Saprophytic Fungi

Rhizosphere fungi were not significantly affected by commercial inoculants (Fig. 4.4a & b). Nonetheless, commercial inoculants marginally increased saprophytic fungi abundance

compared to the uninoculated control (Fig. 4.4a). Soybean variety significantly affected rhizosphere saprophytic fungi abundance (Fig. 4.4b). Afayak stimulated saprophytic fungi abundance in the rhizosphere than Songda (Fig. 4.4a).

In the bulk soil, saprophytic fungi abundance was not significantly affected by soybean variety or commercial inoculants (Fig. 4.4c & d). Saprophytic fungi abundance was slightly higher on fields inoculated with commercial inoculants compared to the uninoculated control (Fig. 4.4d). For soybean variety, Afayak stimulated saprophytic fungi abundance more than Jenguma and Songda in the bulk soil (Fig. 4.4c).

PLFA-Microbial Biomass

In the rhizosphere, PLFA-microbial biomass was significantly affected by soybean variety, commercial inoculant and growth stage main treatment effects (Fig. 4.3c). Biofix and Legumefix inoculants increased the rhizosphere PLFA-microbial biomass compared to the uninoculated control (Fig. 4.3c). Our result suggests that commercial inoculants can induce significant changes in the rhizosphere PLFA-microbial biomass. Similarly, Afayak stimulated rhizosphere PLFA-microbial biomass compared to Jenguma (Fig. 4.3c). Result suggests that the cultivation of improved soybean varieties had significant potential to alter the rhizosphere PLFA-microbial biomass.

In the bulk soil, PLFA-microbial biomass abundance was affected by the interaction of soybean variety and commercial inoculant (Fig 4.6b). Biofix inoculated Afayak had greater bulk soil PLFA-microbial biomass (Fig. 4.6b). While the uninoculated control Afayak had the lower bulk soil PLFA-microbial biomass (Fig.4.6b). Each soybean variety exhibited differential responses to the commercial inoculants (Fig. 4.6b). For Afayak, Biofix significantly altered bulk soil PLFA-microbial biomass compared to the uninoculated control. For Jenguma and Songda,

commercial inoculants did not significantly alter the PLFA-microbial biomass in the bulk soil (Fig.4.6b).

PLFA- microbial biomass in both the rhizosphere and the bulk soil increased with growth stage and significantly peaked at R2 stage, and subsequently declined (Fig. 4.8). In general higher PLFA- microbial biomass was observed at R2 stage compared to V8- stage in both the bulk soil and the rhizosphere (Fig. 4.8). The rhizosphere had greater PLFA- microbial biomass than the bulk soil (Fig. 4.8). This may be attributed to higher root exudation.

Growth Stage Effect on Microbial Community Structure

In general, the rhizosphere had higher microbial community composition or microbial community grouping than the bulk soil in 2017 (Fig. 4.7a & b). Gram-negative bacteria, gram-positive bacteria, fungi and arbuscular mycorrhizal fungi (AMF) in both the bulk soil and the rhizosphere were significantly affected by growth stage (Fig. 4.7a & b). Gram-positive bacteria in both the bulk soil and the rhizosphere peaked at the R2 and R4 stage. Saprophytic fungi generally increased with the growth stage from V8 through R4 stage (Fig. 4.7a & b). In the bulk soil fungi abundance remained stable after V8 growth stage while the rhizosphere saprophytic fungi increased with growth stage peaking at the R4 growth stage (Fig. 4.7a & b). Actinomycete abundance in both the bulk soil and the rhizosphere was significantly lower at R2 growth stage (Fig. 4.7a & b). These microbial communities were highly variable with growth stage.

Commercial Inoculants Impact on Soil Chemical Property

Available soil nitrogen

Soil NH_4^+ concentration was significantly affected by commercial inoculant (Fig. 4.9c & d). Biofix and Legumefix inoculant stimulated higher rhizosphere ammonium (NH_4^+-N) compared to the uninoculated control (Fig. 4.9d). Ammonium (NH_4^+-N) concentration in

rhizosphere was not affected by soybean variety (Fig. 4.9d). Afayak had higher ammonium (NH_4^+-N) concentration than the other varieties although not statistically different (Fig. 4.9c).

Soil nitrate (NO_3-N) concentration in the rhizosphere was not significantly affected by the soybean variety or commercial inoculant and their interaction effects (Appendix Fig. C.1). The rhizosphere nitrate concentration by commercial inoculants ranged from 0.72 mg kg^{-1} with Legumefix to 0.98 mg kg^{-1} with uninoculated control (Appendix Fig. C.1). For the soybean variety, nitrate ranged from 0.77 mg kg^{-1} with Jenguma to 0.86 mg kg^{-1} with Afayak (Appendix Fig. C.1).

Growth stage significantly altered soil NO_3-N and NH_4^+-N concentration in the rhizosphere (Appendix Fig. C.3). The V8 growth stage had greater rhizosphere NH_4^+-N compared to the other growth stages (Appendix Fig. C.3). Similarly, increased rhizosphere NO_3-N was observed at the V8 growth stage compared to the R3 growth stage (Appendix Fig. C.3).

Available soil phosphorus

Rhizosphere phosphorus (P) was significantly affected by soybean variety and commercial inoculant (Fig. 4.9a & b). For the commercial inoculant, Biofix induced greater rhizosphere P than the uninoculated control (Fig. 4.9a & b). For soybean variety, Afayak had greater rhizosphere P than Jenguma (Fig. 4.9a & b). Additionally, significantly greater rhizosphere P was found at R2 growth stage than R6 growth stage (Appendix Fig. C.3). Rhizosphere P at R2 growth stage was 36% more than the R6 growth stage (Appendix Fig. C.3).

Soil N:P

The soil N:P reflects the change or shift in the soil nitrogen to phosphorus. Jenguma had higher soil N:P compared to Songda and Afayak (Fig. 4.10a). Likewise, Songda also had greater soil N:P than Afayak (Fig. 4.10a). Commercial inoculant also affected soil N:P. The

uninoculated control had greater soil N:P than the rest of the other treatment (Fig. 4.10 b). This was followed by both Legumefix and NoduMax while Boifix had lower soil N:P (Fig. 4.10 b). In general higher soil N:P reflects increased soil nitrogen enrichment relative to P enrichment (P acquisition). Thus as available soil N increases, soil available P decreases.

Soil pH

The bulk soil and the rhizosphere pH were not significantly affected by soybean variety or commercial inoculant, and their interaction effects (Appendix Fig. C.2). Nonetheless, pH for both the bulk soil and the rhizosphere were below 6, which is an indication of soil acidity, and has an implication on nutrient availability especially phosphorus (Appendix Fig. C.2). Further, both the bulk soil and the rhizosphere pH were significantly affected by growth stage progression (Appendix Fig. C.3). The rhizosphere pH increased with growth stage (Appendix Fig. C.3). Thus rhizosphere pH was less acidic at R6 stage compared to the V8 stage. The bulk soil pH was less acidic at the V8 and the R4 stages and was more acidic at the R2 stage (Appendix Fig. C.3).

Discussion

Commercial Inoculants Impacts on Microbial Community Structure

The introduction of exogenous organisms into the soil microbial pool either by seed or soil inoculation can induce significant changes in soil microbial community structure. The most significant roles performed by an introduced microorganism include nutrient cyclings such as (1) arbuscular mycorrhizal fungi (AMF) facilitating the uptake of phosphorus and water from the soil, and (2) rhizobium enhancing nitrogen fixation (Trabelsi and Mhamdi, 2013). Inoculation with commercial microbial inoculants ensures that the appropriate microorganisms colonizes the rhizosphere or are introduced into the native soil microbial pool.

In the present study, inoculation of promiscuous nodulating soybean varieties (TGX soybean cultivars) with commercial inoculants altered the microbial community structure and selected chemical properties of both the rhizosphere and the bulk soil. We observed an increase in gram-negative bacteria community, actinomycete, arbuscular mycorrhizal fungi, and PLFA-microbial biomass in the rhizosphere than the bulk soil. These findings corroborate with Huang et al. (2014) who observed different microbial communities in the rhizosphere than the bulk soil. The abundance of these microbial communities in the rhizosphere relative to the bulk soil is due to high substrate availability (root exudation) and favorable environmental conditions.

Further, gram-negative (gram-ve) bacteria and arbuscular mycorrhizal fungi (AMF) abundance in the rhizosphere were influenced by 3-way interaction effects of commercial inoculant, soybean variety, and growth stage. Variability in these PLFA-microbial group suggests selection specificity for soybean variety by commercial inoculant with growth stage in the rhizosphere. Thus soybean variety by commercial inoculant selectivity played a crucial role in increasing and shaping gram-negative bacteria abundance in the rhizosphere as growth progress. For Jenguma, inoculation with Biofix increased rhizosphere gram-negative bacteria abundance. With Afayak, Biofix and Legumefix inoculants stimulated rhizosphere gram-negative bacteria abundance at the different growth stage respectively. In Songda, the uninoculated control and Legumefix increased gram-negative bacteria abundance in the rhizosphere. Thus inoculation with commercial inoculants generally increased the gram-negative bacteria community in the rhizosphere. This finding agrees with Ramakrishnan et al. (2017) who reported an abundance of gram-negative bacteria in chickpea rhizosphere when inoculated with microbial inoculants. The abundance gram-negative bacteria observed in the rhizosphere at the R2 (full flowering) stage can be attributed to higher root exudation which is consistent with other

previous works (Yang et al., 2012). The R2 (full flowering) stage is characterized by rapid root colonization by soil microbes due to increased root exudation. Root exudates provide a rich substrate for gram-negative bacteria which are associated with the nutrient-rich environment. The high gram-negative bacteria in the rhizosphere at the R2 stage could also be attributed to increased growth rate and reproduction by the introduced *Bradyrhizobium japonicum* strains in the commercial inoculant due to the availability of root exduate as a carbon source. *Bradyrhizobium* belongs to the gram-negative bacteria group. However, we did not assess *Bradyrhizobium* growth by soybean growth stage progression in the present study. The non-significant gram-negative bacteria abundance in the bulk soil suggest that gram-negative bacteria were generally uniform or stable regardless of soybean variety and/or commercial inoculants. The low gram-negative bacteria abundance in the bulk soil suggests the bulk soil was nutrient poor relative to the rhizosphere soil. The baseline soil analysis supports the assertion that, the bulk soil was of low quality or fertility. Soil organic carbon (SOC), total N and mineral nutrients (available phosphorus) which are some key drivers of the soil microbial community composition and diversity were generally low in the present study.

We observed high variability in arbuscular mycorrhizal fungi (AMF) abundance in the rhizosphere and the bulk soil. Arbuscular mycorrhizal fungi abundance in the rhizosphere was altered by the interaction effect of soybean variety and commercial inoculants and varied with growth stage. Arbuscular mycorrhizal fungi abundance in the rhizosphere peaked at R2 stage which coincided with full flowering (full bloom; stage associated with increased root exudation or rhizodeposition) and after that declined. The increased AMF abundance in the rhizosphere suggests AMF was perhaps acquiring or obtaining it carbon source from the root exudates. As growth stage progress, each soybean variety induced a variable response to the commercial

inoculants which suggests selection specificity for AMF in the rhizosphere. For Jenguma, NoduMax enhanced AMF abundance in the rhizosphere at the R2 stage. Biofix also stimulated a linear increase in rhizosphere AMF abundance after R2 stage and peaked at R4 stage. This suggests that Biofix inoculated Jenguma could potentially stimulate AMF abundance as growth stage progress. In Afayak, higher AMF in the rhizosphere can be achieved with commercial inoculants. Nonetheless, AMF abundance in the rhizosphere of the uninoculated control Afayak increased linearly with growth stage. In Songda, AMF abundance in the rhizosphere was relatively stable with growth stage. This infers that commercial rhizobium inoculants may not necessarily improve AMF abundance in the rhizosphere of Songda. The present study, therefore, revealed that greater AMF in the rhizosphere of TGX soybean cultivars especially Jenguma and Afayak could be achieved with commercial inoculants.

In our previous work, commercial inoculants stimulated N_2fixation in soybean. The increased AMF abundance in the rhizosphere due to commercial inoculants suggest that enhanced N fixation due to inoculation with commercial inoculants (observed in our previous work) perhaps counteracted phosphorus availability in the rhizosphere. Thus the increased AMF abundance in rhizosphere perhaps facilitated increase P uptake from the soil to the plant, although we did not evaluate AMF colonization on soybean root in the present study. Previous work by Egerton-Warburton et al. (2007) and Wilson et al. (2009) in grassland systems showed that nitrogen enrichment induced phosphorus limitation (deficiency), plants (grasses) therefore meet their P nutritions by depending on AMF colonization (association). Further, since tropical soils are naturally low in available P or generally P deficient (baseline P data is evidence), and sometimes available P easily becomes unavailable due soil reactions, perhaps the blanket 30 kg P ha^{-1} applied was insufficient to meet the P demands of soybean at the full flowering stage. Thus the

significant rhizosphere AMF abundance observed at the R2 (full flowering) stage helped to meet the extra P demands, given that greater soil AMF abundance in rhizosphere would translate into greater potential for AMF root colonization.

In the bulk soil, AMF abundance was also affected by the interaction effect of soybean variety and commercial inoculant. The greatest bulk soil AMF was associated with uninoculated control Jenguma. This suggests that commercial inoculants (Biofix and Legumefix) may not necessarily increase or attract AMF abundance in the bulk soil of Jenguma. On the contrary, Afayak requires commercial inoculant (specifically Biofix) to stimulate higher bulk soil AMF. The increased bulk soil AMF associated with Biofix inoculated Afayak could be attributed to indirect benefits of inoculation via inoculation stimulating biomass production (leaf litter), which dropped and decomposed to supply AMF with a carbon source. That is, inoculation with commercial inoculants enhanced biomass production (such as leaf litter), which later senescence to provide a carbon source for the AMF. While inoculating Songda with commercial inoculants may not significantly improve AMF abundance in the bulk soil. Nonetheless, trends suggest that inoculating Songda with commercial inoculants could marginally increased AMF abundance in the bulk soil.

The increased actinomycete abundance in the rhizosphere due to Biofix and Legumefix inoculants infers that commercial inoculant may be required to improve actinomycete abundance in the rhizosphere. This finding supports the previous work of Trabelsi et al. (2011) who reported that inoculation of *Phaseolus vulgaris* with rhizobium inoculant improved actinomycete (actinobacteria), firmicutes, and alpha and gamma proteobacteria. The actinomycete abundance in the rhizosphere of Afayak can be attributed to increased root exudation although no quantitative assessment was done in this study. The selection of improved soybean cultivar or

modern soybean cultivar would stimulate actinomycete abundance in the rhizosphere over traditional (farmers) cultivars. Additionally, actinomycete abundance in the rhizosphere of Afayak may be associated with nutrient recycling, N-fixation and suppression of soil-borne pathogen (such as *Fusarium* and *Penicillium*) as reported by Yao and Wu (2010). Yao and Wu (2010) reported a significant decline in soil-borne pathogens due to abundance actinomycetes in the rhizosphere of two wilt resistant cucumber cultivars.

In the bulk soil, actinomycete abundance was influenced by the interaction effect of soybean variety and commercial inoculant. The most significant bulk soil actinomycete abundance was achieved with Legumefix inoculated Afayak. Similarly, NoduMax inoculated Afayak and Jenguma also induced some significant increase in bulk soil actinomycete abundance. Commercial inoculants may indirectly improved actinomycete abundance in the bulk soil through enhanced biomass (leaf litter), which fell and recycled to provide substrates for the microbes. Actinomycete abundance at the V8 stage may be helping to suppress soil-borne pathogen as reported in the previous work of Yao and Wu (2010) and Huang et al.(2014).
In general actinomycete abundance in the rhizosphere and the bulk soil may be associated with nutrient recycling (especially total N, available P), and was perhaps triggered by favorable precipitation pattern. Sreevidya et al. (2016) observed that inoculation enhanced actinomycete abundance which subsequently induced a corresponding increase in total N and available P.

Microbial biomass is an important biological indicator of soil health since microbes promote soil fertility. Commercial inoculants increased PLFA- microbial biomass in the rhizosphere. Biofix and Legumefix inoculants significantly altered the microbial community structure in the rhizosphere. This finding agrees with Ramakrishnan et al. (2017) who observed that co-inoculation of chickpea with microbial inoculant increased or altered the PLFA-

microbial biomass in the rhizosphere. PLFA- microbial biomass was very low in the uninoculated control treatments. Thus the introduction of exogenous microorganisms into the soil microbial pool could potentially alter the rhizosphere microbial community composition leading to higher PLFA microbial biomass. Our result also revealed that soybean cultivar selection could significantly alter the rhizosphere PLFA- microbial biomass. We may attribute the superior rhizosphere PLFA- microbial biomass by Afayak over Jenguma to higher root exudation. Thus the selection of modern soybean cultivars or genotype could stimulate greater PLFA-microbial biomass in the rhizosphere. Cobb et al. (2017) observed that the selection of improved sorghum genotypes increased PLFA-microbial biomass and the entire microbial community structure.

In the bulk soil, PLFA microbial biomass increased due to the interaction effect of soybean variety and commercial inoculants. The significant PLFA-microbial biomass achieved when Afayak was inoculated with Biofix and NoduMax was perhaps associated with an indirect effect of inoculation on plant growth. That is inoculating Afayak with commercial inoculants (specifically Bioifx and NoduMax) enhanced biomass production (shoot biomass). The plant litter provided substrates for the microbes, hence the increased PLFA-microbial biomass observed in the bulk soil. Thus the present study revealed that inoculating Afayak with commercial inoculants (specifically Bioifx and NoduMax) could potentially improve the PLFA-microbial biomass of the bulk soil through indirect effects. The other soybean cultivars exhibited variable respond to the commercial inoculants.

In general, greater PLFA-microbial biomass was observed in the rhizosphere compared to the bulk soil due to increased substrate availability (high root exudation). Chaudhary et al. (2012) observed greater microbial biomass in the rhizosphere compared to the bulk soil and attributed it

to increase availability of substrates (root exudation). Since microbial biomass is considered a labile nutrient pool, enhanced microbial biomass can be synonymous to improved biological soil quality or soil fertility.

Gram-positive bacteria abundance in the rhizosphere was not significantly altered by commercial inoculant and soybean variety. This suggests that gram-positive bacteria abundance in the rhizosphere was relatively stable regardless of commercial inoculant and soybean variety. Nonetheless, the increased rhizosphere gram+ve bacteria observed at the R2 and the R4 growth stage may be due to increased root exudation. In the bulk soil, gram-positive bacteria abundance was influenced by the interaction of soybean variety and commercial inoculant. Increased gram-positive bacteria was achieved by inoculating Afayak with Biofix and Legumefix. In general, the increased gram-positive bacteria in the bulk soil was expected as it is nutrients poor compared to the rhizosphere.

Commercial Inoculants Impact on Soil Chemical Properties

Available soil N (NH_4^+ and NO_3-N), P and soil pH are also essential indicators of soil quality. The present study revealed that available P in the rhizosphere was influenced by commercial inoculants and soybean variety. Biofix inoculant had higher available P in the rhizosphere. The increased available P in the rhizosphere due to inoculation with commercial inoculants may be associated with indirect effects. This may be explained as (1) inoculation with commercial inoculants improved plant architecture or biomass production, with corresponding increase in root exudation (contained organic acids). The organic acids from root exudation perhaps stimulated the solubilization of P in the rhizosphere. (2) The organic acids also perhaps altered the pH (less acidic) and ensured applied P and solubilized P remained available in the rhizosphere for uptake by plants. The evidence for this assertion was the increased soil pH with

growth stages, and with a corresponding increased in available P observed in this study. (3) Organic acids or root exudates are also important P sources. Finally, phosphorus assimilated into plant biomass (leave, root and nodules) and microbial biomass was perhaps recycled back into the soil through fallen litters and decaying microbial tissues. Nyoki and Nakidemi (2018) revealed that inoculation of soybean with rhizobium induced greater P availability in the rhizosphere due to improved soil pH and microbial mineralization which solubilized P. On the contrary, Trabelsi et al. (2011) found that rhizobial inoculation of *Phaseolus vulgaris* did not significantly alter P. Soybean cultivar selection also affected P availability in the rhizosphere. The increased available P in the rhizosphere of Afayak may be attributed to organic acid from root exudation, which enhanced P availability in the rhizosphere. Further, an increased in rhizosphere available phosphorus with growth stages progression may be attributed to increased soil pH and root exudation. Results also indicated that as growth progresses, rhizosphere available P increased linearly as soil pH. Improved soil pH perhaps induced P availability in rhizosphere which corroborated with our previous assertions that the root exudation could be stimulating P availability via increased soil pH.

Legumefix and Biofix inoculants increased rhizosphere available NH_4^+-N. The increased rhizosphere NH_4^+-N by Legumefix and Biofix may be attributed to N fixation and a higher rate of mineralization by the microbial community in the rhizosphere. Koranda et al. (2011) observed that inoculation increased NH_4^+-N in the rhizosphere of *Fagus sylvatica (*old beech forest). They also attributed the higher NH_4^+-N concentration in the rhizosphere to increase mineralization by substrates (root exudation). The increased rhizosphere NH_4^+-N concentration at the V8 growth stage (early plant developmental stages) and subsequent declined at the latter growth stage corroborates with Trabelsi et al. (2011), who observed higher NH_4^+-N concentration at an early

developmental stages of *Phaseolus vulgaris.* Trabelsi et al. (2011) attributed the higher NH_4^+-N concentration to increased mineralization. The non-significant rhizosphere NO_3-N concentration may be attributed to high mobility (leaching) in soil solution due to high precipitation experienced during the cropping season.

Soil N:P is an indicator used to determine a shift or a change in the concentration of nitrogen to phosphorus in a system. Our result revealed that the uninoculated control had the highest soil N:P, followed by Legumefix and NoduMax which had intermediate soil N:P, and Biofix had the lowest soil N:P. For soybean variety, Jenguma had greater soil N:P while Afayak had lower soil N:P. High soil N:P infers soil nitrogen enrichment at the expense of P (Egerton-Warburton et al., 2007). Thus high N:P indicates low available P in soils The N-enrichment in the rhizosphere may be from N-fixation, mineralization of decaying plant litters and microbial tissues, and possibly root exudates. Soil N enrichment (such as N fertilization) was found to exacerbates plant P deficiency (high N:P) in grassland ecosystems (Egerton-Warburton et al., 2007 and Wilson et al., 2009). The grasses or plants in the ecosystems depended highly on AMF for P nutrition (Egerton-Warburton et al., 2007 and Wilson et al. , 2009). Thus P acquisition by the grasses or the plants was linked to an association with AMF. Likewise, the high AMF abundance observed in the rhizosphere due to inoculation with commercial inoculants reinforced our earlier argument that N-fixation induced P deficiency in the rhizosphere. Hence, the increased AMF abundance in the rhizosphere was to supplement the P nutrition of the soybean especially at the full flowering stage

Conclusion

Inoculation with commercial inoculants especially with Biofix and Legumefix improved rhizosphere PLFA-microbial biomass which is an active nutrient pool. Soybean cultivar selection was crucial in enhancing PLFA-microbial biomass. Afayak had greater potential to stimulate rhizosphere PLFA-microbial biomass. The total PLFA profile indicated that commercial inoculant, soybean variety and time are all crucial in determining the abundance of gram-negative bacteria and arbuscular mycorrhizal fungi (AMF) in the rhizosphere. The increased gram-negative bacteria abundance in the rhizosphere was consistent with our research hypothesis. The higher arbuscular mycorrhizae fungi perhaps stimulated P uptake by plants, and the gram-negative bacteria abundance could be due to higher root exudation in the rhizosphere. Inoculation with Biofix also enhanced actinomycete abundance in the rhizosphere. In the bulk soil, arbuscular mycorrhizal fungi (AMF), actinomycete, gram-positive bacteria, and PLFA-microbial biomass abundance were also enhanced by the interaction effect of soybean varietal by commercial inoculant type selection specificity.

In general commercial inoculants also improved selected chemical soil quality indicators. Especially available NH_4^+-N and available P were enhanced due to inoculation. Soil pH increased with growth stages. Increased soil pH perhaps induced P availability as growth stage progresses.

Commercial inoculants and soybean varietal selection would play a crucial role in improving soil health. Biofix and Legumefix inoculants exhibited an outstanding performance while Afayak outperformed the other cultivars. We recommend that future research should focus on how co-inoculation of Rhizobium and arbuscular mycorrhizal fungi inoculants will enhance

soil health and plant microbiome in tropical grain legume grains (soybean cowpea, groundnut, Bambara groundnut, and pigeon pea).

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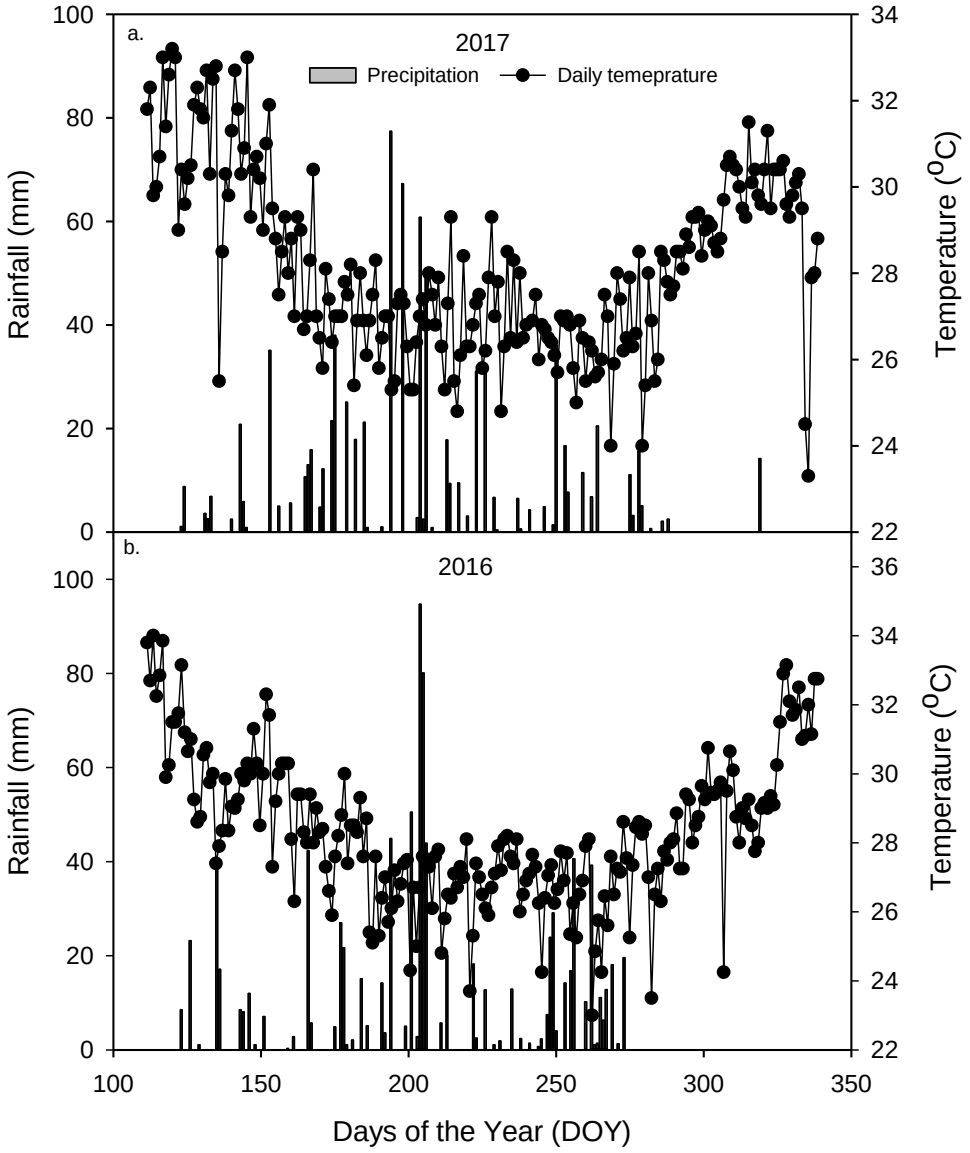
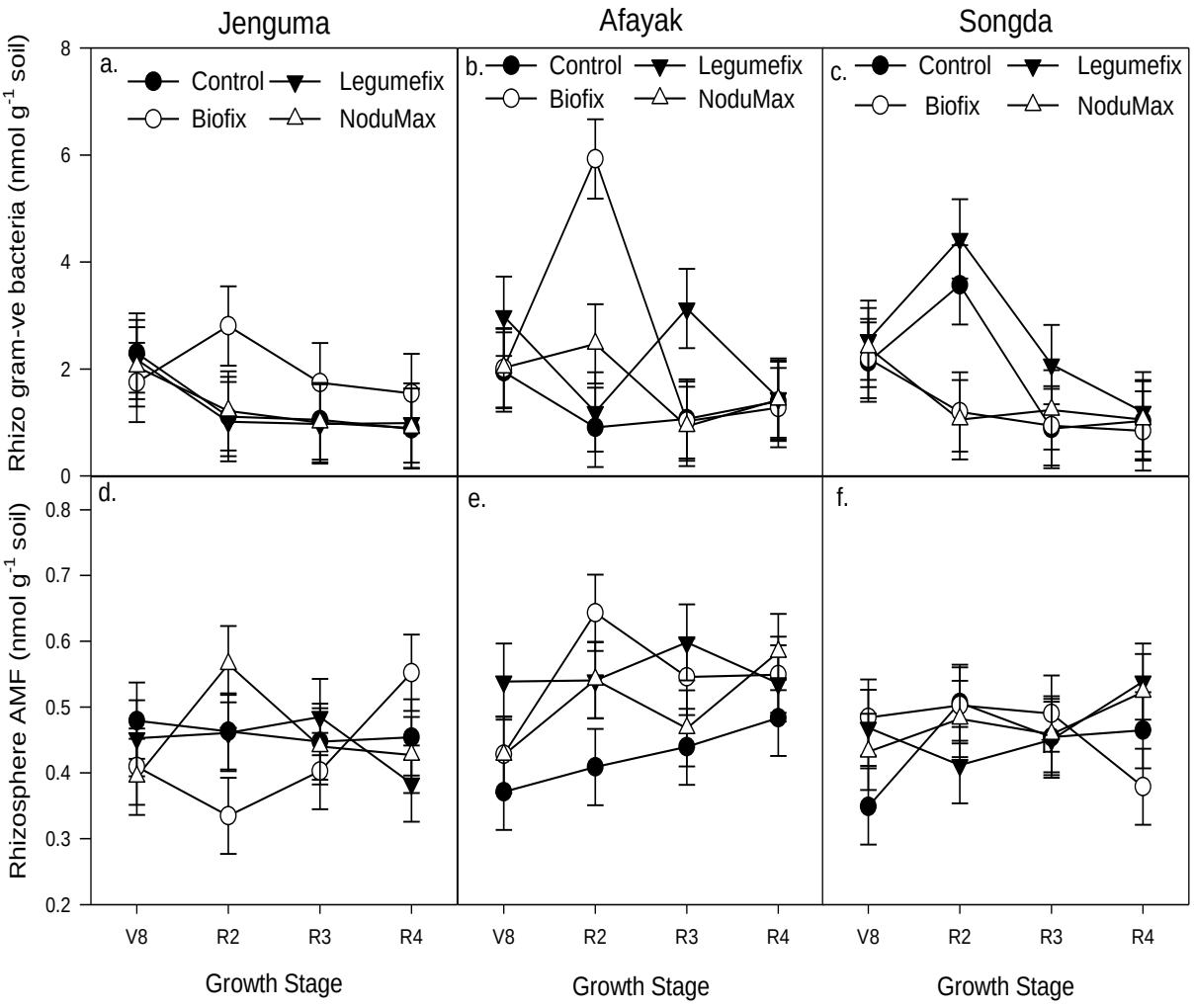


Figure 4.1. Mean rainfall distribution and daily temperature in 2016 and 2017 in Nyankpala, Ghana.



