Comparative analyses of the nitrogen fixing potential of different legume species grown under different agronomic practices

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Dedication

I dedicate this dissertation to all those who directly or indirectly made me reach this far, especially my brothers, Santo and Anita (in memoriam) for being role models and fondness. My parents José Fernando Jorge and Elisa Luis Estrela for their support and love. My mother in South Africa, Mrs Annelie De Beer for enabling me to enrol my studies through SIMMLESA programme in the ARC-GCI. Eng. Domingos Dias and Dr. Carlos Quembo for facilitating the continuation of my studies. My girlfriend Manuela Domingos for encouragement, patience, love and affection. My siblings Agostinho, Maria Fernanda, Maria Alice, Ana Maria, Helena and Joana for being there for me and for having trust in me towards completion of this study.

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Declaration

I, Custódio José Fernando Jorge, declare that, this dissertation for the MSc degree in Crop Science at the North West University-Mafikeng Campus, has not been submitted by me for a degree at any other University.

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Custódio José Fernando Jorge
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List of Acronyms

A	Area
B-value	Fractionation given by the nitrogenase and the 15N distribution in the plant
BNF	Biological Nitrogen Fixation
C	Carbon
LSD	Least Significant Differences
N	Nitrogen
Ndfa	Nitrogen derived from atmosphere
NTN	Number of total nodules
NVN	Number of non- viable nodules
P	Amount of seed
SAS	Statistical analyses system
VN	Viable nodules
Y	Yield

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Abstract

Biological nitrogen fixation (BNF) is an ecologically and economically beneficial process that can reduce the use of costly conventional inorganic nitrogen (N) fertilisers.This study was therefore conducted to assess the N fixing potential of four different legumes grown under different agronomic practices such as inoculation and fertilisation under both glass house and field conditions. One rainfed field trial and one glasshouse experiment were conducted during 2013/14 summer cropping season at the Agricultural Research Council- Grain Crops Institute (ARC-GCI), Potchefstroom. Treatments consisted of four legume species namely, cowpea, dry bean, groundnut and soya bean. These legumes were fertilised, inoculated, and fertilised + inoculated. Control plots with neither fertiliser nor inoculation were also included as standard checks. The different legumes were fertilised at optimum recommended rates for P and K, while N was only applied to specific treatments to determine the minimum accretion of whether nitrogen would have influence on nodulation and productivity. The legumes were inoculated with the rhizobium inoculant registered for each respective crop. The sources of N, P and K were limestone ammonium nitrate, superphosphate and potassium chloride, respectively. All the treatments were replicated four times and were arranged in a randomised complete block designin factorial arrangement (4 legumes x 2 inoculants x 2 fertiliser = 16 treatments) in all experiment. Data collected included total number of nodules, viable and non-viable nodules, %N, %C, %Ndfa, total amount of nitrogen in the plant, total amount of nitrogen fixed and seed yield. The treatment effects showed statistically significant differences ($P<0.05$) on the number of nodules, percentage of nitrogen from atmosphere and total amount of nitrogen fixed per plant. Significantly higher total number of nodules, viable nodules, percentage of nitrogen from atmosphere and total amount of nitrogen fixed per plant were observed from inoculated plants irrespective of the legume species under both field and glasshouse conditions. Under field conditions, groundnut (48.5) and soya bean (42.9) recorded significantly higher average total number of nodules at full flowering, while dry bean (83.2) and groundnuts (70.6) produced significantly higher total number of nodules at physiological maturity. The crops studied showed that fertiliser application had a depressive effect on nodulation, but promoted nodulation when the different crops were inoculated comparable to when used in combinations. Nodulation in groundnuts was higher in both samplings, but lower with cowpea. Nonetheless, the response of dry bean and soya bean was infrequent across the sampling. Dry bean showed lower percentage of nitrogen (%N) from the atmosphere and total amount of nitrogen fixed across the cycle under field and glasshouse conditions. Groundnut showed higher percentage of nitrogen from atmosphere and total amount of nitrogen fixed across the cycle under field and glasshouse conditions. Cowpea had higher percentage of nitrogen from atmosphere and total amount of nitrogen fixed at 100% flowering and 100% pod formation, while for soya bean that was achieved at 100% pod formation and physiological maturity in the field. The results highlight that groundnuts among other legumes has been noted to be more recommendable for use as crop that can fix nitrogen. This crop has been noted that it can fix N throughout its cycle. It has also been proven that it contributes to the improvement of chemical, physical and biological soil caractreisticas

Keywords: Fertiliser, inoculants, leguminous plants, nodulation, seed yield

CHAPTER 1 : INTRODUCTION

1.1 BACKGROUND STATEMENT

Among the most important elements in plant nutrition is nitrogen (N) and is most needed in large quantities (Resende *et al.,* 2003). Besides, N is generally considered the most limiting nutrient for plants' growth in their natural environment. However, due to the complexity of chemical and biological reactions, the dependence of environmental conditions and their effect on crop yields, N remains the most difficult element to manage in agricultural production even in technically oriented properties (Hungria and Vargas, 2000). Nitrogen as a primary macronutrient represents the most extracted and exported by crops (Smithson and Giller, 2002). Therefore, its use in agriculture is essential for plants to fulfil their life cycle. Plants and/or soil N demand has been met solely through N fertilisation from both organic and inorganic N-containing sources. Although N is the most abundant nutrient in the atmosphere, representing 78% of the earth's atmosphere and not a constituent of any terrestrial rock (Schlesinger, 1997). Perhaps, it is for this reason that it is the most expensive fertiliser because its formation is needed for diverse chemical reaction that requires a lot of energy (Cantarella and Marcelino, 2008).

Biological nitrogen fixation (BNF) is considered one of the most important biological processes, in which some genera of bacteria fix nitrogen (N) from the air, making it available to the plants. Leguminous plants are frequently used in various cropping system programs in order to improve soil nitrogen content due to their efficient fixation ability of atmospheric N in the soil (Hungria *et al.*, 2001). Besides, legumes have a competitive advantage over other plants since they are able to fix atmospheric N through a specific association of microorganisms that colonize the roots of the plant (Coelho *et al.*, 2003). This advantage enables farmers to possibly partially and/or totally replace conventional inorganic N fertiliser application. For BNF to effectively occur, it is imperative that most legume seeds should be inoculated with microorganism, i.e. rhizobium that will improve atmospheric nitrogen fixation efficiency (Zill *et al.*, 2009).

1.2 PROBLEM STATEMENT AND JUSTIFICATION OF STUDY

Agriculture remains an integral component for food production and for sustaining livelihoods of the majority of the populace in the African continent (Ashley and Maxwell 2001). Nonetheless, agricultural food production has not been an easy exercise due to persistently low crop yields obtained in farmlands resulting from the absence of nutrient replenishment after crop harvests, particularly on smallholder farmlands where cereal grains are produced (Lopes and Guilherme, 2007). The lack of fertiliser application in farmlands is mostly because of their high cost, thus impacting negatively on crop production and productivity particularly under resource limited farmers' fields. Smallholder farmers often do not apply fertiliser N and when they do, they apply at sub-optimal rates due the technical limitations (Moreira and Sequeira, 2006). Nitrogen is considered as the most needed mineral element in large quantities due to its effect in plant metabolism, and that it has been noted to be generally deficient in most crop lands (Resend *et al.*, 2003).

The use of leguminous green manure provides N and consequently improves soil productive capacity by improving its physical, chemical and biological properties, thus providing a healthy environment for microbial activity (Bertoni and Neto, 2008). Therefore, the use of BNF by leguminous plants represents a low cost alternative to meet N demand by crops for optimal production, and also reduce the possible negative impacts associated with use of conventional inorganic fertiliser that directly or indirectly affects the environment (Hungriaand Vargas, 2000; Gliessman, 2001). It was expected that identification of the most efficient legume would contribute to the improvement of crop productivity and therefore alleviate rural food insecurities in South Africa as well as in Africa at large.

1.3 GENERAL AND SPECIFIC OBJECTIVE

1.3.1 General objective

Evaluate the N contribution of the inoculated and fertilised legumes under different agronomic practices.

1.3.2 Specific Objectives

1. To assess the nodulation efficiency of the different legumes under both glass house and field conditions.

2. To quantify the Total nitrogen (TN) by the different leguminous species with and without inoculation and fertiliser applicationunder both glass house and field conditions.

3. To quantify the amount of fixed N in the soil by different legumes with and without inoculation and fertiliser application under both glass house and field conditions.

1.4 Hypotheses

1. Basal nitrogen application and rhizobium inoculation will promote higher N fixation by the different legumes.
2. The soya bean crop is more BNF efficient than other legumes *inter alia* cowpea, dry bean and groundnut.

CHAPTER 2 : Background and Literature

2.1 Classification and characteristics of leguminous plants

Legumes are part of a group of plant species that belong to the family Fabaceae, which is also known as leguminosae. They have widespread geographical distribution, although there are few exceptions, and a typical characteristic of the presence of fruit-shaped pod (Franco *et al.*, 2003). The great diversity of legume species relates to their important role of supplying N to the ecosystems through incorporation of plant materials to the soil and also soil cover (Wojciechowski *et. al.*, 2004). Hence, plants in this botanical family are efficient in reducing the use of N fertiliser and improve soil structure (Wojciechowski *et. al.*, 2004). Among the species include the family of grain legumes such as soya beans, cowpea, groundnut, and dry bean that are the most commonly used because they form symbiotic associations with N-fixing bacteria (Perin *et al.*, 2003) thereby contributing to the nutrition of the succeeding crops (Andreola *et al.*, 2000; Zotarelli, 2000). Another important characteristic of legumes is the low C/N ratio when compared to other plant families coupled with the large presence of soluble compounds that favors their rapid decomposition and mineralization by soil microorganisms and ultimately promotes nutrient recycling (Zotarelli, 2000).

Legumes stand out as important cover crops or green manure by reducing soil erosion, promote water conservation in the soil and enhance nutrient recycling (Rao and Mathuva, 2000; Aita *et al.*, 2001). However, the major benefit of growing legumes is in increasing crop yield of the follow up crop, which is attributed to the increased N availability (Rao and Mathuva, 2000; Aita *et al.*, 2001). This contributes to the reduction in costs of mineral N fertilisers (Bohlool *et al.*, 1992) and the environmental impacts generated by the industrial production of mineral fertilisers (Amado *et al.*, 2001; Zanatta *et al.*, 2007). The use of legumes in cropping systems associated with no-tillage system has also been reported to contribute to increased total N stocks in the soil (Diekow*et al.*, 2005) resulting from higher N soil input by BNF plus the lower rate of mineralisation of organic N (Lovato *et al.*, 2004).

Hence, plants in this botanical family are efficient in reducing the use of nitrogen fertilisers and improving soil structure (Ribeiro, 1999). The great diversity of legume species is allied to the important role of supplying nitrogen to ecosystems and incorporation of plant material to the soil and soil cover (Ribeiro, 1999).

2.1.1 Cowpea

Cowpea (*VignaunguiculataL.* Walp) is one of the crops with social importance due their contribution on food security and economic importance owing to their contribution on family income. They are known to have originated from Africa (Zilli *et al.*, 2009). Beside grain production, cowpeas have the potential for use as green manure because of their ability to fix atmospheric N into a form that is available to plants. Castro *et al.* (2004) on their studies reported that this characteristic favours its introduction as low input technology in the management of agro-ecosystems, contributing to the increase to soil fertility for subsequent crops. (Rumjanek *et al.*, 2005; Gulter *et al.*, 2011) in their investigationreported that Inoculating cowpea with efficient bacterial strains enables the crop to achieve high levels of productivity and greater amount of N from BNF.

2.1.2 Dry bean

The *Phaseolus* genus has about 55 species that include dry bean (*Phaseolus vulgaris* L,). This bean is considered to be the oldest species that is grown and is an important source of food source worldwide (Borém *et al.*, 2006; Embrapa, 2010). The story of dry bean domestication is explained by different assumptions and its origins. Several factors influence optimum production of dry beans and these include adequate supply of nutrients particularly N and K (Rosolem, 1987). Hungria and Vargas (2000); Gliessman (2001) in their work also argued that, typically, when N is not available, crop production is compromised. In this way BNF is a sustainable source of N and therefore promoting sustainable land management practice. The association of dry beans with bacteria of the genus Rhizobium, which is capable of fixing atmospheric N to a form that is available to plants, is a biological mechanism that partially replaces N fertilisation and this results in the reduction of N fertiliser costs. Furthermore, this may also increase crop productivity, and also minimize nitrate leaching into ground waters (Hungria*et al.,* 1997).

2.1.3 Groundnut

Groundnut (*Arachishypogaea* L.) belongs to the genus *Arachis*, family *Fabaceae,* and subfamily of *Papilionaceae*, and includes more than 70 species (Krapovickas and Gregory, 1994; Yang*et al.*, 2008). It is a plant that originated from South America and its natural distribution is restricted to Brazil, Bolivia, Paraguay, Argentina and Uruguay (FAO, 2012). It is believed that the center of origin of this legume is Brazil but is now widely grown in various regions such as Asia, Africa and North America (Allen and Allen, 1991).Groundnut is a legume with high nutritional and caloric values and is commonly used in human and animal food. Its stover can also be added to the soil as green manure (Carneiro, 2006). In a studyof biological nitrogen fixation (Siddique and Bal, 1991), concluded that from the BNF point of view and in contrast to most other legumes, groundnut is a privileged plant because it can sustain N fixation under conditions of little supply of photosynthate such as prolonged periods of darkness. Moreira and Siqueira, (2006)also argued that generally, application of N fertiliser is not recommended in most groundnut cropping systems because it generally fixes its own N through the BNF process.

2.1.4 Soya bean

Soya bean (*Glycine max L.*) originated from China and it is one of the oldest crops planted. It is known to have been domesticated about 5000 years ago. Its cultivation has spread from China to throughout the world through the English travelers, as well as Japanese and Chinese immigrants (Chung and Singh, 2008). Hungria *et al.* (2006) in their investigation reported that BNF in this crop provides up to 94% of N required by the crop, while the absence of symbiosis could promote the rise in N costs. This has been considered a factor that can affect competitiveness of soya bean production (Hungria*et al.*, 2006). Seed inoculation with N-fixing bacteria in soya bean is an indispensable technology for its production (Zilli *et al.*, 2012). The BNF in soya bean is performed by *Bradyrhizobium* (rhizobium group) that establishes a symbiosis with the plant and results in the formation of nodules on the roots. The bacteria fix atmospheric N and convert it to a form that is available to plants (Zilli, 2012) making it possible to cultivate the crop without fertilisation (Alves *et al.*, 2003).

2.2 Planting of legumes in crop rotation

Legumes are normally included in crop rotation sequence because they have both commercial and ecological benefits (Aita *et al.*, 2001). This makes crop types to be highly preferred by both small scale farmers and commercial farmers.Aita *et al.*, (2001) In their studiesreported that regarding the choice of cultivars for the recovery of soil fertility and the environment, legumes are often included in rotational sequences entirely for the fact that they are fixing N from the air in large quantities. Perin *et al.*, (2004) also argued that furthermore, legumes have remarkably long roots that help in ripping open compacted soil, recycle nutrients and bring the nutrients from deeper soil layers to the upper surface layer. They also contribute to increases in soil organic matter, enhance microbial activity, and provide protection against erosion processes (Heinzmann, 1985; Spagnollo *et al.*, 2002; Perin *et al.*, 2004). Thus, thispractice may be one of the most ecologically appropriate agricultural practices that can contribute to development of a sustainable production system. In the economic analyses of the use of legumes in rotation with maize, Spagnollo *et al.* (2001) in their workconcluded that the growing of leguminous cover crops proved viable alternative to significantly increase the net revenue of cereal crops.

2.3 Nitrogen as anutrient

Nitrogen is amply recognized for its importance in crop growth and constitutes one of the key drivers of global agricultural production (Liu *et al.*, 2014). Despite being the most abundant nutrient in the earth's atmosphere (78% of the atmosphere), N is not a constituent of any terrestrial rock. Although there is a large N reservoir (earth's atmosphere) it is not directly available to living organisms (Vance, 2001). Maybe it is for this reason that it is the most expensive fertiliser because they have to be factory manufactured and therefore requiring various inputs such energy and various chemicals. For example, the energy cost of forming NH_3 is estimated to be 16800 Kcal / kg. Obtaining atmospheric N requires the breaking of a covalent triple bond of exceptional stability between the two N atoms ($N \equiv N$) to produce ammonia (NH_3) or nitrate (NO_3^-) (Hubbell and Kidder, 2009).

2.4 Industrial Fixation

Under high temperature (about 450°C) and high pressure (about 200 atmospheres kpa), N_2 combines with hydrogen (H^+) forming NH_3, this is the product base for obtaining nitrogenous fertilisers. This fixation of N, known as Haber-Bosch process is the starting point for the manufacture of many products for the industry and agriculture. Industries throughout the world produce more than 80 x 10^{12} gram per year of N fertilisers, representing about 20% of all fixed N per year (Faostat, 2001). The difficult synthesis reaction is ratifying, in part by processing of the ration gas N_2, a super stable compound. The N_2 molecule contains a triple covalent that bond is very stable and 2.2 x 105 Kcal / Kmol energy is needed to break it. In addition to this, the formation reaction of ammonia (NH_3), a most important compound for the production of N fertilisers, requires a high energy cost, as shown in the reaction below. Finally, the energy cost of manufacture relies on the use of NH_3 H^+ electrolytic originated from natural gas, naphtha, residual gas or asphaltic residue as described by Faostat (2001) presented under equation 2.1.

$$N_2 + 3H_2 \underline{450^oC} N_2 \quad > 2NH_3 \qquad (2.1)$$

200 atm

Catalyst

Equation 2.1: Chemical formula displaying industrial N fixation (Haber process)

2.5 Biological nitrogen fixation and its benefits

Biological nitrogen fixation (BNF), discovered by Beijerinck in 1901 is carried out by a specialised group of prokaryotes. These organisms utilize the enzyme nitrogenase to catalyze the conversion of atmospheric nitrogen (N_2) to ammonia (NH_3). These prokaryotes include aquatic organisms, such as cyanobacteria, free-living soil bacteria, such as *Azotobacter*, bacteria that form associative relationships with plants, such as *Azospirillum*, and most importantly, bacteria, such as Rhizobium and Bradyrhizobium that form symbioses with legumes and other plants and outlined by Postgate (1982) in Figure 2. 1.

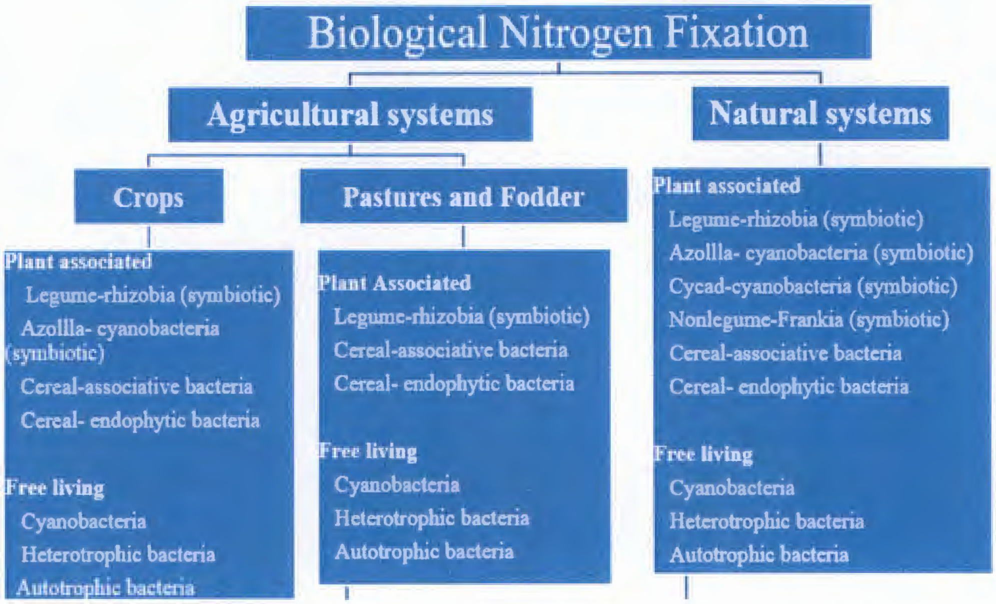

Figure 2.1: Nitrogen-fixing organisms found in agricultural and natural systems (Adapted from Postgate, 1982)

Biological nitrogen fixation (BNF) is considered one of the most important biological processes in which some genera of bacteria capture atmospheric N, making it available to plants (Hungria and Campo, 2006). Leguminous plants are commonly used for this purpose because they are more efficient at the fixation of atmospheric N in the soil (Hungria *et al.,* 2001). Bacteria of the genus Rhizobium when in contact with the roots of legumes infect them and result in root hairs forming nodules. Inside the nodules, an enzyme called dinitrogenase present in prokaryotes breaks the triple bond of atmospheric N that cause reduction to ammonia (NH_3). Microorganisms that fix nitrogen require 16 moles of adenosine triphosphate (ATP) to reduce each mole of nitrogen (Hubbell and Kidder, 2009). These organisms obtain this energy by oxidizing organic molecules. Non-photosynthetic free-living microorganisms must obtain these molecules from other organisms, while photosynthetic microorganisms, such as cyanobacteria, use sugars produced by photosynthesis. Associative and symbiotic nitrogen-fixing microorganisms obtain these compounds from their host plants' rhizosphere (National Research Council, 1994, Hubbell and Kidder, 2009). Schlesinger, (1997) in his work concluded that, the natural processes produce approximately 190 x 10^{12} gram per year of nitrogen through the following processes (Table 2.1).

Table 2.1: Process of the biogeochemical cycle of nitrogen

Process	Definitions	Taxa (10^{12} g ano^{-1})
Industrial Fixation	Photochemical conversion and the lightings of molecular nitrogen into nitrate	80
Atmospheric fixation	Photochemical conversion of molecular nitrogen by lightning to nitrate	19
Biological fixation	Conversion of molecular nitrogen into ammonia by prokaryotic Acquisition by plants	170
	Absorption and assimilation of ammonium or nitrate by plants	1200
Immobilisation	Absorption and assimilation of ammonium or nitrate by microorganisms	N/C
Ammonification	Action of bacteria and fungi in the catabolism of soil organic matter into ammonium	N/C
N nitrification	Oxidizing bacteria (Nitrosomonas sp.) Of ammonium into nitrite and the subsequent oxidation by bacteria (Nitrobacteria sp.) Of nitrite to nitrate	N/C
Mineralisation	Action of bacteria and fungi in the catabolism of soil organic matter in mineral nitrogen through the ammonification and nitrification	N/C
Volatilisation	Physical loss of ammonia gas to atmosphere	100
Ammonium fixation	Physical connection ammonium to soil particles	10
Denitrification	Bacterial conversion of nitrate to nitrous oxide and molecular nitrogen	210
Leaching of nitrate	Physical flow nitrate dissolved in the ground water in the upper soil layers and eventually to the oceans	36

(Schlesinger, 1997)

N/C = unknown

2.5.1 Advantages of BNF

Coelho *et al.* (2003) in their investigation reported that BNF promotes several benefits to agricultural crops, among which stand out:

- The lower use of N fertilisers, which results in savings to the producer;
- The characteristic of contributing to the self-supply of nitrogen used for growth of the crop, minimizes the potential effects of mineral nitrogen fertiliser on the environment;
- The use of leguminous green fertilisers through BNF will efficiently provide N to the soil and improves its physical, chemical and biological properties;
- Increased productivity, especially in soils deficient in available N;
- BNF is an environmentally friendly process, enriching the soil with C, and generally promotes the growth and development of higher plants.

2.6 Benefits of leguminous N to poaceae crops

Since the cultivation of gramineae crops (e.g. maize) is very demanding in terms of soil fertility, the use of leguminous crops can provide an efficient source of N to increase the yield of these crops (Queiroz, 2006). This generally results in increased revenue due to increased productivity and improved product quality, soil conservation and a reduction of production cost.

Alcantara *et al.* (2000) in their work highlighted that the use of green manure is a viable way to mitigate the impacts of modern agriculture such as high cost and negative effect on the environment, bringing sustainability to agricultural soils. The increased organic matter (OM) content, the greater availability of nutrients, and the high cation exchange capacity are some of advantages and the beneficial effects of green manure on soil fertility(Alcantara *et al.*, 2000). This favours the production of organic acids required for the solubilisation of minerals, the reduction of the content of exchangeable Al, and the increased ability to mobilize and recycling of leachate or less soluble nutrients. The latter, are in the deeper profile which can make it beneficial to other crops such as poaceae crops that are grown in rotation with the legumes (Calegari *et al.*, 1993).

Rao and Mathura (2000) in their studies reported that the contribution of N by legumes to other crops in intercropping depends on the legume species, BNF and growth of legumes, which is determined by the climate, the soil and the management of waste. These authors highlighted that the dual purpose legumes that produce food for humans, such as cowpea, groundnut, soya bean, dry bean, peas and fodder for animals such as *Stylosanthes*, are more attractive to small-scale farmers who practice the cropping system in intercropping. Leguminous crops bring the ecological benefits such as the improvement in the physical and chemical characteristics to cereal crops grown in rotation (Rao and Mathura, 2000).

2.7 Factors affecting BNF

The study of BNF usually places a lot of emphasis on aspects related to the intrinsic characteristics of the bacterial species, quality and application of inoculants (Soares *et al.*, 2006a, b). In a long-term study of factors affecting BNF numerous authors, concluded that the successful formation of a functional symbiosis is dependent on many factors such as physical, environmental, nutritional and biological factors (Hungria and Vargas, 1997; Soares *et al.*, 2006a). Other important factors include plant cultivars and the inoculant strain as well as their interaction. The BNF capacity of dry bean has been reported not to be effective as compared to cowpea and soya bean (Martins *et al.*, 2003; Xavier *et al.*, 2007; Hungria *et al.*, 1991; Mendes *et al.*, 2008). To obtain economic yield under poor soil conditions, N fertilisation is usually essential for a while until the nodulation is fully established (Oliveira *et al.*, 2003). According to Graham and Temple (1984) and various other workers (Kamicker and Brill,1986), conditions such as low soil fertility and high N levels, the effect of the rhizosphere, water tension, soil pH, salinity, temperature, toxins and predators can also affect nodulation and/or N fixation of legumes together with the variety or strain of Rhizobium. Among the most important environmental factors that affect the BNF process is the occurrence of water deficit, i.e., drought during the crop cycle. This has a negative effect on different stages of nodulation and nodule activity and it also affects survival of rhizobium in the soil. (Mendes *et al.*, 2008) High temperature also affects the survival of rhizobia in the soil, the infection process, formation of nodules, and even the activity of BNF (Graham and Temple, 1984).

2.8 Methods of measuring biologically fixed nitrogen

2.8.1 ^{15}N Natural abundance method

Application of the ^{15}N natural abundance method is not always straightforward. It does not work if the $\delta^{15}N$ of the legume does not fall between the **B** value and the $\delta^{15}N$ of the reference plant. This problem will be indicated by %Ndfa being <0% or >100%. If this happens, a greater investigation into **B** values of reference plants may be warranted. The 'B' values of most legume shoots tend to lie between 0 and –1‰. The 'B' value is best determined on plants grown in a glasshouse in sand culture, and using the same strain(s) of rhizobia responsible for N2 fixation at the field site(s) under study. The rhizobia strain involved in the symbiosis can also influence the 'B' value. It is not fully understood why this occurs, but it could be through impacts on nodule mass. Analyses of $\delta^{15}N$ require highly sophisticated and well-maintained equipment and skilled operators. The cost of analyses is high but it is always worthwhile to include a few replicates of individual samples to check for within-sample variability. Unkovich *et al.* (1994) and Pate *et al.* (1994) provide fuller description of the procedure.

This method is performed on effectively nodulated legumes exposed to a medium free of combined N (from mineral N and organic N) completely reliant upon symbiotic N_2 fixation for growth, the isotopic composition of the legume would be expected to be similar to that of atmospheric N ($\delta^{15}N$ 0‰). Conversely, if a non N_2-fixing plant is grown in a soil containing mineral N, its $\delta^{15}N$ value should resemble that of the soil mineral N taken up by the plant. In the case of a nodulated legume or other N_2-fixing plant that is using a combination of atmospheric N (N_2) and soil mineral N for growth, the $\delta^{15}N$ of the legume should lie between the values of the two possible N sources, soil and atmospheric N. The percentage of the legume can then be calculated from its $\delta^{15}N$ value using equation described by Unkovich *et al.* 2008 (2.2).

$$\%Ndfa = \frac{\delta^{15}N \text{ of soil N} - \delta^{15}N \text{ of } N_2 \text{ fixing legume}}{\delta^{15}N \text{ of soil} - \delta^{15}N \text{ of } N_2} \times 100 \qquad (2.2)$$

While estimates of N_2 fixation could theoretically be derived from such direct measures of the ^{15}N abundance of soil mineral N. It is technically less challenging, and often more convenient to use a non-N_2-fixing plant such as a non-legume to sample the $\delta^{15}N$ of soil mineral N. In this case, the equation described in Unkovich *et al.* (2008) can be revised as in Equation 2.3.

$$\%Ndfa = \frac{\delta^{15}N \text{ of reference plant} - \delta^{15}N \text{ of } N_2 \text{ fixing legume}}{\delta^{15}N \text{ of reference} - \delta^{15}N \text{ of } N_2} \times 100 \qquad (2.3)$$

2.8.2 Dilution method using ^{15}N isotope

The basis of the method is essentially the same as for ^{15}N natural abundance, except that the soil is artificially enriched with ^{15}N above the background ^{15}N abundance. No account of isotope fractionation is needed (i.e. B value) since the enrichments used greatly exceed natural variations in ^{15}N. The principal assumption is that the ^{15}N enrichment of the non-N_2 fixing reference plant accurately reflects the ^{15}N enrichment of soil N taken up by the legume. The amount taken up from the soil by the reference plants does not have to be the same as the legume. For this to be valid, the ^{15}N enrichment of the soil N would need to be relatively constant over-time and space, or the time course and depth of soil N uptake by the reference and N_2-fixing plants the same. When applying the ^{15}N isotope dilution methodology, most effort is focused on these two aspects (Fried and Middleboe, 1977). To determine the potential suitability of the various techniques for quantifying N_2 fixation in agriculture systems, legume and non N_2-fixing reference plants are grown in soil receiving the same amount of ^{15}N-labelled fertiliser (Unkovich *et al.*, 2008). The total N in shoots is then analyzed for ^{15}N, and the percentage of N derived from the atmosphere (%Ndfa) by the legume is calculated using the following equation (Fried and Middleboe, 1977).

$$\%Ndfa = 1 - \frac{(\%atom\ ^{15}N \text{ ref.} - \%atom\ ^{15}N \text{ legume})}{atom\ ^{15}N \text{ ref.}} \times 100 \qquad (2.4)$$

Where ^{15}N leg is the number of atoms in the leguminous plant and $^{15}Nref$ is the number of atoms in the reference plant grown in the same soil legume

The amount of N fixed is computed using the following equation (Hardarson *et al.*, 1987):

$$N \text{ fixed} = \frac{\%Ndfa \times \text{total N in fixing crop}}{100} \qquad (2.5)$$

Table 2.2: Methods of quantification of biological nitrogen fixation and their suitability in agricultural system (More X the suitable is the methodology)

Specie	System	Non-isotopic methods				N isotopic methods	
		N Balance	N difference	Ureide	C_2H_2 reduction	^{15}N Natural abundance	^{15}N isotope dilution
Crop Legume	Monocrop	-1	XX	XXX	-	XXX	XX
	Intercrop	-1	-3	XXX	-	XXX	XX
Pasture	Monocrop	X2	X	XXX	-	XXX	XX
legume	Intercrop	X2	X	XXX	-	XXX	XX
Tree	Monocrop	-	X	XXX	-	XX	X
legumes	Intercrop	-	-	XXX	-	XX	X
	Native forest	-	-	5	-	X	
Grasses/cereals	Mono/ intercrop	X2	X	-	-	X	XX7
Azolla		X2	-	-	X	XXX	XX

(Adapted from Unkovich, *et al.*, 2008)

1 For short-term experiment is not suitable

2Suitable if is long-term experiments

3 Not suitable if legume proportion in mixture is small

4 only for ureide producing species

5 Not known

6 Notfor quantification but is useful for activity assessing

7 Only with relatively stable labeled soil

Table 2.3: Methods of quantification of biological nitrogen fixation and its characteristics (The more R the greater the cost)

Method	**Non-isotopic method**				**Isotopic methods**				
Characteristics	N balance	N difference	Ureide	C_2H_2 reduction	^{15}N natural abundance	^{15}N isotope dilution	^{15}N enriched A-value	Gas ($^{15}N_2$)	^{13}N
Direct								X	X
Indirect	X	X	X	X	X	X	X		
Time integrated	X	X			X	X	X	X	
Reference plant needed		X			X	X[4]	X		
Non-destructive			X[2]		X[2]				
%Ndfa measured		X[1]	X		X	X	X	X	
Quantify kg N/ha fixed	X	X	X		X	X	X		
Laboratory			X	X		X		X	X
Glasshouse	X	X	X	X	X	X	X	X	
Field	X	X	X		X	X	X		
Possibility to assess fate of fixed N in system						X			
Short-term			X	X	X	X	X	X	X
Long-term	X	X	X	X	X	X	X		
Precision	Low	Low	Good	Low	Low-good	Medium	Low	Low	Low
Costs	RR	R	R	R	R	RR	RR	RRR	R

(Unkovich, *et al.*, 2008)

1 possible calculate indirectly

2 When %Ndfa is required only

3 Depending on natural enrichment of soil

4 Is not possible when cultivated in N-free media.

2.9 Assessment of Nodulation

Nodulation is generally assessed by examining the roots of a number of plants from each respective treatment. Measurements include earliness of nodulation, root nodule number, mass and color, distribution and longevity of the nodule population, and visual nodulation score. It is recommended that nodulation be assessed during mid-late vegetative growth when it is still relatively easy to uproot the bulk of the root system. Nodule mass per plant is the most informative measure but can be very time consuming for species with high nodule numbers. Practical alternative is to assess nodule number or, if nodule numbers are large, to use a scoring method. This particular system scores nodule from 0 to 5, taking into account the number of nodules, size, pigmentation and distribution (Corbin *et al.,* 1977).