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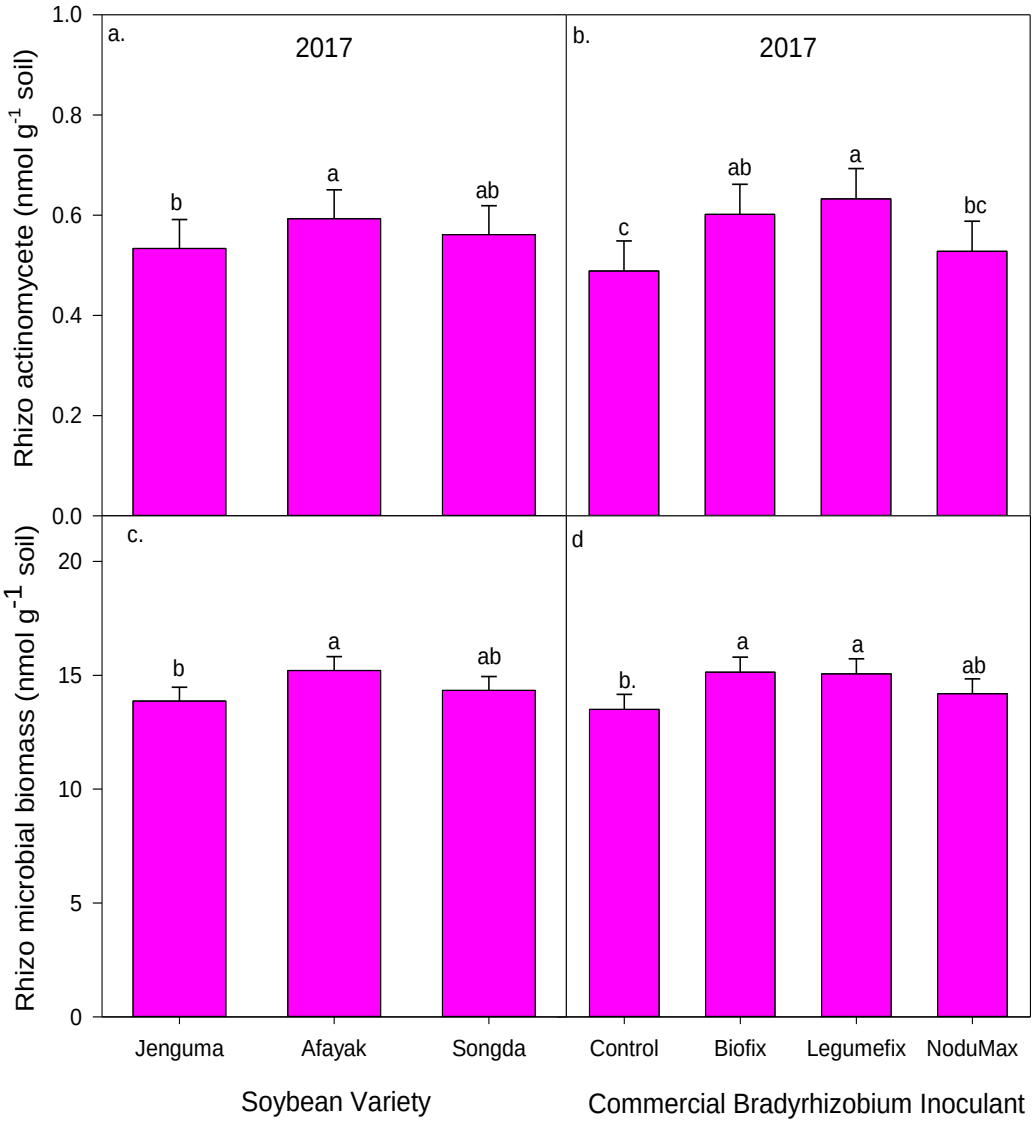

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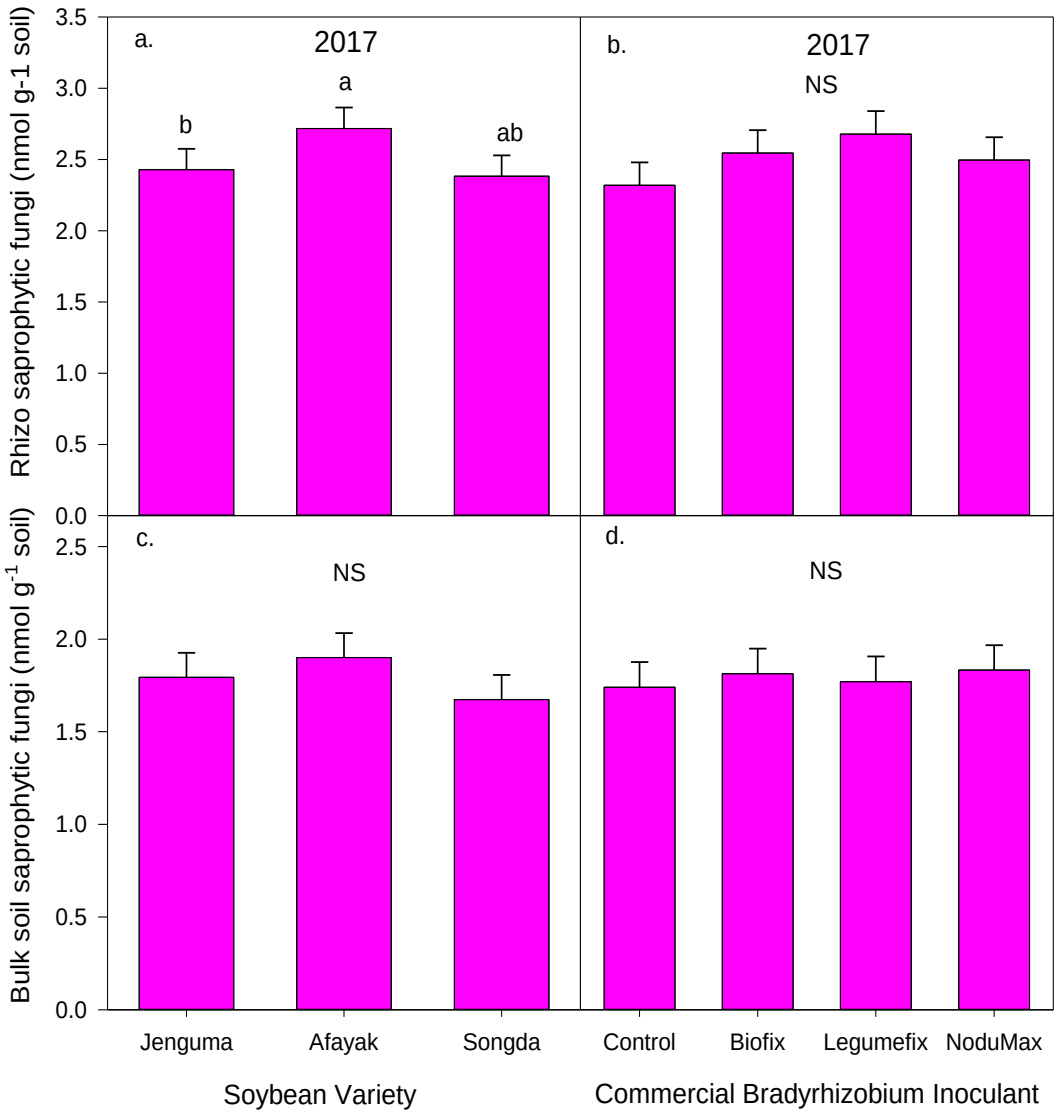


Figure 4.4. (a & b) Rhizosphere (Rhizo) saprophyte fungi and (c & d) bulk soil saprophyte fungi affected by soybean variety and commercial rhizobium inoculant main treatment effects in 2017 in Nyankpala, Ghana. Different letters indicate significant differences at p < 0.1. Error bar is a standard error (SE).

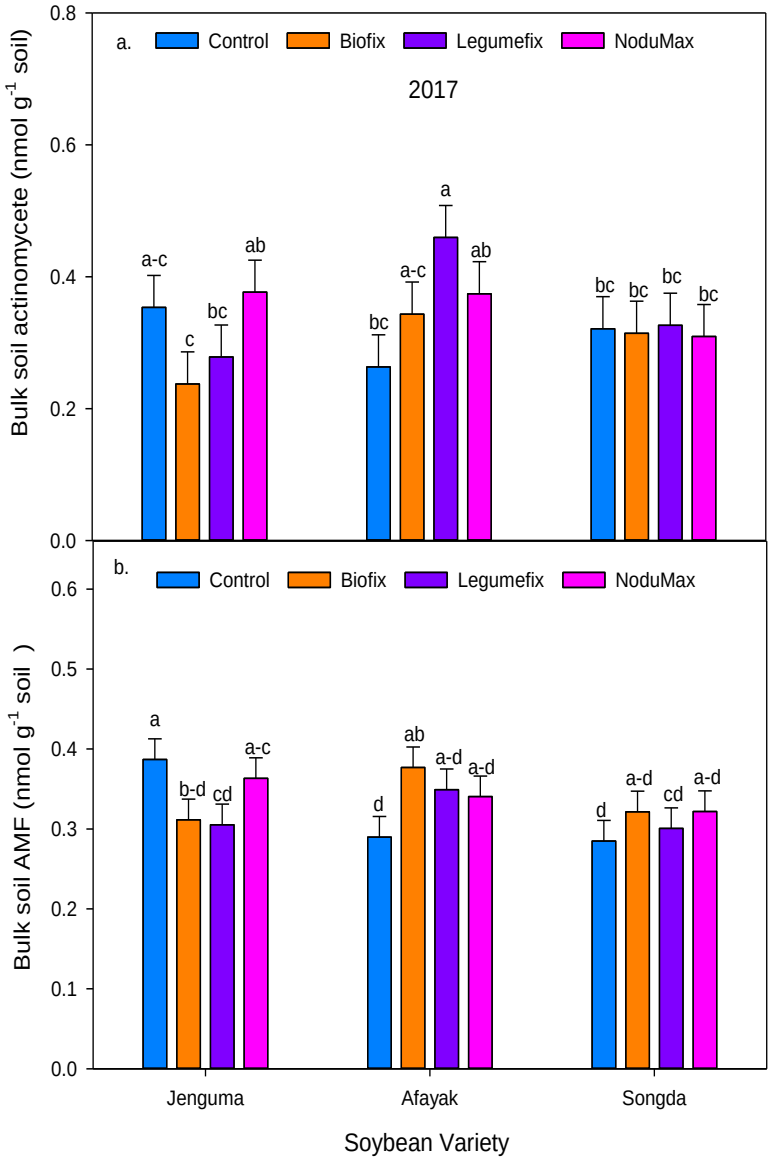
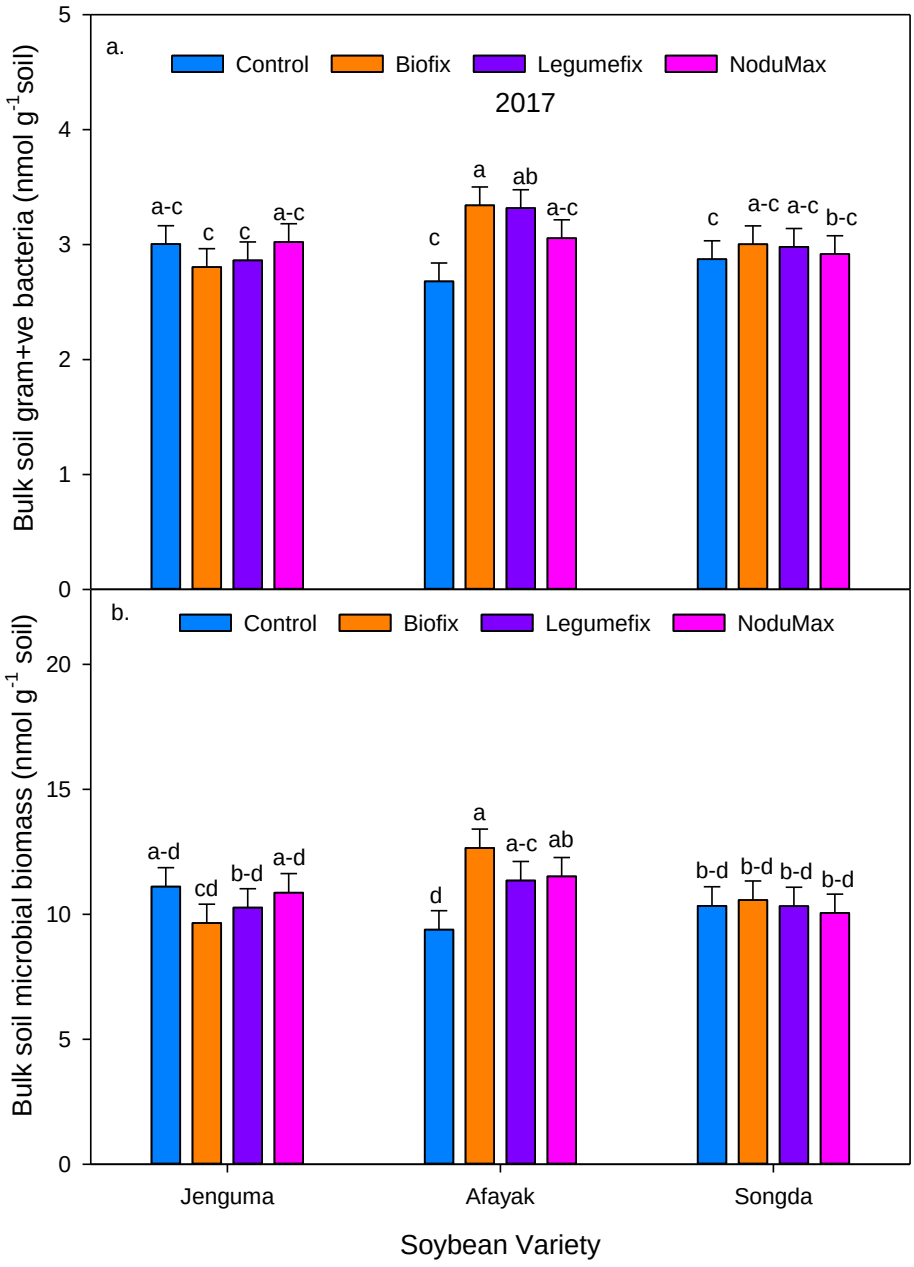


Figure 4.5. Bulk soil actinomycete (a&b) and arbuscular mycorrhizal fungi (c&d) affected by the interaction of soybean variety and commercial rhizobium inoculant in 2017 in Nyankpala, Ghana. Different letters indicate significant differences at p < 0.1. Error bar is a standard error (SE).



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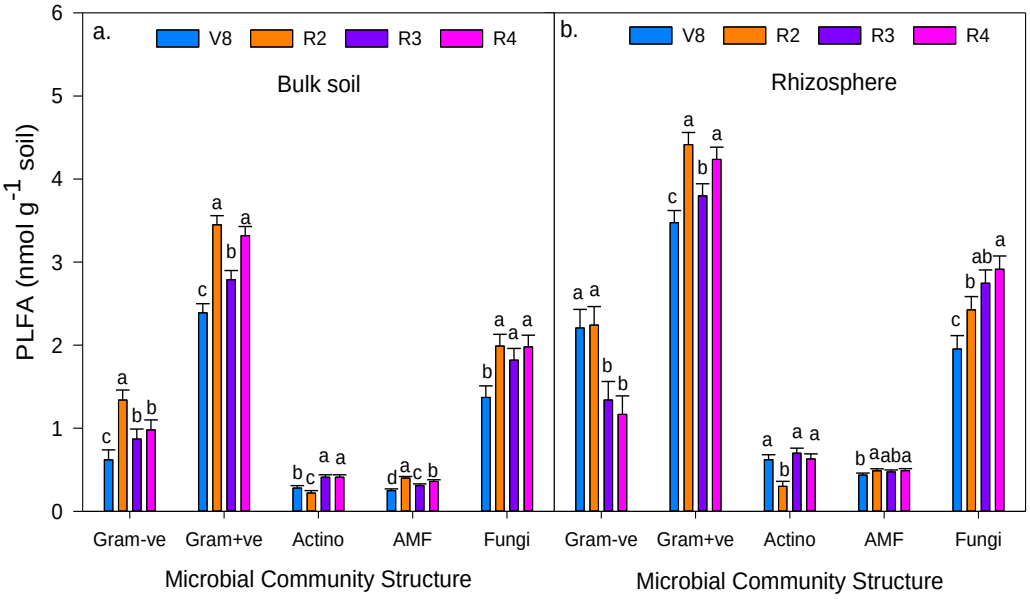
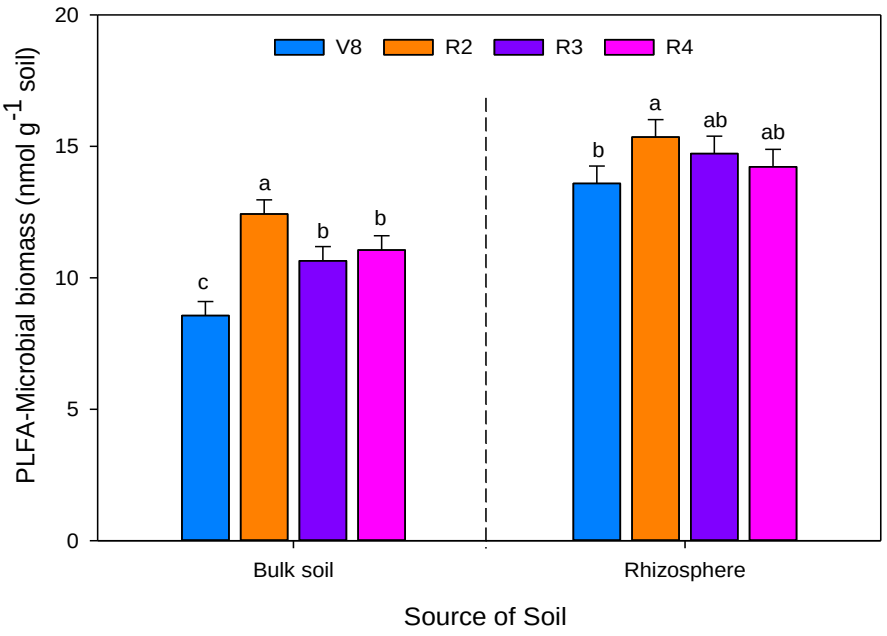


Figure 4.7. Microbial community structure of (a) bulk soil (b) rhizosphere affected by growth stage in 2017 in Nyankpala, Ghana. Different letters indicate significant differences at p < 0.1. Error bar is a standard error (SE).



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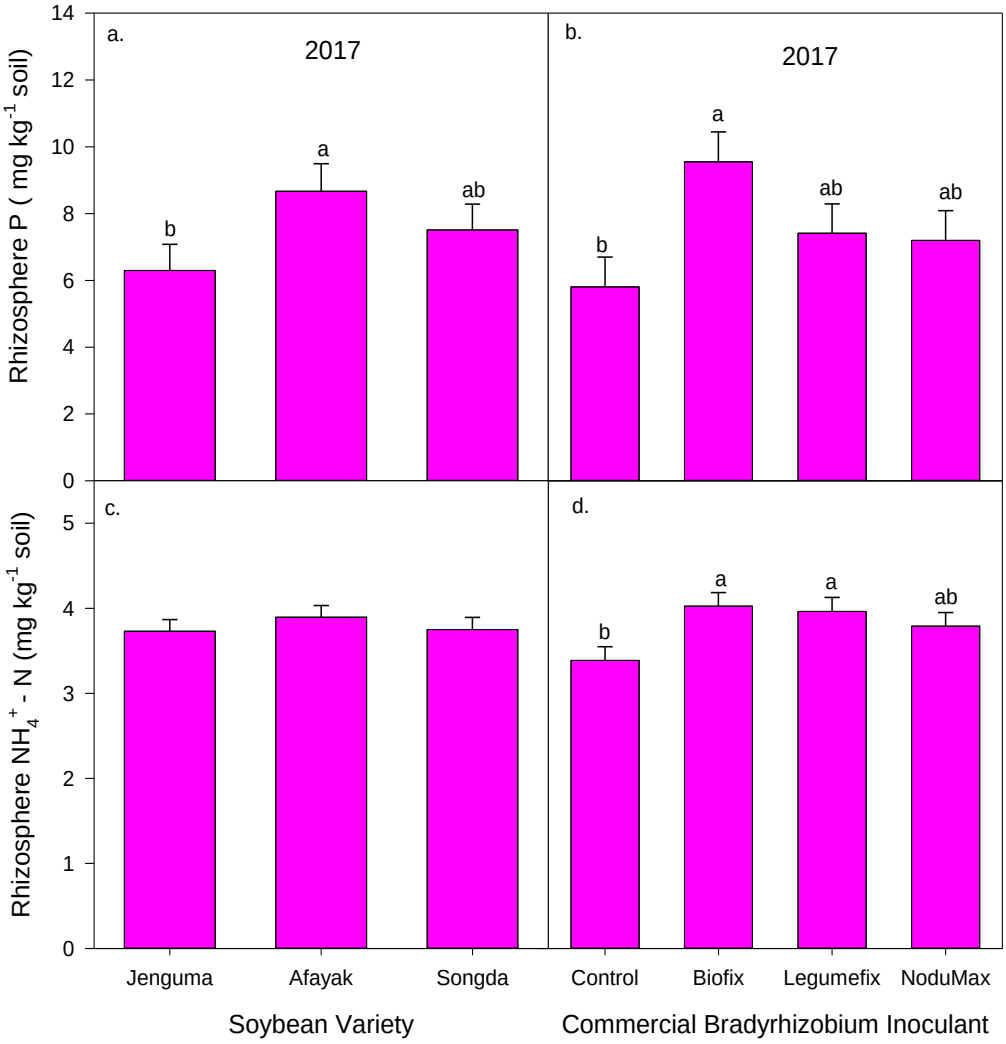

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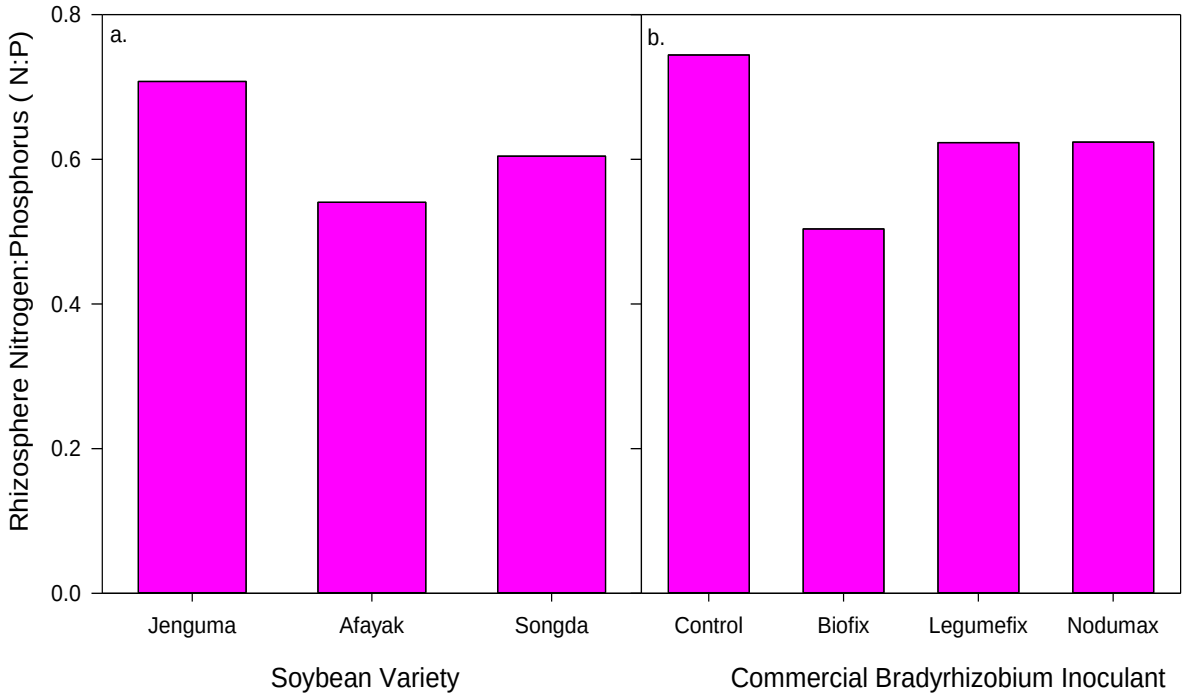

Figure 4.10. Rhizosphere nitrogen: phosphorus affected by main treatment effects of soybean variety and commercial rhizobium inoculant in 2017 in Nyankpala, Ghana.

Table 4.1. Physico-chemical baseline soil analysis for the experimental site at Akukayili in Nyankpala in 2016 and 2017 cropping season in Nyankpala, Ghana.

Depth : 0-15 cm	**Site - 2016**	**Site - 2017**
Soil Class (USDA Soil Taxonomy)	Typic-plinthic Paleustalf	Typic-plinthic Paleustalf
Soil pH (Soil : H_2O : 1: 5)	6.5	6.2
SOC (g C kg^{-1})	3.2	3.8
Total N (g N kg^{-1})	0.37	0.92
NH_4^+- N (mg N kg^{-1})	5.4	20.7
NO_3-N (mg N kg^{-1})	1.8	1.4
Soil N (NH_4^+- N + NO_3-N) (mg kg^{-1})	7.1	20.7
Available Bray-1 P (mg kg^{-1})	6.3	8.2
CEC (meq /100 g)	13.7	11.4
Sand (%)	69.0	67.4
Silt (%)	29.0	25.9
Clay (%)	2.0	6.7
Texture class	Sandy loam	Sandy loam

Chapter 5 - Short-Term Impacts of Cropping Systems on Yield and Soil quality

Abstract

Inoculation of TGX soybean cultivars with commercial inoculant is a new technology in Northern Ghana's cropping systems. Nonetheless, the extent to which previous commercial inoculants affect the subsequent crops is not well documented. A field study was conducted to determine how previous Bradyrhizobium inoculants affected the subsequent soybean and maize crop and as well as soil quality. The study started in 2016 as a randomized complete block in split-plot design where the main plot consisted of three promiscuous soybean cultivars: Jeguma (TGX1448-2E), Afayak (TGX1834-5E) and Songda (TGX 1445-3E). The subplot consisted of three different types of commercial *Bradyrhizobium japonicum* inoculant: Biofix (*USDA 110 strain*), Legumefix (*USDA 532c strain*) and Nodumax (*USDA 110 strain*) plus an uninoculated control. In 2017 each plot (4 x 4 m^2) was divided into two (2) halves (2 x 2 m^2). One half was cropped to soybean without inoculation, and the other half cropped to hybrid maize (*Zea mays* var Wangdata). Additional mineral N fertilizer rates (0 N, 50 N and 100 N kg ha^{-1}) was also introduced using urea. Assessment included nodulation, shoot dry matter, grain yield, harvest index, N-fixation, and residual N balance for soybean. For maize, the assessment included dry shoot matter, grain yield, harvest index, and N uptake. In the soybean phase, previous Legumefix enhanced greater nodulation (nodule number and mass) than the other treatments. Biofix produced greater soybean grain yield compared to NoduMax. In the maize phase, Biofix yielded greater shoot dry matter and grain yield compared to Legumefix. Maize grain yield from the rotation system was comparable to those that received 50 kg N ha^{-1} mineral N fertilizer. Conclusively, inoculating soybean with commercial inoculants would decrease the quantity of

mineral N fertilizer required by the subsequent maize by ~50%. To enhance higher N-fixation and grain yield yearly inoculation of soybean is necessary.

Introduction

Grain legumes-cereal rotation systems promote diversification and intensification technology for restoring soil quality and enhancing crop productivity. Grain legume such as soybean establishes a symbiotic association with *Rhizobia* and fix N for plant uptake thereby contributing to the N economy of the field (Franke et al., 2018). Soybean seeds are inoculated with commercial *Bradyrhizobium* inoculants to enhance nodulation, N-fixation, biomass production and grain yield in fields with no soybean history or with a low population of soybean *Bradyrhizobium spp*. Meanwhile, there is also considerable controversy or debate on whether re-inoculation would be beneficial to fields previously cropped to soybean. The controversy often centered on (1) whether there is an adequate population of *Bradyrhizobium japonicum* persisting in soils from fields previously cropped to soybean to enhance effective nodulation and grain yield for the present soybean crop (Elkins et al., 1976); and (2) how competitive is the introduced *Bradyrhizobium japonicum* strain to subsequently produce and occupy a greater proportion of nodules compared to the native rhizobia (Elkins et al., 1976). Previous work by Abel and Erdman (1964) and Elkins et al. (1976) showed no yield and other auxiliary growth responses for inoculated and uninoculated treatments on fields previously cropped to soybean. Nonetheless, Elkins et al. (1976) stated that inoculation should be practiced as an inexpensive insurance policy as the survival and persistent of rhizobia in the soil, are influenced by several factors (Zengeni et al., 2006; Peoples et al., 2009). Obapton et al. (2002) observed that *Bradyrhizobium japonicum* could survive and persist a for at least five years in a field previously cropped to soybean before re-inoculation., Revellin et al. (1996) recommended re-inoculation for a new soybean crop after

5.5 and 18 years without soybeans in calcareous and non-calcareous soils in France, respectively. Thus the cropping history of soybean can affect the dynamics of *Bradyrhizobium japonicum* populations. Nonetheless, in northern Ghana cropping systems, the persistence of commercial *Bradyrhizobium* inoculant strains under field condition is poorly understood.

Soybean also provides additional rotation benefits to the subsequent crops in rotation. The rotational effect of soybean on the subsequent crops was classified as N effects and non-N effects. The N effects were attributed to residual N balance not uptake by the soybean and those from decayed litters (SOM). The amount of N fixed depends on the environment, management, soybean genotype, the rhizobia strains and their symbiosis association (Franke et al., 2018). However, a significant amount of the fixed N, accumulate in the grain and the stover and removed at harvest. Therefore the field N balance for soybean at harvest may be close to zero or negative (Singh et al., 2003; Franke et al., 2018). The amount of N-fixed by the soybean for the subsequent crop is usually, reported as the N replacement values (NFRV) or the fertilizer equivalence. Numerous NFRV had been documented for soybean across the Guinea savanna of West Africa. Carsky et al. (2003) reported 20 to 45 kg N ha^{-1} for soybean, Ogoke et al. (2003) reported -17 to 5 kg N ha^{-1} and Singh et al. (2003) reported 10 and 20 kg N ha^{-1} for surfaces applied and incorporated soybean residues respectively. Again, Ogoke et al. (2003) reported 14.4 kg N ha^{-1} when no P was applied, and 21 kg N ha^{-1} and 19.5 kg N ha^{-1} when 30 kg P ha^{-1} and 60 kg P ha^{-1} were applied, respectively. Sanginga et al. (1997a) also stated that N contributions from soybean were variable, ranging between −8.0 and 43 kg N ha^{-1} depending on N analytical procedure employed. The N-effect from grain legumes to the subsequent crops in the rotation can affect the quantity of mineral N fertilizer to apply. Even at very low residual N balance from soybean, the subsequent maize grain yield improved (Osunde et al., 2003a; Franke et al., 2008;

Yusuf et al., 2009b). Sanginga et al. (2002), also observed improve maize grain yield on previous promiscuous soybeans field with low N net balance of 10-22 kg N ha^{-1}contributed by the soybean residues. The improved maize yield was not attributed to only N effect but also to non-N-effect (Sanginga et al., 2002).

Non-N effects of legumes are attributed to effects other than N. Improvement in soil health indicators such as soil pH, soil water holding capacity, soil organic matter (SOC) and nitrogen (SON), increased microbial diversity and abundance, improved soil structure (aggregate stability and bulk density) are examples of the non-N effect of legume-cereal rotation systems. Others include a decline in diseases and pests (Kelley et al., 2003), reduction in toxic substance in crop residues, weed suppression (such as *Striga hematica*) and release of growth-promoting substances (Lynch and Hobbie,1988).

Assessing the impact of non-N effects on legume-cereal rotation systems have been neglected in sub-Saharan Africa West Africa (SSWA) cropping systems (Franke et al., 2018) Impact assessment of legumes such as soybean in legume-cereal rotation systems had focused on measuring on the N dynamics indicators such as N_2-fixation rates, legume N field balances, and uptakes by the subsequent crop (Franke et al., 2018). Nonetheless, the few studies that exist documented that the non-N effect can have a significant impact on the soil quality and yield of the subsequent crop in the rotation (Horst and Härdter, 1994; Yusuf et al., 2009; Franke et al., 2008, 2018). In the Northern Guinea Savanna of Nigeria, Yusuf et al. (2009) observed improved grain yield of maize in legume-cereal rotation compared to cereal monocropping system due to enhanced microbial biomass. Drinkwater et al. (1998), documented an enhance SOC and SON in legume-cereal rotation systems in the USA. The benefits of crop rotation over mono-cropping are also well documented. In Northern Ghana, maize yield improved on fields previously

cropped to legumes than those in monoculture and maize/legume intercrop systems (Horst and Härdter, 1994). Subsequently, maize yield in the monoculture system declined over several cropping seasons (Horst and Härdter, 1994). Nonetheless in northern Ghana cropping systems, previous commercial *Bradyrhizobium* inoculant effects on non-nitrogen effect under field condition are not well documented

The impact of legume-cereal crop rotation on microbial community structures has been well investigated (Alvey et al., 2003; Marschner et al., 2004; Vargas Gil et al., 2011; Zhang et al., 2014). Previous research conducted on West Africa soils also revealed that crop rotation could alter the rhizosphere microbial community structures, microbial diversity, and abundance, thereby promoting plant growth (Alvey et al., 2001; Marschner et al., 2004). Nonetheless, in the Northern Guinea Savanna of Ghana, there is insufficient information on cropping systems impact microbial community structure and soil quality. However, crop yield in West Africa farming systems depends on inherent soil fertility and on microbial processes that regulate the mineralization and the mobilization of nutrients required for plant growth and development (Alvey et al., 2003). In-depth research is needed to understand how management and cropping systems affect soil health, microbial community structures, and crop production. This would provide a better approach to addressing differential yield gap in crop production. This research sought to determine (1) the impact of previous season *Bradyrhizobium inoculation* on double-cropped soybean cropping systems; (2) how residual N contribution from the previous soybean crops affect the subsequent maize in rotation; and (3) how the previous soybean crop and commercial inoculants affected the soil microbial structure and selected soil health indicators.