To test whether nodules are active or not, the nodule is chipped gentle to examine internal colour. A positive test result exhibits a pink-red colour due the presence of the oxygen carrier leghemoglobin, which is essential for legume N_2 fixation. White, greenish or dark colours are indicative of ineffective nodulation and might correlative with low N_2 fixation rates. The procedure involves carefully digging up an appropriate number of plants at random across a crop or from a replicate plot and scoring each plant using the criteria below. The score from all plants are added and then divided by the number of plants to obtain a mean nodule score. A mean nodule score of:

4-5 represents excellent nodulation; 3-4 indicates good nodulation; 2-3 represents fair nodulation; 0-2 Indicates poor nodulation.

CHAPTER 3 : MATERIALS AND METHODS

3.1 Glasshouse Experiment

A glasshouse pot trial was conducted during 2013/2014-summer growing season at the ARC-Grain Crops Institute, Potchefstroom to assess the nitrogen fixation potential of inoculated and fertilised four leguminous species. The soil used for the glasshouse study was collected from the topsoil (0-20 cm) in the plot where the field trial was established. The trial was conducted concurrently with the field trials. The composition of the treatment trial was four legumes namely, cowpea, dry bean, groundnut and soya bean used as test crops. Maize was used as a reference plant and was planted 5 m away from the trial. Sixteen treatment combinations were employed as shown in Table 3.2. Pots (21 cm high x 24 cm in diameter) were filled with sieved soil passed through 2 mm stainless sieve. The soil in the pots was watered with distilled water until field capacity prior to planting any of the test crops. The treatments and test crops in the glasshouse were similar to those used in the field study (Table 3.1). A total of 16 treatments were replicated four times and laid out in complete randomized block designin factorial arrangement (4 legumes x 2 inoculants x 2 fertiliser = 16 treatments)

Each treatment, including conventional inorganic fertilizer, was thoroughly mixed with soil in the pots. All pots containing treatments were carefully arranged in the glasshouse in a completely randomized design. Three uniform sized seeds for each legume were sown in each pot at a depth of 3 cm. The temperature of the glasshouse was maintained at between 18 and 27°C throughout the four-month study period using an electric fan and wet wall cooling system. Weed control was carried out manually when necessary. Data collection was carried out for the following parameters: total number of nodules, viable and non-viable nodules as well as the amount of fixed nitrogen.

3.2 Field Experiment

3.2.1 Description of the study area

The field study was conducted at the ARC-Grain Crop Institute Research station, Potchefstroom during 2013/14 summer cropping season. The Potchefstroom site lies at a latitude of 27°09'and longitude of 27°7'with an altitude of 1355 m above sea level. The long-term (1948) average annual rainfall is 622.2 mm with daily temperature range from 9.1 to 25.2°C during planting (ARC-ISCW, 2013).

3.2.2 Soil characterisation

Prior to trial establishment, soil sampling was randomly carried out across the field using a soil auger and the soil was analysed to determine nutrient composition, organic matter; pH; N; P; K; Ca; Na; cation exchange capacity and particle size distribution using standard procedures (Table 3.1).

Table 3.1: Analytical data of the surface soil at Potchefstroom prior to field trial in 2013/14 planting season

Soil properties		Potchefstroom
Sand	(%)	66.7
Silt	(%)	8
Clay	(%)	26
Textural class		Sandy clay loam
% Organic C		0.85
pH	(H_2O)	7.39
N	(mg kg^{-1})	4.55
P	(mg kg^{-1})	2.75
K	(mg kg^{-1})	128
Ca	(mg kg^{-1})	1260
Mg	(mg kg^{-1})	568
Na	(mg kg^{-1})	43
CEC cmol (+) kg^{-1}		11.5

3.2.3 Description of treatments and experimental design

The four legumes: cowpea, dry bean, groundnut and soya bean were used as test crops. Maize was used as a reference plant and was planted 5 m away from the trial. Sixteen treatment combinations were used (Table 3.2). The trial was laid-out in a complete randomized block design in factorial arrangement (4 legumes x 2 inoculants x 2 fertiliser = 16 treatments) with four replications. The total area for the experiment was 2565 m^2 (95 m x 27 m). The plot sizes were 27 m^2 (5.4 m x 5 m) giving the total area for each replication of 513 m^2. Each plot comprised of six rows of 5 m each.

3.2.4 Seeding

Seeding was done manually and the following varieties were used for each:

- ✓ Bechuana white for cowpea;
- ✓ RS 6 for dry bean;
- ✓ PAN 1454R for soya bean
- ✓ Tufa for groundnut

To ensure that plant population was the same for each crop, plants were planted at adensity of 111111 plants ha^{-1} for each crop determined using equation 3.1

$$p/ha = \frac{np/m \times 10\,000}{sp\ (m)} \qquad 3.1$$

Where np is number of plant per meter and sp is spacing

Table 3.2: Treatment combinations (4 legumes x 2 inoculants x 2 fertiliser) of the four legumes that was employed in the field and glasshouse experiments

Observations	Treatments	Designate	Description
1	T1	So	Soya beans with no inoculant and N
2	T2	Sin	Soya beans with inoculant and no N
3	T3	SNt	Soya beans with N fertiliser and no inoculants
4	T4	SinNt	Soya beans with inoculant and N fertilizer
5	T5	CPo	Cowpea with no inoculant and N fertilizer
6	T6	CPin	Cowpea with inoculant and no N
7	T7	CPNt	Cowpea with N fertiliser and no inoculants
8	T8	CPinNt	Cowpea with inoculant and N fertilizer
9	T9	DBo	Dry bean with no inoculant and N
10	T10	DBin	Dry bean with inoculant and no N
11	T11	DBNt	Dry bean with N fertiliser and no inoculants
12	T12	DBinNt	Dry bean with inoculant and N fertilizer
13	T13	Go	Groundnut with no inoculant and N
14	T14	Gin	Groundnut with inoculant and no N
15	T15	GNt	Groundnut with N fertiliser and no inoculants
16	T16	GinNt	Groundnut with inoculant and N fertilizer
17	-	Prefs	Reference plant

3.2.5 Rhizobium inoculation and seeding

Inoculation was done only in treatments where this was required (Table 3.2) and all legumes were inoculated with the inoculant recommended for the respective legume (solid) namely,

- ✓ Stimulplant® for soya bean
- ✓ Stimulplant®dry Bean
- ✓ Stimulplant® for groundnut
- ✓ Stimulplant for cowpea

3.2.6 Fertiliser application

All treatment combinations received phosphorus at optimal levels, while K was reported adequate according to the soil analyses results. Nitrogen was only applied to specific treatments to determine the minimum accretion of whether N fertiliser will influence the BNF. The sources of N and P were ammonium sulphate and superphosphate, respectively. Fertilisers were applied at planting time at rates of 33 kg P ha^{-1} and 70 kg N ha^{-1} for dry bean; 31 kg P ha^{-1} and 40 kg N ha^{-1} for soya bean, 0 kg P ha^{-1} and 35 kg N ha^{-1} respectively for groundnut and cowpea.

3.3 Data collection

3.3.1 Total number of nodules

The total number of nodules (TNN) was collected from seven plants per plot in two outer rows. This was done in order to prevent excessive harvesting of the plants in the two middle rows which were for seed yield. Collection of the nodules was carried out the during the following crop growth stages: at full flowering, full pod formation, and at physiological maturity. Plants were carefully dug out to avoid losing some nodules in the soil with a spade and care was taken to ensure that the roots were intact. Harvested plants were packed in plastic bags and transported in a cooler box to the laboratory where the roots were cleaned and the nodules carefully removed. The number of viable (VN) and non-viable (NVN) nodules were determined from the same plant. However, for the determination of viability, the nodules were bisected with stylus and a pinkish colour was used to determine competence and non-pinkish colour for non-competence.

3.3.2 Biomass production

One of the main sources of organic matter in a farm is crop biomass. The organic matter consists of all plant residues (stems, leaves and roots), animal manure and microbes in different stages of decomposition, until the formation of humus, which is a very stable part of decomposed materials. One of the main soil quality indicators is organic matter. Soils with satisfactory levels of organic matter are more suitable for the cultivation of plants, because of the better physical, chemical and biological characteristics (Guchert and Roussenq, 2007).Crop biomass was determined at full flowering, full pod formation and physiological maturity in the two outer rows. Seven plants were carefully dug out and cleaned in the laboratory, thereafter partitioned in root and shoot and their fresh mass determined. Sub-samples were collected for oven-drying at 65°C to a constant weight to determine dry biomass in the field. Sampling for biomass production during pod formation under field conditions was determined only in shoots.

3.3.3 Determination of amount of fixed nitrogen in the soil by different legumes

For the determination of ^{15}N, seven randomly selected plants per plot were collected during full flowering, full pod formation and physiological maturity by natural abundance technique of the ^{15}Nisotope (Shearer and Kohl, 1986). Plants were divided into shoots and roots, and then were weighed separately and dried in an oven at 65°C until a constant weight was attained. After drying the samples were milled in a Wiley grinder and passed through a 0.853 mm sieve, to determine ^{15}N in mass spectrometer in the laboratory using 2 mg of plant material. The reference plant was collected at each stage of legumes sampling, and dried in an oven at 65°C, there after it was milled in a Wiley grinder (Figure 3.1) to pass through a 0.853 mm sieve. Aliquots of approximately 1.2 mg of plant material were weighed into tin capsule that were pre-cleaned in toluene to determine ^{15}N in mass spectrometer.

Figure 3.1: Wiley grinder for oven-dried plant samples

The proportion of N in plants fixing N from the Air (% Ndfa) by BNF process was calculated using the equation 2.7 (Shearer and Kohl 1986; (Unkovich *et al.* 2008):

$$\%Ndfa = \frac{\delta^{15}N \text{ of reference plant} - \delta^{15}N \text{ of } N_2 \text{ fixing legume}}{\delta^{15}N \text{ of reference} - B} \times 100 \qquad (2.7)$$

'Where: B' is the δ15N of shoots of legumes that are fully dependent upon N2 fixation and sampled at the same growth stage as the field plants. The B value replaces the value of atmospheric N_2 as it incorporates the isotopic fractionation associated with N_2 fixation (Unkovich *et al.*, 2008). In this study, the B values that were used were −1.35‰ for groundnuts (Okito *et al.*, 2004), -1.5 ‰ for cowpea (Nguluu *et al.*, 2001) -1.3‰ for soya bean (Bergersen *et al.*,1989) and -2.5‰ for dry bean(Yoneyama *et al.,* 1986).

The amount of N-fixed per hectare was estimated as the product of fixed N in shoots + pods + roots per plant and plant density per hectare. The plant density in each treatment was estimated by counting the number of plants in an area of $4m^2$. The amount of N fixed was computed using the following equation:

$$N\ fixed = \frac{\%Ndfa \times total\ N\ in\ fixing\ crop}{100} \qquad (2.8)$$

The tissue N content of each plant was determined as the product of %N and tissue masses as described by Pausch *et al.* (1996), using the formula beneath:

Total N (mg $plant^{-1}$) = dry matter of plant x % N in plant tissue

3.3.4 Statistical analyses

Data collected were subjected to analyses of variance using SAS Software (SAS, 1999). Shapiro-Wilks test was performed on the standardised residuals to test for deviations from normality (Shapiro-Wilk, 1965). Means of significant effects were compared using Student's t-LSD (Least Significant Differences) at the 5% level of significance. Analyses of variance and all the above analyses were performed using SAS 9.2 Statistical Software.

The correlation test was performed at a significance level of 5% to verify if there was any association (negative or positive) between total number of nodules, number of viable nodules, number of non-viable nodules, and total nitrogen in the plant, fixed nitrogen and percentage of nitrogen from atmosphere. Interpretation of the results was based on Karl Person correlation values (Table 3.3) table

Table 3.3 Coefficient of correlation of Karl Pearson

Coefficient values (r)	Intepretation
$r = -1$	The relationship is perfect and negative (there is an inverse proportionally
$-1 < r < -0,5$	The relationship is negative and strong
$-0,5 \leq r < 0$	The relationship is negative and weak
$r = 0$	Indicates the absence of relationship
$0 < r \leq 0,5$	The relationship is positive and weak
$0,5 < r < 1$	The relationship is positive and strong.
$r = 1$	The relationship is perfect and positive (there is direct proportionality)

CHAPTER 4 : RESULTS

4.1 Glasshouse Study

4.1.1 Number of nodules

4.1.1.1 Interaction effecton nodulation

The effects of interaction (crop x treatment) was notstatistically significant($P<0.05$) across the different sampling intervals for the total number nodules and viable nodules, but significant on the number of non-viable nodules during 100% pod formation and physiological maturity stages for dry bean and groundnut (Table 4.1). Significantly lower number of non-viable nodules per plant at 100% pod formation was observed from fertilised groundnut and dry bean in the combined treatment (inoculation x N fertiliser). During physiological maturity, a significantly higher total number of non-viable nodules was observed from plants without inoculation and N application for groundnut and plants that received N fertiliser for dry beans.

Table 4.1: Interaction effects of treatments and crops on nodulation at different sampling periods

		100% flowering			100% pod formation			Physiological maturity		
	Treatments	**Total nodules**	**Viable nodules**	**Non-viable nodules**	**Total nodules**	**Viable nodules**	**Non-viable nodules**	**Total nodules**	**Viable nodules**	**Non-viable nodules**
Cowpea	**No inoculation**	6	4	2	4	2	2c	8	2	6f
	Inoculation	28	22	6	18	13	5c	8	3	5f
	N fertiliser	10	9	1	10	1	9c	3	1	2f
	Inoculation x N fertiliser	20	14	6	21	11	10c	5	2	3f
	Mean	**16**	**12.3**	**3.8**	**13.3**	**6.6**	**6.5**	**6**	**2**	**4**
Dry bean	**No inoculation**	14	7	7	27	10	17bc	13	3	10fed
	Inoculation	57	22	35	75	24	51a	18	11	7fe
	N fertiliser	7	2	5	36	14	22bc	32	2.5	29.5abc
	Inoculation x N fertiliser	28	16	12	4	2	2c	22	6	16fedc
	Mean	**26.5**	**11.8**	**14.8**	**35.5**	**12.5**	**23**	**21.3**	**5.6**	**15.6**
Groundnut	**No inoculation**	17	11	6	53	23	40ba	62	20	42a
	Inoculation	43	31	12	76	53	23bc	63	31	32ba
	N fertiliser	17	13	4	37	22	15c	47	22	25bac
	Inoculation x N fertiliser	38	33	5	61	36	25bc	57	29	28bac
	Mean	**28.8**	**22**	**6.8**	**56.8**	**33.5**	**25.8**	**57.3**	**25.5**	**15.8**
Soya bean	**No inoculation**	12	9	3	17	7	10c	21	5	16fedc
	Inoculation	29	27	2	43	40	3c	35	14	21bedc
	N fertiliser	13	12	2	15	13	3c	14	4	10fed
	Inoculation x N fertiliser	13	12	1	29	26	3c	26	10	17fedc
	Mean	**16.8**	**15**	**2**	**26**	**21.5**	**4.8**	**24**	**8.3**	**16**
Means		22	15,5	6.8	32.9	18.6	15.0	27.1	10.3	15.8
CV(%)		29.2	26.8	24.1	23.2	26.2	30.1	28.9	27.1	22.9
$LSD_{T(0.05)}$		26.49	16.77	14.08	31.27	21.59	23.33	16.77	8.42	15.02

Means within the column followed by the same letter are not significantly different at 5% probability level

4.1.1.2 Effects of treatments on nodulation

Significant differences ($P<0.05$) were observed amongst treatments at both 100% flowering and 100% pod formation sampling stages (Table 4.2). A higher total number of nodules and viable nodules were observed from inoculated plants irrespective of the legume species. Equally, significantly higher numbers of non-viable nodules were observed only during 100% flowering when crops were inoculated and in the combined use of inoculant x N fertiliser. At physiological maturity, the effect of N application and inoculation as well as their interaction did not exert statistically significant effects on the total number of nodules and non-viable nodules per treatment. In the same sampling stage, significantly higher numbers of viable nodules were observed when the different crop species were inoculated relative to the other treatments.

4.1.1.3 Crop effect on nodulation

At the 100% flowering stage, the effect of crop (species) did not appear toexert statistically significant effects on total number of nodules, but was significant ($P <0.05$) for the number of viable and non-viable nodules (Table 4.3). Generally, a higher total number of nodules across the crops was achieved with groundnuts, while cowpeas yielded the lowest. Significantly higher total number of viable nodules was observed from groundnuts and soya beans, while dry beans yielded significantly superior total number of non-viable nodules (Table 4.3). At 100% pod formation and physiological maturity sampling stages, significant differences ($P <0.05$) were observed amongst the crops. Generally, significantly more total number of nodules, viable and non-viable, were obtained from groundnuts, while it was consistently significantly lower with cowpea.

Table 4.2: Effect of inoculation and fertiliser on nodulation of different legume species at different sampling intervals

	100% flowering			100% pod formation			Physiological maturity		
Treatments	**Total nodules**	**Viable nodules**	**Non-viable nodules**	**Total nodules**	**Viable nodules**	**Non-viable nodules**	**Total nodules**	**Viable nodules**	**Non-viable nodules**
No inoculation	12b	8b	4b	28b	11b	17	26	8b	18
Inoculation	39a	25a	14a	53a	33a	20	31	15a	16
N fertiliser	12b	9b	3b	24b	12b	12	24	7b	17
Inoculation x N fertiliser	25b	19a	6ba	29b	19b	10	27	11ba	16
Mean	22.0	15.3	6.8	33.5	18.8	14.8	27.0	10.3	16.8
CV (%)	28.2	25.8	24.1	23.2	28.2	30.1	29.3	24.7	29.9
$LSD_{T (0.05)}$	13.24	8.38	7.04	15.636	10.79	11.67	8.39	4.21	7.51

Means within the column followed by the same letter are not significantly different at 5% probability level

Table 4.3: Effect of legume species on nodulation at different sampling intervals

	100% flowering			100% pod formation			Physiological maturity		
Crops	**Total nodules**	**Viable nodules**	**Non-viable nodules**	**Total nodules**	**Viable nodules**	**Non-viable nodules**	**Total nodules**	**Viable nodules**	**Non-viable nodules**
Cowpea	16	12b	3.5b	13c	7c	6b	6c	2c	4c
Dry bean	27	12b	15a	35b	12cb	23a	21b	6cb	15b
Groundnut	28	22a	6b	57a	33a	24a	58a	26a	32a
Soya bean	17	15ba	2b	26cb	21b	5b	24b	8b	16b
Mean	22	15.3	6.6	32.8	18.3	14.5	27.3	10.5	16.8
Cv(%)	28.2	25.8	24.1	23.2	28.2	30.1	29.3	24.7	29.9
$LSD_{T (0.05)}$	13.24	8.38	7.04	15.64	10.79	11.67	8.39	4.21	7.51

Means within the column followed by the same letter are not significantly different at 5% probability level

4.1.2 Effects of inoculation and N fertiliser on BNF

4.1.2.1 Interaction effects

The effects of interaction (crop x treatment) was statistically significant($P<0.05$) for the %Ndfa across the three sampling intervals, while no interaction effects were found on %N and %C (Table 4.4). Higher %Ndfa was observed from inoculated plants irrespective of the leguminous species, while lower values were obtained from treatments without inoculant and N application for groundnut, soya bean and cowpea, and with N application for dry bean at 100% flowering gave lower %Ndfa. At 100% pod formation, lower %Ndfa resulted from uninoculated groundnut, soya bean and dry bean, and with N application for cowpea. At physiological maturity, lower %Ndfa were observed from plants without inoculation and N application.

The interaction effect of crops x treatments was significant on the total amount of N and total N fixed at 100% flowering and 100% pod formation (Table 4.5). At 100% flowering, significantly higher amounts of total N from shoots and roots was achieved. Similarly, total N fixed was also achieved when crops were inoculated irrespective of the legume. At 100% pod formation, higher total amounts of N fixed were observed from inoculated plants irrespective of the legume species, than when used in combination.

At physiological maturity, there was marginally higher total N from shoot and root when crops were inoculated for groundnuts and cowpeas; and when crops were treated with rhizobium inoculant and N fertiliser for soya beans and dry beans. Equally, marginally higher total N fixed was achieved from inoculated groundnuts, dry beans and cowpeas as well as from soya beans that were inoculated and N fertilised. At both sampling stages, the effect of interaction (crop X treatment) in the same crop was not statistically significant on C/N ratio (Table 4.5).

Table 4.4: Interactive effect of treatments x crops on the %Ndfa, %N and %C at three sampling periods under glasshouse conditions

		100% flowering			100% pod formation			Physiological maturity		
	Treatments	%Ndfa	%N	%C	%Ndfa	%N	%C	%Ndfa	%N	%C
Cowpea	No inoculation	16.8f	3.1	36.8	38.2cde	0.8	38.4	48.6de	2.4	38.2
	Inoculation	55.0abcde	3.3	35.3	48.9bcd	1.0	37.3	73.2bcd	2.4	37.9
	N fertiliser	31.6cdef	3.2	37.0	28.2de	1.0	37.4	49.7de	2.4	37.3
	Inoculation x N fertiliser	45.4bcdef	3.3	37.5	47.9bcd	1.1	37.1	49.4de	2.8	38.1
	Mean	**37.2**	**3.2**	**36.7**	**40.8**	**0.98**	**37.6**	**55.2**	**2.5**	**37.9**
Dry bean	No inoculation	64.6abc	2.1	36.6	27.0de	0.9	37.4	33.8e	2.1	37.9
	Inoculation	86.2a	2.3	35.8	60.4bac	1.5	38.3	68.5bcde	2.2	36.4
	N fertiliser	37.5cdef	2.7	37.4	25.1de	1.2	36.4	53de	1.8	36
	Inoculation x N fertiliser	61.2abcd	2.6	37.4	58.7abc	1.4	37.8	65.7bcde	1.8	36.8
	Mean	**62.4**	**2.4**	**36.8**	**42.8**	**1.25**	**37.5**	**55.3**	**2.0**	**36.8**
Groundnut	No inoculation	67.8abc	2.7	38.0	69.0ab	1.3	37.0	56cde	3.0	43.2
	Inoculation	87.0a	3.2	36.8	78.3ª	1.2	39.4	136.3a	3.1	42.6
	N fertiliser	79.5ba	2.7	38.3	71.5ab	1.2	38.8	99.3b	3.1	43.2
	Inoculation x N fertiliser	84.3a	2.6	35.7	77.7ª	1.3	38.2	97.2b	3.0	43.2
	Mean	**79.7**	**2.8**	**37.2**	**74.1**	**1.25**	**38.4**	**97.2**	**3.1**	**43.1**
Soya bean	No inoculation	25.8def	3.4	40.3	29.1de	0.9	42.4	89.5cb	3.3	40.8
	Inoculation	57.7abcd	3	38.9	69.9ab	1.3	41.7	95.9b	3.6	42.3
	N fertiliser	42.1cdef	2.8	39.4	56.3abc	0.9	39.8	95.5b	3.2	41.1
	Inoculation x N fertiliser	19.5ef	2.6	39.5	19.0e	0.9	39.3	93.9b	4.1	42.3
	Mean	**36.3**	**3.0**	**39.5**	**43.6**	**1**	**40.8**	**93.7**	**3.6**	**41.6**
Mean		53.9	2.9	37.5	52.7	1.1	38.5	75.5	2.8	39.9
CV(%)		27.5	15.4	4.2	35.0	35.9	4.7	33.8	19.4	3.9
$LSD_{T\,(0.05)}$		36.46	0.625	2.2724	25.10	0.57	2.58	36.29	0.767	2.23

Means within the column followed by the same letter are not significantly different at 5% probability level

Table 4.5: Interactive effects of crop x treatment on the total N, total N fixed and C/N ratio during three sampling periodsunder glasshouse conditions

		100% flowering			100% pod formation			Physiological maturity		
	Treatments	Total N in shoot +root ($kgha^{-1}$)	Total N fixed N in shoot +root ($kg\ ha^{-1}$)	C/N	Total N in shoot +root ($kg\ ha^{-1}$)	Total N fixed N shoot +root ($kg\ ha^{-1}$)	C/N	Total N in shoot +root ($kg\ ha^{-1}$)	Total N fixed N shoot +root ($kg\ ha^{-1}$)	C/N ratio
Cowpea	No inoculation	70.25b	11.0bc	12.1	34.8	9.37bcde	18.0	113.1	70.2	16.3
	Inoculation	141.98a	51.0a	10.7	45.4	22.365a	16.1	111.9	76.9	16.0
	N fertiliser	96.65ba	19.5bc	11.7	32.3	9.43bcde	16.7	54.4	25.9	15.5
	Inoculation x N fertiliser	79.11bc	29.7b	11.7	34.2	13.5abcd	29.1	96.2	51.0	14.4
	Mean	**97.0**	**27.8**	**11.6**	**36.7**	**13.7**	**20.0**	**93.9**	**56.0**	**15.6**
Dry bean	No inoculation	8.34e	4.57c	17.8	3.53	1.88de	22.8	10.3	4.10	17.2
	Inoculation	10.59e	8.59c	16	7.81	5.38de	19.4	10.6	6.96	17.6
	N fertiliser	8.58e	3.65c	14.2	6.99	17.8abc	18.9	5.85	2.62	19.0
	Inoculation x N fertiliser	10.16e	5.08c	14.8	12.5	19.885ab	24.0	16.5	4.92	22.0
	Mean	**9.4**	**5.5**	**15.7**	7.7	**11.2**	**21.3**	**10.8**	**4.7**	**19.0**
Groundnut	No inoculation	7.12e	6.04c	12.3	27.6	20.2ab	17.0	76.3	59.0	12.7
	Inoculation	14.34de	11.37bc	13.8	31.7	22.5a	17.6	96.1	91.1	14.1
	N fertiliser	10.44e	7.06c	14.2	25.5	17.8abc	14.4	64.6	83.8	13.7
	Inoculation x N fertiliser	35.30ecd	30.4ab	12.5	27.4	19.9ab	23.1	81.4	78.2	14.6
	Mean	**16.8**	**13.7**	**13.2**	**28.1**	**20.1**	**18.0**	**79.6**	**78.0**	**13.8**
Soya bean	No inoculation	30.06cde	6.37c	12.2	18.3	4.96de	14.5	32.6	29.6	12.4
	Inoculation	33.11cde	13.95bc	12.9	28.2	20.1ab	14.7	43.6	35.3	12.0
	N fertiliser	21.57cdef	4.77c	14.4	15.6	6.8cde	16.9	26.9	25.4	13.0
	Inoculation x N fertiliser	23.03cde	8.19c	15.7	15.3	3.95de	17.7	46.9	43.7	13.71
	Mean	**26.9**	**8.3**	**13.8**	**19.4**	**9.0**	**16.0**	**37.5**	**33.5**	**12.8**
Mean		35.6	13.4	13.5	22.9	13.8	18.8	55.4	40.0	15.3
CV(%)		22.7	29.6	16.7	28.9	23.2	30.1	26.5	24.5	17.1
$LSD_{T\ (0.05)}$		57.193	20.662	2.0141	25.775	11.69	9.2994	62.01	44.319	3.5804

Means within the column followed by the same letter are not significantly different at 5% probability level

4.1.2.2 Treatment effects on %Ndfa, %N and %C

The %Ndfa differed significantly ($P < 0.05$) between treatments during 100% flowering and 100% pod formation under glasshouse conditions (Figures 4.1 and 4.2). At 100% flowering, the inoculated plants showed higher values %Ndfa. At 100% pod formation, significantly higher percentage of Ndfa was obtained when plants were inoculated. With respect to % N and %C, the main effect of N fertiliser application and inoculation as well as their combination exerted statistically significant effects only on %C at 100% flowering stage. A lower %C was observed from inoculated treatments and higher values from un-inoculated plants (Figure 4.3). Generally, treatments that were inoculated had slightly higher concentrations of N (%N). More so, the combined use of inoculant and N fertiliser showed slightly higher concentrations of carbon.

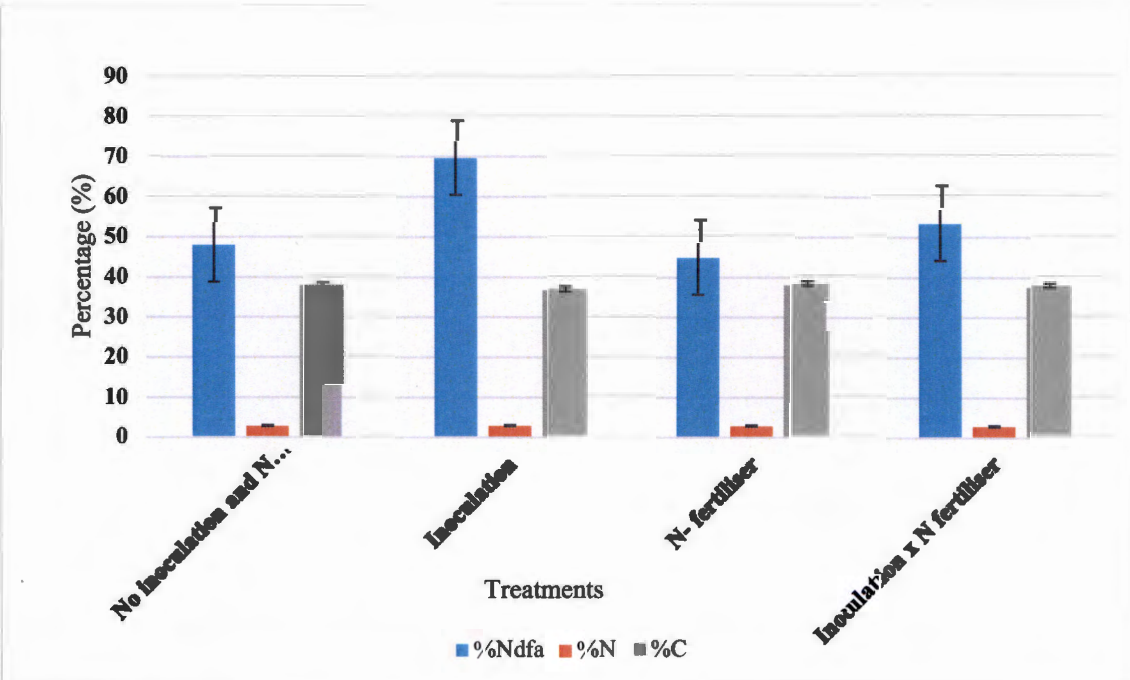

Figure 4.1: Effect of treatment %Ndfa, percentage of N, %N and percentage of carbon (%C) during 100% flowering under glasshouse conditions

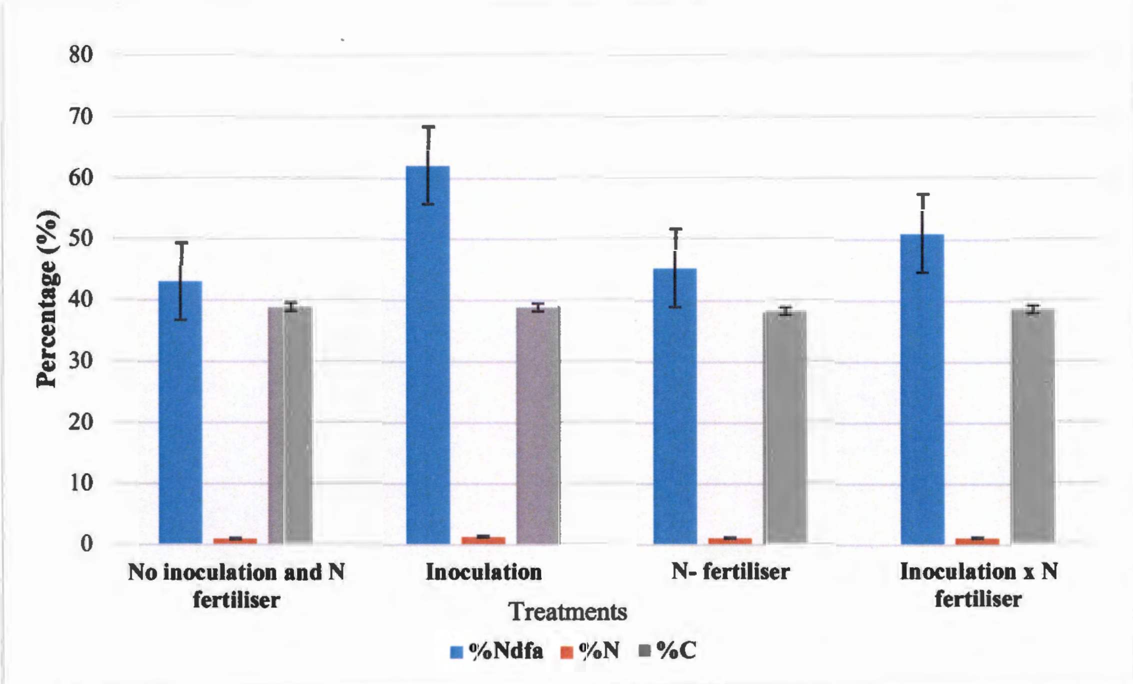

Figure 1.2: Effect of treatment on the Percentage of N derived from atmosphere (%Ndfa), Percentage of N (%N) and percentage of carbon (%C) during full pod formation