Materials and Methods

Study Site

The study was conducted at the Savanna Agricultural Research Institute (SARI) located in Nyankpala in the Northern Ghana part of the West Africa Guinea Savanna. The climate is characterized by 5-6 humid months, with an annual mean precipitation of 1095 m, classified as a summer-humid dry climate (Horst and Härdter, 1994).

The soil at the experimental site was a well-drained sandy loam (69 % sand, 29% silt and 2% clay) with pH 6.4, and as classified as a typic-plinthic Paleustalf according to the US Soil Taxonomy. The initial background soil sample analysis in 2016 was Soil organic C = 3.19 g C kg^{-1}, Total N = 0.37 g N kg^{-1}, Soil available N (NH_4-N + NO_3-N) = 7.12 mg kg^{-1}, Soil available P (Bray-1) = 6.34 mg kg^{-1} and CEC was 10.2 meq 100 g^{-1} at the 0-15 cm horizon.

The field study was initiated in 2016. The field was previously cropped to soybean to in 2016 where different soybean cultivars and commercial *Bradyrhizobium* inoculants were evaluated. After harvest, the field was left fallow for about six months. Results presented here primarily refers to the experimental year of 2017 since that was when the rotational effect was evaluated. In 2017, the peak rainfall was in July and September resulting in very moist condition during growth. There was also 1-1.5 weeks of a short spell of drought in August

Experimental Design

The experiment was designed as a split-plot using a randomized complete block design (RCBD) with four replications. The main plot consisted of three promiscuous soybean cultivars: Jeguma (TGX1448-2E), Afayak (TGX1834-5E) and Songda (TGX 1445-3E). The subplot consisted of three different types of commercial inoculant: Biofix (contained *Bradyrhizobium*

japonicum strain USDA 110) obtained from Kenya, Legumefix (contained *Bradyrhizobium japonicum* strain USDA 532c) obtained from the UK, and Nodumax (contained *Bradyrhizobium japonicum strain* USDA 110) from Nigeria, in addition to an uninoculated control. Maize was also cropped as a reference crop. Each experimental plot measured 16 m^2 (4 x 4 m^2) with eight hand-made ridges at 50 cm part. These were the treatments applied in 2016 before the initiation of the crop rotation systems in 2017. In 2017, each plot (experimental units) (4 x 4 m^2) was divided into two halves with each measuring (2 x 2 m^2) with four hand-made ridges. The first half was cropped to the same soybean cultivars without inoculation representing double-cropped soybean cropping systems. This cropping system would allow for evaluation of the persistent of the inoculant introduced in 2016. The second half was cropped to hybrid maize (*Zea mays* var Wangdata) obtained from SARI-Maize Section. This cropping system represents soybean-maize cropping system and would allow for the evaluation of the residual N from BNF for the succeeding crop in the rotation. Plots previously cropped to maize as a reference crop in 2016 was still to cropped maize in 2017. However different N-rates (0 N, 50 N and 100 N kg ha^{-1}, respectively) were applied using urea (46% N). This system represents maize monocropping and would allow for comparing the performance of the maize under the different soybean varieties (with or without inoculation) to those receiving the mineral N fertilizer (urea).

Agronomic Management

Prior to planting roundup (glyphosate), herbicide was used to kill both monocotyledon and dicotyledon weeds. Fours soybean seeds were sown per hill on ridges at 50 cm apart and inter-hill distance of 10 cm. Emerged seedlings were later thinned to two stands at 13 days after sowing (DAS). For maize, three seeds were sown per hill on ridges at 50 cm part, and inter-hill distance of 60 cm and later thinned to two stands per hill. Sowing was done on July 3, 2017.

Plant establishment data for both soybean and maize were taken at 19 DAS. The entire plant population per plot was counted and recorded.

The soybean plots received 30 kg K ha^{-1} and 30 kg P ha^{-1} from Muriate of Potash (MoP) and Triple Super Phosphate (TSP), respectively at 16 days after sowing (DAS). The maize plots received 60 kg K ha^{-1} and 60 kg P ha^{-1} from MoP and TSP respectively at 20 DAS. The fertilizers were banded at 3-5 cm away from the plants and at 5 cm depth. The mineral N (urea) fertilizer was also applied at 20 DAS as a single dose. The mineral N fertilizer rate consisted of 0 N, 50 N and 100 N kg ha^{-1}, respectively. The mineral N fertilizer was also banded 3-5 cm from the plants and at 5 cm depth on the ridges.

After sowing, pre-emergence herbicide (Basagran) was applied to control weeds. After that, three manual weedings were done using a hoe. In all weedings, separate hoes were used to prevent cross-contamination of inoculants.

Soil Sampling

The baseline soil sample was collected from each plot at the 0-15 cm depth before planting in 2017. The soil samples were divided into two halves; one half was air dried for (4) days. The air-dried soil was passed through 2 mm sieve size-mesh and then bagged in the ziplock for further analysis. The other half was kept in a refrigerator at – 4^{o}C. The frozen soil samples were freeze-dried for 48 hr and sieved with 1 mm size-mesh sieve to ensure homogenized mixing of samples. Afterward, the freeze-dried samples were kept in -40^{o}C freezer for microbial analysis.

Biomass Sampling

Sampling for soybean biomass was done at 45, 54, and 76 DAS representing VS (pre-flower or vegetative), R2 (full flower), R4 (full pod) stages, respectively. Ten hills (representing

an area of 0.5 m^2) consisting of 20 soybean plants were randomly sampled. Plants were gently uprooted using a spade, and the soil around the root was gently shaken off. The rhizosphere soil was also collected as detailed in our earlier chapter. Roots with nodule were detached from the plants and bagged separately in Ziplock bags. Any nodule that fell during the sampling was collected and bagged separately. To avoid inter-inoculants contamination, a spade was assigned to each treatment. Also, to eliminate sampling bias, samplers were rotated at the end of each replication. At the laboratory, shoot biomass was washed with water to remove soil particles and air-dried for about 20-30 min in a cool place. After that, the shoot biomass was weighed, oven dried at 70 ^oC for about 48 hr, and re-weighed. Shoot dry matter (Shoot DM) was then expressed as Mg ha^{-1} . Roots with nodules were placed on top of a sieve (< 0.53 mm mesh size) and washed under running water several times to remove any soil particles. This procedure allowed fallen nodules to be captured on top of the sieve. After that, the roots plus nodules were also air dried for 20 mins in the Lab. Nodules were then detached leaving the root biomass. Nodulation data, which consisted of nodule number and weight were also recorded. After that, root weight was also recorded. Both root and nodules were oven dried at 70 ^oC for 48 hrs and then weighed again.

For maize, three sampling was also done at (a) VT stage, i.e., late vegetative stage to early tasseling stage (b) R1-R2 stage, i.e., silking to blister stage (3) R3-R4 stage, i.e., milking to dough stage. The plant establishment in the sampling area (4 m^2) were noted or determined. After that, three hills consisting of five plants were randomly sampled by gently uprooting the whole plant with a spade. The soil around the root was gently shaken off, and later the rhizosphere soil was captured as previously described. Roots were detached from the plants and separately bagged in a ziplock bag. Both shoot and root biomass were washed several times under running water to remove soil. Shoot and root fresh weight were recorded, oven dried at 70

^{o}C for about 72 hr and then re-weighed. Maize shoot and root dry matter (DM) were extrapolated and expressed as kg ha^{-1}. Precautionary measures taken during soybean sampling were also repeated for maize to avoid cross-contamination and bias. Plant height was randomly taken on five tagged plants for both soybean and maize plants at each sampling time. Plant height was recorded with a wooden metric ruler.

At harvest plant population within the harvest area (3 m^2) were determined. Maize cobs were harvested at full maturity and dried before shelling. The entire stover within the harvest area were sampled by cutting the shoots at the soil level, and the fresh weight was recorded. A subsample of the grain and the stover were oven dried (i.e. 60 ^{o}C for 72 hrs.), weighed and ground. Grain and stover yield for maize were expressed as kg ha^{-1} after adjusting to moisture content 10 %.

The soybean plant (pod and stover) was removed from the harvest area (3 m^2) in conformity with the farmers' practice in the area (Osunde et al., 2003). Soybean plants were harvested at full maturity by uprooting and carrying the entire plants to the Lab. A subsample of 20 plants was taken for assessing pod load, pod dry weight, haulms dry weight and a1000 seed weight. The subsample pods and haulms were oven dried (i.e. 60 ^{o}C for 72 hrs.), weighed and ground (Osunde et al., 2003). Harvested soybean plants (pod and stover) were manually threshed and winnowed to obtain clean grain yield, and later weighed and dried. Grain yield moisture content was adjusted to 12%. Grain, haulm and pod yields for soybean were expressed as Mg ha^{-1}. Pod load was expressed on per plant basis. Harvest index was also determined for both maize and soybean.

Plant Analyses

Ground plant samples (shoot, root, halums, stover, and grain) were analyzed for total N by dry combustion method using Carlo Erba elemental analyzer EA112 (Thermo Fisher Scientific). The % N of shoot, root, grain, stover (including haulm) dry matter was multiplied by their respective dry matter (kg ha^{-1}) and expressed in kg N ha^{-1}. The amount of total N fixed in kg N ha^{-1} was estimated as the N content in the whole soybean plant (kg N ha^{-1}) subtracted from the reference plant N content (kg N ha^{-1}). ie. Amount of N fixed (kg N ha^{-1}) = whole soybean plant N content (kg N ha^{-1}) – the reference plant (maize) N content (kg N ha^{-1}). The N balance was estimated as the difference between the N fixed and the total N exported in the grain (+stover) or total aboveground biomass (−stover) (Osunde et al., 2003).

Soil Analyses

Soil bulk density was assessed after harvest at 0-15 cm following the procedure reported by Horst and Härdter (1994). Soil pH (1:5, soil: H_2O) was also measured on the soil samples collected prior to field preparation for the 2017 cropping season following the procedure reported (Meriles et al., 2009). Briefly, 2g of soil was added to 10 mL of nanopure water and shaken. The suspension was allowed to stand for 15 mins, then shaken again and allowed to settle before the final reading was taken with Orin Thermo-Scientific pH meter. Soil organic C and total N was assessed by dry combustion (Hurisso et al., 2016). Briefly, soil was ground and sieved with 0.25 mesh size diameter sieve, and ~ 100 mg was weighed into aluminum foil and folded. The sample was then analyzed using Carlo Erba elemental analyzer EA112 (Thermo Fisher Scientific). Soil available N (NH_4-N and NO_3-N) was assessed on soil samples collected before planting. Briefly, 10 g of soil was extracted with 20 mL of 1MKCl solution and shaken on a digital shaker (VWR) for 1 hr at 325 rev min^{-1}. The slurry was then filtered using Whatman

filter paper size 42 (110 mm diameter size). The filtrate was frozen at -20 ^{o}C and later analyzed for NH_4-N and NO_3-N on an Alpkem auto-analyzer (OI Analytical at K-State, Agronomy Dept. Soil testing Lab) colorimetrically (Maul and Drinkwater, 2010; Hirzel et al., 2012).

The Potentially Mineralizable N was estimated using the aerobic incubation method in the laboratory as reported by Hirzel et al. (2012). Briefly, 10 g of soil was weighed and adjusted to 60% water-filled pores space (WFPS) and then placed in an Erlenmeyer flask, sealed with a stopper and incubated at 40◦C for 28 days. The available soil N (NH_4-N and NO_3-N) was extracted with 20 mL of 1M KCl solution and shaken on a digital shaker (VWR) at 325 rev min^{-1} for 1hr. The slurry was filtered using Whatman filter paper size 42 (110 mm diameter size). The filtrate was frozen at -20 ^{o}C and later analyzed for NH_4^+-N and NO_3-N on an Alpkem auto-analyzer (OI Analytical at K-State, Agronomy Dept. Soil testing Lab) calorimetrically (Maul and Drinkwater, 2010; Hirzel et al., 2012). Potentially N mineralizable was estimated as the difference between inorganic N concentration (ammonium and nitrate concentrations) of the incubated soil and initial soil samples (Nadelhoffer et al., 1984).

Mineralizable carbon (C min) was estimated using aerobic incubation method in the laboratory as reported by Hirzel et al. (2012). Briefly, 10g of air-dried soil was adjusted to 60% water-filled pores space (WFPS) placed in an Erlenmeyer flask, sealed with a stopper and pre-incubated at 25◦C in the dark in the controlled environmental room for 7 days. After that, water content for the sample was adjusted and placed in a 990 mL Mason jar, with 10 mL of de-ionized water to maintain a humidified atmosphere and sealed. An empty mason jar with 10 ml of water was also included as a control. The samples were placed in a controlled environment room and incubated for 28 days. The moisture levels of the soil in the jars (60% of soil WHC) were checked weekly by measuring weight loss and adjusting the weight with deionized water when

necessary to maintain constant moisture. Time course gas sampling was done at T0, T1, and T2 which corresponds to 1, 14 and 28 days. T0 gas was collected 3-hr after incubation. About 0.5 mL of the gas was analyzed for C-carbon-dioxide (CO_2) for using Shimadzu GC-8A Gas Chromatograph HPLC. Potentially mineralizable C was calculated as the difference between CO2-C captured in the incubated soil (sample traps) and in the blanks (Nadelhoffer et al., 1984).

Phospholipid fatty acid (PLFA) extraction was carried following the procedure of Bligh and Dyer (1959) as described by Bossio and Scow (1998). Briefly, lipids were extracted in a single-phase chloroform-methanol-phosphate buffer system. Phospholipids were separated from neutral lipids and glycolipids on solid phase extraction columns (Supelco, Inc., Belle fonte, PA, The USA). After methylation of the polar lipids, PLFA methyl esters were analyzed using both an HP 5890 gas chromatograph (Agilent Inc., Palo Alto, CA) equipped with a flame ionization detector (FID) and an HP 6890 gas chromatograph–mass spectrometer (GC–MS). An HP-5 MS 5%-phenyl methylpolysiloxane (25 mm) capillary column (Agilent Inc., Palo Alto, CA) was used for both the GC and GC–MS analysis (Zelles et al., 1992). The MIDI Sherlock Microbial Identification System (Microbial ID Inc., Newark, NJ, USA) was used to identify fatty acids. Nonadecanoic acid methyl ester (19:0, Sigma) was added as internal standard and used to convert fatty acid peak areas to absolute abundance. Thirty individual PLFAs consistently presented in the samples were used for data analysis. The sum of all PLFAs was used to indicate total microbial biomass. The sum of i14:0, a15:0, i15:0, i16:0, a17:0, and i17:0 was to used represent gram-positive bacteria (Gram +ve) and the sum of 16:1 2OH, 16:1ω7c, 16:1ω9c, cy17:0, 17:1ω8c, 18:1ω7c, and cy19:0 represent gram-negative bacteria (Gram -ve) (Zogg et al., 1997; Bossio and Scow, 1998; Liang et al., 2014). The sum of 10Me16:0, and 10Me18:0 was used to represent actinomycetes. We used 16:1ω5c to indicate arbuscular mycorrhizal fungi

(AMF) and the sum of 18:1ω9c and 18:2ω6c to represent saprotrophic fungi (SF). The sum of 16:1ω5c, 18:1ω9c, 18:2ω6c to was used indicate the total fungi (Vestal and White, 1989). Fungi:Bacteria (F:B) ratios were calculated using PLFA Percent proportion of fungi relative to bacteria as reported by (Malik et al., 2016), and was expressed as below:

$$\%\ proportion\ F{:}B = \frac{Fungal\ index}{Bacterial\ index}\ x\ 100$$

Statistical Analysis

Data were subjected to normality test using shirpo-wilk in Sigmaplot 13.0. Data were analyzed using SAS Proc Mixed Model procedures version 9.4 . Copyright © 2014 SAS Institute Inc., Cary, NC, USA (SAS Institutes, 2014) for Analysis of Variance (ANOVA). Inoculant, Variety, and Growth Stage were considered as a fixed effect. Block (replication), and Interaction of Block and Variety were also considered as random effect. Growth stage was fitted as repeated measurement and with the slice effect option. Unless otherwise stated significant difference among treatments was declared at $\alpha = 0.05$ probability level. Means were separated using Fisher's least significant difference (Fisher's LSD).

Results

Impacts of Previous Commercial Inoculants and Soybean on the Subsequent Soybean

Nodulation data which consisted of nodule number and nodule dry mass per plant and specific nodule weight were affected by the main treatment effect. Afayak produced greater number of nodules per plants compared to Jenguma and Songda (P = 0.09) (Table 5.1). Previous Legumefix increased the number of nodules per plants compared to Biofix and NoduMax (Table 5.1). Greater number of nodules per plants were produced at full flowering (R2) relative to the vegetative stage (VS) and full podding stage (R4) (Table 5.1). For nodule dry mass, Afayak and Songda produced nodules with greater mass of 42% and 37% more than Jenguma respectively (Table 5.1). Nodule mass from previous Legumefix was significantly greater than the other treatments (Table 5.1). Increased nodule dry mass was produced at the R2 stage compared to the R4 stage and the VS. Similarly, nodule mass at the R4 stage was also greater than the VS (Table 5.1).Nodules produced by Songda had increased specific nodule weight of 23% and 27% more compared to those produced by Afayak and Jenguma respectively (Table 5.1). Inoculation had no significant effect on specific nodule weight (Table 5.1). While specific nodule weight tended to significantly increase with growth stage (Table 5.1).

Shoot and root dry matter were significantly affected by the interaction of soybean variety and commercial inoculant and commercial inoculant and growth stage interactions (Appendix Table D.1). The different soybean variety showed variable response to the previous commercial inoculants. For Songda, the uninoculated control produced increase shoot and root dry matter than Legumefix and NoduMax (Fig. 5.1a & b). For Jenguma, the previous Legumefix yielded greater shoot dry matter than the uninoculated control. For root dry matter, Legumefix

outperformed the uninoculated control and NoduMax (Fig. 5.1a & b). For Afayak, no significant difference existed among the treatments for dry shoot matter. However, an enhanced root dry matter was produced by Legumefix compared to previous Bioifx and NoduMax (Fig. 5.1a & b). Across all soybean varieties, the previous uninoculated control Songda and previous Legumefix inoculated Jenguma yielded the greatest shoot dry matter (Fig. 5.1a & b). Shoot dry was a generally lower on previous uninoculated control Jenguma, and previous Bioifx and NoduMax inoculated Songda (Fig. 5.1a & b). Likewise, greater root dry matter was obtained with the previous uninoculated control Songda and previous Legumefix inoculated Jenguma and Afayak respectively (Fig. 5.1a & b). Root dry matter was generally low on all previous NoduMax inoculated soybean varieties, the previous uninoculated control Jenguma, and the previous Bioifx inoculated Afayak (Fig. 5.1a & b). For interaction effect of commercial inoculant and growth stage, shoot and root dry matter significantly increased with growth stage (Fig. 5.2a & b). Shoot and root dry matter peaked at R4 stage, and NoduMax produced the least shoot and root dry matter compared to the other treatments (Fig. 5.2a & b).

Plant height was significantly affected by the interaction of soybean variety and commercial inoculant (Appendix Table D.1). Generally, plant height was variable due to the interaction effects of the treatments. For Songda, the uninoculated control produced plants with greater height than the other treatments (Fig. 5.5). For, Jenguma, the previous Boifix, and Legumefix induced greater plants height compared the previous uninoculated control and NoduMax (Fig. 5.5). For Afayak, there was no significant increase in plant height due to inoculation (Fig. 5.5). Across all treatments, NoduMax inoculated Afayak, and the uninoculated control Afayak and Songda had higher plant heights (Fig. 5.5).

The number of pods (pod load) per plant was not affected by previous soybean variety and commercial inoculants (Appendix Table D.2). Pod dry wt was significantly affected by the previous soybean variety. Pod dry wt produced by Afayak and Jenguma was 21% and 18% greater than those produced by Songda respectively (Appendix Table D.2). However, commercial inoculant did not significantly affect pod dry wt.. Halum dry matter had no significant effect on previous soybean variety and commercial inoculant (Appendix Table D.2). Grain yield was not affected by the previous season soybean variety (Fig. 5.6). For commercial inoculants, grain yield from previous uninoculated control and previous Biofix was significantly greater than those from previous NoduMax (Fig. 5.7).

Soybean Shoot and Root Total Nitrogen Content

The total nitrogen content of shoot and root dry matter was affected by the interaction effect of previous soybean variety and commercial inoculant, and previous commercial inoculant and growth stage interaction effect (Appendix Table D.1). For Jenguma, increased shoot total N was associated with the previous Legumefix compared to the uninoculated control (Fig. 5.3a & b). Also, previous the Legumefix produced greater root total N relative to the uninoculated control and NoduMax (Fig. 5.3a & b). Within Afayak, shoot total N was not affected by the previous commercial inoculant (Fig. 5.3a & b). Nonetheless, the root total N by the previous Legumefix was significantly higher compared to previous Bioifx and NoduMax (Fig. 5.3a & b). For Songda, the previous uninoculated control produced significantly greater shoot and root total N than previous Legumefix and NoduMax (Fig. 5.3a &b).

For all the soybean varieties assessed, the previous uninoculated control Songda and previous Legumefix Jenguma yielded the highest shoot total N concentration (Fig. 5.3a & b). Also, the previous uninoculated control Jenguma, Legumefix inoculated Afayak, and as well as

Songda inoculated with Biofix and NoduMax produced shoot dry matter with lower total N concentration (Fig. 5.3a &b). Overall root total N due to interaction effect was significantly enhanced with the previous uninoculated control Songda and Legumefix inoculated Jenguma and Afayak respectively (Fig. 5.3a & b). Likewise, the uninoculated control Jenguma, Boifix inoculated Afayak, and NoduMax inoculated with all soybean varieties had the least significant root total N (Fig. 5.3a & b).

Shoot and root total N was improved due to the interaction of commercial Bradyrhizobium inoculant and growth stage (Appendix Table D.1). The total N content of shoot and root significantly increased with growth stage reaching climax at the R4 stage (Fig. 5.4a & b). The difference in the shoot and root total N was obvious at the R4 stage with the least effect associated with NoduMax compared to the other treatments (Fig. 5.4a & b).

Soybean N Fixed

Total N fixed, was affected by the interaction effect of previous soybean variety and commercial inoculant and the interaction effect of the growth stage and commercial inoculant (Appendix Table D.1). Among the interaction effect, N fixation varied among the different soybean variety and with some level of host specificity. With Jenguma, the N fixed by the previous Legumefix was higher than the previous uninoculated control (Fig. 5.3c). For Afayak, there was no significant difference in the amount of N fixed by the different treatments (Fig. 5.3c). While with Songda, the amount of N fixed by the uninoculated control was significantly greater compared to the previous NoduMax (Fig. 5.3c.). Across the soybean varieties, the previous uninoculated control Songda and previous Legumefix inoculated Jenguma fixed greater nitrogen (Fig. 5.3c). Whereas, the amount of N fixed by the previous uninoculated control Jenguma, and previous Biofix and NoduMax inoculated Songda was significantly lower (Fig.

5.3c). Similarly, the interaction effect of growth stage and commercial inoculants showed that N fixation increased with growth stage, reaching a maximum at full podding (R4) stage (Fig. 5.4c). At the R4-stage, there was a significant difference in the amount of N fixed by the previous commercial inoculants, but NoduMax fixed the least total N (Fig. 5.4c).

Soybean Grain N Uptake, Haulm N Uptake, Total N Uptake, and Residual N balance

There were no significant differences in grain N uptake, haulm N uptake, total N uptake, residual N balance and N harvest index with the previous soybean variety and previous commercial inoculant and their interaction effects (Appendix Table D.2). Nonetheless, residual N balance for budget 1 and 2 were negative for all the soybean varieties. In both budgets, Afayak had greater negative N balance than the other soybean varieties (Appendix Table D.2). For commercial inoculants, the residual N balance for both budget 1 and 2 indicated negative values for all the previous inoculation treatments (Appendix Table D.2). Legumefix had lower negative value than the other treatments (Appendix Fig. D.2). Nitrogen harvest index (NHI) was not affected by the previous soybean variety and commercial inoculant main treatment effects (Appendix Table D.2). Mean NHI ranged from 0.88 to 0.90 (88 % to 90 %) (Appendix Table D.2).

Impacts of Previous Commercial Inoculants and Soybean on the Subsequent Maize

Maize shoot dry matter was significantly affected by the previous commercial inoculant (Appendix Table D.3). The previous Biofix yielded greater maize shoot and root dry matter than previous Legumefix (Fig. 5.8). However, the previous soybean variety did not affect shoot dry matter for the subsequent maize crop. (Fig. 5.8). Maize plant height varied due to the interaction effect of commercial inoculant and soybean variety (Appendix Table D.3). Among the soybean varieties, previous Jenguma inoculated with Legumefix produced significantly taller plants than

uninoculated control Jenguma, and Jenguma inoculated with Biofix and NoduMax (Fig. 5.12). The uninoculated control Afayak and Biofix inoculated Afayak had an intermediate maize plant height than Legumefix inoculated Afayak (Fig. 5.12). Finally, previous Boifix inoculated Songda produced taller maize plants compared to the previous Legumefix inoculated Songda (Fig. 5.12). Overall, greater plant heights were associated with Jenguma inoculated with Legumefix while shorter plant height was associated with uninoculated control Jenguma, and Legumefix inoculated Afayak and Songda (Fig. 5.12). Maize plant height, and dry mattter (DM) of shoot and root increased with a corresponding increase in the growth stage (Fig. 5.13).

Plant height, shoot DM and root DM were affected by the interaction effect of mineral fertilization and growth stage (Appendix Table D.3). Mineral N enhanced maize plant height, shoot DM and root DM compared to the control (Appendix Fig. D.1, D.2, and D.3). Maize plant height, shoot DM and root DM increased with growth stage (Appendix Table D.6). In general maize shoot DM produced from previous soybean plot with the main inoculation treatments were comparable to those that received the half recommended mineral N fertilizer rate and ~ about 1000% than the unfertilized control treatment.

Maize Grain Yield

Maize grain yield was significantly enhanced ($P < 0.1$) from previous Biofix compared to the uninoculated control and Legumefix (Table 5.2 & Fig. 5.13). Maize grain yield from previous Bioifix varied by approximately 22 %, 29 % and 40 % over the previous NoduMax and Legumefix and the uninoculated control treatment respectively (Table 5.2 & Fig. 5.13). Maize grain yields under the previous soybean variety were not significantly ($P < 0.1$) different although Jenguma produced greater grain yield than Songda (2.5 %) and Afayak (12 %) (Table 5.2 & Fig. 5.13).

Application of mineral N fertilizer increased maize grain yield compared to unfertilized control treatment. It was evident that maize grain yield increased with the corresponding increment in mineral N fertilizer rate (Table 5.2 & Fig. 5.13). In general, maize grain yield from previous inoculated and uninoculated treatments, and as well as previous soybean variety were comparable to the grain yield from half recommended mineral N fertilizer rate (50 kg N ha^{-1}) (Table 5.5 & Fig. 5.13 & 5.14).

Maize stover yield and harvest index were not significantly ($P < 0.1$) affected by previous commercial inoculant and soybean variety (Table 5.2). However mineral N fertilizer application produced greater stover yield (175 %) than the unfertilized control treatment (Table 5.2).

Maize Shoot Nitrogen Uptake

Shoot N content was affected by the interaction effects of previous soybean variety and commercial inoculant and commercial inoculant and growth stage respectively (Appendix Table D.3). Shoot N content varied with soybean variety and commercial inoculant (Table 5.2 & Fig. 5.10). The overall shoot N content showed that previous Biofix inoculated Afayak had greater shoot N uptake of ~13.7 kg N ha^{-1} (Fig. 5.10). While the uninoculated control Jenguma, and Legumefix inoculated Afayak and Songda had lower shoot N uptake of 7.3, 7.0 and 6.0 kg N ha^{-1}, respectively (Table 5.2 & Fig. 5.10).

Shoot N content increased and varied with commercial inoculant and growth stage peaking at the R2 stage before declining (Fig. 5.11). At the R2 stage, greater shoot N uptake was associated with Biofix and NoduMax compared to Legumefix (Fig. 5.11). Meanwhile, shoot N uptake by NoduMax was also significantly higher than the uninoculated control (Fig. 5.11).

Shoot N content peaking at the R2 stage was expected since it coincided with silking and kernel blister of the maize, a developmental stage which requires significant available N.

Maize Total Nitrogen Uptake

The total N uptake by maize grain, stover, and total biomass was not affected by the previous soybean variety (Table 5.2). Grain N uptake ranged from 12.2 kg N ha^{-1} to 14.3 kg N ha^{-1} with Jenguma and Songda respectively (Table 5.2). Stover N uptake also ranged from 6.0 kg N ha^{-1} with Songda to 7.4 kg N ha^{-1} with Jenguma (Table 5.2). The overall total biomass uptake was 21.8 kg N ha^{-1}, 18.5 kg N ha^{-1} and 19.5 kg N ha^{-1} with Jenguma Afayak and Songda respectively (Table 5.2).

Previous year's commercial inoculant had a significant effect on total N uptake by maize grain, total biomass but not stover (Table 5.2). Previous year's Biofix treatment stimulated greater grain N uptake ($P < 0.1$) and total N uptake ($P < 0.1$) compared to the uninoculated control and the Legumefix (Table 4.2). The performance of NoduMax regarding grain N uptake and total N uptake was intermediate. Although stover N uptake was not significant; mean values range from 6.37 kg N ha^{-1} with NoduMax to 6.57 kg N ha^{-1} for the uninoculated control (Table 5.2).

Mineral N fertilizer increased grain N uptake, stover N uptake and total biomass N uptake than control (Non-fertilization) (Table 5.2). Grain N uptake, stover N uptake, and total biomass N uptake increased with a corresponding increase in mineral N fertilizer rate (Table 5.2). The difference between 50 N and 100 N kg ha^{-1} with respect to grain N uptake, stover N uptake and total biomass N uptake was about 1.5 fold (Table 5.2).

Maize Harvest Index (HI) and Nitrogen Harvest Index (NHI)

Harvest index (HI) and nitrogen harvest index (NHI) for maize were not significantly affected by the previous soybean variety main treatment effect (Table 5.2). Regardless of previous soybean variety, average HI was about ~ 45 %, and NHI was ~ 60 % (Table 5.2). Harvest index (HI) and N harvest index (NHI) for maize were significantly affected by commercial inoculant (Table 5.2). Biofix had greater HI and NHI of 49 % and 68% than Legumefix with 39 % and 55 %, respectively (Table 5.2). Higher HI implies greater conversion of biomass matter into grain yield and while greater NHI suggests higher conversion of N uptake by biomass matter into N uptake by grain yield. Similarly, mineral N fertilizer application also affected both HI and NHI index of maize (Table 5.2). Both HI and NHI index increased with a corresponding increase in mineral N fertilizer rates. The 100 kg N ha^{-1} siginificantly affected HI and NHI (Table 5.2).

Soil Health Indicator Assessment

The key soil health indicators assessed included soil organic C (SOC), soil total N (STN), soil available N (NH_{4+}-N and NO_3-N), potentially mineralizable N, soil pH, soil bulk density (Appendix Table D.4) and soil respiration (soil mineralizable C) (Appendix Table D.4). Except for soil bulk density, all the soil health indicators were not significantly affected by the soybean variety and commercial inoculant main treatments and their interaction effect. Soil organic C (SOC) ranged from 7.06 Mg C ha^{-1} with Songda to 7.82 Mg C ha^{-1} with Jenguma. For commercial inoculant, SOC ranged from 6.61 Mg C ha^{-1} with Legumefix to 8.34 Mg C ha^{-1} with NoduMax (Appendix Table D.4). While soil total N (STN) averaged for soybean variety ranged from 0.8 Mg N ha^{-1} with Afayak to 0.88 Mg N ha^{-1} with Jenguma, and for commercial Inoculant, ranged from 0.82 Mg N ha^{-1} with Legumefix to 0.95 Mg N ha^{-1} with NoduMax (Appendix Table

D.4). In general SOC and STN were low due to the short duration of the study coupled with residue removal at harvest. For available soil N and potentially mineralizable N values ranged between 4.80 mg kg^{-1} and 2.66 mg kg^{-1} with Songda to 5.47 mg kg^{-1} and 3.58 mg kg^{-1} with Jenguma, respectively (Appendix Table D.4). Available soil N due to commercial inoculant was 4.99 mg kg^{-1} with Biofix to 5.47 mg kg^{-1} with Legumefix (Appendix Table D.4). For potentially mineralizable N, the control had lower mean value of 2.79 mg kg^{-1} while Legumefix had the greater mean value of 3.54 mg kg^{-1} (Appendix Table D.4). In general, inoculation tends to enhanced PMN availability compared to uninoculated control although not statistically significant.