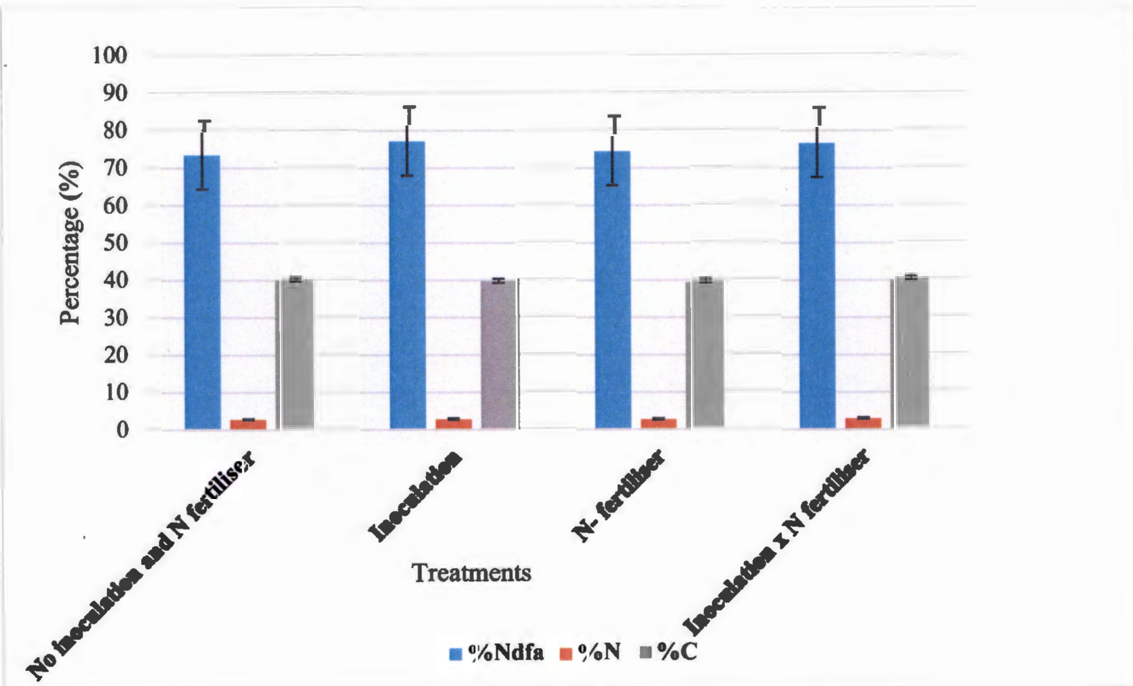

Figure 4.3: Effect of treatment on %Ndfa, %N and %C during physiological maturity under glasshouse conditions

The total amount of N from shoots and roots did not differ significantly among treatments across all sampling intervals under glasshouse conditions (Figures 6 to 7). Generally, the total N from treatments that received inoculation was consistently higher relative to when no inoculation and N fertiliser were applied. The total amount of nitrogen fixed by different crops differed significantly ($P<0.05$) across the sampling periods, except at physiological maturity (Figures 4.4 to 4.5). As shown in Figure 4.4, at 100% flowering, higher amounts of total nitrogen fixed (21.22 kg ha^{-1}) were achieved from treatments with inoculant, while lower values (6.99 kg ha^{-1}) were achieved from treatments without application of inoculant and N fertiliser. At 100% pod formation, significantly higher amounts of total N fixed (16.9 kg ha^{-1}) were achieved from treatments with inoculant application while lower (8.96 kg ha^{-1}) were achieved from treatment with N application.

Table 4.6: Treatment effect on the amount of fixed N during 100% flowering under glasshouse conditions

	100% flowering		100% pod formation	
Treatments	Total N shoot+root (kg/ha)	Total N fixed shoot+root (kg/ha)	Total N shoot+root (kg/ha)	Total N fixed shoot+root (kg/ha)
No inoculation	28.9	7.0c	21.1	9.1b
Inoculation	47.5	21.2[a]	27.2	16.9a
N fertiliser	34.3	8.8bc	20.1	9.0b
Inoculation x N fertiliser	39.4	18.3ba	23.4	11.8ba
Mean	37.5	13.8	23.0	11.7
Cv(%)	28.5	24.5	31.2	29.3
$LSD_{T\,(0.05)}$	28.6	10.3	12.9	5.8

Means within the column followed by the same letter are not significantly different at 5% probability level

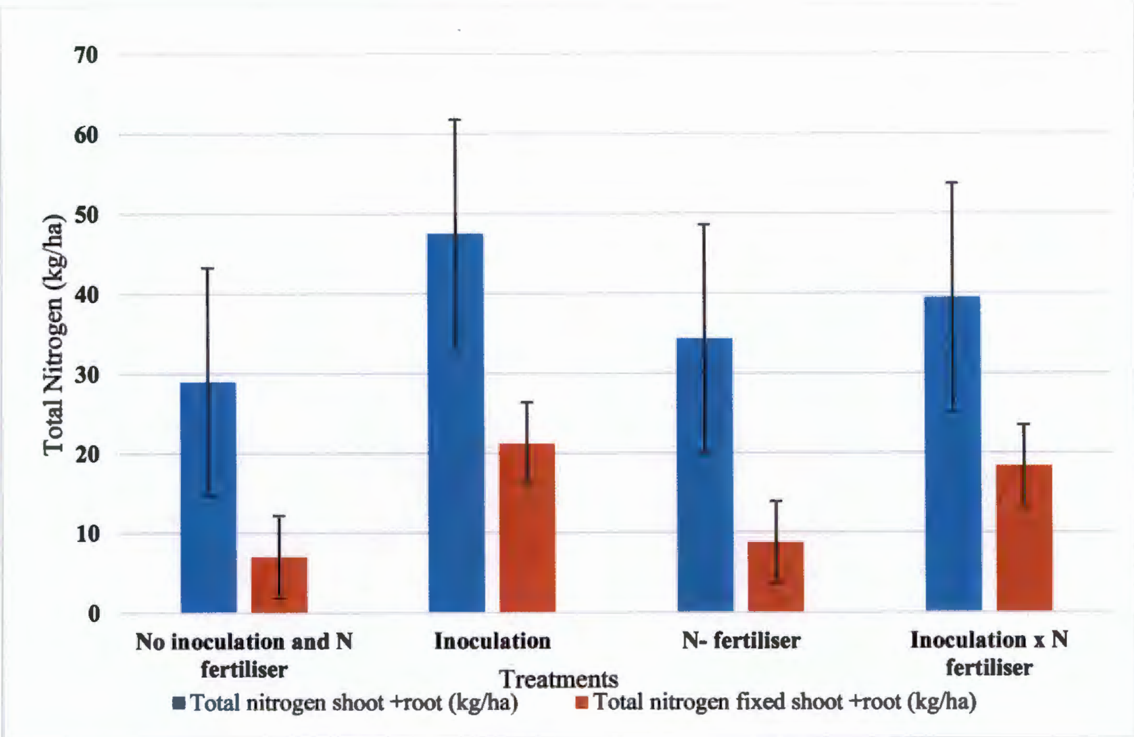

Figure 4.4: Treatment effect on the amount of fixed N during 100% flowering under glasshouse conditions.

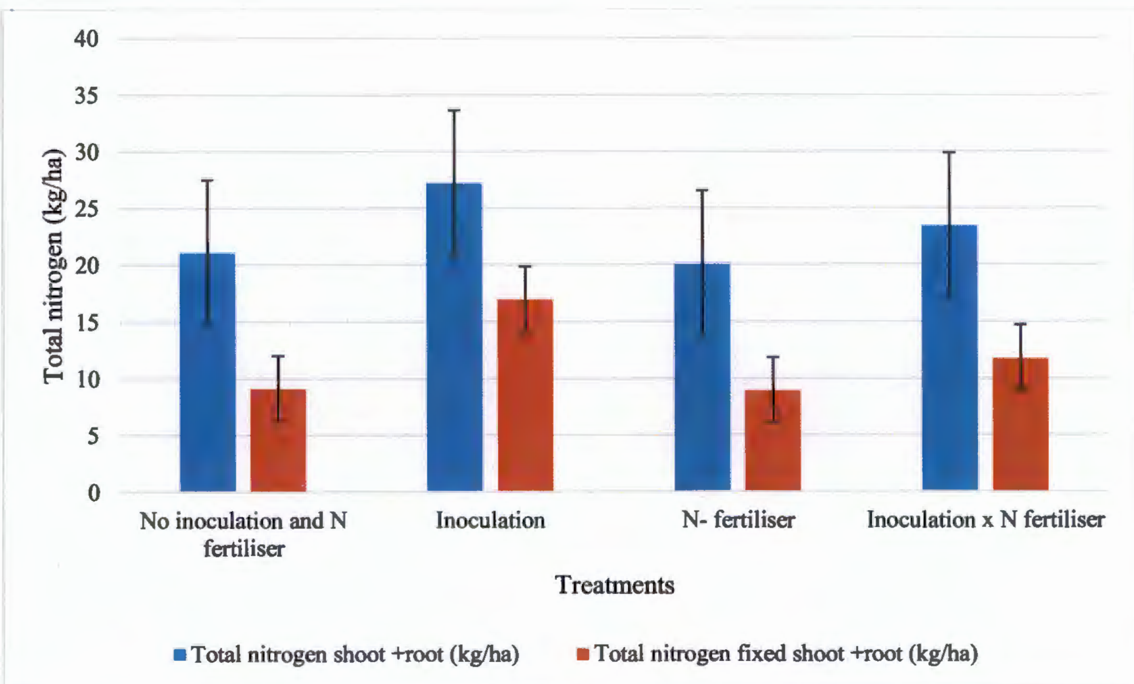

Figure 4.5: Total N and total N fixed in shoots + roots during 100% pod formation under glasshouse conditions

At physiological maturity, the effect of N application and inoculation as well as their interaction did not exert statistically significant effects on total N and total N fixed (Table 4.7). Generally, inoculated treatments had a mean of 58.1 kg ha^{-1} of total N (shoot + root) while 57.7 kg ha^{-1} of total N (shoots + roots) was obtained from plants without application of inoculant and N fertiliser across all crop types. A higher amount of total nitrogen fixed (51.84 kg ha^{-1}) was achieved from treatment with inoculant application while a lower value (36.4 kg ha^{-1}) was achieved from treatment without application of inoculant and N fertiliser.

Table 4.7: Total N and total N fixed N at physiological maturity under glasshouse conditions

Treatments	Total N in shoot +root (kg ha^{-1})	Total N fixed N in shoot +root (kgha^{-1})
No inoculation	57.7	36.4
Inoculation	58.1	51.8
N fertiliser	45.8	34.4
Inoculation x N fertiliser	60.2	44.4
Mean	55.5	41.8
Cv(%)	28.5	24.5
LSD	31.0	22.2

4.1.2.3 Treatment effect on C/N ratio

The effect of treatment was not significant on the C/N ratio during different sampling intervals (Table 4.8). The C/N ratios varied between 13.1 and 14.2 at 100% flowering, 19.5 and 20.9 at 100% pod formation, while they varied between 20.5 and 22.5 at physiological maturity.

Table 4.8: Effect of different treatments on C/N ratios at three sampling periods under glasshouse conditions

Treatments	C/N ratios		
	100% flowering	100% pod formation	Physiological maturity
No inoculation	14.2	20.9	20.5
Inoculation	13.8	19.5	21.3
N fertiliser	14.1	19.9	22.5
Inoculation x N fertiliser	13.1	20.3	21.2
Mean	13.8	20.1	21.4
CV(%)	16.7	30.1	17.1
$LSD_{T(0.05)}$	**1.03**	**2.02**	1.89

4.1.2.4 Crop effect on %Ndfa, %N and %C

There were significant ($P<0.05$) differences on the %Ndfa, %N and %C between crops across the sampling intervals, with the exception of %C during flowering and %N at 100% pod formation under glasshouse conditions (Ffigures 4.6 to 4.8). At 100% flowering, the %Ndfa from groundnuts and dry beans was significantly higher than soya beans and cowpeas. During pod formation, the %Ndfa from groundnuts was significantly higher than soybeans, cowpeas and dry beans, while at physiological maturity, %Ndfa from groundnuts and soya beans was significantly higher than cowpeas and dry beans.

With regard to the %N, significantly higher values were observed from cowpeas and soya beans respectively, while lower %N were observed from dry beans at 100% flowering stage. At physiological maturity, significantly higher values were observed from soya beans and groundnuts respectively, while lower values were from dry beans. On the %C at 100% pod formation, significantly higher %C values were observed from soya bean than all the other crops. At physiological maturity, significantly higher values were observed from soya bean and groundnut respectively, while lower from dry bean.

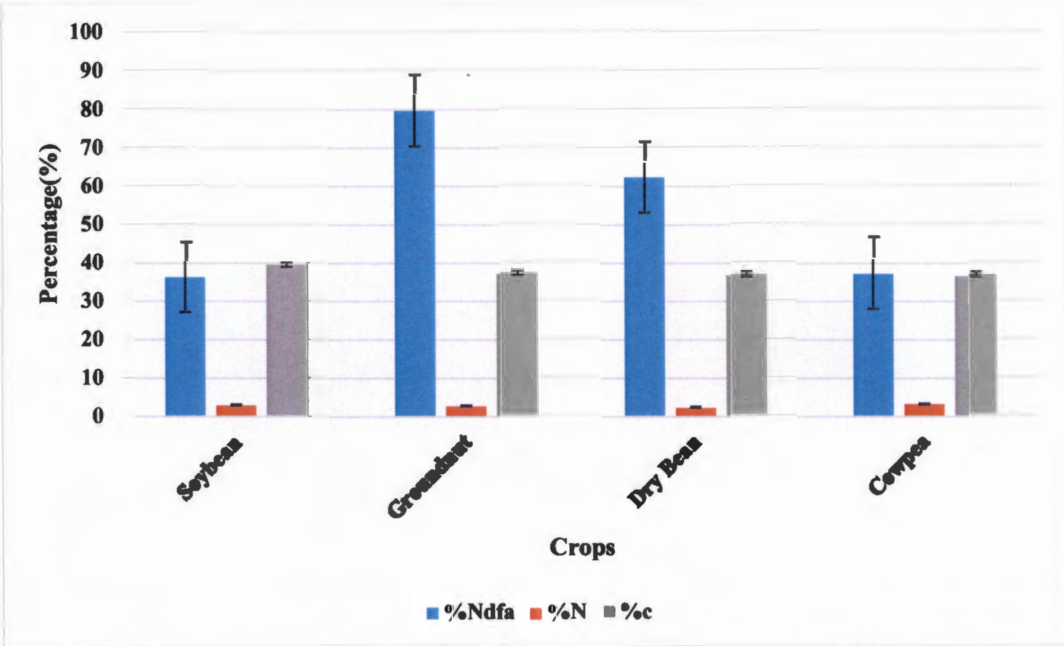

Figure 4.6: Crop effect on %Ndfa, %N and %C during 100% flowering under glasshouse conditions

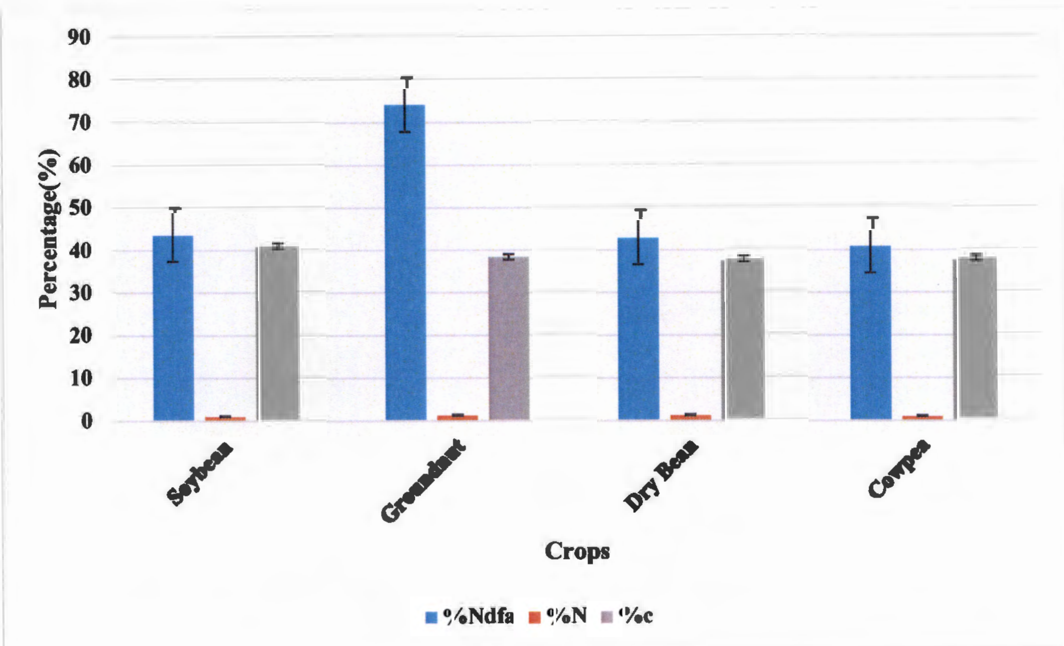

Figure 4.7: Crop effect on %Ndfa, %N and %C during full pod formation under glasshouse conditions

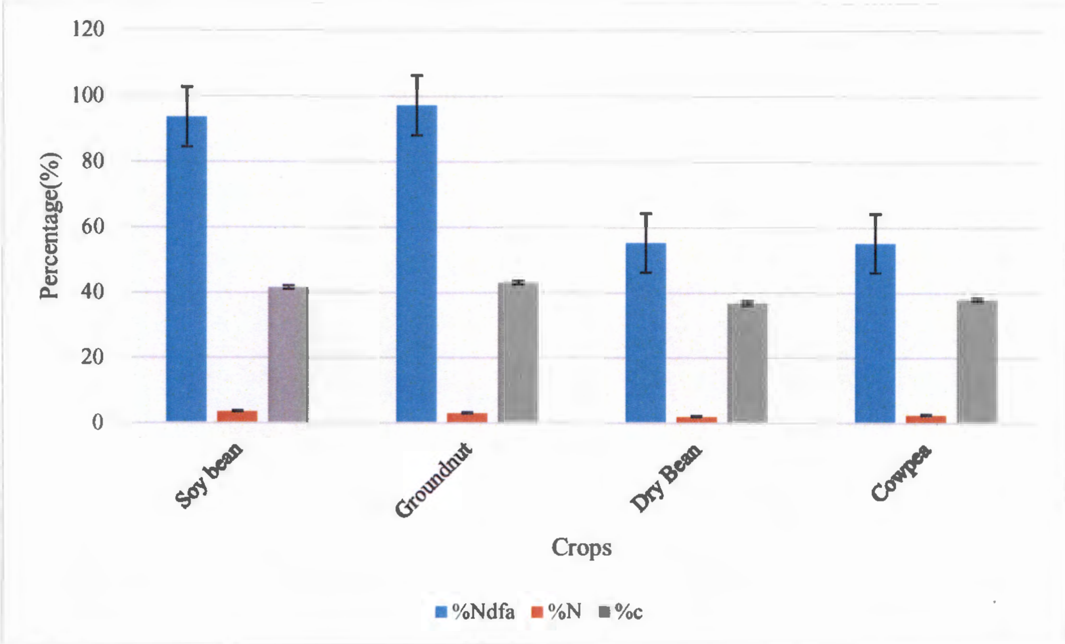

Figure 4.8: Crop effect on %Ndfa, %N and %C during physiological maturity under glasshouse conditions

There were significant ($P<0.05$) differences on the total amount of N (shoot and root), and total amount of N fixed across all sampling intervals under glasshouse conditions (Figures 4.9 to 4.11). At 100% flowering, the total amount of N (shoot and root) and total amount of N fixed from cowpeas was significantly higher than from soya beans, groundnuts and dry beans. During 100% pod formation, the total amount of N (shoot and root), and total amount of N fixed from cowpeas and groundnuts were significantly higher than soya beans and dry beans. At physiological maturity, the total amount of N (shoot and root), and total amount of N fixed from cowpeas and groundnuts were significantly higher than soya beans and dry beans.

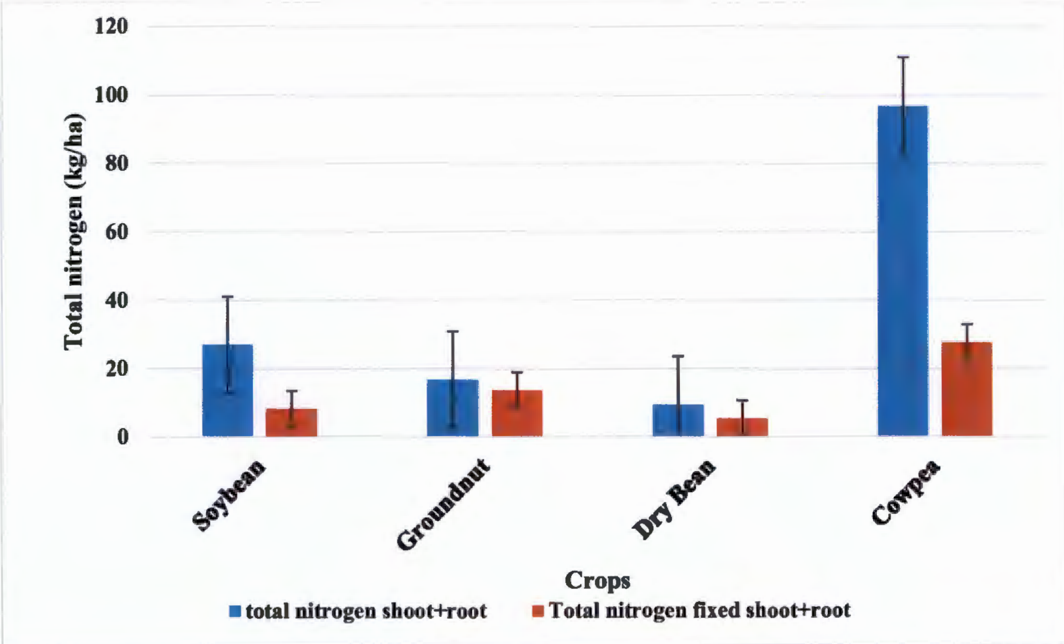

Figure 4.9: Crop effect on total N and total N fixed during full flowering under glasshouse conditions

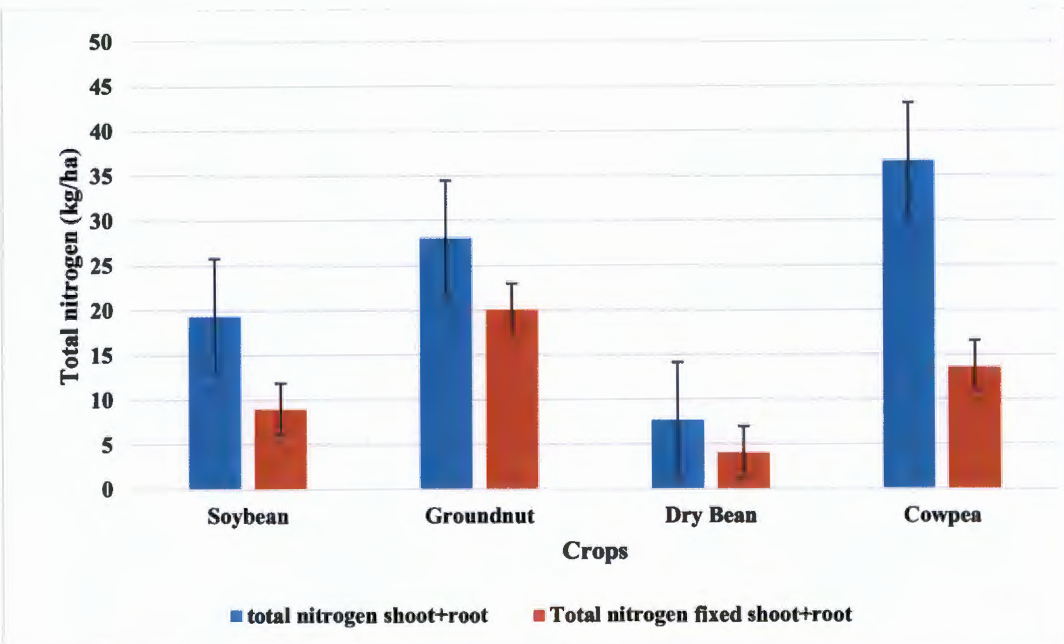

Figure 4.10: Crop effect on total N and total N fixed during 100% pod formation under glasshouse conditions

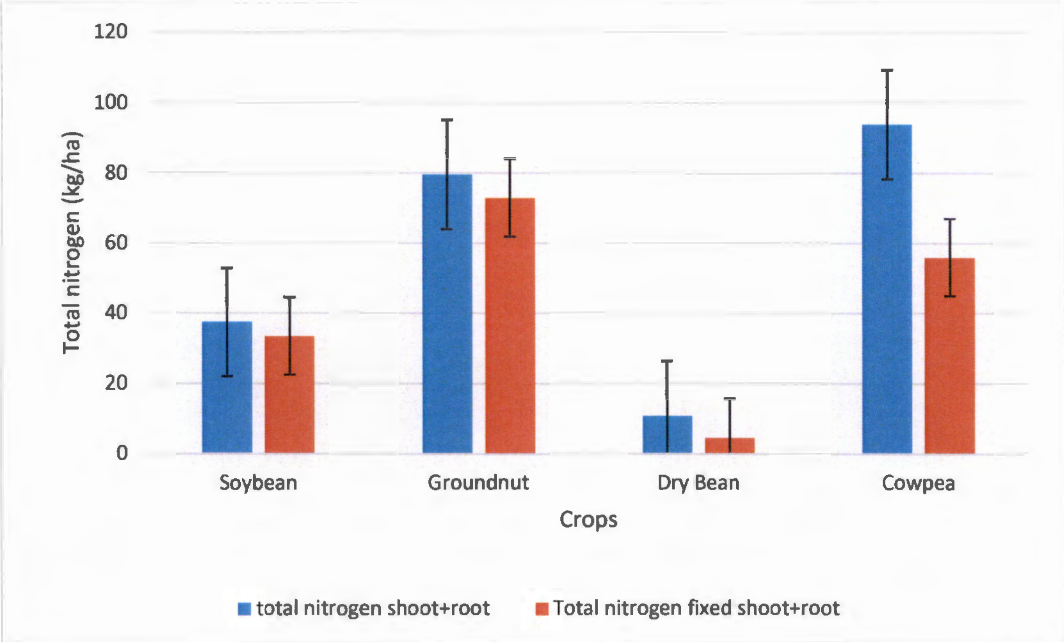

Figure 4.11: Crop effect on total N and total N fixed during physiological maturity under glasshouse conditions

4.1.2.5 Crop effect on C:N ratio

There were significant ($P<0.05$) differences on C/N ratio at both sampling periods under glasshouse conditions (Table 4.9). TheC/N ratio from cowpeas was significantly higher across the sampling stages than from the other crops.

Table 4.9: Effect of crop on C/N ratio at three sampling periods under glasshouse conditions

	C/N		
Crops	**100% flowering**	**100% pod formation**	**Physiological maturity**
Cowpea	16.5a	23.4a	27.5a
Dry bean	14.7b	24.0a	18.5c
Groundnut	12.7c	19.1b	24.3b
Soya bean	11.3d	14.2c	15.1d
Mean	13.8	20.1	21.4
CV(%)	16.1	30.1	17.1
$LSD_{T\,(0.05)}$	1.0321	2.015	1.8898

Means within the column followed by the same letter are not significantly different at 5% probability level.

4.1.2.6 Correlation between BNF assessment parameters

The results showed positive correlations between nodulation and biological N fixation components. As is shown in Table 4.10 strong and positive correlations were observed between TNN, VN and %Ndfa; or in all sampling interval, the higher number of nodules (total nodules and viable nodules) increased as the percentage of N from atmosphere and the amount of N was fixed by plant. Equally there were weak and negative correlations between non-viable nodules and percentage of N from the atmosphere as well as the amount of N fixed in the plant. This means that when the number of non-viable nodule increases the amount of total N fixed and % Ndfa decreases.

Table 4.10: Correlation coefficients between parameters of biological N Fixation under glasshouse conditions

100% flowering	Total N	N fixed	%Ndfa
TNN	0.12	0.24	0.77
VN	0.05	0.46	0.67
NVN	-0.20	-0.06	-0.45
100% pod formation	Total N	N fixed	%Ndfa
TNN	0.10	0.39	0.58
VN	0.13	0.58	0.63
NVN	-0.27	-0.08	-0.34
Physiological maturity	Total N	N fixed	%Ndfa
TNN	0.14	0.47	0.58
VN	0.28	0.61	0.69
NVN	-0.04	0.04	-0.21

TNN Total number of nodules

VN Total number of viable nodules

NVN Non-viable nodule

N Nitrogen

%Ndfa percentage of derived nitrogen from atmosphere.

4.2 Field Experiment

Glasshouse experiments cannot provide estimates of amounts of N_2 fixed that can be extrapolated to the field. Thus, any quantification of ergonomically relevant N_2 fixation must be undertaken in the field. Additionally, any practical testing of a management treatment should be done in the field.

4.2.1 Number of nodules

4.2.1.1 Interaction effect

The effects of interactions were not significant for the total number of nodules and viable nodules (Table 4.11) at 100% flowering. However, at physiological maturity, the effect of interaction amongst legumes species showed significant difference ($p < 0.05$) on number of nodules for groundnuts and dry beans. A higher number of total nodules was observed when crops were inoculated irrespective of the legume species at both 100% flowering and physiological maturity sampling stages. The effects of interactions amongst legumes species showed significant differences ($p < 0.05$) on non-viable nodules for dry beans and groundnuts, respectively. A higher number of non-viable nodules was observed when groundnuts, soybean and cowpea were not inoculated as compared to dry beans that exhibited higher number from inoculated treatment.

Table 4.11: Interaction effect of crops and treatments on nodulation of different legume species at 100% flowering and physiological maturity sampling periods

	Treatments	100% flowering			Physiological maturity		
		Total nodules	Viable nodules	Non-viable nodules	Total nodules	Viable nodules	Non-viable nodules
Cowpea	**No inoculation**	11	8	3f	7h	4ef	3e
	Inoculation	13	10	3f	8h	5f	3e
	N fertiliser	4	2	2f	9h	6f	3e
	Inoculation x N fertiliser	5	2	2f	4h	2f	2e
	Mean	**8.3**	**5.5**	**2.5**	**7**	**4.3**	**2.8**
Dry bean	**No inoculation**	20	4	16[a]	52cdef	18de	34cd
	Inoculation	29	13	16[a]	135a	49a	86a
	N fertiliser	10	2	8d	63cde	35bc	28d
	Inoculation x N fertiliser	11	3	8d	84bc	25cd	59b
	Mean	**17.5**	**5.5**	**12**	**83.3**	**31.8**	**51.8**
Groundnut	**No inoculation**	51	36	15.a	68bcde	18de	50bc
	Inoculation	57	47	10dc	88b	45ab	43bcd
	N fertiliser	44	30	14ab	56def	20d	36cd
	Inoculation x N fertiliser	43	29	14ba	71cbd	33bc	38cd
	Mean	**48.8**	**535.5**	**13.3**	**70.8**	**29**	**41.8**
Soya bean	**No inoculation**	40	30	10bcd	47gef	8ef	39bcd
	Inoculation	46	36	10bcd	51defg	14def	37cd
	N fertiliser	46	38	8d	33f	2f	31cd
	Inoculation x N fertiliser	40	33	7ed	38fg	3f	35cd
	Mean	**43**	**34.3**	**8.8**	**42.3**	**6.8**	**35.5**
Mean		29.4	20.2	9.1	50.9	17.9	32.9
Cv (%)		28.6	28.2	30.5	31.0	26.8	30.0
$LSD_{T\ (0.05)}$		11.93	11.07	4.54	22.30	11.83	20.08

Means within the column followed by the same letter are not significantly different at 5% probability level

4.2.1.2 Treatment effect

The number of nodules showed significant differences ($p<0.05$) amongst treatments at both 100% flowering and physiological maturity sampling stages (Table 4.12). At 100% flowering, significantly higher total number of nodules and viable nodules per plant were observed irrespective of the legume species from inoculated plants than when inoculation and N fertiliser are used in combination. At physiological maturity, higher total numbers of nodules and viable nodules were observed from inoculated plants, while lower numbers of total nodules and of viable nodules were observed when plants received N fertiliser and not inoculated, respectively (Table 4.12). Significantly higher numbers of non-viable nodules were observed from inoculated and un-inoculated treatments during 100% flowering and physiological maturity, respectively.

Table 4.12: Differences in the number of nodules at 100% flowering and physiological maturity among four treatments across the different leguminous species

	100% flowering			Physiological maturity		
Treatments	Total nodules	Viable nodules	Non-viable nodules	Total nodules	Viable nodules	Non-viable nodules
No inoculation	30b	19b	11ª	43b	12b	31b
Inoculation	37.a	27a	10ab	70a	28a	42a
N fertiliser	26b	18b	8b	40b	15b	25b
Inoculation x N fertiliser	25b	17b	8b	49b	16b	33ab
Mean	29.5	20.3	9.3	50.5	17.8	32.8
CV (%)	28.6	28.2	30.5	31.0	26.8	30.0
$LSD_{T\ (0.05)}$	5.96	5.53	2.27	11.15	5.91	10.04

Means within the column followed by the same letter are not significantly different at 5% probability level.

4.2.1.3 Crop effect

As shown in Table 4.13, at 100% flowering, significantly higher total number of nodules and viable nodules were observed from groundnuts and soya beans respectively, while higher number of non-viable nodule were observed from groundnuts and dry beans. At physiological maturity, the crop effect showed significant difference on number of nodules per plant. Average total number of nodules obtained from dry beans and groundnuts was significantly higher than soya beans and cowpeas. Dry beans and groundnuts showed higher numbers of viable and non-viable nodules respectively per plant at physiological maturity.

Table 4.13: Differences in the number of nodules at 100% flowering and physiological maturity among four legumes species

	100% flowering			Physiological maturity		
Crops	Total nodules	Viable nodules	Non-viable nodules	Total nodules	Viable nodules	Non-viable nodules
Cowpea	8c	6b	2c	7d	4b	3c
Dry bean	18b	6b	12a	83a	32a	51a
Groundnut	48a	35a	13a	71b	29a	42ab
Soya bean	43a	34a	9b	42c	7b	35b
Mean	29.3	20.3	9.0	50.8	18.0	32.8
CV (%)	28.6	28.2	30.5	31.0	26.8	30.0
$LSD_{T\ (0.05)}$	5.96	5.53	2.27	11.15	5.91	10.04

Means within the column followed by the same letter are not significantly different at 5% probability level

4.2.2 Biomass

4.2.2.1 Effects of different treatment interactions on the biomass of the respective legume species

The effect of interaction within crops was not significant for fresh biomass and dry biomass (Table 4.14) at 100% flowering. However, in the same stage the effect of interaction showed significant difference (P<0.05) on percentage of dry biomass for soya bean. The higher percentage of dry biomass was observed from plants without inoculation and N application, while lower percentages were observed from inoculated plant.