Soil pH was not significantly different but range between 5.5 to 5.6 for both soybean variety and commercial inoculant main treatment effects (Appendix Table D.4). Low pH value is an indicator of soil acidity. Soil pH values documented in this study were consistent with those typically observed in the Guinea Savanna of West Africa.

Microbial biomass assessed by PLFA was not affected by the previous season soybean variety (Appendix Table D.4). However, the previous commercial inoculant affected PLFA-microbial biomass. Previous NoduMax and the uninoculated control produced greater microbial mass compared to the previous Legumefix (Appendix Table D.4).

Cumulative evolved CO_2 was not statistically different for soybean variety and commercial inoculants. However, Jenguma had higher mean value for cumulative evolved CO_2 than the other soybean varieties (Appendix Table D.5). Also, inoculation with commercial inoculants had greater mean value for cumulative evolved CO_2 compared to uninoculated control but not statistically different (Appendix Table D.5). Trends for cumulative evolved CO_2 due to

commercial inoculants probably suggest an enrichment of the soil with an introduced soil microbe could induce greater mineralization of soil organic matter.

Discussion

Inoculation of grain legumes such as a soybean with commercial Bradyrhizobium inoculant is relatively a new technology in sub-Saharan West Africa. Inoculation of grain legumes promotes plant growth, nodulation, symbiotic N fixation and also improve grain yield and grain protein. Therefore the efficiency or effectiveness of inoculum in inoculant are assessed using these symbiotic indicators (growth, nodulation, N-fixation, grain yield, and grain protein). Our results revealed that the previous Legumefix produced superior nodulation (nodule number and nodule mass) than NoduMax and Biofix. Enhanced nodulation by Legumefix can be attributed to *Bradyrhizobium strains* used as the inoculum in the inoculants. Legumefix inoculant was formulated with *Bradyrhizobium japonicum* strain 532c while NoduMax and Biofix had *Bradyrhizobium japonicum* strain USDA 110. Improved nodulation by Legumefix possibly suggests that it took some time for the *Bradyrhizobium japonicum* strain USDA 532c to adapt, grow and colonized the host in its new environment. In our previous work, where we inoculated annually, NoduMax and Biofix had better nodulation than Legumefix (with strain USDA 532c). Therefore, the superior performance of USDA 532c was perhaps masked by climate and edaphic factors in its new environment, thereby favoring *Bradyrhizobium japonicum* strain USDA 110 in preceding studies. Zhang et al. (2003) observed that the superior performance of *Bradyrhizobium japonicum* strain could be altered in a new environment which was different from their natural environment. Further, nodulation also increased with growth stage peaking at R2-stage before declining at R_4-stage. Peak nodulation coincided with full flowering, a stage where N-fixation is assumed to reach a maximum. Nodulation declined at the R4-stage (full pod) was expected, since

nodules start to deteriorate once pods set. Our result corroborates with Chowdhury et al. (1983), who observed nodulation decline in inoculated promiscuous soybeans cultivar at full pod in Tanzania. Zhang et al. (2003) also observed a decrease in nodulation (number and mass) in inoculated soybean after R4-stage (full pod) in Canada. For soybean variety, Afayak still maintained superior nodulation performance.

Soybean shoot dry matter, root dry matter, shoot total nitrogen, root total nitrogen, and nitrogen fixation were influenced by the interaction of the previous soybean variety and commercial inoculants. In general, the interaction effect gives an indication of genotype by commercial inoculant host specificity. Although the interaction effect was highly variable and not consistent, across all treatments, the previous uninoculated (control) Songda yielded the greatest soybean shoot and root dry matter, shoot total N, root total N, and N-fixations. This observation suggests that sequential double cropping of Songda soybean genotype can potentially enhance the symbiotic capabilities of the native *Rhizobium spp.* compared to *Bradyrhizobium japonicum* strains in the commercial inoculant regarding dry matter production, shoot and root total N, and N-fixation. With Jenguma, the previous Legumefix stimulated increase shoot and root dry matter, shoot and root total N and N-fixation. Thus inoculating Jenguma with Legumefix in a preceding season may induce a greater residual benefit to the subsequent Jenguma crop.

Plant height was also affected by the interaction of the previous soybean variety and commercial inoculant. The interaction effect of Afayak and previous commercial inoculants produced the greatest plant height across all treatments. Similarly, the uninoculated control Songda also produced plants with significant height. Plant height correlate linearly with shoot

dry ($R= 0.693$, $P < 0.001$). Thus it is apparent that increased biomass (shoot and root) production may be associated with plant height.

Residual N balance was negative for both the previous soybean variety and the commercial inoculants regardless of the estimation approach. Nonetheless, residual N balance was more negative when both grain and stover yield were exported. This finding agrees with Osunde et al. (2003b) who reported negative residual N balance for promiscuous soybean cultivars cropped on previous soybean fields inoculated with *Bradyrhizobium japonicum* the in southern Guinea savanna zone of Nigeria. Nonetheless, the average negative residual N balance documented in their work was two-fold greater than what we observed. Similarily, Adu-Gyamfi et al. (2007) documented greater negative N residual balance when both stover and grain yields were exported in maize-pigeon systems in Malawi. The negative residual N balance observed in this work implies that sequential double cropping of soybean would lead to further depletion of soil available N. Since available N uptake by the soybean plant exceeded the amount of N fixed by the soybean plant.

Harvest index for nitrogen (NHI) ranged between 86-90% though not significant. This high NHI indicates that a significant proportion of the N uptake by the plant was translocated or assimilated into grain yield. The N harvest index (NHI) documented in this study is similar to those (74 -84 %) reported by Singh et al. (2003). On the contrary, about 1-1.5 fold lower than the NHI by reported Sanginga et al. (1997a, 2002). Thus soybean grain yield removal at harvest contributed to significant nutrient removal. The high NHI also suggest that soybean variety and commercial inoculant contributed marginally or nothing to the soil N nutrition. Hence the negative residual N balance provides clear evidence of how doubling cropping of soybean and

previous year's Bradyrhizobium inoculant did not contribute to soil N nutrition but rather enhanced N depleting from the available soil N pool.

Total N content of grain and haulm dry matter was not statistically significant. Nonetheless, trends for grain N and haulms N was similar to grain yield and halum dry matter. The previous uninoculated control and Biofix showed a higher tendency to produced grain and haulm dry matter with greater N content.

The previous uninoculated control and Biofix produced significantly greater soybean grain yields. The enhanced grain yield by the previous uninoculated control suggests an increased in the native Rhizobium population with better symbiotic efficiency due to the previous soybean crop. Chowdhury et al. (1983) also observed greater soybean grain yield on previous uninoculated control fields than previous inoculated fields. The increased grain yield by the previous Biofix may be attributed to a greater persistent of it *Bradyrhizobium japonicum strain* compared to the other commercial inoculants. Nonetheless, we are unable to provide a detail explanation for the significant grain yield difference between previous Biofix and NoduMax as both inoculants contain the same *Bradyrhizobium japonicum strain (USDA 110).* We speculate that the poor performance of NoduMax was perhaps due to quality control and handling. Although grain yield from the previous soybean variety was not significant, Afayak produced higher grain yield than the other varieties. Halums dry matter was not significant for both the previous soybean variety and commercial inoculant main treatment effect.

In general, grain yield was higher when we inoculated annually than when we did not inoculate (data not shown) except the uninoculated control where we witnessed a marginal increase, perhaps due to carry over effect. This observation seems highly likely to reinforce the

conclusion from our previous work that yearly inoculation of soybean is necessary to enhance sustainable grain production and greater soil productivity.

Impacts of Previous Commercial Inoculants and Soybean on the Subsequent Maize

Several authors have documented the impact of soybean on the subsequent maize crop in crop rotation (Escuro, 1992; Sanginga et al., 1997b, 2002; Osunde et al., 2003a; Singh et al., 2003). Ogoke et al. (2003), Osunde et al. (2003a) and Singh et al. (2003) documented that soybean contributed to net negative residual N balance to the soil N pool. Sanginga et al. (2002) and Ennin et al. (2004) documented that soybean contributed net positive residual N balance to the soil N pool. In the present study, the N contributed by the previous soybean crop before maize was variable (data not shown). The net residual N balance contributed by the main treatments when grain was removed ranged between 32-46 kg N ha^{-1} for soybean variety and 3-93 kg N ha^{-1} for commercial inoculant (Chapter 3; data not shown). While the net residual N balance contributed when both grain and haulm were removed ranged between 3-24 kg N ha^{-1} for soybean variety and -25-66 kg N ha^{-1} for commercial inoculant (Chapter 3; data not shown). In both scenarios, the soybean varieties contributed positively to the net residual N balance. This observation agrees with Sanginga et al. (2002) who documented net positive residual N balance of 11-43 kg N ha^{-1} for different promiscuous soybean varieties in the Southern Guinea Savanna of Nigeria.

On the other hand, residual N balance for previous commercial inoculant was largely variable. In the second scenario, Legumefix, and Biofix contributed a net negative residual N of -7 kg N ha^{-1} and -25 kg ha^{-1} respectively, while the uninoculated control and NoduMax contributed a net positive residual N of 26 kg N ha^{-1} and 67 kg N ha^{-1} respectively (Chapter 3; data not shown). Net negative residual N balance (values) indicate net removal of soil available

N. Remarkably, the significant residual N balance of 67 kg N ha^{-1} contributed by NoduMax did not translate into increase grain yield of the succeeding maize crop. Rather, the previous Biofix produced the greatest maize grain yield. The enhanced maize grain yield (1132 kg ha^{-1}) by the previous Biofix could be due to N sparing effect and other rotation effects' since the contribution from residual N balance was a net negative (-25 kg N ha^{-1}). Thus it was apparent that a -25 kg N ha^{-1}cannot produce a grain yield of 1132 kg ha^{-1}. This finding corroborates with a previous work by Sanginga et al. (2002) who observed an increase in maize grain yield from fields previously cropped to soybean with a low net residual N balance and even negative net residual N balance in some cases. The "other effects" may be that the maize plant was able to exploit the soil better when rotated with the soybean than maize monoculture as reported by Sanginga et al. (2002).

Further, maize grain yield also increased due to mineral N fertilization, with significant or pronounced effect associated with 100 kg N ha^{-1} (full recommended rate), and then followed by 50 kg N ha^{-1}(half recommended rate). Enhanced maize grain yield due to mineral fertilization was ~ 25 times more with 50 kg N ha^{-1} and ~ 43 times more with 100 kg N ha^{-1} compared to the 0 kg N ha^{-1} (control) respectively. This result contradicts the work of Ennin et al. (2004) who found no significant difference in average maize yield between 45 kg N $ha^{-1}$and 90 kg N ha^{-1} in Ejura, transitional forest zone of Southern Ghana. In general, average maize grain yield from the soybean-maize rotation systems are comparable to maize grain yield from the mineral N fertilizer. Maize grain yield from the previous Biofix was comparable or the same as maize grain yield from 50 kg N ha^{-1} (half recommended rate). While grain yield from the previous uninoculated control and NoduMax inoculant was 25 % and ~ 20 % less than those from 50 kg N ha^{-1} (half recommended rate), respectively. Grain yield produced by Legumefix was ~ 39 % less than 50 kg N ha^{-1}. Therefore inoculating soybean with commercial inoculant especially with

Biofix, and to some extent, NoduMax, or growing soybean alone by producers can save up to 50 % of mineral fertilizer cost for the succeeding cereal (maize) crops. Maize grain yield from previous soybean variety main treatment effects did not increase statistically but ranged between 807- 921 kg ha^{-1}. Average grain yield by Afayak, Songda, and Jenguma was 28%, 19% and 17%, less than the grain yield from 50 kg N ha^{-1} respectively.

In general average grain yield from both soybean variety and commercial inoculant main treatment effects were ~ 19.5 times (fold) higher than grain yield from the 0 N kg ha^{-1} and ~ 2.5 times (2.5 fold) lower than grain yield from 100 kg N ha^{-1} (full mineral fertilizer recommend) respectively. Therefore introducing or inclusion of promiscuous nodulating soybean cultivars into legume-cereal crop rotation systems can improve or increase the grain yield of the subsequent cereal crop than continuous cereal monoculture. Osunde et al. (2003a) documented 3 t ha^{-1} of maize grain yield from a 2-yr double-cropped soybean field rotated to maize. Maize grain yields in this study were low due to insufficient N supply, poor plant stands, and disease and pest attack during early stages. Insufficient N supply was evident when most of the plants had yellow leaf coloration and stunted growth in the field. While pest attack such as Fall armyworm *(Spodoptera frugiperda)* invasion at the early stages of plant development was evident but controlled through frequent spraying. Maize stover yield though not statistically significant was similar to maize grain yield for both previous soybean variety and previous commercial inoculant main treatment effect. Mineral N fertilization enhanced stover dry matter production.

Maize harvest index and NHI were significant due to previous commercial inoculants. Greater HI and NHI were associated with the previous Biofix and NoduMax compared to Legumefix. The efficiency of both Biofix and NoduMax inoculants in the partitioning of dry

matter (total biomass DM) into grain yield was ~ 46 % and their nitrogen translocated from dry matter into grain (grain N uptake) of about ~ 67% respectively. The NHI obtained in this work are similar to those reported by Sanginga et al. (2002). The high NHI suggests that a significant proportion of the N fixed by the preceding soybean crop or soil N available was translocated or partitioned into the grain of the succeeding crop. This was evident by the high grain N content associated with the previous Biofix and NoduMax in this study.

Increased maize shoot and root dry matter were also produced from the previous Boifix and NoduMax fields. Shoot and root dry matter also increased with growth *s*tage. The improved shoot and root dry matter by previous NoduMax possibly suggest that a significant proportion of its residual N balance (67 kg N ha^{-1}) was directed into dry matter production at the expenses of grain yield. Hence the enhanced shoot and root dry matter but not grain yield. Similarly, mineral N fertilization enhanced maize shoot and root dry matter production. Pronounced maize shoot and root dry matter were associated with 50 kg N ha^{-1} (half fertilizer recommended rate) at R2-stages and 100 kg N ha^{-1} (full fertilizer recommended rate) at R4-stage respectively. Average shoot and root dry matter produced from the previous soybean variety and commercial inoculant main treatment effects were three times (fold) greater than those from 0 kg N ha^{-1} (control) while shoot dry matter from independent mineral N fertilizer (50 N kg ha^{-1} and 100 N kg ha^{-1}) was ~ two-fold greater than those from the soybean rotation study.

Maize plant height and shoot N content were affected by the interaction of soybean variety and commercial inoculant. This suggests soybean variety by commercial inoculant selection specificity for both plant height and shoot N content. The different soybean varieties induced variable responses to previous commercial inoculants regarding plant height and shoot nitrogen content. Significant shoot N was observed when Afayak was inoculated with Biofix,

followed by Biofix inoculated Jenguma and NoduMax inoculated Songda. Likewise, an enhanced maize plant height was achieved when Jenguma was inoculated with Legumefix and Songda inoculated Boifix respectively.

Impacts of Previous Commercial Inoculants and Soybean on Soil Quality and Soil Microbial Community Structure

Legume-cereal rotation system has been documented to positively affect both soil and plant health. Improvement in soil organic carbon (SOC) and total N (TN), mineralizable C and N, microbial biomass, soil available N, soil bulk density and pH are some influence of soybean-maize rotation on soil health. While the reduction in weed infection (such as *Striga hermatica*), plant toxicity, and pest and diseases attack are some impact of soybean-maize rotation on the impact plant health. Apart from microbial biomass (PLFA), all the soil quality parameters assessed were not affected by the previous soybean crop variety and commercial inoculant. Results for the soil quality parameters were largely variable and inconsistent perhaps due to the short duration of the experiment. Yusuf et al. (2009a) observed a significant increase in both soil quality chemical and biological indicator in 3-yr legume-maize rotation systems in the Guinea Savanna Zone of Nigeria.Drinkwater (1998) reported an increase in soil organic carbon (SOC) and total N (TN) in 14-yr legume-cereal rotation. The high microbial biomass (PLFA) observed with the uninoculated control, and NoduMax can be attributed to the increase biomass produced in the preceding year. The same argument could hold for SOC and total N as the trend was similar to microbial biomass. Regarding the soybean varieties, Jenguma exhibited a greater tendency to increased SOC, SON, soil available N, potentially mineralizable N and mineralizable C than the other soybean varieties.

Conclusion

Previous Legumefix stimulated greater nodulation while nodulation declined on the previous Biofix and NoduMax. We attribute this observation to the persistent of *Bradyrhizobium japonicum strain* used as inoculum in the two inoculants. For the soybean variety, Afayak maintained superiority nodulating capacity. Shoot and root dry matter, and their N concentration were influenced by the interaction effect of soybean variety by previous commercial inoculant, an indication for host specificity. We observed that the uninoculated control Songda had a superior performance due to interaction effect. Enhanced soybean grain yield was observed on previous Biofix and uninoculated control. Improved soybean grain yield by previous Biofix may be due to increased persistent of *Bradyrhizobium* strain. For the previous uninoculated control, improved yield was perhaps the a result of enhanced symbiotic efficiency of the native soil *Rhizobium*. Adoption of double-cropped soybean systems would induce further depletion of soil available N. The negative residual N balance signify that soil N uptake exceeded N-fixation. Therefore to enhance soybean grain production with subsequent improvement in soil quality, annual (yearly) inoculation with commercial inoculant is needed. Inoculation can serve as insurance against low yield and possible low N-fixation due to reduced symbiotic efficiency of the introduced rhizobium or the native rhizobium population.

When maize was rotated to the previous soybean inoculated fields, greater shoot dry matter and grain yield were observed with Biofix. Improved maize grain from the previous Biofix was perhaps due to other rotation effects. Maize grain yield in the rotation phase was generally low due to insufficient N supply, and pest and disease attack. Further, maize grain yield from soybean rotation study was comparable to grain yield from independent mineral N fertilizer (50 kg N ha^{-1}). We also observed that both Biofix and NoduMax yielded greater harvest

index and nitrogen harvest index, indicating increased ability to partition total dry matter into grain matter and N uptake.

Apart from microbial biomass (PLFA), the other soil quality indicators assessed were not affected by the previous soybean variety and the commercial inoculants. The apparent lack of significant differences in the other soil quality indicators can be attributed to the short duration of the study.

Finally, we recommended yearly inoculation of soybean since it will enhance higher N-fixation and grain yield, translating into greater economic returns. Adoption of soybean-maize rotation system could significantly decrease (50%) the amount of mineral N fertilizer required by the subsequent maize in the rotation. The impact of soybean-cereal rotation on soil health may not be obvious in one rotation cycle.

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List of Tables and Figures

Double Cropped Soybean

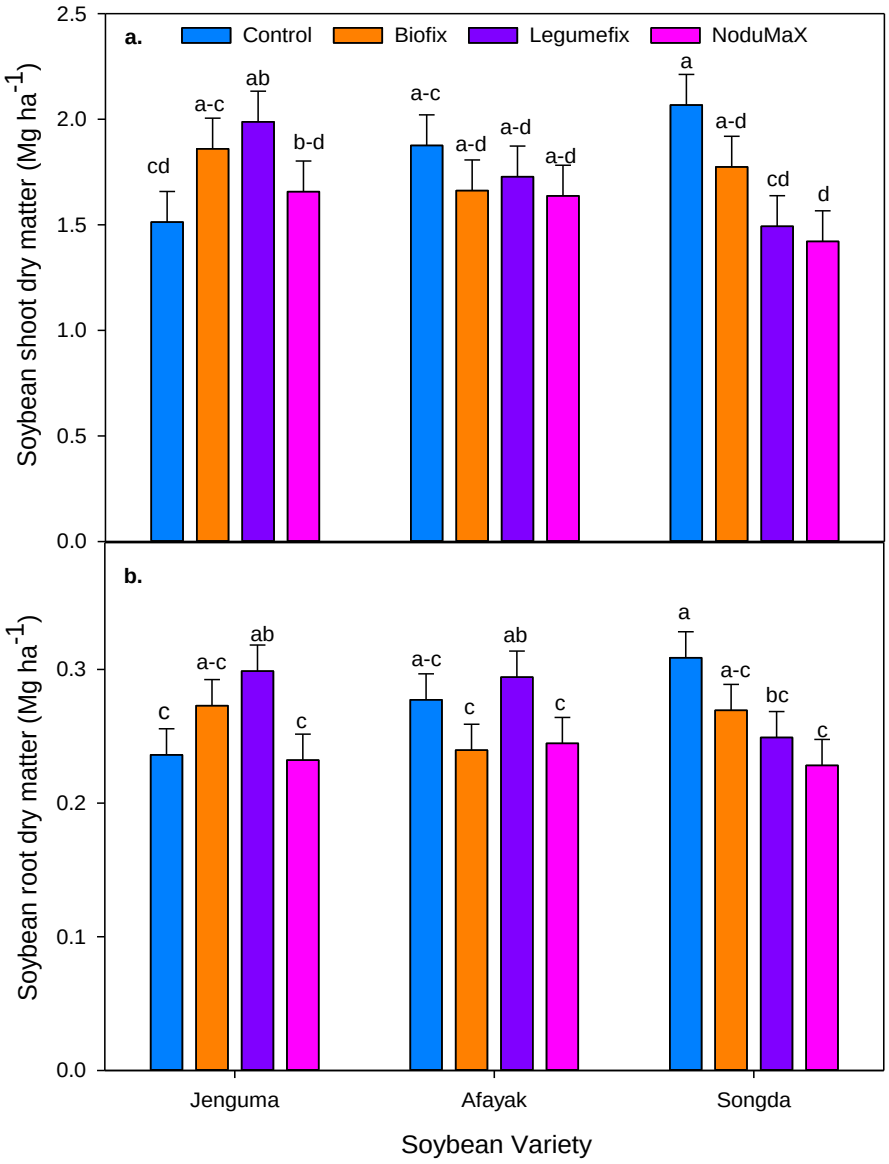

Figure 5.1. Interaction effect of previous commercial Bradyrhizobium inoculant and soybean variety on (a) shoot dry matter and (b) dry matter in double-cropped soybean in Nyankpala, Ghana, 2017. Different letters indicate significant differences at $p < 0.05$. Error bar is a standard error (SE).

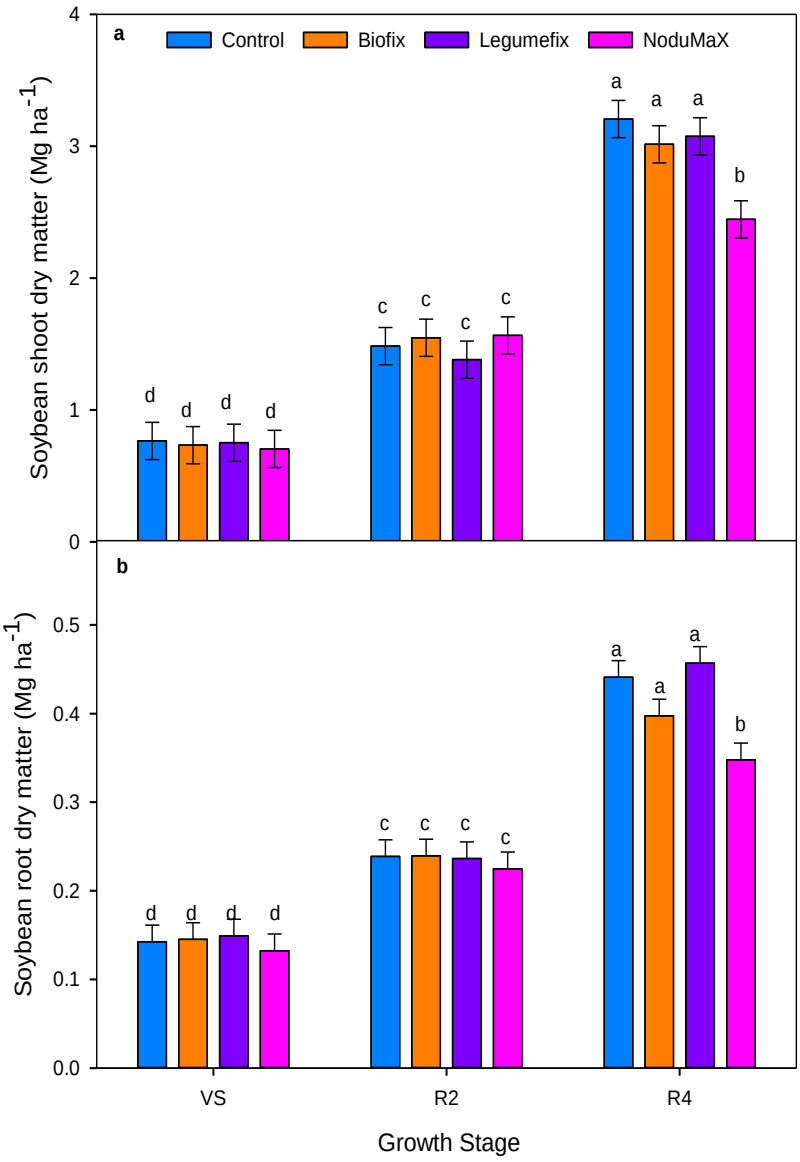

Figure 5.2. Interaction effect of previous commercial Bradyrhizobium inoculant and growth stage variety on (a) shoot dry matter and (b) dry matter in double-cropped soybean in Nyankpala, Ghana, 2017. Different letters indicate significant differences at p < 0.05. Error bar is a standard error (SE).

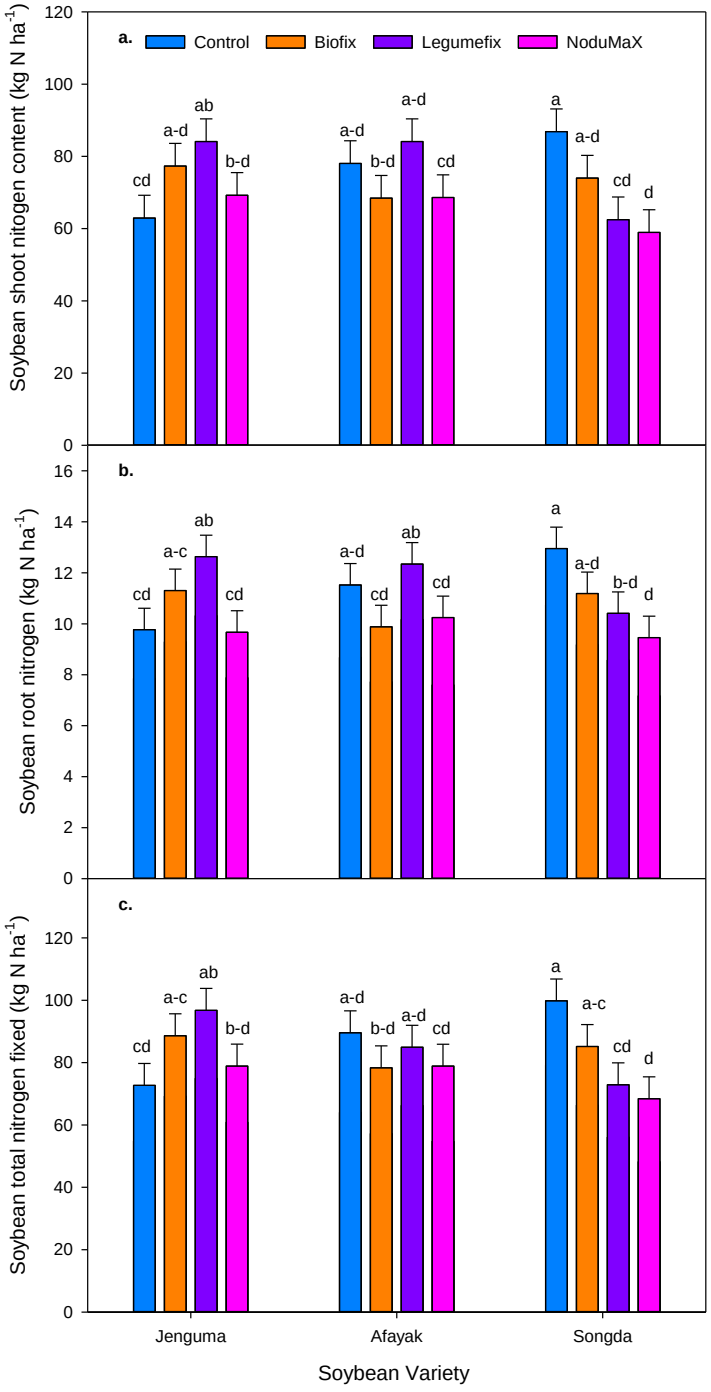

Figure 5.3. Interaction effect of previous Bradyrhizobium inoculant and soybean variety on (a) shoot N (b) root N (c) total N fixed in double-cropped soybean in Nyankpala, Ghana, 2017. Different letters indicate significant differences at $p < 0.05$. Error bar is a standard error (SE).

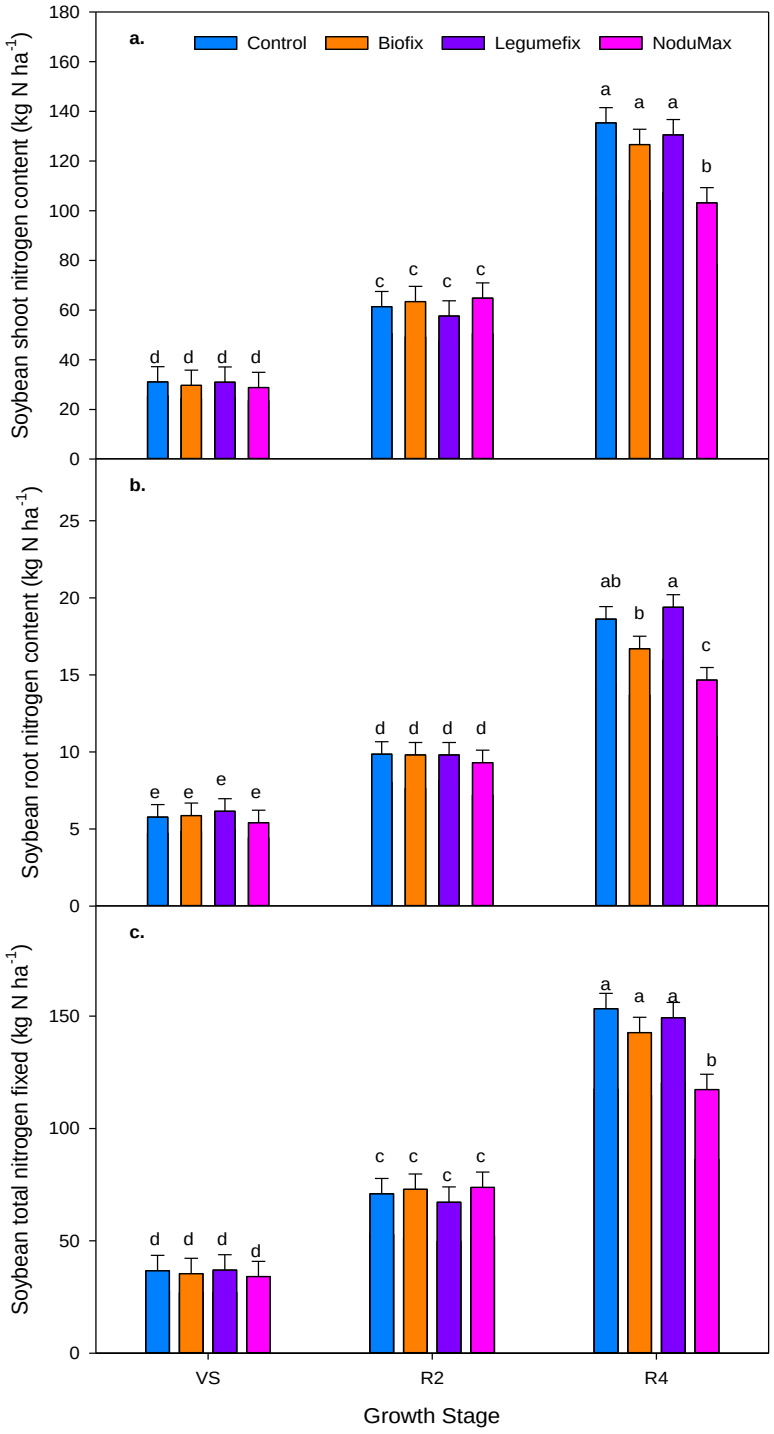

Figure 5.4. Interaction effect of previous commercial Bradyrhizobium inoculant and growth stage on (a) shoot N (b) root N (c) total N fixed in double-cropped soybean in Nyankpala, Ghana, 2017. Different letters indicate significant differences at $p < 0.05$. Error bar is a standard error (SE).