There was an interaction effect within crops on fresh biomass and dry biomass during 100% pod formation. Higher amounts of fresh biomass were observed when cowpeas received N fertiliser and not inoculated. Higher dry biomass was observed from fertilised and uninoculated cowpea, and inoculated and unfertilised soya bean, while lower amounts of dry biomass were observed from cowpeas without inoculation and N fertiliser, and when soya beans received N fertiliser and not inoculated.

At physiological maturity sampling stages, the effect of interaction (crop x treatment) within crop showed significant difference ($P<0.05$) only on cowpeas fresh biomass. Lower amounts of fresh biomass were observed on uninoculated and fertilised cowpeas, while no significant effects were observed for other treatments. The results of interaction in the same crop exerted statistically significant effects on dry biomass only for soya beans. However lower amounts of dry biomass were observed from soya bean without inoculation and N fertilisers, while for other treatments it was not observed as a significant difference. The effect of interaction in the same crop did not exert any statistical difference on percentage of dry biomass at full pod formation and physiological maturity stage

Table 4.14: Interactive effect of crop x treatment biomass during three sampling periods under field conditions

		100% flowering			100% pod formation			Physiological maturity		
	Treatments	Fresh biomass (kg/ha)	Dry biomass (kg/ha)	Dry biomass (%)	Fresh biomass (kg/ha)	Dry biomass (kg/ha)	Dry biomass (%)	Fresh biomass (kg/ha)	Dry biomass (kg/ha)	Dry biomass (%)
Cowpea	**No inoculation**	41445	8434	20.4d	46685b	9842ed	21.3	32290bdc	8781efd	27.4
	Inoculation	46138	8791	19.3d	61063ba	12435dc	20.4	44696a	12537bcd	29.2
	N fertiliser	45756	8985	19.9d	72076a	13643bac	19.2	36857ba	10202ecd	27.6
	Inoculation x N fertiliser	44446	10896	21.2dc	59357ba	12097dc	20.6	35035bac	10436ecd	29.9
	Mean	**44446.3**	**9276.5**	**20.2**	**59795.3**	**12004.3**	**20.4**	**37219.5**	**10489**	**28.5**
Dry bean	**No inoculation**	7360	1539	20.9d	16102c	4454g	27.4	13381f	7833efd	42.4
	Inoculation	13887	3018	19.6d	16983c	4946fg	29.0	22571fed	4689f	35.0
	N fertiliser	14958	2919	19.7d	15863c	4518g	29.0	18214f	6607ef	36.0
	Inoculation x N fertiliser	15268	2647	18.8d	17864c	4733g	26.5	19119fe	7020ef	37.5
	Mean	**12868.3**	**2530.8**	**19.8**	**16703**	**4662.8**	**28**	**18305.5**	**6537.3**	**37.7**
Groundnut	**No inoculation**	8337	1958	23.6bdac	20389c	5995fg	29.5	21161fed	8984efd	42.7
	Inoculation	15768	3061	19.9d	25563c	8005ef	31.2	30222bedc	12660bcd	41.9
	N fertiliser	10075	2200	22.6bdc	21485c	6841efg	32.2	20309fe	8415efd	42.1
	Inoculation x N fertiliser	12052	2433	21.4dc	20746c	6887efg	33.5	24934fedc	10531ecd	43.4
	Mean	**11558**	**2413**	**21.9**	**22045.75**	**6932**	**31.6**	**24156.5**	**10147.5**	**42.5**
Soya bean	**No inoculation**	11505	3128	27.8a	46066b	13335bc	29.6	32718bdc	14281bc	42.2
	Inoculation	16316	3730	21.8bdc	57190ba	16647a	29.0	38951ba	20430a	51.6
	N fertiliser	19841	5163	26bdc	46995b	11745dc	30.2	36816ba	17578ba	47.4
	Inoculation x N fertiliser	15244	4080	26.6ba	49567b	15766ba	31.8	35857bac	15894ba	46.1
	Mean	**15726.5**	**4025.3**	**25.6**	**49954.5**	**14373.3**	**30.2**	**36085.5**	**17045.75**	**46.8**
Means		21149.8	4561.4	21.9	37124.6	9493.1	27.6	28941.8	11054.9	38.9
CV(%)		27.7	28.85	16.1	29.0	23.0	16.4	28.1	24.3	17.5
$LSD_{T (0.05)}$		12249	2979.1	5.01	15731	3158.6	6.45	11613	5157.3	9.74

Means within the column followed by the same letter are not significantly different at 5% probability level

4.2.2.2 Treatment effects on biomass

The effect of N application and inoculation as well as their combination did not exert statistically significant effects on fresh biomass and percentage of dry biomass amongst treatments at both 100% flowering, 100% pod formation and physiological maturity sampling stages (Table 4.15). The amount of dry biomass showed significant differences ($P<0.05$) amongst treatments at 100% pod formation and physiological maturity sampling stages (Table 4.15). At 100% pod formation and at physiological maturity, significantly higher amounts of dry biomass were observed when plants received inoculants and N fertilisers than for uninoculated and unfertilised plants.

4.2.2.3 Crop effects on biomass

There were significant ($P<0.05$) differences on the fresh biomass, dry biomass and percentage of dry biomass between crops across the three sampling intervals (Table 4.16). At both sampling intervals, the fresh biomass from cowpeas was significantly higher than soya beans, groundnuts and dry beans.

During the100% flowering stage, cowpeas showed higher amounts of dry biomass than soya bean, groundnut and dry bean. The amount of dry biomass from soya bean was significantly higher than cowpea, groundnut and dry bean at 100% pod formation and physiological maturity.

Table 4.15: Effect of inoculation and fertilisers on biomass of different legume species at different sampling intervals

	100% flowering			100% pod formation			Physiological maturity		
Treatments	Fresh biomass (kg/ha)	Dry biomass (kg/ha)	Dry biomass (%)	Fresh biomass (kg/ha)	Dry biomass (kg/ha)	Dry biomass (%)	Fresh biomass (kg/ha)	Dry biomass (kg/ha)	Dry biomass (%)
No inoculation	20175	4392.2	22.2	32531	8529.6b	27.3	25672	9587b	39.6
Inoculation	20746	4281.4	20.9	38775	10105.6ba	27.6	31230	11296ba	37.4
N fertiliser	20574	4308.0	22.5	39105	9186.8ba	27.6	28049	10701ba	38.3
Inoculation x N fertiliser	23105	5263.9	21.8	38088	10150.5a	27.5	30832	12636a	40.2
Mean	21150	4661.4	21.8	37124.8	9493.1	27.5	28945.8	11055	38.9
CV (%)	27.7	28.9	16.1	29.0	23.0	16.4	28.1	24.3	17.5
$LSD_{T\,(0.05)}$	6124.7	1489.5	2.50	7865.7	1579.3	3.22	5806.6	2578.6	4.87

Means within the column followed by the same letter are not significantly different at 5% probability level

Table 4.16: Effect of legume species on biomass at different sampling intervals

	100% flowering			100% pod formation			Physiological maturity		
Crops	Fresh biomass (kg/ha)	Dry biomass (kg/ha)	Dry biomass (%)	Fresh biomass (kg/ha)	Dry biomass (kg/ha)	Dry biomass (%)	Fresh biomass (kg/ha)	Dry biomass (kg/ha)	Dry biomass (%)
Cowpea	44446a	9276.7a	20.2b	59795a	12004.3b	20.4c	37220a	10489b	28.5c
Dry bean	12868b	2530.8c	19.8b	16703c	4662.7d	28.0b	18321c	6537c	37.7b
Groundnut	11558b	2413.0c	21.9b	22046c	6931.9c	31.6a	24156b	10147b	42.5ba
Soya bean	15727b	4025.1b	25.5a	49955b	14373.5a	30.2ba	36085a	17046a	46.8a
Mean	21149.8	4561.4	21.9	37124.8	9493	27.6	28945.5	11054.8	38.9
CV(%)	27.7	28.85	16.1	29.0	23.0	16.4	28.1	24.3	17.5
$LSD_{T\,(0.05)}$	6124.7	1489.5	2.50	7865.7	1579.3	3.22	5806.6	2578.6	4.87

Means within the column followed by the same letter are not significantly different at 5% probability level

4.2.3 Effect of inoculation and N fertiliserson BNF

4.2.3.1 Interaction effect

At 100% flowering, the effect of interaction (crop by treatment) did not exert statistically significant effects on percentage of N derived from the atmosphere (%Ndfa), percentage of N (%N) and percentage of carbon (%C) within crops (Table 4.17).

At 100% pod formation, the effect of interaction was significant on %Ndfa, %N and %C within crop species (Table 4.17). Higher values of %Ndfa were observed from inoculated plants and also from plants that received N fertilisers for soya beans, while for groundnuts, cowpeas and dry beans the effects of interactions did not exert significant difference. Higher %N was recorded from inoculated soya beans. Groundnuts fertilised by N showed lower %C than other treatment combination for groundnuts.

During physiological maturity, the effect of interaction in the same crop (crop x treatment) showed significant difference on %Ndfa and N (Table 4.17). The %Ndfa was observed to be significantly higher from plants treated with inoculants than plants without inoculants and N for soya beans, while dry beans inoculated and fertilised showed higher %Ndfa than dry beans without inoculant and N. Effect of interaction (crop by treatment) did not exert statistically significant effects on the percentage of N derived from the atmosphere (%Ndfa) for cowpeas and groundnuts.

Table 4.17: Interaction effect of crop x treatment on %Ndfa, %N and %C at three sampling periods under field conditions

		100% flowering			100% pod formation			Physiological maturity		
	Treatments	**%Ndfa**	**%N**	**%C**	**%Ndfa**	**%N**	**%C**	**%Ndfa**	**%N**	**%C**
Cowpea	**No inoculation**	61.4	2.4	36.8	61.6bdc	1.95gf	41.9b	58.8dec	1.6	47.3
	Inoculation	63.1	2.5	35.3	64.4bac	2.2def	41.4b	72.9bdac	1.9	46.4
	N fertiliser	47.3	2.3	37.0	51.2bedc	2.0gef	40.0b	61.3dc	1.6	47.0
	Inoculation x N fertiliser	41.7	2.3	37.5	53.8bedc	2.1gef	40.5b	57.0dec	1.8	47.4
	Mean	**53.4**	**2.4**	**36.7**	**57.8**	**2.1**	**41.0**	**62.5**	**1.7**	**40.0**
Dry bean	**No inoculation**	33.9	2.4	36.6	20.0h	1.6750f	38.8b	36.1fde	2.5	45.8
	Inoculation	37.7	2.8	35.8	35.3fegh	2.1gef	39.8b	56.0g	2.5	45.1
	N fertiliser	31.9	2.6	37.4	26.0hg	2.1gef	39.6b	37.2fg	2.4	45.7
	Inoculation x N fertiliser	50.7	2.7	37.4	29.8fhg	2.0gef	40.6b	41.2feg	2.3	46.2
	Mean	**38.6**	**2.6**	**36.8**	**27.8**	**2.0**	**39.7**	**42.6**	**2.4**	**45.7**
Groundnut	**No inoculation**	44.0	3.2	38.0	39.3fegh	2.5de	41.2b	57.2dec	2.7	61.5
	Inoculation	46.5	3.2	36.8	47.6fedc	2.7de	40.3b	57.7dec	2.7	61.5
	N fertiliser	46.5	2.7	38.3	40.2feg	2.0gef	34.8e	75.7bac	2.4	60.7
	Inoculation x N fertiliser	43.0	3.1	35.7	42.3fedg	2.5de	38.975b	60.4dec	2.4	60.2
	Mean	**45.0**	**3.1**	**37.2**	**42.4**	**2.4**	**38.8**	**62.8**	**2.6**	**61.0**
Soya bean	**No inoculation**	22.2	3.7	40.3	51.5bedc	3.5ba	41.4b	90.2ba	3.5	53.9
	Inoculation	28.1	4.2	38.9	67.8ba	3.8a	41.6ba	91.9a	3.7	54.0
	N fertiliser	21.2	3.7	39.4	82.0a	3.5ba	44.9b	71.9bdc	3.5	52.9
	Inoculation x N fertiliser	29.3	4.0	39.5	26.9bg	3.0bc	40.4b	63.7dc	3.7	53.7
	Mean	**25.2**	**3.9**	**39.5**	**57.1**	**3.5**	**42.1**	**79.4**	**3.6**	**53.6**
Mean		40.5	3.0	37.5	46.2	2.5	40.4	61.8	2.6	51.8
CV (%)		33.3	13.1	5.0	30.2	14.7	5.9	22.3	12.6	2.7
$LSD_{T (0.05)}$		20.147	0.5594	2.8514	19.848	0.5182	3.4157	19.638	0.4602	1.9619

Means within the column followed by the same letter are not significantly different at 5% probability level

At 100% flowering and pod formation, the interaction effect of crop x treatment did not exert statistically significant effects on total N (shoot and root), total N fixed and C/N within crop (Table 4.18). Inoculated groundnuts and soya beans generally showed higher amounts of N, while the combined use of inoculation and N fertilisers gave higher values of total N in cowpeas and dry beans. Generally, higher total amounts of N fixed were achieved when crops were inoculated with bacteria for groundnuts, soya beans and cowpeas. With respect to the C/N ratios, higher proportions were observed when crops received N fertilisers for soya beans, without inoculant and N for dry beans and cowpeas, and the combined application of inoculant and N fertilisers for groundnuts. Inoculated crops in generally showed higher amounts of total N, with the exception of dry beans that showed higher amounts of total N when N fertiliser was applied. The values of C/N were higher from un-amended plots for cowpeas and dry beans, and they were higher for inoculated groundnuts and soya beans.

At full pod formation, the interaction effect of crop X treatment in the same crop exerted statistically significant effects on total N fixed (Table 4.18). Significantly a higher total amount of N fixed by crops was realized from treatments with inoculation irrespective of the legume species. At physiological maturity, the effects of interaction (crop and treatment) within crop did not exert statistically significant effects on total N (shoot and root), total N fixed and C/N within the same crop (Table 4.18).

Generally, higher amounts of total N and total N-fixed were achieved when crops were inoculated irrespective of the legume, while the lower total N was achieved when groundnuts and cowpeas received N fertilisers, without amendments N for dry beans, and when the plants were inoculated and N fertilised for soya bean. Generally lower total N fixed was achieved when crops were untreated with inoculant and fertilised with N for cowpeas, dry beans and groundnuts, while for soya beans, lower total N fixed was achieved when crops were untreated with inoculants and N. With regard to C/N, general higher ratios were observed when crops were untreated with inoculant and N for soya beans, when treated with inoculants and N for groundnuts, and untreated for cowpeas and dry beans.

Table 4.18: Interactive effects of treatments x crop on total N, total N fixed and C/N ratio during three sampling periods under field conditions

		100% flowering			100% pod formation			Physiological maturity		
	Treatments	Total N in shoot +root ($kg\ ha^{-1}$)	Total N fixed N in shoot +root ($kg\ ha^{-1}$)	C/N ratio	Total N shoot +root ($kg\ ha^{-1}$)	Total fixed N in shoot +root ($kg\ ha^{-1}$)	C/N ratio	Total NN in shoot +root ($kg\ ha^{-1}$)	Total fixed N in shoot +root ($kgha^{-1}$)	C/N Ratio
Cowpea	**No inoculation**	165.03	91.37	17.878	160.88	100.15cbd	2.250	147.70	85.41	25.3
	Inoculation	185.43	111.83	15.748	230.45	140.13b	22.350	171.80	124.41	28.8
	N fertiliser	175.83	81.49	16.195	226.83	115.73bc	23.150	143.83	88.69	29.2
	Inoculation x N fertiliser	230.25	96.81	16.115	211.55	102.78cbd	22.725	159.23	89.02	26.6
	mean	**189.1**	**95.4**	**16.5**	**207.4**	**114.7**	**17.6**	**155.6**	**96.9**	**27.5**
Dry bean	**No inoculation**	34.83	15.21	15.328	67.80	14.75e	26.100	99.18	52.81	18.3
	Inoculation	60.93	21.83	14.300	76.43	24.63e	22.625	163.70	58.32	18.3
	N fertiliser	65.00	19.12	14.593	80.23	22.65e	23.150	151.65	56.87	19.8
	Inoculation x N fertiliser	69.85	36.49	14.500	79.65	23.43e	23.925	133.35	54.53	17.8
	mean	**57.7**	**23.2**	**14.7**	**76.0**	**21.4**	**24.0**	**134.0**	**55.6**	**18.6**
Groundnut	**No inoculation**	67.70	31.04	12.118	130.63	55.50ced	18.275	203.90	114.83	22.5
	Inoculation	80.03	35.07	13.260	144.63	59.98ced	20.350	231.95	133.24	23.4
	N fertiliser	50.20	23.98	13.220	114.00	44.53ed	20.350	165.73	127.28	25.7
	Inoculation x N fertiliser	53.13	25.09	12.158	163.95	70.58ced	18.700	246.13	147.56	25.8
	Mean	**62.8**	**28.8**	**12.7**	**138.3**	**57.6**	**19.4**	**211.9**	**130.7**	**24.5**
Soya bean	**No inoculation**	159.23	27.36	11.555	397.73	242.55a	13.875	464.78	324.81	15.8
	Inoculation	128.08	37.37	10.195	471.65	271.45a	14.175	555.83	382.05	14.7
	N fertiliser	102.23	28.11	12.285	354.90	297.98a	12.975	527.23	366.53	15.1
	Inoculation x N fertiliser	138.40	40.21	11.173	397.53	112.75bc	15.650	437.08	329.94	14.7
	mean	**132.0**	**33.3**	**11.3**	**405.5**	**270.7**	**14.2**	**496.2**	**350.8**	**15.1**
Mean		110.38	45.15	13.79	206.80	106.22	18.79	250.19	158.52	21.36
CV(%)		22.4	24	10.5	27.9	21.3	14.0	28.3	23.5	16.5
LSDT (0.05)		66.622	36.645	2.0642	82.033	62.403	4.03	155.55	109.21	3.7795

Means within the column followed by the same letter are not significantly different at 5% probability level

4.2.3.2 Treatment effects on %Ndfa, %N and %C

The effect of inoculation and N application as well as their combination was not statistically significant on %Ndfa, %N and %C during the 100% flowering sampling period (Table 4.19). Generally, higher %Ndfa, %N and %C were observed from inoculated plants and treatments which received N fertilisers.