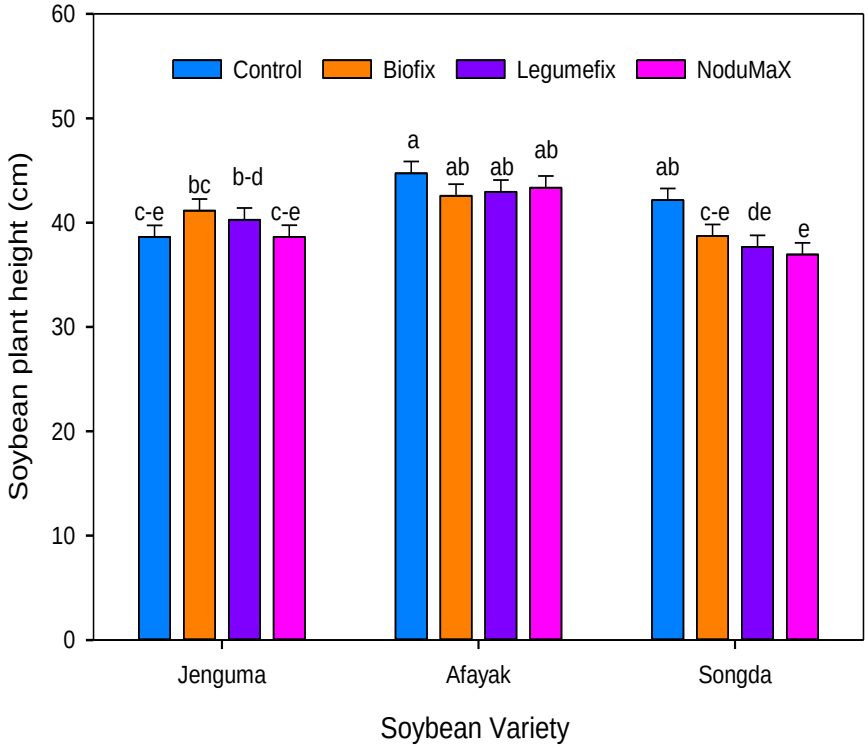

Figure 5.5. Interaction effect of previous commercial Bradyrhizobium inoculant and growth stage on plant height in double-cropped soybean in Nyankpala, Ghana, 2017. Different letters indicate significant differences at $p < 0.05$.

Error bar is a standard error (SE).

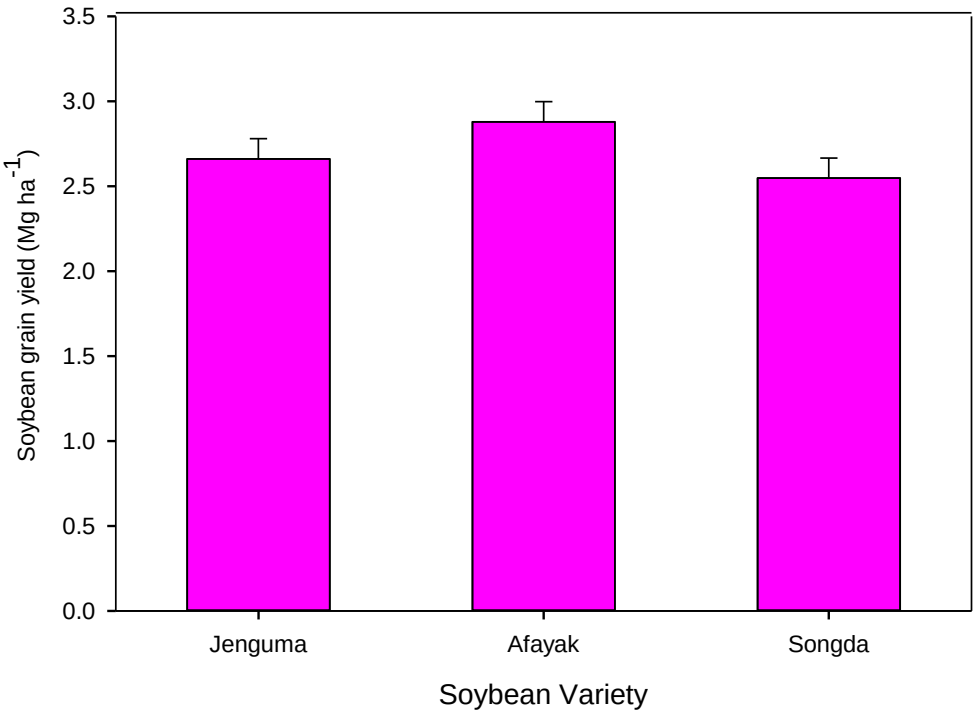

Figure 5.6. Effect of previous soybean variety on soybean grain yield in double-cropped soybean in Nyankpala, Ghana, 2017.

Error bar is a standard error (SE).

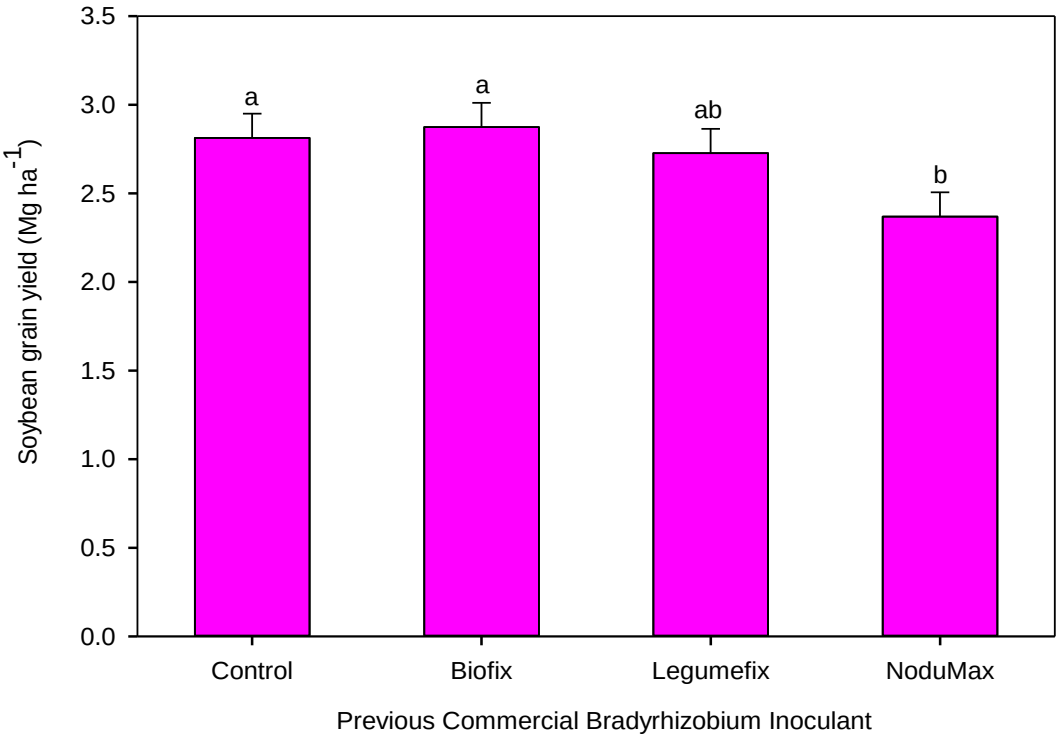

Figure 5.7. Effect of previous commercial Bradyrhizobium inoculant on soybean grain yield in double-cropped soybean in Nyankpala, Ghana, 2017.

Different letters indicate significant differences at $p < 0.05$. Error bar is a standard error (SE).

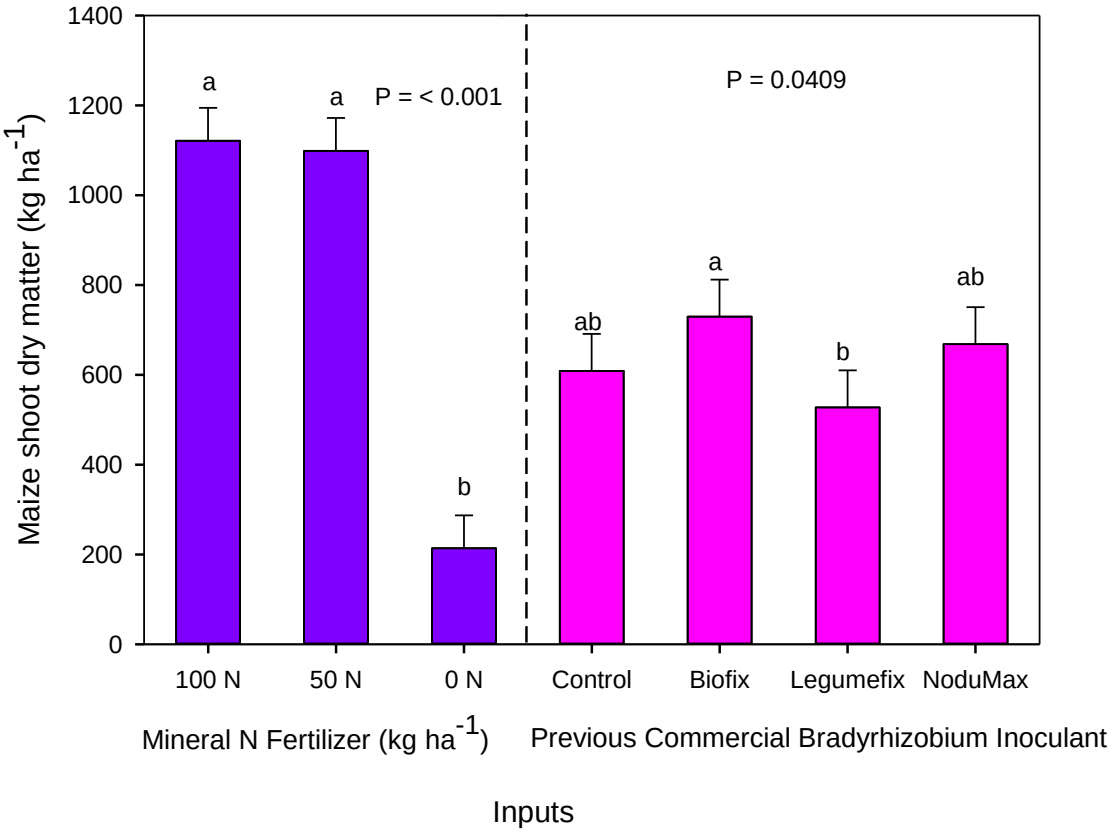

Figure 5.8. Maize shoot dry matter affected mineral N fertilizer and previous commercial Bradyrhizobium inoculant in Soybean-Maize rotation systems in Nyankpala, Ghana, 2017. Different letters indicate significant differences at p < 0.05. Error bar is a standard error (SE).

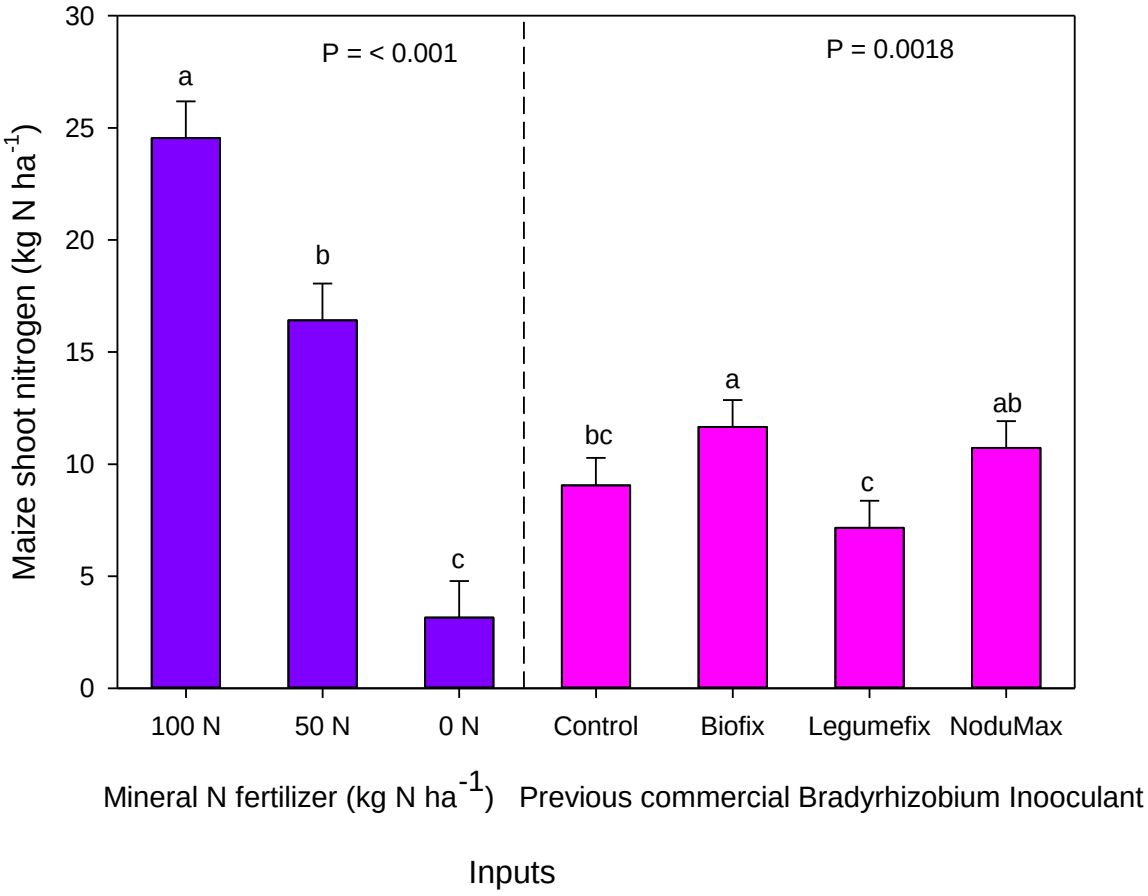

Figure 5.9. Maize shoot nitrogen content affected mineral N fertilizer and previous commercial Bradyrhizobium inoculant in Soybean-Maize rotation systems in Nyankpala, Ghana, 2017. Different letters indicate significant differences at $p < 0.05$. Error bar is a standard error (SE).

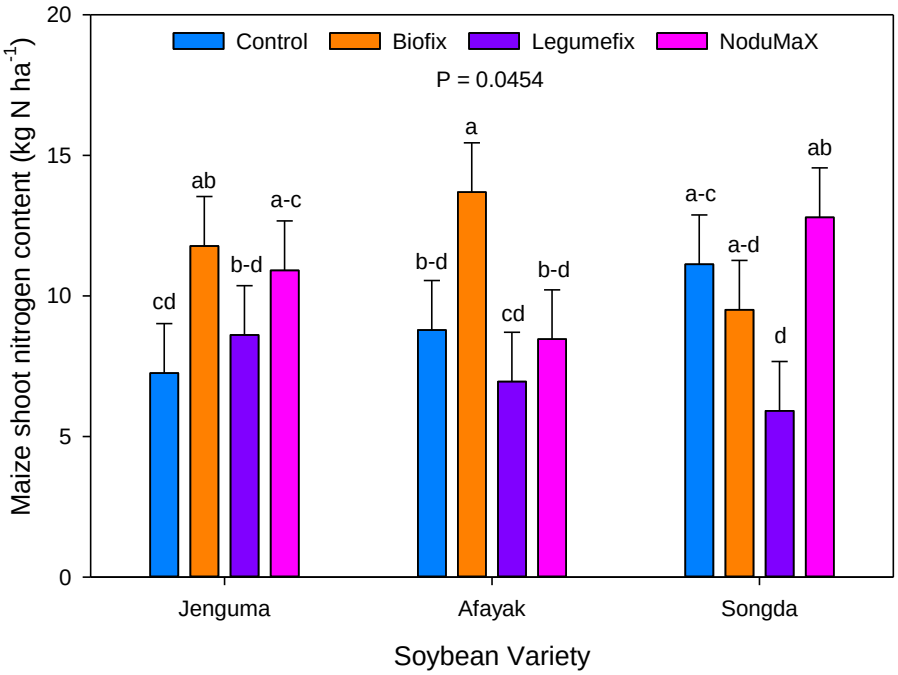

Figure 5.10. Maize shoot N affected by the interaction of previous soybean variety and commercial Bradyrhizobium inoculant in Soybean-Maize rotation systems in Nyankpala, Ghana, 2017. Different letters indicate significant differences at p < 0.05. Error bar is a standard error (SE).

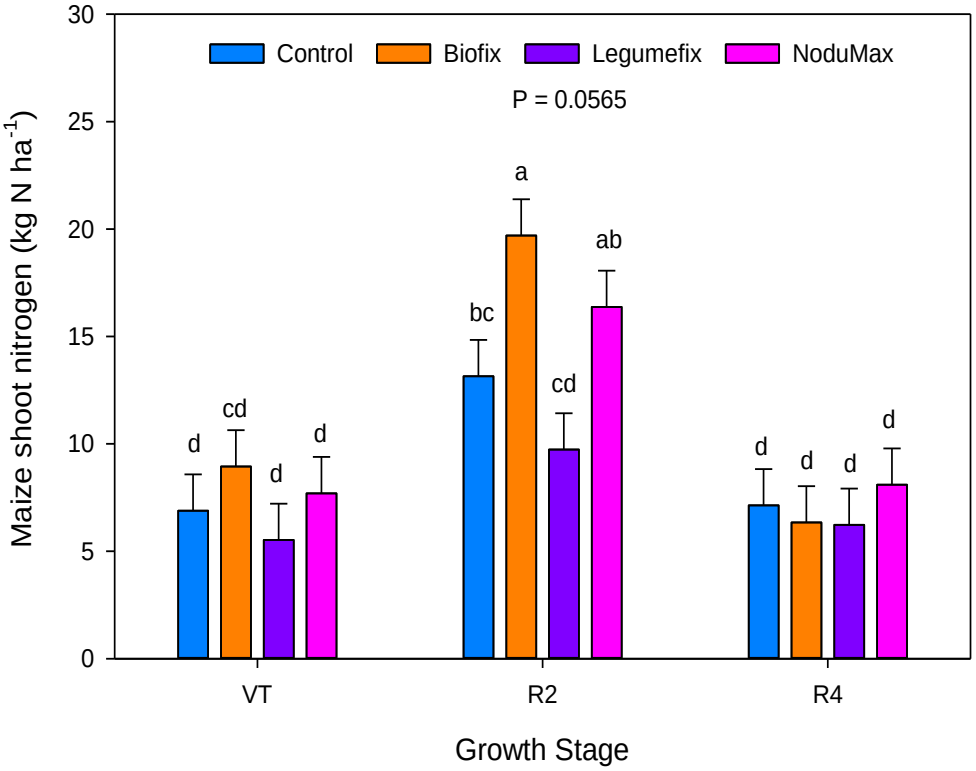

Figure 5.11. Maize shoot nitrogen affected by the interaction of growth stage and previous commercial Bradyrhizobium inoculant on in Soybean-Maize rotation systems in Nyankpala, Ghana, 2017. Different letters indicate significant differences at p < 0.1. Error bar is a standard error (SE).

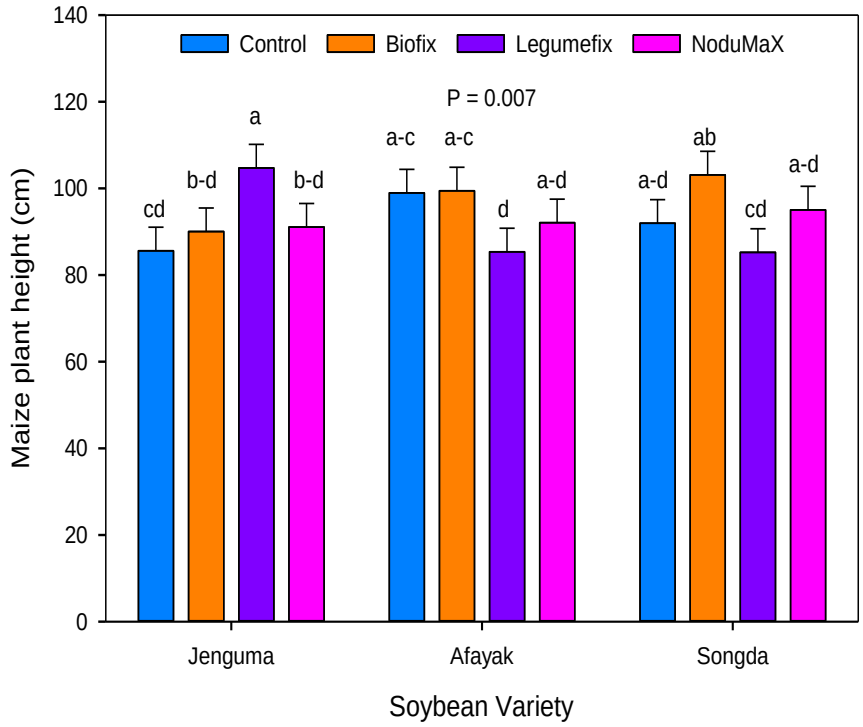

Figure 5.12. Maize plant height affected by previous soybean and commercial Bradyrhizobium inoculant in Soybean-Maize rotation systems in Nyankpala, Ghana, 2017. Different letters indicate significant differences at $p < 0.05$. Error bar is a standard error (SE).

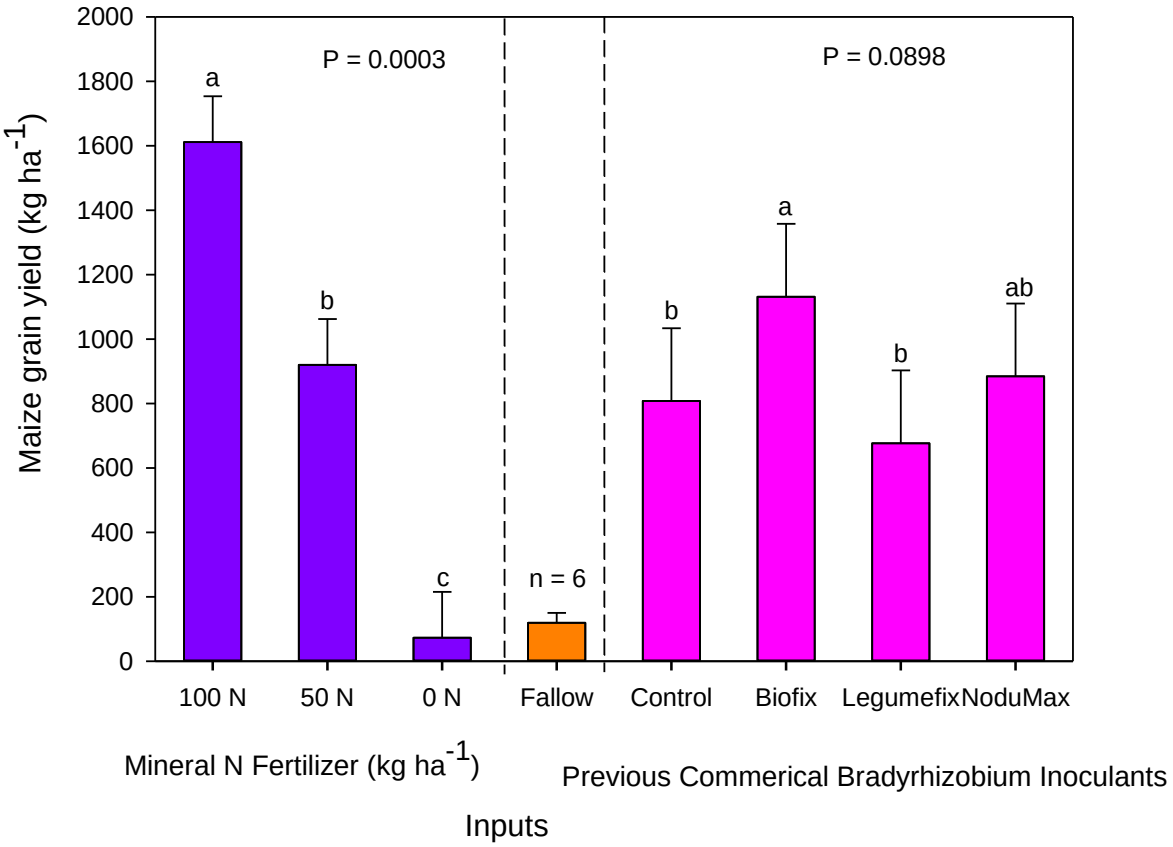

Figure 5.13. Maize grain yield affected by mineral N fertilizer and previous commercial Bradyrhizobium inoculant in Soybean-Maize rotation systems in Nyankpala, Ghana, 2017. Different letters indicate significant differences at $p < 0.05$ and $p < 0.1$. Error bar is a standard error (SE).

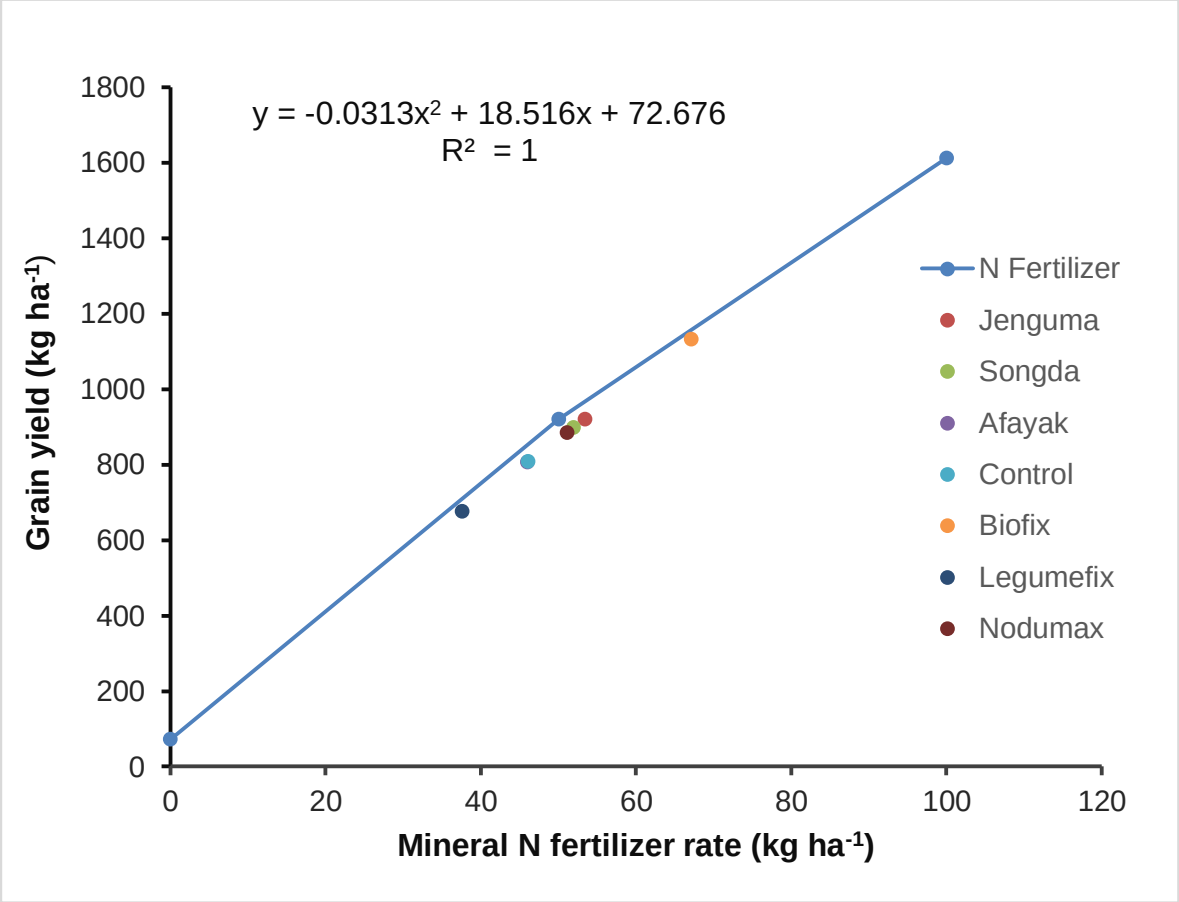

Figure 5.144. Relationship between maize grain yield from mineral N fertilizer and previous commercial Bradyrhizobium inoculant in Soybean-Maize rotation systems in Nyankpala, Ghana, 2017.

Table 5.1. Nodulation affected by previous soybean variety and commercial Bradyrhizobium inoculants at a different growth stage in Nyankapala, Ghana in 2017.

Treatment	Nodule number	Nodule dry wt.	Specific nodule dry wt.
	plant^{-1}	(mg plant^{-1})	(mg nodules $^{-1}$)
Variety			
Jenguma	18 b	106 b	6.46 b
Afayak	23 a	145 a	6.72 b
Songda	19 b	150 a	8.24 a
Inoculant			
Control	20 ab	131 b	6.97
Biofix	19 b	119 b	6.68
Legumefix	23 a	154 a	7.38
NoduMax	18 b	132 b	7.54
Stage			
VS	18 b	106 c	6.12 c
R2	26 a	168 a	6.87 b
R4	16 b	128 b	8.43 a

Effects	Pr. > F		
Variety	0.031	0.001	0.013
Inoculant	0.043	0.002	0.320
Stage	<.0001	<.0001	<.0001
Variety x Inoculant	0.481	0.531	0.161
Variety x Stage	0.621	0.186	0.807
Inoculant x Stage	0.285	0.361	0.959
Var x Inoc x Stage	0.117	0.782	0.358

Values within a column followed by the same (letter) are not significantly different at p < 0.05. 20 plants mean nodules
VS = vegetative stage, R2 = full flower and R4 = full pod

Table 5.2. Maize grain yield, stover dry matter, total dry matter (total biomass), harvest index and their total nitrogen contents affected by previous soybean variety and commercial Bradyrhizobium inoculant in Nyankapala, Ghana in 2017.

Treatment	Grain yield	Stover yield	Total yield	Harvest index	Grain N	Stover total N	Total biomass N	N harvest index
	dry matter (kg ha^{-1})				dry matter N content (kg N ha^{-1})			
Variety								
Jenguma	921	1061	1982	0.45	14.3	7.42	21.8	0.62
Afayak	807	967	1774	0.42	13.7	6.31	18.5	0.60
Songda	899	902	1800	0.47	12.2	6.04	19.7	0.65
Inoculants								
Control	808 ^{b}	980	1788	0.42 ^{bc}	11.3 ^{b}	6.57	17.3	0.59 ^{bc}
Biofix	1132 ^{a}	1043	2175	0.49 ^{a}	18.4 ^{a}	7.3	25.7	0.68 ^{a}
Legumefix	677 ^{b}	930	1607	0.39 ^{c}	10.0 ^{b}	6.13	16.2	0.55 ^{c}
NoduMax	885 ^{ab}	952	1837	0.47 ^{ab}	13.9 ^{ab}	6.37	20.3	0.66 ^{ab}
N-fertilizer (kg N ha^{-1})								
0	45 ^{c}	283 ^{b}	329 ^{c}	0.15 ^{c}	0.6 ^{c}	2.4 ^{c}	3.0 ^{c}	0.20 ^{c}
50	1115 ^{b}	1750 ^{a}	2865 ^{b}	0.39 ^{b}	17.6 ^{b}	14.2 ^{b}	31.8 ^{b}	0.55 ^{b}
100	1933 ^{a}	1958 ^{a}	3891 ^{a}	0.50 ^{a}	31.5 ^{a}	16.9 ^{a}	48.4 ^{a}	0.65 ^{a}
Effect				Pr. > F				
Variety	0.7312	0.661	0.7357	0.3388	0.7594	0.5088	0.6797	0.9015
Inoculant	0.0898*	0.8793	0.2715	0.0679*	0.0957*	0.5261	0.1603	0.0263
Variety*Inoculant	0.9094	0.6207	0.7369	0.7468	0.8954	0.2455	0.8937	0.6877
N-fertilizer	0.0001	0.0001	0.0001	<.0001	0.0001	0.0002	0.0006	<.0001

Values within a column followed by the same (letter) are not significantly different at p < 0.05 and p < 0.1. NS = Not significantly different.*

Sub-sample 5 plants was taken, multiply by the plant establishment per area (plot) and later extrapolated into ha basis.

Chapter 6 - General Conclusion

Promiscuous nodulating soybeans cultivars (Tropical *Glycine max crosses, TGX*) are seldom inoculated with commercial *Bradyrhizobium* inoculants as they nodulate with the native rhizobium. In the present study, we assessed commercial *Bradyrhizobium* inoculants impacts on promiscuous nodulating soybean varieties with regards to (1) plant growth, symbiotic performance, nitrogen fixation, and grain yield, (2) soil microbial community structure and soil chemical properties, and (3) we also evaluated the impacts of the previous season commercial *Bradyrhizobium* inoculants on the subsequent crops.

Inoculating promiscuous nodulating soybean varieties with commercial inoculants enhanced shoot dry matter, nodulation (nodule number and nodule mass), grain yield, grain protein, total N fixation, nitrogen uptake, and residual N balance. Net returns on grain yield due to inoculation with commercial Bradyrhizobium inoculants ranged between 20-25%. Our results suggest that commercial inoculants formulated with *Bradyrhizobium japonicum* strain USDA110 (especially NoduMax) consistently outperformed those with *Bradyrhizobium japonicum* strain USDA 532c (especially Legumefix). Thus in the tropical Guinea Savanna zone of West Africa, commercial inoculants with *Bradyrhizobium japonicum* strain USDA110 seem to be the best candidate.

Regarding soybean cultivar, Afayak, one of the improved soybean lines also outperformed Songda and Jenguma. Thus Afayak could be a potential candidate for inoculation with commercial inoculants. Inoculation may be an insurance against low yield and reduced N-fixation due to poor symbiotic efficiency by the native rhizobia. Additionally, the exportation of haulm and grain yield at harvest contributed to a significant nutrient loss. Negative N balance was observed for some of the commercial inoculants (especially with Biofix and Legumefix)

when whole plants (haulms + grain) were exported in 2016. The negative N balance signifies that soil N uptake surpassed N-fixation. Therefore, for the succeeding crop to benefit from residual N balance from the previous legume (soybean) crop, residues need to be retained.

The soil microbial community structure and soil chemical property were also altered by commercial inoculants and soybean varietal selection. Both Biofix and Legumefix inoculants improved rhizosphere PLFA-microbial biomass, an active nutrient pool. Afayak also produced greater rhizosphere PLFA-microbial biomass due to increase exudation. The total PLFA profile revealed that gram-negative bacteria and arbuscular mycorrhizal fungi (AMF) abundance in the rhizosphere were also affected by the interaction of soybean variety by commercial rhizobium inoculant and growth stage. Further, commercial inoculants improved selected soil quality chemical indicators. That is, commercial inoculants increased the availability of NH_4^+-N and phosphorus. Rhizosphere phosphorus increased with growth stage progression due to root exudation and favorable soil pH (less acidic pH). The current study revealed that commercial Bradyrhizobium inoculants and soybean varietal selection would play a crucial role in improving the soil microbiome and soil health.

In assessing the previous commercial inoculants impacts on the subsequent soybean crop, results revealed that previous Legumefix inoculant induced greater nodulation while nodulation declined on previous Biofix and NoduMax inoculants. The difference in nodulation could be attributed to the persistence of *Bradyrhizobium japonicum strain* used as inoculum in the two commercial inoculants. Afayak still maintained superiority nodulating efficiency. Further, previous Biofix and uninoculated control produced greater soybean grain yield. The increased soybean grain yield by previous Biofix may be due to enhanced persistence by the introduced *Bradyrhizobium japonicum strain* while that of the previous uninoculated control may be

attribute to enhanced symbiotic efficiency of the native soil *Rhizobium* from the previous soybean crop. In general, grain yield from previous commercial inoculants fields was lower than grain yields from field annually inoculated with commercial inoculants. Therefore, yearly inoculation of soybean with commercial inoculant is necessary to sustain higher grain yield and N-fixation.

When maize was rotated on previous soybean fields inoculated with commercial inoculants, previous Biofix produced greater shoot dry matter and grain yield. Maize grain yield was generally low due to insufficient N supply. Nonetheless, maize grain yield from the rotation phase was comparable to maize grain yield from the half recommended independent mineral N fertilizer (50 kg N ha^{-1}) rate. Thus the adoption of soybean-maize rotation system could significantly reduce (50%) the amount of mineral N fertilizer required by the subsequent maize crop. It is apparent that commercial inoculants and soybean varietal selection are crucial to enhancing soybean productivity and sustaining soil quality.

Finally, we recommend that future research should focus on co-inoculation of soybean with Rhizobium and arbuscular mycorrhizal fungi inoculants, and how they affect symbiotic plant performance, soil microbiomes, and soil health. The same research could be extended to other legumes such as cowpea (*Vigna ungui*culata), groundnut (*Arachis hypoga*ea), Bambara groundnut (*Vigna subterranean*), and pigeon pea (*Cajanus cajan*) which are currently not inoculated with commercial inoculants. There is also the need to continue the search for elite native Rhizobium strains which could be used as potential inoculum for commercial inoculants.

Appendix A - List of Figures and Tables for Chapter 2

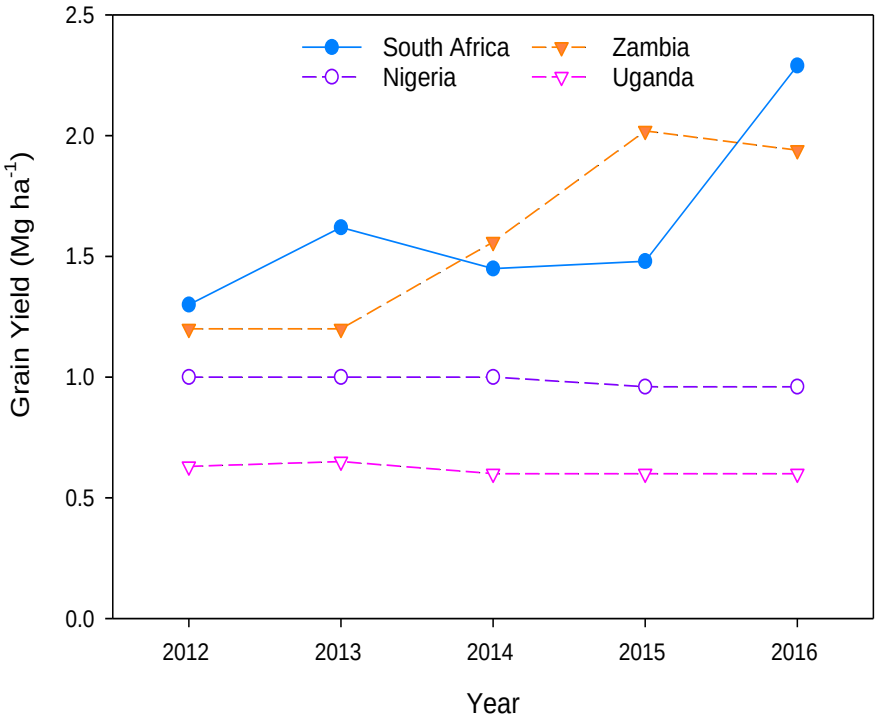

Figure A.1. Average soybean grain yield from the top five countries in SSA from 2012 to 2016 (Graphed using data from (Khojely et al., 2018).

Appendix B - List of Figures and Tables for Chapter 3

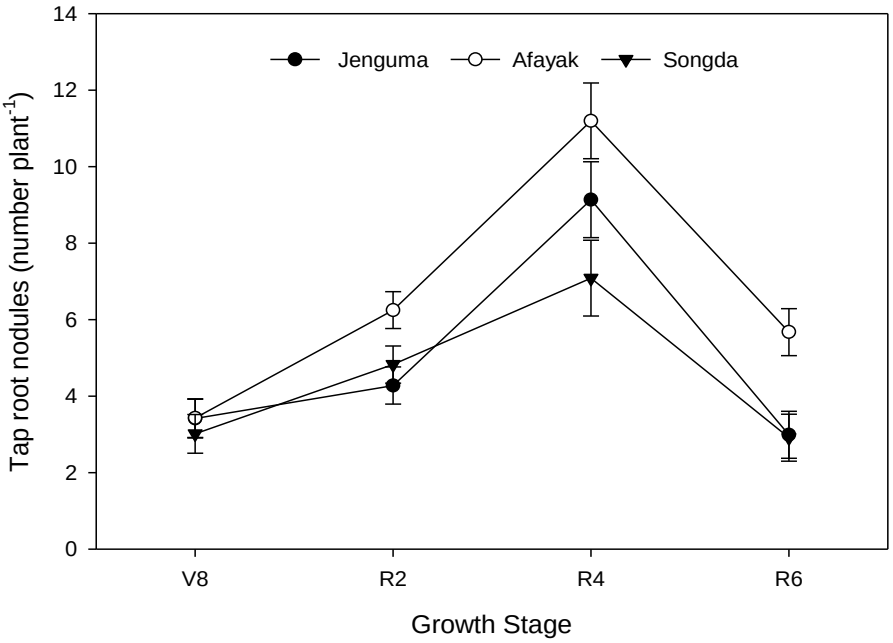

Figure B.1. Interaction effect of growth stage and soybean variety on the number of nodules on tap roots in 2016 in Nyankpala, Ghana. Mean value ± standard error of four replicates

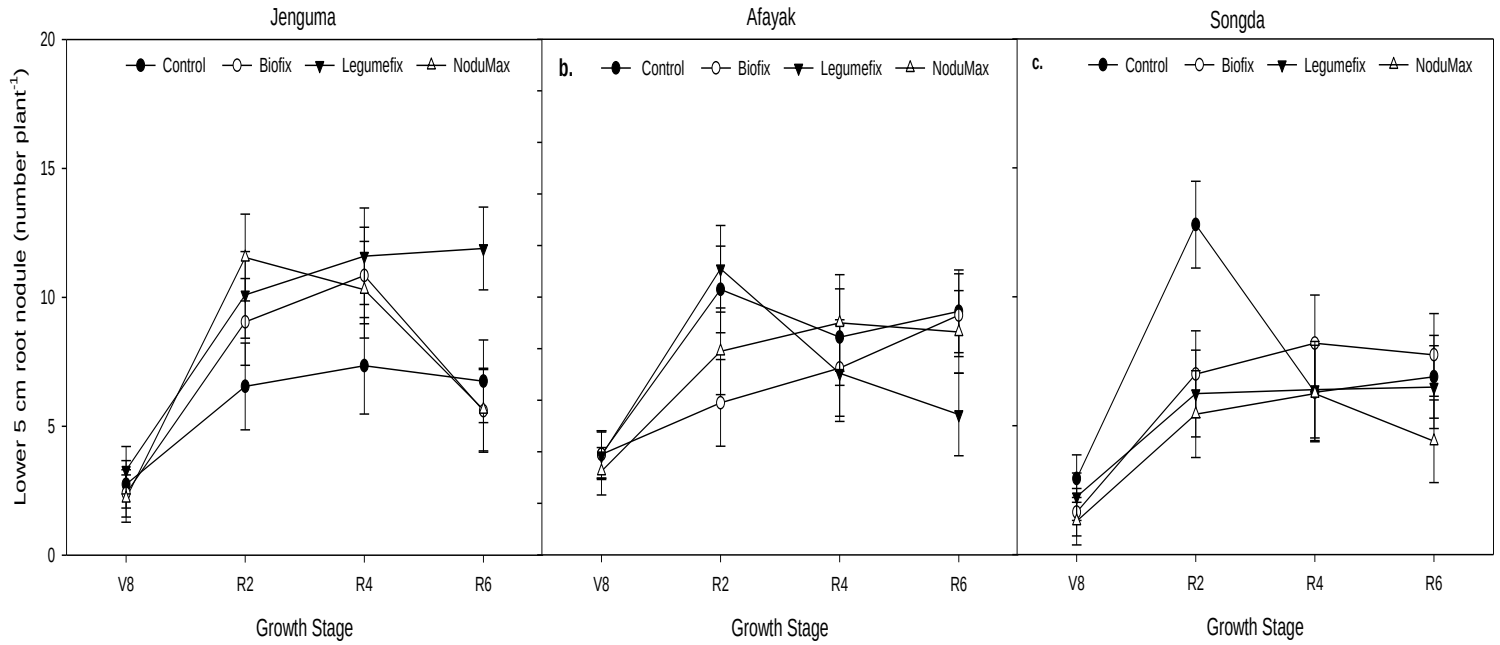

Figure B.2. I**nteraction effect of soybean variety, commercial Bradyrhizobium Inoculants and growth stage on number of nodules on Lower 5 cm root in 2016 in Nyankpala, Ghana. Mean value ± standard error of four replicates**

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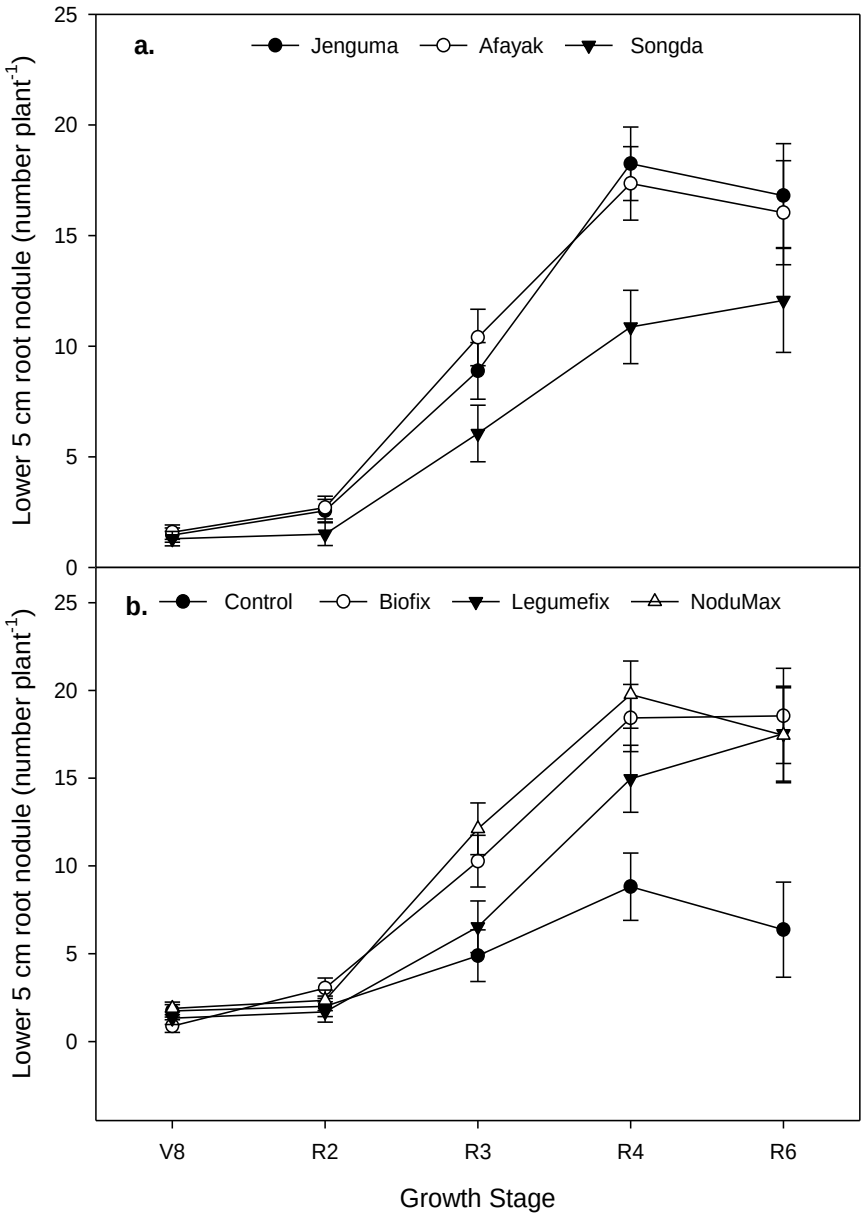


Figure B.3. Interaction effect of growth stage and soybean variety on (a) number of nodules on lower 5 cm root segment (fig. a) and Interaction effect of growth stage and commercial Bradyrhizobium inoculant on the number of nodules on lower 5 cm root segment (fig. b) in 2017 in Nyankpala, Ghana. Mean value ± standard error of four replicates

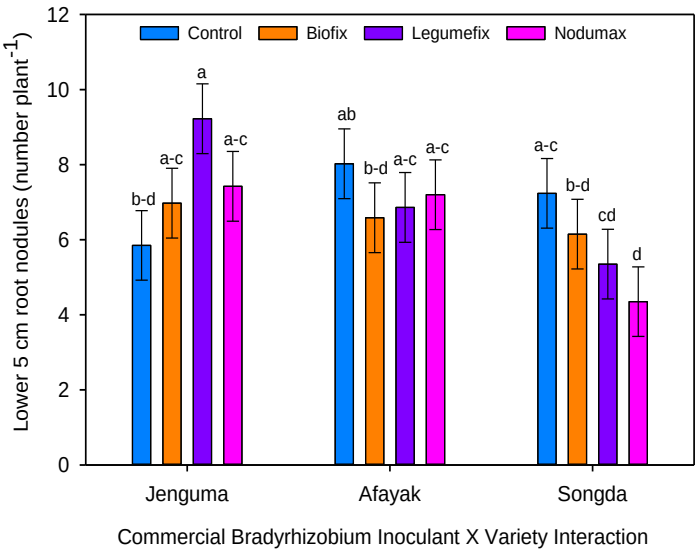


Figure B.4. Interaction effect of soybean variety and commercial Bradyrhizobium inoculants on number of nodules on lower 5 cm root in 2017 in Nyankpala, Ghana. Lower case letters indicate significant differences at $p < 0.05$. Error bar is a standard error (SE).

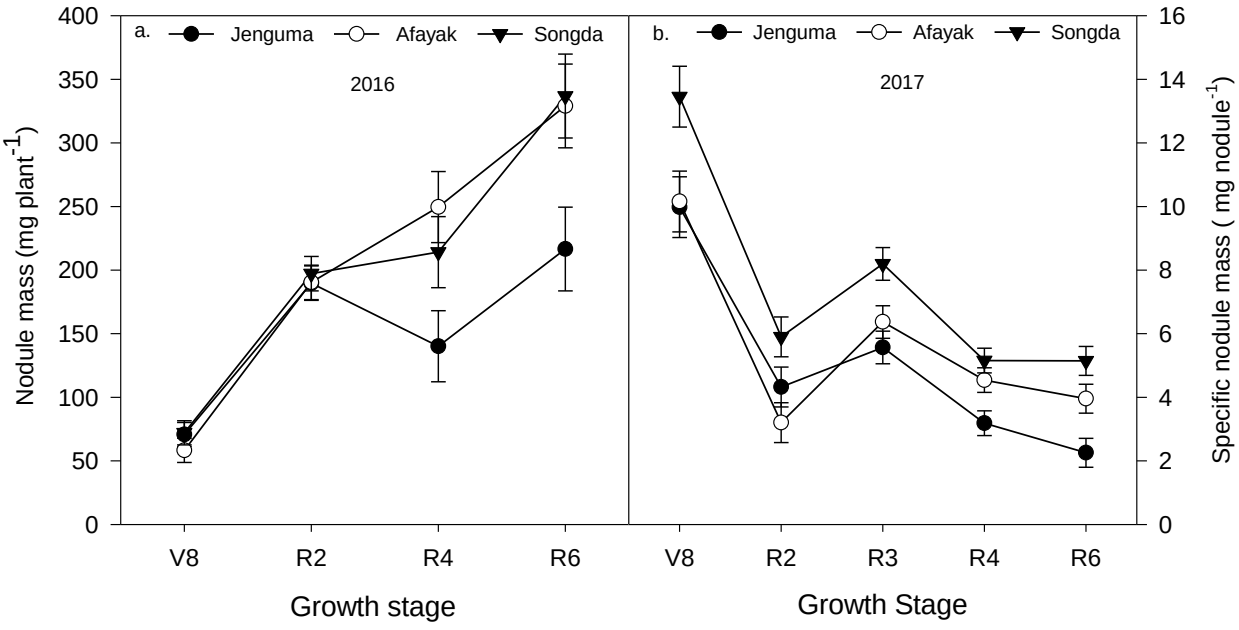


Figure B.5. Interaction effect of growth stage and soybean variety on (a) nodule mass in 2016 (fig. a), and (b) specific nodule mass in 2017 (fig. b) in Nyankpala, Ghana. Mean value ± standard error of four replicates

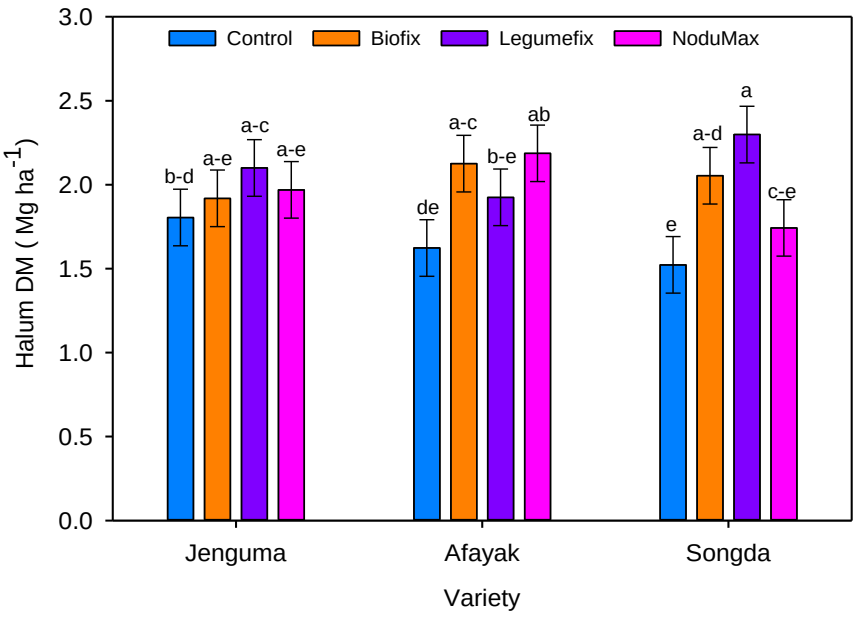


Figure B.6. Haulm dry matter affected by interaction effect of commercial Bradyrhizobium inoculants and soybean variety in 2016 in Nyankpala, Ghana. Lower case letters indicate significant differences at $p < 0.05$. Error bar is a standard error (SE).

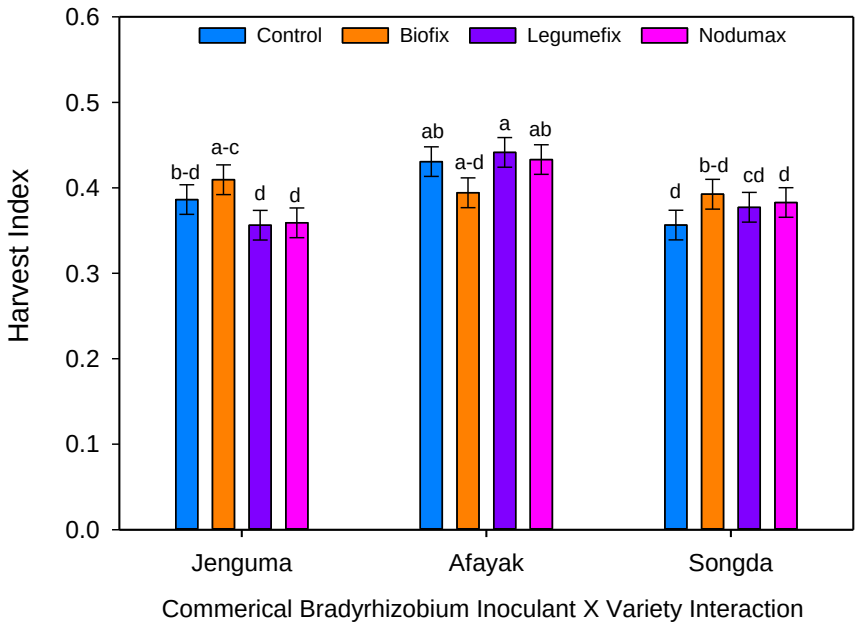


Figure B.7. Harvest index affected by the interaction of of commercial inoculants and soybean variety in 2017 in Nyankpala, Ghana. Lower case letters indicate significant differences at p< 0.05. Error bar is a standard error (SE).

Table B.1. Main effects of soybean variety, commercial Rhizobium inoculant, and growth stage on specific nodule weight, upper 5cm root and Lower 5 cm root segment in 2016 and 2017 in Nyankpala, Ghana.

Main Effects	Upper 5 cm root		Lower 5 cm root		Whole root		Specific nodule wt.	
	Nodule number plant^{-1}						mg nodule^{-1}	
	2016	2017	2016	2017	2016	2017	2016	2017
Variety								
Jenguma	17.8 ^{ab}	19.1 ^{b}	7.4 ^{a}	9.6 ^{a}	25.1 ^{a}	28.4 ^{a}	7.3	5.1 ^{b}
Afayak	19.6 ^{a}	22.2 ^{a}	7.2 ^{a}	9.6 ^{a}	26.8 ^{a}	31.8 ^{a}	7.4	5.7 ^{b}
Songda	16.3 ^{b}	14.8 ^{c}	5.8 ^{b}	6.4 ^{b}	22.1 ^{b}	21.1 ^{b}	8.3	7.6 ^{a}
							NS	
Inoculant								
Control	12.5 ^{b}	9.6 ^{c}	7	4.8 ^{c}	19.6 ^{b}	14.4 ^{c}	9.0 ^{a}	7.0 ^{a}
Biofix	19.5 ^{a}	21.5 ^{a}	6.6	10.2 ^{a}	26.1 ^{a}	31.7 ^{a}	7.1 ^{b}	5.9 ^{b}
Legumefix	20.4 ^{a}	17.7 ^{b}	7.1	8.4 ^{b}	27.5 ^{a}	26.1 ^{b}	7.1 ^{b}	5.7 ^{b}
Nodumax	19.1 ^{a}	26.0 ^{a}	6.3	10.7 ^{a}	25.4 ^{a}	36.3 ^{a}	7.4 ^{b}	5.7 ^{b}
			NS					
Growth Stage								
V8	8.4 ^{c}	9.9 ^{c}	2.8 ^{c}	1.5 ^{a}	11.2 ^{c}	11.3 ^{e}	6.5 ^{b}	11.2 ^{a}
R2	16.7 ^{b}	17.8 ^{b}	8.7 ^{a}	2.3 ^{c}	25.3 ^{b}	19.5 ^{d}	7.4 ^{b}	4.5 ^{c}
R3	.	21.2 ^{a}	.	8.5 ^{b}	.	29.6 ^{c}	.	6.7 ^{b}
R4	30.2 ^{a}	25.1 ^{a}	8.3 ^{ab}	15.5 ^{a}	38.5 ^{a}	40.6 ^{a}	7.3 ^{b}	4.3 ^{c}
R6	16.3 ^{a}	19.5 ^{b}	7.4 ^{b}	15.0 ^{a}	23.7 ^{b}	34.5 ^{b}	9.3 ^{a}	3.8 ^{c}
Year	Pr. > F (P-value)							
Variety	0.009	<.0001	0.027	0.001	0.002	<.0001	0.235	0.0003
Inoculant	<.0001	<.0001	0.622	<.0001	<.0001	<.0001	0.047	0.004
Variety*Inoculant	0.226	0.693	0.061	0.009	0.109	0.253	0.923	0.383
Stage	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001	0.0004	<.0001
Variety*Stage	0.393	0.25	0.368	0.039	0.437	0.038	0.569	0.071
Inoculant*Stage	0.064*	0.018	0.742	<.0001	0.088*	<.0001	0.771	0.936
Variet*Inocula*Stage	0.323	0.838	0.058*	0.415	0.195	0.758	0.722	0.487

Values within a column followed by the same alphabet (letter) are not significantly different at $p < 0.05$ and $p < 0.1$.*

10 plants mean nodules

Table B.2. Main effects of soybean variety and commercial Bradyrhizobium inoculant on pod load per plant and pod yield and plant height in 2016 and 2017 in Nyankpala, Ghana.

Year	Pod load plant^{-1}		Pod yield (Mg ha^{-1})		Plant height (cm)
Variety	2016	2017	2016	2017	2017
Jenguma	57 ^{ab}	46 ^{a}	4.8 ^{b}	5.4 ^{a}	51.9 ^{b}
Afayak	59 ^{a}	38 ^{ab}	6.1^{a}	4.7 ^{ab}	58.0 ^{a}
Songda	50 ^{b}	30 ^{b}	3.8 ^{c}	3.5 ^{b}	47.3 ^{b}
Inoculant					
Control	52	38	4.4	4.2	49.4 ^{c}
Biofix	58	36	5.3	4.8	54.7 ^{ab}
Legumefix	55	34	4.8	4.5	49.9 ^{bc}
NoduMax	57	44	5.1	4.6	55.5 ^{a}
LSD	NS	NS	NS	NS	1.9
			Pr. > F (P-value)		
Year	2016	2017	2016	2017	2017
Effect	Pod load plant^{-1}		Pod yield (Mg ha^{-1})		Plant height (cm)
Variety	0.031	0.016	0.000	0.045	0.012
Inoculant	0.111	0.135	0.102	0.743	0.044
Variety*Inoculant	0.514	0.274	0.189	0.434	0.629

Values within a column followed by the same (letter) are not significantly different t at p < 0.05. NS = Not significantly different

Table B.3. Main effects of soybean variety and commercial Bradyrhizobium inoculant on Haulm dry matter (DM), harvest index, A 1000 seed weight, Seed nitrogen (N) content and Grain protein content in 2016 and 2017 in Nyankpala, Ghana.

	Haulm DM (Mg ha^{-1})		Harvest Index (g g^{-1})		A1000 seed wt. (g)		Seed Nitrogen (gg^{-1})		Grain protein (Mg ha-1)	
Variety	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**	**2016**	**2017**
Jenguma	1.9	2.3	0.60 ^{b}	0.38 ^{b}	74 ^{b}	108 ^{b}	58.5	30.5	1.10 ^{a}	0.51
Afayak	2.0	2.2	0.66 ^{a}	0.42 ^{a}	106 ^{a}	114 ^{a}	57.7	29.9	1.40 ^{a}	0.52
Songda	1.9	2.0	0.57 ^{b}	0.38 ^{b}	80 ^{b}	107 ^{b}	58.3	30.6	0.93 ^{b}	0.47
LSD	NS	NS					NS	NS		
Inoculant										
Control	1.7	2.0	0.61	0.39	81	110	57.2	30.2	0.96^{b}	0.42^{b}
Biofix	2.0	2.3	0.62	0.40	91	109	59.1	30.0	1.23 ^{a}	0.51^{ab}
Legumefix	2.1	2.1	0.59	0.39	87	109	57.6	29.9	1.13 ^{ab}	0.47^{b}
NoduMax	2.0	2.3	0.62	0.39	88	109	58.9	31.2	1.21 ^{a}	0.60^{a}
LSD	NS	NS	NS	NS	NS	NS	NS	NS		
Effect					Pr. > F (P-value)					
Variety	0.773	0.481	0.001	0.001	0.001	0.047	0.931	0.550	0.003	0.611
Inoculant	0.106	0.560	0.485	0.930	0.375	0.988	0.676	0.411	0.051	0.005
Variety*Inoculant	0.050	0.422	0.863	0.056*	0.560	0.414	0.524	0.495	0.943	0.104

Values within a column followed by the same alphabet (letter) are not significantly different at $p < 0.05$ and $p < 0.1$. NS = Not significantly different*

Appendix C - List of Figures and Tables for Chapter 4

Commercial inoculants impact on soil biological and chemical properties in 2016 and 2017

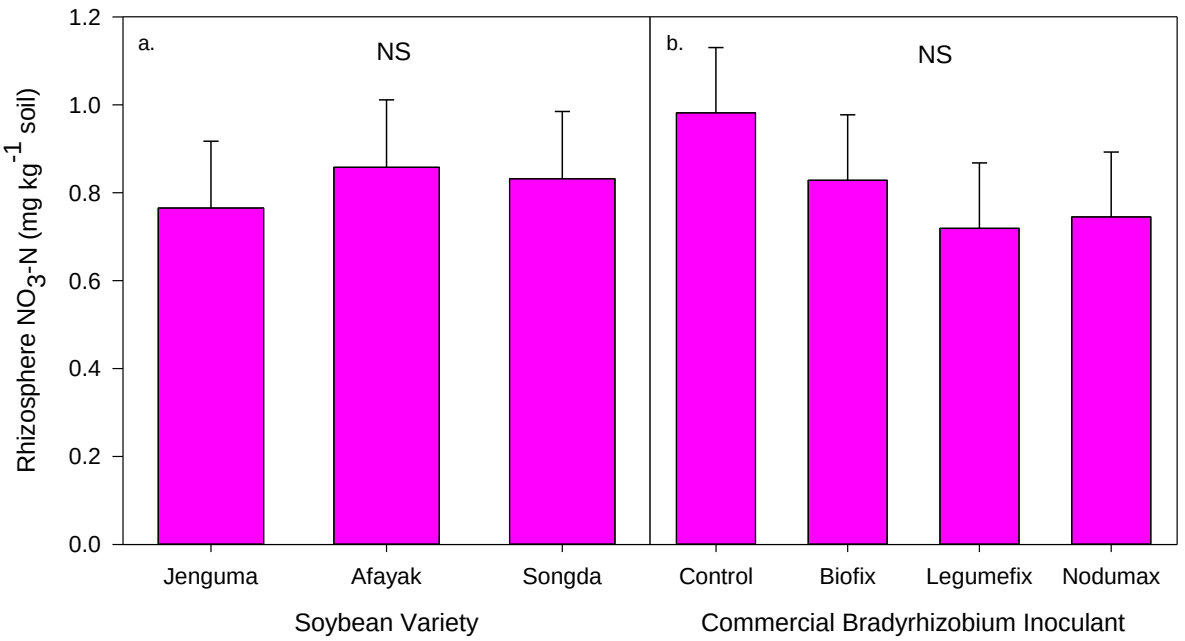


Figure C.1. Rhizosphere nitrate (NO_3-N) affected by main treatment effects of (a) soybean variety and (b) commercial rhizobium Inoculant in 2017 in Nyankpala, Ghana.

Error bar is a standard error (SE). NS = Not significantly different at $p < 0.1$.

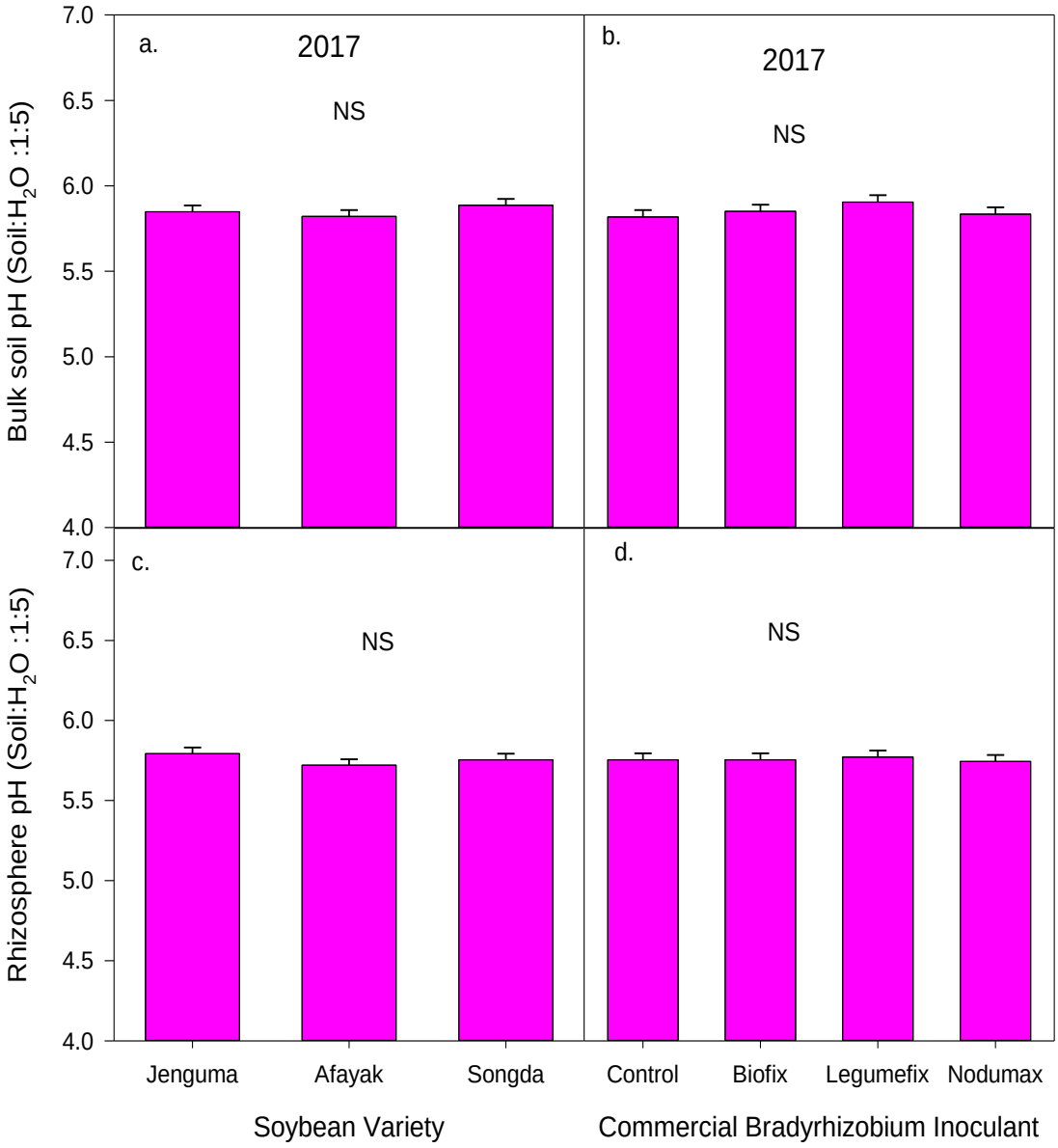


Figure C.2. pH of bulk soil (a & b) and rhizosphere (c & d) affected by main treatment effects of soybean variety and commercial rhizobium inoculant in 2017 in Nyankpala, Ghana.

Error bar is a standard error (SE). NS = Not significantly different at $p < 0.1$.

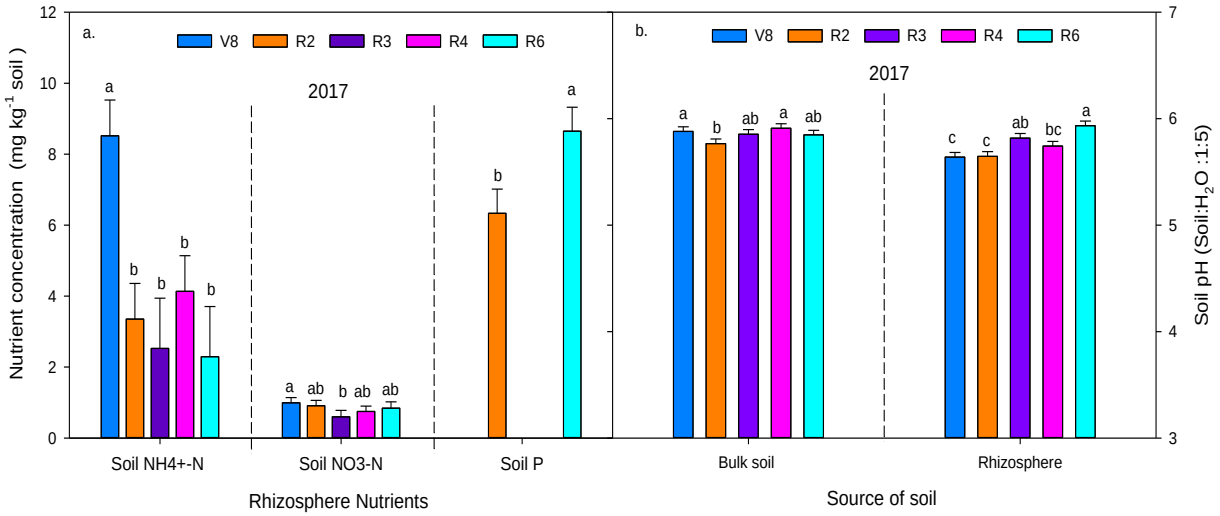


Figure C.3. Growth stage effect on (a) rhizosphere nutrients (NH_4^+-N, NO_3-N, and Phosphorus concentration) and (b) soil pH of bulk soil and rhizosphere in 2017 in Nyankpala, Ghana. Different letters indicate significant differences at $p < 0.1$. Error bar is a standard error (SE).

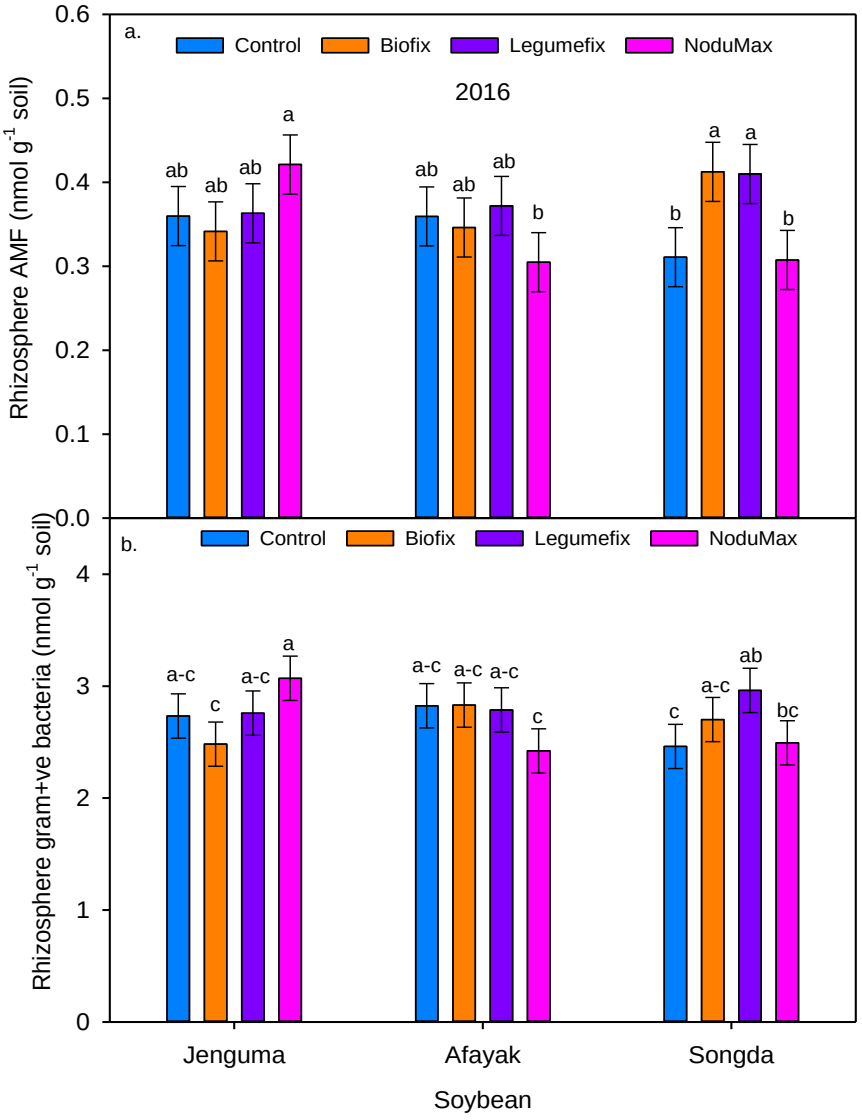


Figure C.4. Rhizosphere arbuscular mycorrhizal fungi (a) and Rhizosphere gram-positive bacteria (b) affected by the interaction of soybean variety and commercial rhizobium Inoculant in 2016 in Nyankpala, Ghana. Different letters indicate significant differences at $p < 0.1$. Error bar is a standard error (SE).

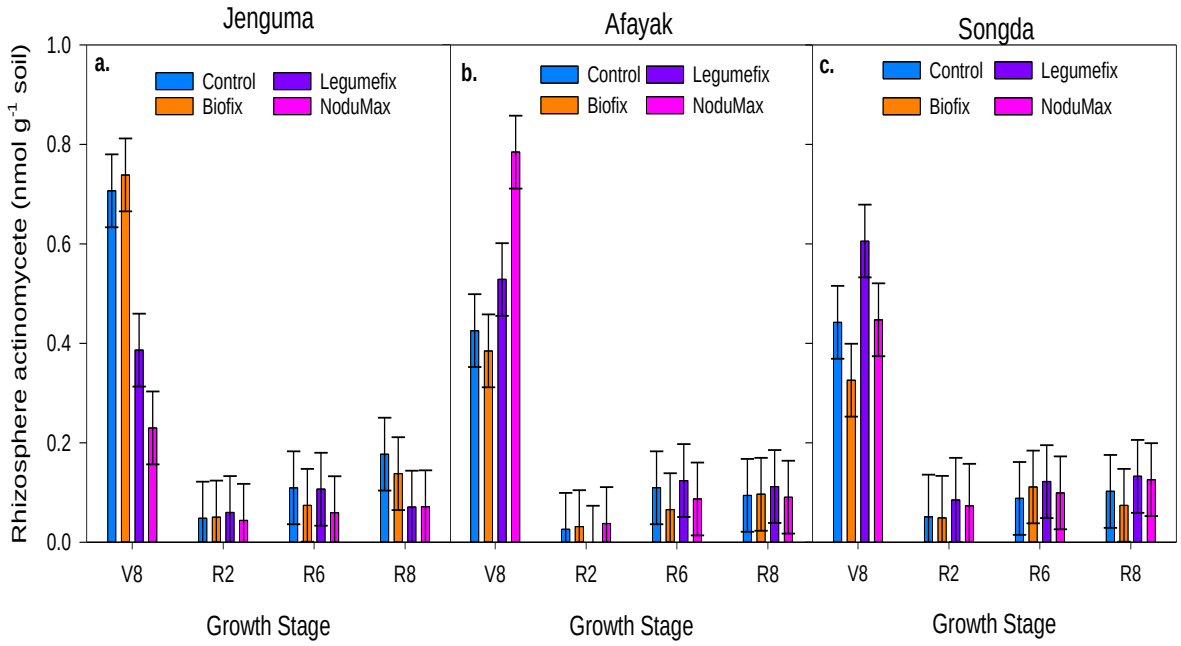


Figure C.5. Rhizosphere actinomycete (a, b &c) affected by the interaction of soybean variety, commercial rhizobium Inoculant and growth stage in 2016 in Nyankpala, Ghana. Error bar is a standard error (SE).

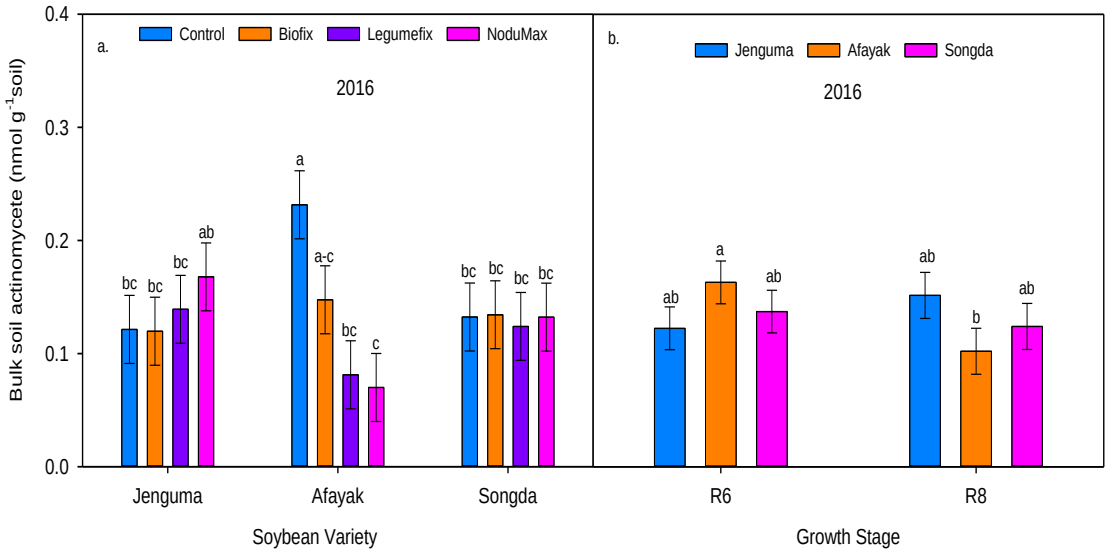


Figure C.6. Bulk soil actinomycete affected by the interaction of (a) commercial rhizobium inoculant and soybean variety (b) soybean variety and growth stage in 2016 in Nyankpala, Ghana. Different letters indicate significant differences at $p < 0.1$. Error bar is a standard error (SE).

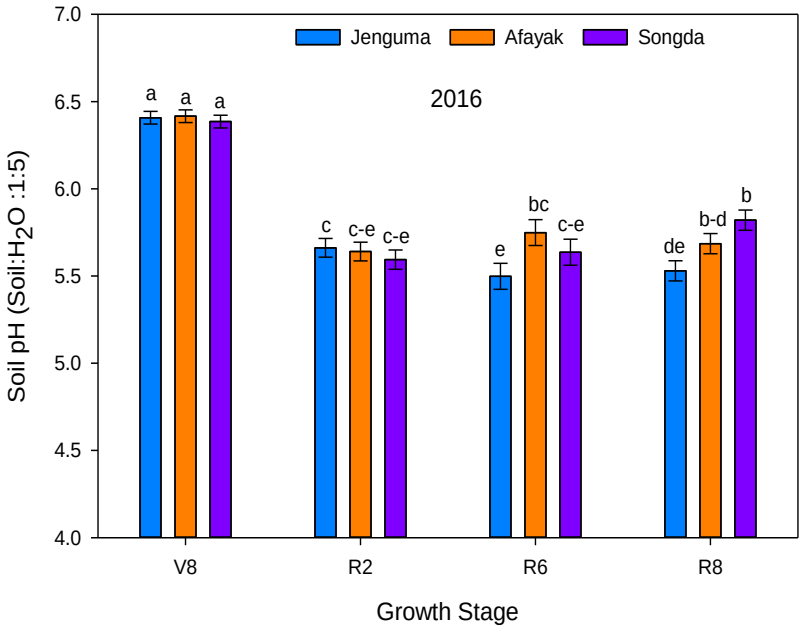


Figure C.7. Rhizosphere pH affected by the interaction of growth stage and soybean variety in 2016 in Nyankpala, Ghana. Different letters indicate significant differences at p < 0.1. Error bar is a standard error (SE).

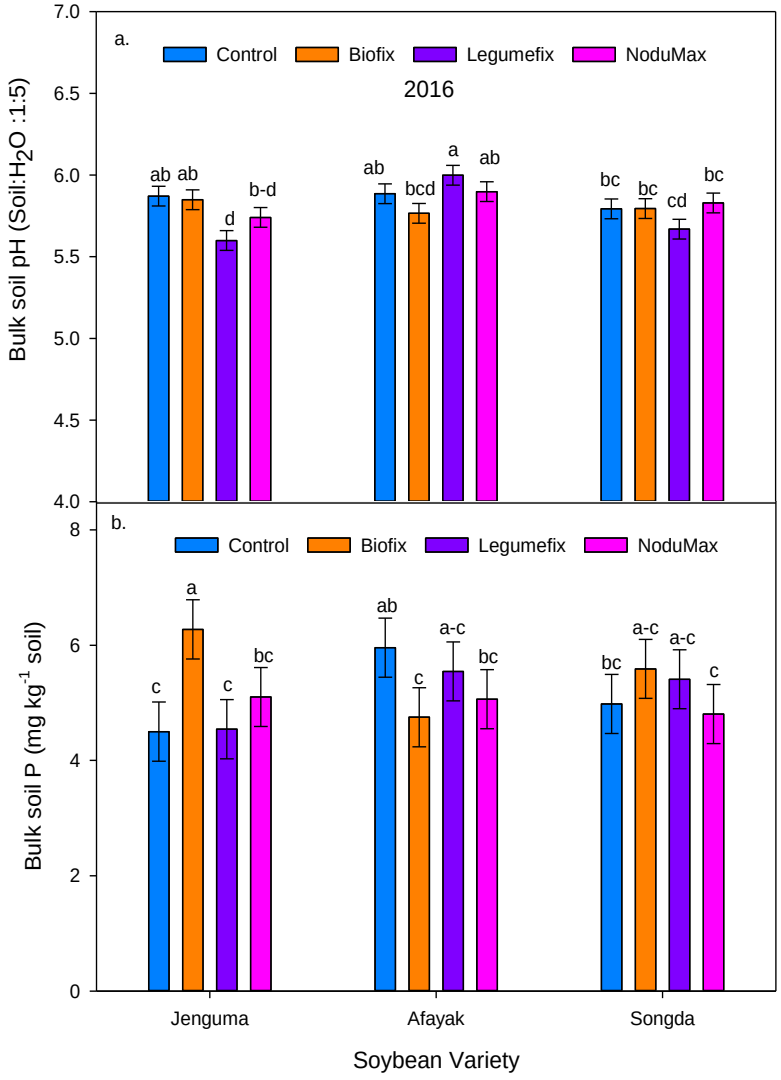


Figure C.8. Interaction effect of commercial rhizobium inoculant and soybean variety on (a) soil pH (b) Soil phosphorus (P) in 2016 in Nyankpala, Ghana. Different letters indicate significant differences at p < 0.1. Error bar is a standard error (SE).

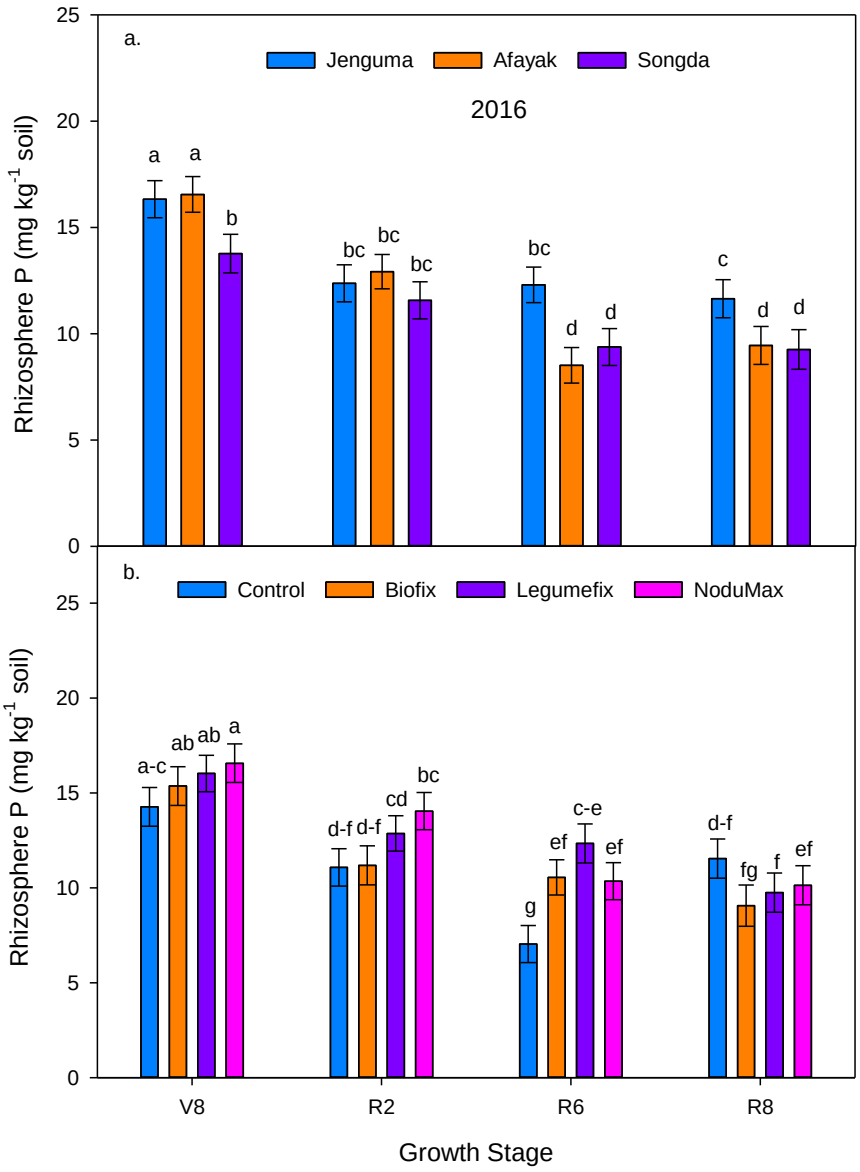


Figure C.9. Rhizosphere phosphorus (P) affected by the interaction of growth stage and (a) soybean variety and (b) commercial rhizobium inoculant in 2016 in Nyankpala, Ghana. Different letters indicate significant differences at p < 0.1. Error bar is a standard error (SE).

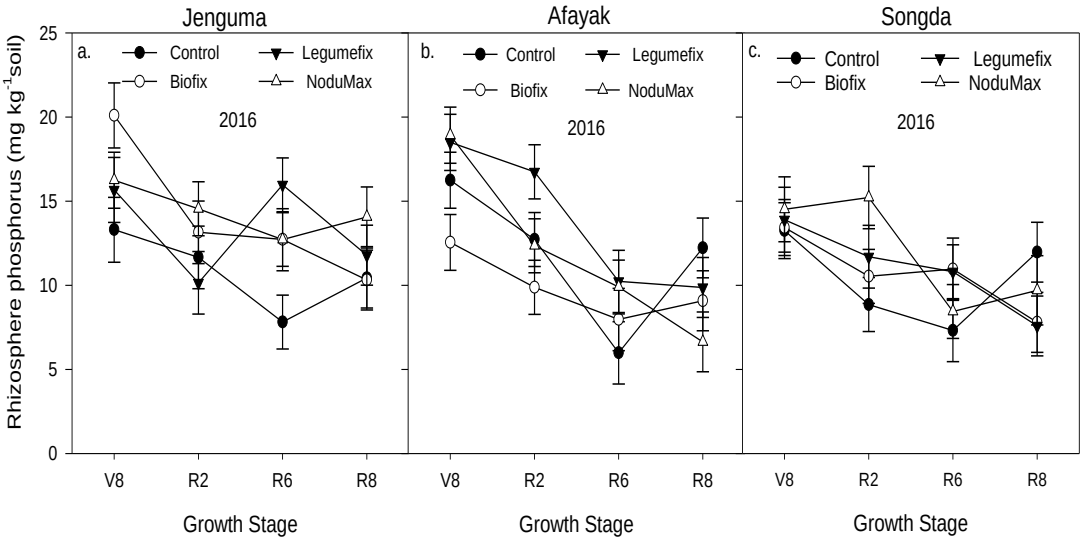


Figure C.10. Rhizosphere phosphorus (P) affected by the interaction of growth stage and (a) soybean variety and (b) commercial rhizobium inoculant in 2016 in Nyankpala, Ghana. Mean value ± standard error of four replicates

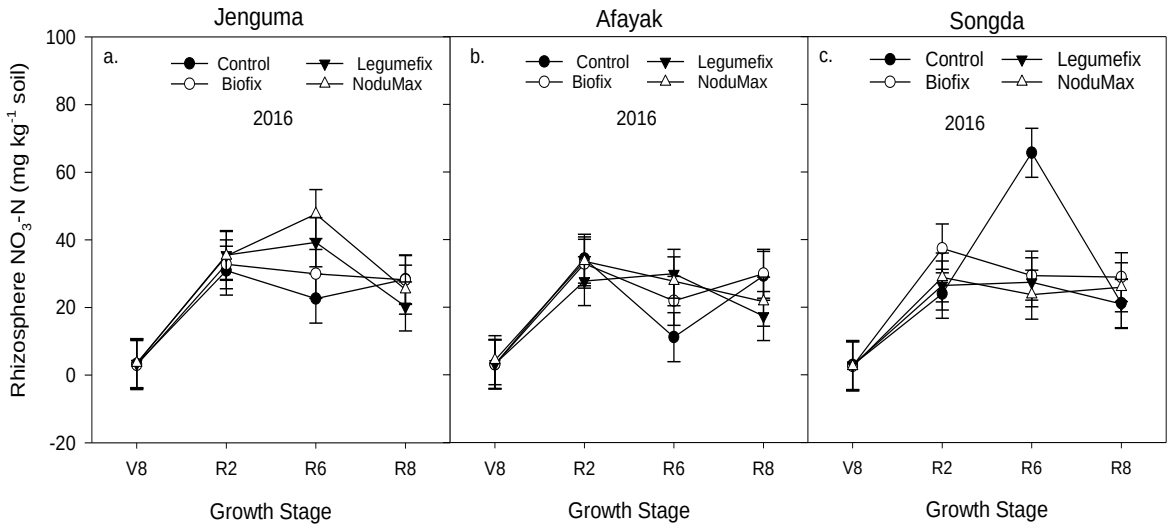


Figure C.11. Rhizosphere NO_3-N affected by the interaction of commercial rhizobium inoculant, soybean variety and growth stage in 2016 in Nyankpala, Ghana. Mean value ± standard error of four replicates

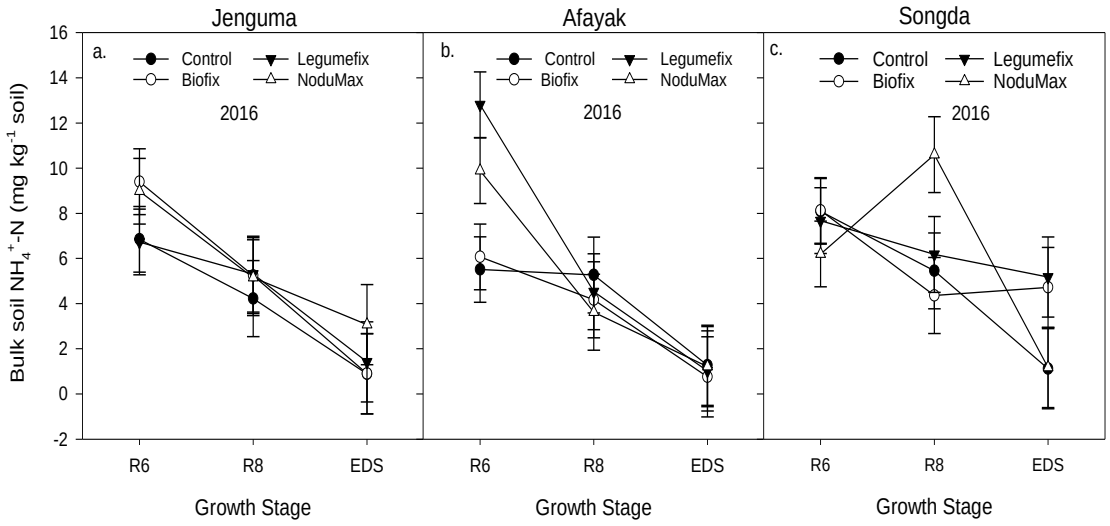


Figure C.12. Bulk soil NH_4^+-N affected by the interaction of commercial rhizobium inoculant, soybean variety, and growth stage in 2016 in Nyankpala, Ghana. Mean value ± standard error of four replicates

Table C.1. The microbial community structure of bulk soil (bulk) and rhizosphere (rhizo) as affected by commercial Bradyrhizobium inoculants, soybean variety and growth stage in 2016 in Nyankpala, Ghana.

Treatment	Bulk	Rhizo	Bulk	Rhizo	Bulk	Rhizo	Bulk	Rhizo	Bulk	Rhizo	Bulk	Rhizo
	Gm-ve bact.		Gm+ve bact.		Sap fungi		AMF		Actinomycete		Microbiomass	
Variety	nmol g^{-1} soil											
Jenguma	0.43	1.08	1.84	2.76	0.43	1.87	0.33	0.37	0.14	0.19	3.9	9.39
Afayak	0.35	1.13	1.42	2.72	0.4	1.79	0.19	0.35	0.13	0.19	3.75	9.42
Songda	0.31	1.09	1.08	2.66	0.31	1.72	0.1	0.36	0.13	0.18	3.46	9.41
Sed	0.07	0.09	0.39	0.14	0.09	0.11	0.13	0.02	0.02	0.02	0.25	0.45
Inoculant												
Control	0.32	1.1	1.26	2.67	0.41	1.76	0.18	0.34	0.16	0.2	3.95	9.18
Biofix	0.29	1.06	1.4	2.67	0.33	1.74	0.15	0.37	0.13	0.18	3.32	9.59
Legumefix	0.49	1.24	1.18	2.84	0.31	1.91	0.11	0.38	0.11	0.19	3.83	9.79
NoduMax	0.35	1.01	1.95	2.66	0.48	1.75	0.39	0.34	0.12	0.18	3.72	9.06
Sed	0.08	0.08	0.44	0.15	0.09	0.1	0.15	0.02	0.02	0.02	0.27	0.44
Stage												
V8		1.99		3.96		3.01		0.72		0.5		14.87
R2		0.54		2.07		1.07		0.17		0.05		5.97
R6	0.37	0.84	1.4	1.94	0.56	1.13	0.15	0.2	0.14	0.1	3.59	7.37
R8	0.35	1.05	1.49	2.87	0.21	1.96	0.26	0.33	0.13	0.11	3.81	9.41
Sed	0.06	0.09	0.32	0.15	0.07	0.12	0.11	0.03	0.01	0.02	0.19	0.43

Gm-ve bact. = Gram negative bacteria, Gm+ve bact. = Gram positive bacteria, Sap fungi = Saprophytic fungi, AMF = Arbuscular mycorrhizae fungi, and Micro. Biomass = Microbial biomass, Bulk = Bulk soil, Rhizo = Rhizosphere

Table C.2. Analysis of variance table (P-value) for soil microbial community structure and soil chemical property in the rhizosphere and bulk soil in 2017.

Effect	Var	Ino	Var*Ino	Stage	Var*Sta	Ino*Sta	Var*Ino*Sta
Microbial Group			**Pr. > F (P-value)-Bulk soil -2017**				
Gram-negative bact.	0.986	0.664	0.451	<.0001	0.759	0.310	0.978
Gram-positive bact.	0.176	0.289	0.073	<.0001	0.982	0.440	0.599
Saprophtytic fungi	0.263	0.882	0.154	<.0001	0.866	0.924	0.549
AMF	0.217	0.492	0.014	<.0001	0.632	0.147	0.773
Actinomycete	0.280	0.340	0.082	<.0001	0.282	0.725	0.468
Microbial biomass	0.109	0.607	0.027	<.0001	0.862	0.310	0.136
Chemical property							
Soil pH	0.227	0.225	0.237	0.055	0.685	0.280	0.340
Microbial Group			**Pr. > F (P-value) -Rhizosphere- 2017**				
Gram-negative bact.	0.176	0.176	0.100	0.000	0.785	0.400	0.019
Gram-positive bact.	0.164	0.179	0.150	<.0001	0.381	0.826	0.527
Saprophtytic fungi	0.083	0.287	0.416	<.0001	0.379	0.530	0.857
AMF	0.010	0.220	0.137	0.079	0.886	0.787	0.088
Actinomycete	0.385	0.015	0.332	<.0001	0.647	0.294	0.639
Microbial biomass	0.087	0.065	0.206	0.083	0.795	0.451	0.276
Chemical property							
Soil NH_4^+-N	0.652	0.025	0.597	<.0001	0.777	0.460	0.369
Soil NO_3-N	0.821	0.131	0.419	0.098	0.585	0.713	0.914
Soil P	0.074	0.018	0.480	0.005	0.129	0.835	0.171
Soil pH	0.299	0.944	0.363	<.0001	0.602	0.951	0.996

Var = Variety, Ino = Commercial rhizobium inoculant and Sta = Stage, AMF = Arbuscular mycorrhizae fungi, Gram-negative bact. = Gram-negative bacteria, Gram-positive bact. = Gram positive bacteria.

Table C.3. Analysis of variance table (P-value) for soil microbial community structure and soil chemical property in the rhizosphere and bulk soil in 2016.

Effect	Var	Ino	Var*Ino	Stage	Var*Sta	Ino*Sta	Var*Ino*Sta
Microbial Group			**Pr. > F (P-value)-Bulk soil -2016**				
Gram-negative bact.	0.502	0.245	0.852	0.781	0.463	0.197	0.917
Gram-positive bact.	0.442	0.579	0.322	0.840	0.165	0.210	0.423
Saprophtytic fungi	0.602	0.554	0.205	0.000	0.165	0.345	0.518
AMF	0.500	0.590	0.492	0.446	0.284	0.394	0.570
Actinomycete	0.956	0.277	0.043	0.282	0.042	0.864	0.653
Microbial biomass	0.409	0.393	0.347	0.398	0.553	0.752	0.998
Chemical property							
Soil NH_4^+-N	0.260	0.293	0.789	<.0001	0.226	0.875	0.046
Soil NO_3-N	0.642	0.491	0.343	<.0001	0.958	0.177	0.708
Soil P	0.764	0.422	0.013	<.0001	0.430	0.779	0.293
Soil pH	0.045	0.479	0.064	0.000	0.885	0.440	0.459
Microbial Group			**Pr. > F (P-value)-Rhizosphere- 2016**				
Gram-negative bact.	0.899	0.182	0.594	<.0001	0.311	0.128	0.944
Gram-positive bact.	0.712	0.499	0.049	<.0001	0.151	0.940	0.641
Saprophtytic fungi	0.634	0.495	0.227	<.0001	0.219	0.736	0.845
AMF	0.681	0.344	0.046	<.0001	0.124	0.740	0.431
Actinomycete	0.942	0.850	0.005	<.0001	0.791	1.000	0.005
Microbial biomass	0.999	0.508	0.284	<.0001	0.194	0.934	0.512
Chemical property							
Soil NH4+-N	0.508	0.980	0.792	<.0001	0.322	0.993	0.896
Soil NO3-N	0.941	0.294	0.483	<.0001	0.843	0.274	0.030
Soil P	0.031	0.028	0.040	<.0001	0.115	0.052	0.099
Soil pH	0.154	0.205	0.374	<.0001	0.002	0.923	0.818

Var = Variety, Ino = Commercial rhizobium inoculant and Sta = Stage. AMF = Arbuscular Mycorrhizae fungi, *Gram-negative* bact. = Gram-negative bacteria, Gram-poistive bact. = Gram positive bacteria

Appendix D - List of Figures and Tables for Chapter 5

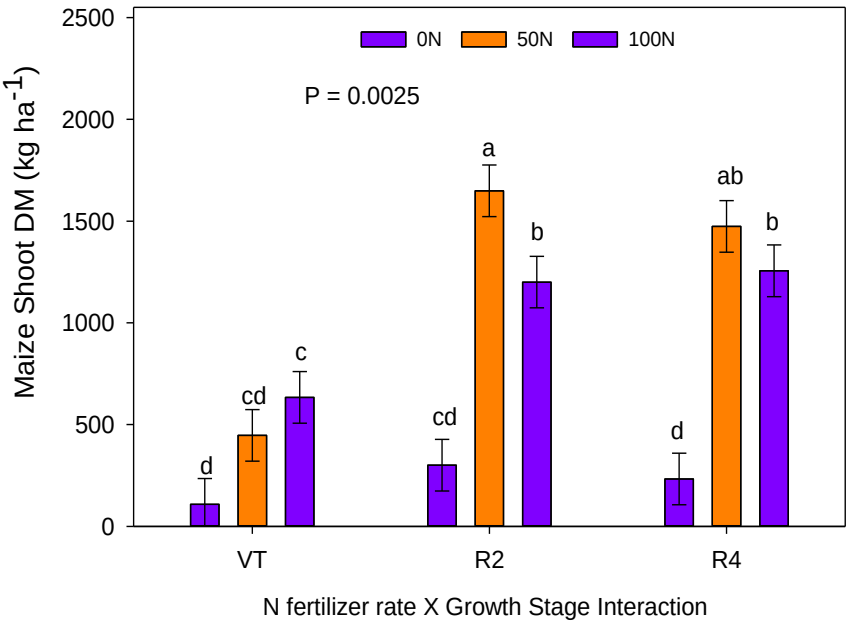


Figure D.1. Maize shoot dry matter affected by the interaction of growth stages and different nitrogen fertilizer level in Nyankapala, Ghana, 2017. Different letters indicate significant differences at p < 0.05. Error bar is a standard error (SE).

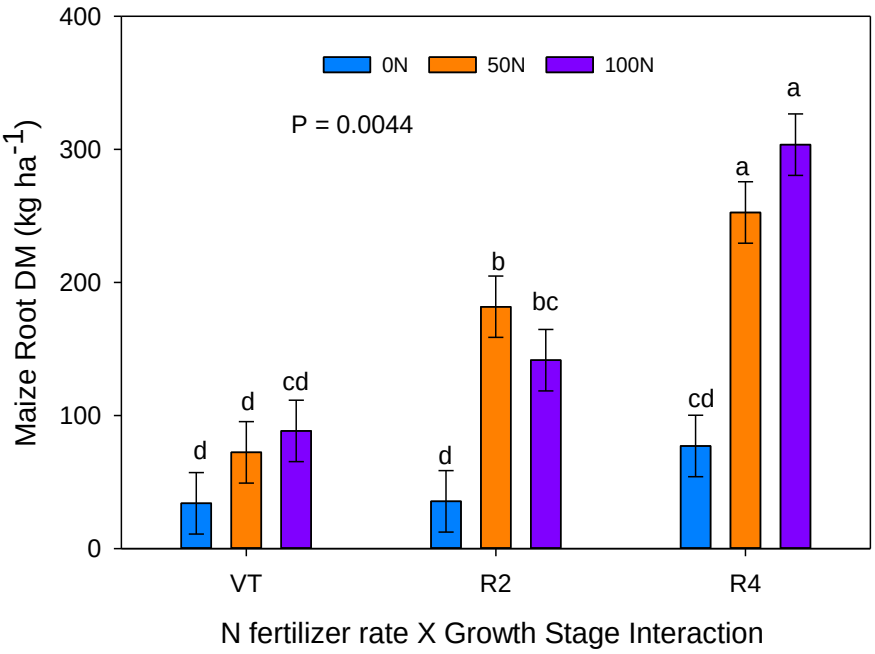


Figure D.2. Maize root dry matter affected by the interaction of growth stages and different nitrogen fertilizer level in Nyankapala, Ghana, 2017. Different letters indicate significant differences at p < 0.05. Error bar is a standard error (SE).

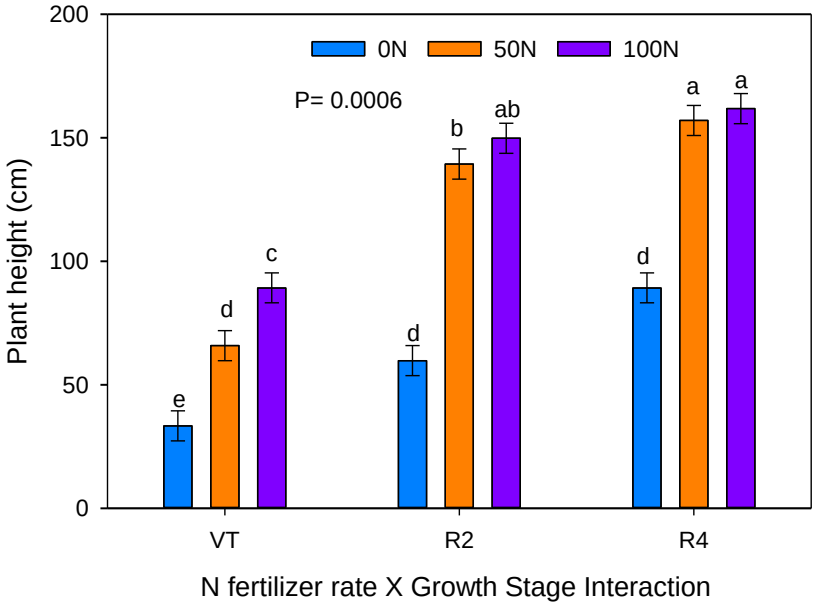
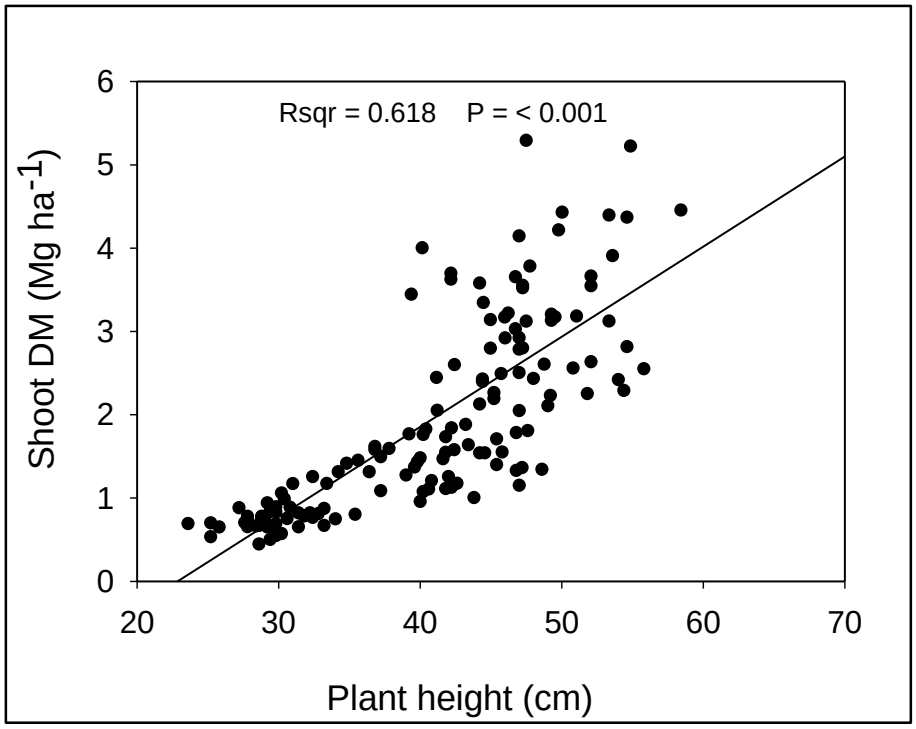


Figure D.3. Maize plant height affected by the interaction of growth stages and different nitrogen fertilizer level in Nyankapala, Ghana, 2017. Different letters indicate significant differences at p < 0.05. Error bar is a standard error (SE).



Relationship between Shoot DM and plant height (cm)

Equation that best fit: Shoot DM (Mg/ha) = -2.471 + (0.108 * Plant Height)

Figure D.4. Relationship between shoot dry matter and plant height in double soybean systems in Nyankapala, Ghana, 2017.

Table D.1. Analysis of variance (ANOVA) table for double-cropped soybean study

Effect	Pr. > F (P-value)					
	Shoot DM	Root DM	Shoot N	Root N	Total N	Plant height
	Mg ha^{-1}		kg ha^{-1}			cm
Variety	0.847	0.955	0.623	0.797	0.653	0.006
Inoculant	0.152	0.006	0.134	0.004	0.101	0.083
Variety*Inoculant	0.012	0.010	0.035	0.028	0.032	0.029
Stage	<.0001	<.0001	<.0001	<.0001	<.0001	<.0001
Variety*stage	0.925	0.972	0.823	0.856	0.841	0.560
Inoculant*stage	0.040	0.042	0.080	0.067	0.078	0.996
Var*Inoculant*stage	0.556	0.736	0.821	0.910	0.832	0.825

Table D.2. Pod load (pod number plant), pod dry matter (DM), grain yield, haulm dry matter (DM), total nitrogen (N) uptake of grain (grain N) and haulm (haulm N), total N uptake, residual N and harvest index N (Har.N) affected by previous soybean variety and commercial Bradyrhizobium inoculant in Nyankpala, Ghana, 2017.

Treatments	Pod load	Pod DM	Grain DM	Haulm DM	Grain N uptake	Haulm N uptake	Total N uptake	Residual N1	Residual N 2	Har.N Index
	No.plt[1]	Mg ha^{-1}			kg N ha^{-1}			kg N ha^{-1}		
Variety										
Jenguma	48.85	4.71 [a]	2.66	1.39	132	15.2	147	-33.3	-18.1	0.90
Afayak	45.46	4.81 [a]	2.88	1.58	137	18.3	155	-51.6	-33.4	0.88
Songda	43.38	3.99 [b]	2.55	1.45	124	18.8	143	-31.1	-12.3	0.87
	NS		NS	NS	NS	NS	NS	NS	NS	NS
Inoculant										
Control	45.47	4.59	2.81 [a]	1.55	136	19.1	155	-38.0	-18.9	0.88
Biofix	43.24	4.45	2.87 [a]	1.52	140	19.4	159	-44.4	-25.0	0.88
Legumefix	47.74	4.64	2.73 [ab]	1.42	129	15.9	145	-24.3	-9.0	0.89
NoduMax	47.14	4.34	2.37 [b]	1.40	118	15.3	134	-47.9	-32.0	0.88
	NS	NS		NS	NS	NS	NS	NS	NS	NS
Effect				Pr. > F (P-value)						
Variety	0.419	0.051	0.149	0.416	0.347	0.198	0.462	0.241	0.263	0.127
Inoculant	0.785	0.878	0.059*	0.724	0.154	0.244	0.107	0.427	0.482	0.534
Variety*Inoculant	0.944	0.338	0.891	0.759	0.971	0.786	0.993	0.297	0.244	0.619

Values within a column followed by the same (letter) are not significantly different at p < 0.05 and p < 0.1. NS = Not significantly different*

1. Residual N budget 1= Total N fixed –Total N uptake
2. Residual N budget 2= Total N fixed – Grain N uptake,

Table D.3. Analysis of variance (ANOVA) table for soybean-maize rotation study

Effect	Pr. > F (P-value)			
	Shoot DM	Root DM	Shoot total N	Plant height
	kg ha^{-1}		kg N ha^{-1}	cm
Variety	0.499	0.507	0.965	0.962
Inoculant	0.041	0.712	0.002	0.420
Variety*Inoculant	0.175	0.540	0.045	0.007
Stage	<.0001	<.0001	<.0001	<.0001
Variety*Stage	0.464	0.833	0.513	0.913
Inoculant*Stage	0.256	0.249	0.057	0.990
Variety*Inoculant*Stage	0.945	0.867	0.939	0.972
Nitrogen fertilizer	<.0001	<.0001	<.0001	<.0001
Stage	<.0001	<.0001	<.0001	<.0001
Nitrogen Fertilizer*Stage	0.0025	0.0044	0.244	0.0006

Table D.4. Bulk density (BD), Soil organic C (SOC), Soil total N (STN), Microbial biomass (PLFA-MB), Soil available nitrogen (Soil N), potentially mineralizable nitrogen (PMN) and soil pH affected by the previous soybean variety and commercial Bradyrhizobium inoculant in Nyankapala, Ghana, 2017.

Treatment	BD	SOC	STN	PLFA-MB	Soil N	PMN	Soil pH
	Mg ha^{-1}	Mg C ha^{-1}	Mg N ha^{-1}	$nmol^{-1}$ g^{-1} soil	mg N kg^{-1} soil		H_2O (1:10)
Variety							
Jenguma	1.41	7.82	0.88	7.86	5.47	3.58	5.51
Afayak	1.42	7.12	0.80	8.62	5.40	3.17	5.50
Songda	1.45	7.06	0.87	7.51	4.80	2.66	5.60
LS Means	NS	NS	NS	NS	NS	NS	NS
Inoculant							
Control	1.41	7.43	0.86	8.99 [a]	5.27	2.79	5.53
Biofix	1.41	6.95	0.82	7.70 [ab]	4.99	3.24	5.52
Legumefix	1.41	6.61	0.78	6.41 [b]	5.47	3.54	5.51
NoduMax	1.47	8.34	0.95	8.88 a	5.18	2.96	5.57
	NS	NS	NS	NS	NS	NS	NS
Effect				Pr. > F (P-value)			
Variety	0.264	0.419	0.582	0.745	0.457	0.937	0.182
Inoculant	0.216	0.113	0.165	0.053*	0.746	0.979	0.822
Variety*Inoculant	0.092**	0.337	0.443	0.392	0.365	0.923	0.617

Values within a column followed by the same (letter) are not significantly different at $p < 0.05$ and $p < 0.1$ NS = Not significantly different*

Table D.5. Cumulative evolved CO2 and Mineralizable carbon (C) affected by the previous soybean variety commercial Bradyrhizobium inoculant and Time in Nyankapala, Ghana, 2017

Treatment	Cumulative evolved CO_2	Mineralizable C
	μg C O_2-Cg^{-1} Soil	μg C O2-C g^{-1} Soil
Variety		
Jenguma	606	114
Afayak	571	79
Songda	585	92
	NS	NS
Inoculant		
Control	582	90
Biofix	591	99
Legumefix	587	95
NoduMax	590	97
	NS	NS
Time		
T0	173	31
T1	693	43
T2	896	211
	NS	NS

Effect	Pr. > F (P-value)	Pr. > F (P-value)
Variety	0.2398	0.2398
Inoculant	0.9806	0.9806
Variety*Inoculant	0.3482	0.3482
Time	<.0001	<.0001
Variety* Time	0.7454	0.7454
Inoculant* Time	0.9951	0.9951
Variety*Inoculant* Time	0.7995	0.7995

T0 = 1- day incubation (1-2 hr.), T1 = 14- days incubation period, T2 =28- days incubation period. NS = Not significantly different.

Table D.6. Maize shoots and root dry matter, plant height and shoot total nitrogen affected by Commercial Bradyrhizobium inoculant and growth stage in Nyankapala, Ghana, 2017.

Treatment	Plant height (cm)	Shoot DM	Root DM	Shoot total N
Fertilizer (kg N ha^{-1})		kg ha^{-1}		kg N ha^{-1}
0	60.8 b	214 b	0.88 b	3.152 c
50	120.8 a	1099 a	3.20 a	15.003 b
100	126.5 a	1121 a	3.25 a	26.898 a
Stage				
V8	55.6 c	396 b	1.17 c	12.215 b
R2	116.3 b	1069 a	2.20 b	21.044 a
R4	136.1 a	969 ab	3.95 a	11.793 b

Values within a column followed by the same (letter) are not significantly different at $p < 0.05$

Appendix E - Growth Chamber Study in Manhattan, Kansas

Growth Chamber Experiment Conducted in Manhattan, Kansas, June, 2018

Objective: To enumerate the native soil *Bradyrhizobium* populations and compare their symbiotic performance (nodule formation and pattern) to a known *Bradyrhizobium japonicum* strains.

Data Analysis: Data were subjected to normality test using Shapiro Wilk and test of equal variance using Brown-Forsynthe test in SigmaPlot 13. Nodulation and biomass data were transformed using log $1+\sqrt{x}$ function to fit the test of normality and equal variance. Analysis of variance done using Proc-Mixed model in SAS 9.4 at alpha (α) = 0.05 probability level. Means were separated using Fisher's least significant difference (Fisher's LSD).

Results: Results represent the pool means of three growth chambers

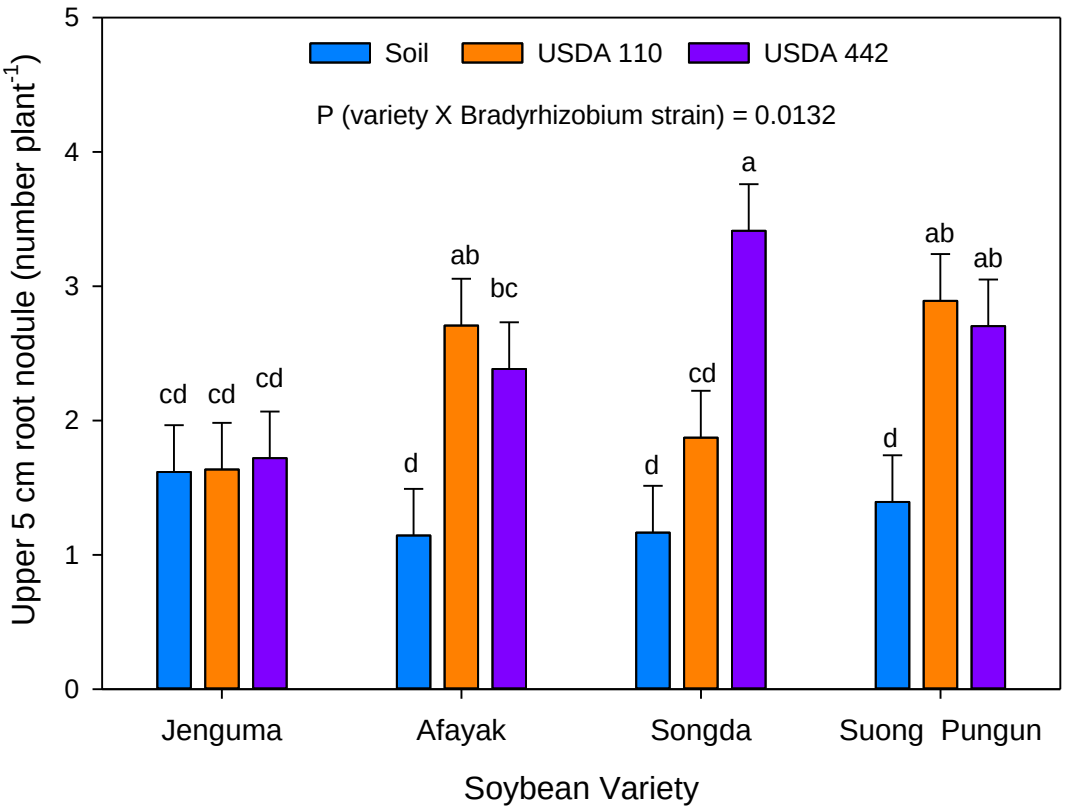


Figure E.1. Upper 5 cm root nodules affected by the interaction of soybean variety and different *Bradyrhizobia japonicum* strain. Different letters indicate significant differences at p < 0.05. Error bar is a standard error (SE).

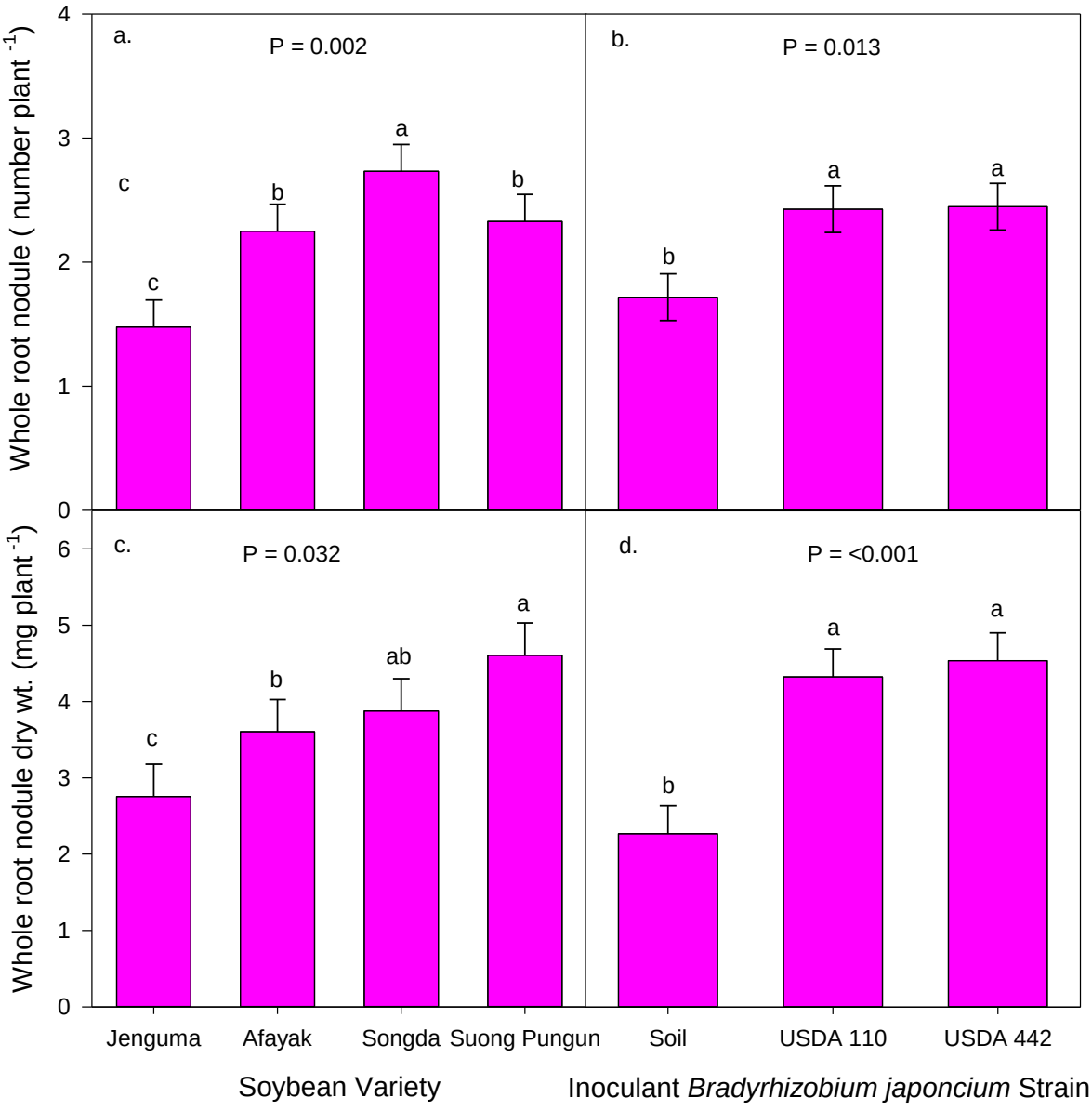


Figure E.2. Soybean variety and *Bradyhizobium japoncium* strain main effects on whole roots nodulenumber and nodule dry mass. Different letters indicate significant differences at p < 0.05. Error bar is a standard error (SE).

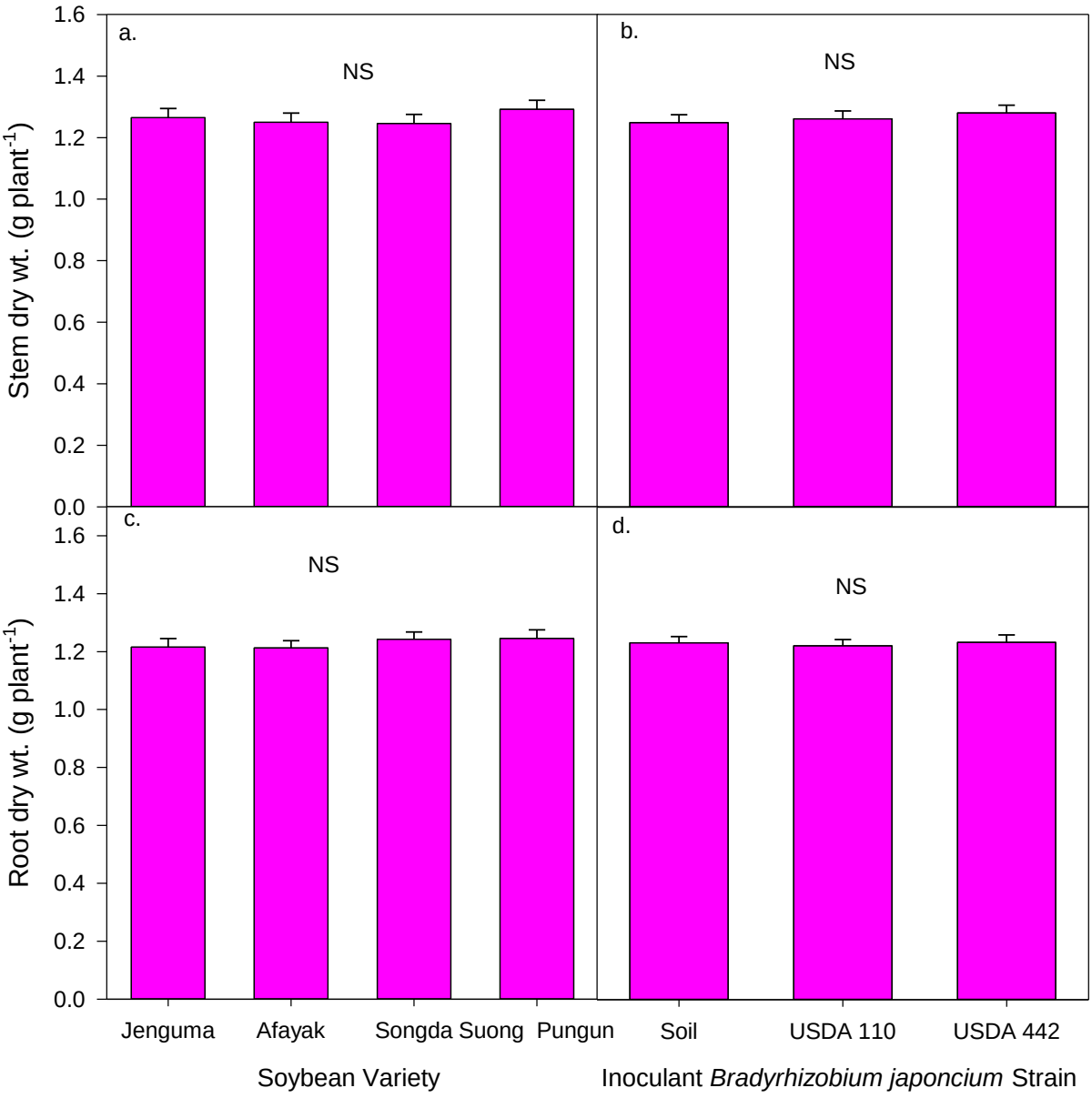


Figure E.3. Soybean variety and *Bradyhizobium japoncium* strain main effects on dry matter of stem and root.

Error bar is a standard error (SE). NS= Not significantly different at $p < 0.05$.

Data below were not transformed.

Table E.1. The number of Rhizobia in inoculant with different strains and native soil in a growth chamber study, 2018.

Bradyrhizobium japonicum strain	The population of Rhizobia g^{-1} inoculant (MPN)
Native soil Bradyrhizobium	5.8×10^{2}
USDA 110	1.7×10^{8}
USDA 442	1.7×10^{8}

Means of three (3) replications (N)

Table E.2. Population of natural Bradyrhizobium found in soil under different soybean varieties in Nyankpala, Ghana 2017.

Source of soil	Population of Bradyrhizobium (g^{-1}soil)	
	Most Probable Number (MPN)	Colony Forming Unit (CFU)
Baseline	5. 8 $x10^2$	5.0 x 10^2
Jenguma	5. 8 x 10^2	5.5 x 10^4
Afayak	5. 8 x 10^2	3.5 x 10^4
Songda	5. 8 $x10^3$	2.5 $x10^5$

Means of three (3) replications (N)