Table 4.19: Treatment effect on %Ndfa, %N and %C at 100% flowering period under field conditions

	100% flowering		
Treatments	%Ndfa	%N	%C
No inoculation	40.9	2.96	40.3
Inoculation	41.2	3.04	40.4
N fertiliser	36.7	2.81	39.1
Inoculation x N fertiliser	43.8	3.14	39.9
Mean	40.6	3.0	39.9
CV (%)	33.4	13.1	5.0
$LSD_{T (0.05)}$	10.07	0.28	1.43

At 100% pod formation, the effect of inoculation and N fertilisers as well as their combination showed statistically significant effects on % Ndfa (Figure 12). Significantly higher %Ndfa was observed when plants were inoculated and this was significantly lower in the combined treatments (inoculation andN fertiliser). The %N and %C did not differ between different treatments during 100% pod formation (Figure 4.12).

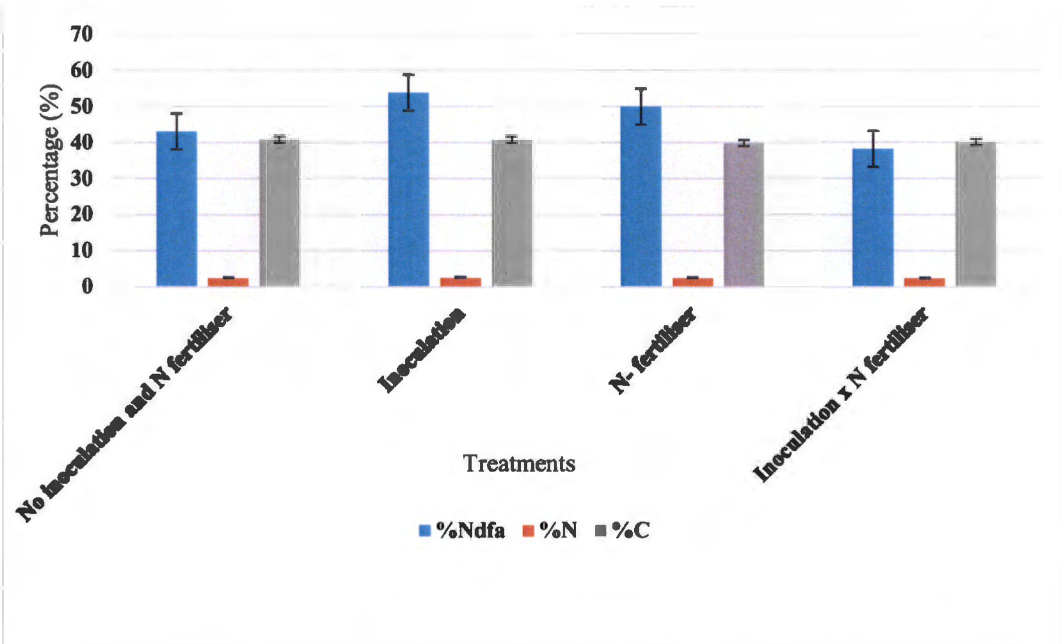

Figure 4.12: Effect of treatment on %Ndfa, %N and %C at 100% pod formation under field conditions

At physiological maturity, the effects of treatments showed significant differences on the percentage of N derived from the atmosphere (%Ndfa), while no significant effects were observed for the %N and %C (Figure 4.13). Higher percentages were observed from inoculated treatments, while lower percentages were observed from treatments with inoculant and N application.

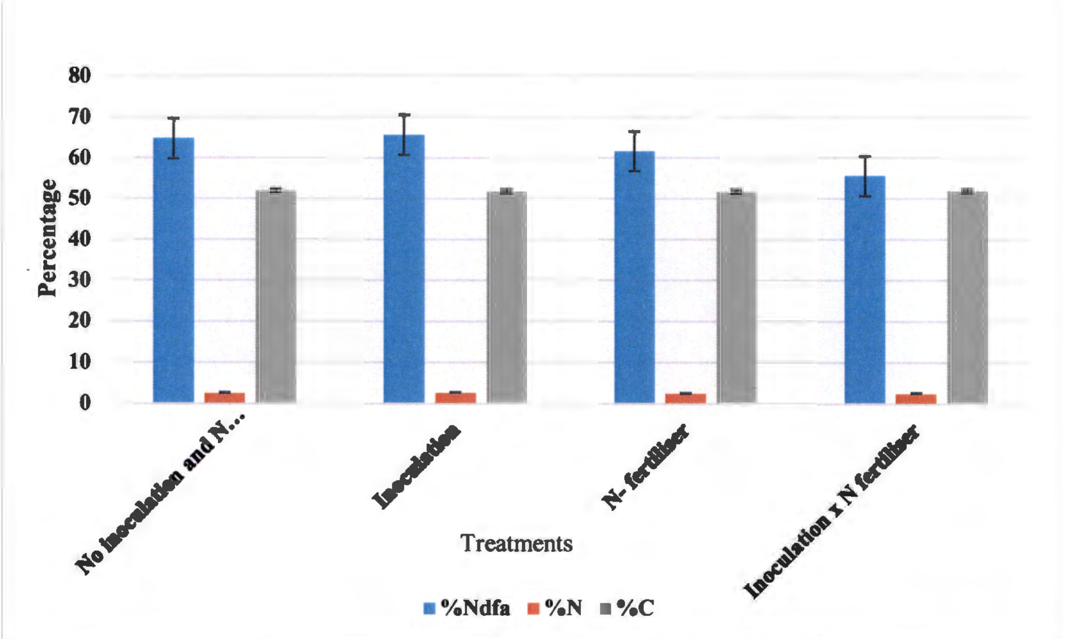

Figure 4.13: Treatment effects on %Ndfa, %N and %C during physiological maturity under field conditions

4.2.3.3 Effects of treatments on total N and total N fixed in shoots and roots

During100% flowering, the effect of N application and inoculation as well as their combination did not exert statistically significant effects on the total N (shoot and root) and total N fixed (Table 4.20). Generally, a higher amount of total N and total N fixed per treatment were observed from inoculated plants.

At 100% pod formation, the effects of N application and inoculation as well as their combination showed statistically significant ($P < 0.05$) effects on the total N (shoot and root) and total N-fixed (Figure 4.14). Higher amounts of total N and total N fixed per treatment were observed from the inoculated plants. However, lower total N (shoot and root) was observed from treatments without inoculation and N application (untreated). Conversely, significantly lower total N fixed in the shoot was obtained when plants were inoculated and fertilised.

Table 4.20: Treatment effect on total N (shoot &root) and total N fixed (shoot & root) at 100% flowering stage under field conditions

	100% flowering	
Treatments	**Total N in shoot +root (kg ha^{-1})**	**Total fixed N in shoot +root (kg ha^{-1})**
No inoculation	106.7	51.2
Inoculation	129.6	52.1
N fertiliser	98.3	38.2
Inoculation x N fertiliser	106.9	49.0
Mean	110.4	47.6
CV (%)	22.4	24.0
LSD$_{T (0.05)}$	33.3	18.3

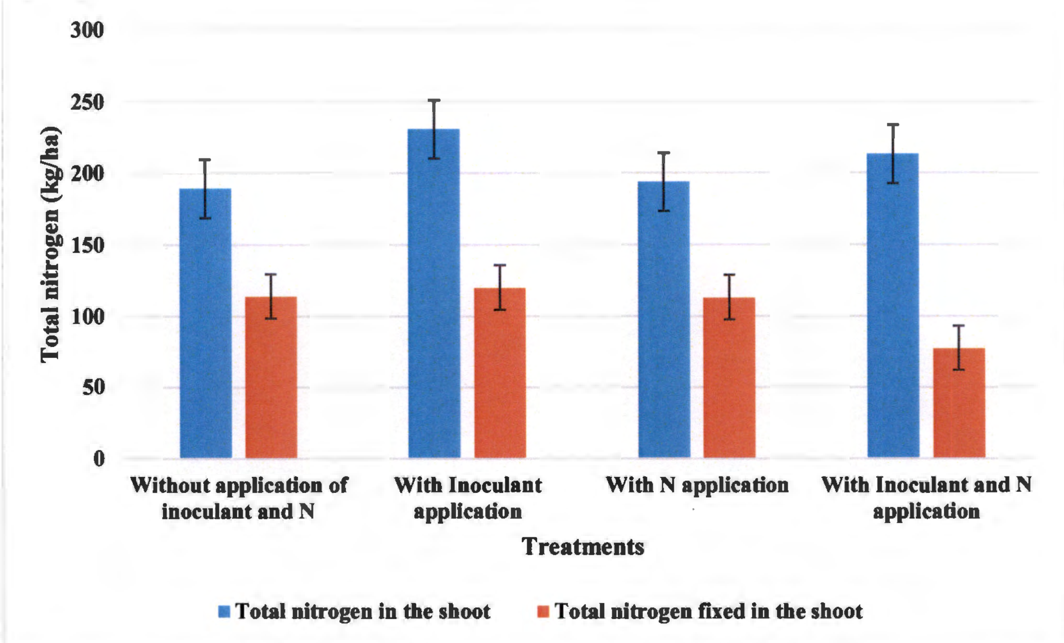

Figure 4.14: Effect of treatment on total N and total N fixed in shoots during 100% pod formation under field conditions

The total amount of N (shoot &root) and total amount of N fixed in the shoot and root at physiological maturity was not significantly affected by the application of N fertilisers and inoculation as well as their combination (Table 4.21).

Table 4.21: Treatment effect on total N in shoot +root and total N fixed shoot +root at physiological maturity under field conditions

	100% flowering	
Treatments	Total N shoot +root (kg ha^{-1})	Total N fixed shoot +root (kg ha^{-1})
No inoculation	228.9	169.5
Inoculation	251.1	174.5
N fertiliser	247.1	159.8
Inoculation x N fertiliser	273.6	155.3
Mean	250.2	164.8
CV (%)	23.80	26.54
$LSD_{T\ (0.05)}$	77.775	54.603

4.2.3.4 Treatment effects on C:N ratio

At all sampling stages, the effect of N application and inoculation as well as their combinations did not exert statistically significant effects on C/N ratio (Table 4.22). The ratio average of

C/N were 15.4, 20.2 and 24.9 at 100% flowering, 100% pod formation and physiological maturity stages, respectively

Table 4.22: Treatment effects on C/N ratio at three sampling intervals under field conditions

	C/N ratio		
Treatments	100% flowering	100% pod formation	Physiological maturity
No inoculation	16.6	20.9	23.9
Inoculation	15.3	19.5	24.8
N fertiliser	16.4	19.9	26.2
Inoculation x N fertiliser	13.1	20.3	24.7
Mean	15.4	20.2	24.9
CV (%)	10.5	14.0	16.2
$LSD_{T(0.05)}$	1.208	2.015	2.203

4.2.3.5 Crop effects on %Ndfa, %N and %C

Significant differences were displayed for all crops on the %Ndfa, %N and %C in all sampling intervals (100% flowering, 100% pod formation and physiological maturity, (Figures 4.15 to 4.17). At 100% flowering, higher %Ndfa was observed from cowpeas, while soya beans showed higher values of %N and %C (Figure 4.15). At 100% pod formation, higher %Ndfa was observed from soya beans and cowpeas, while higher values of %N and %C were observed from soya bean and cowpea, respectively (Figure 4.16). Equally, at physiological maturity, higher %Ndfa and %N were observed from soya beans, while groundnuts showed higher values of %C (Figure 4.17).

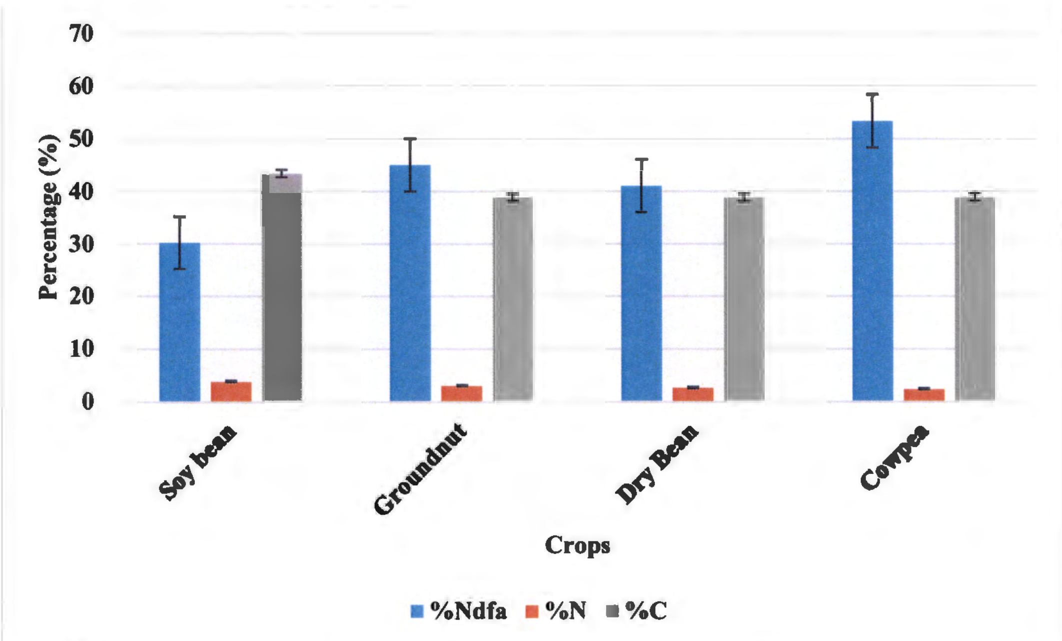

Figure 4.15: Crop effects on %Ndfa, %N and %C at 100% flowering under field conditions

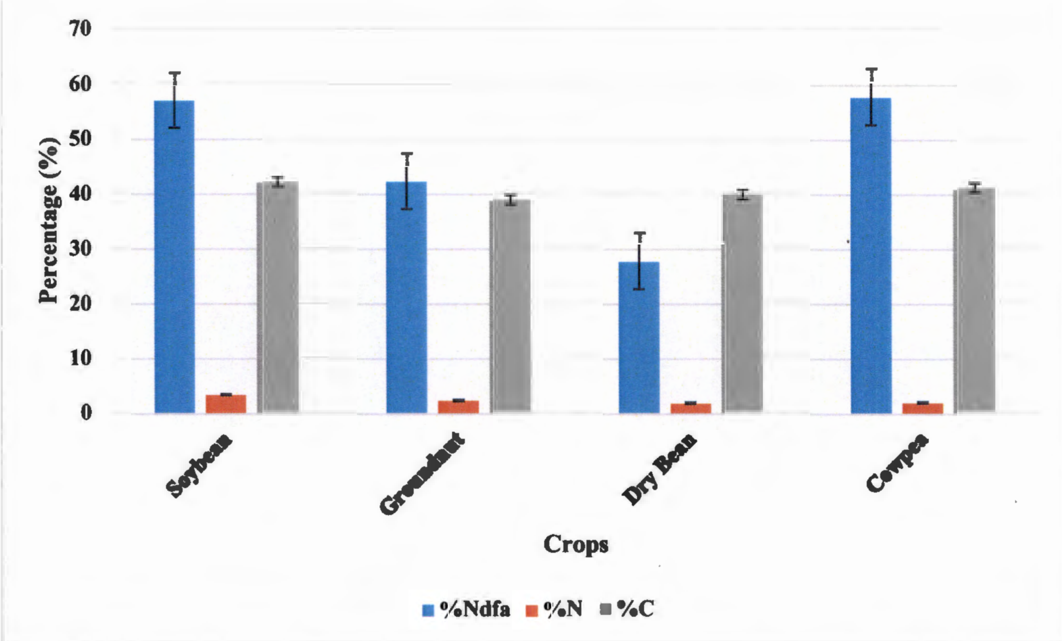

Figure 4.16: Crop effects on %Ndfa, %N and %C at 100% pod formation under field conditions

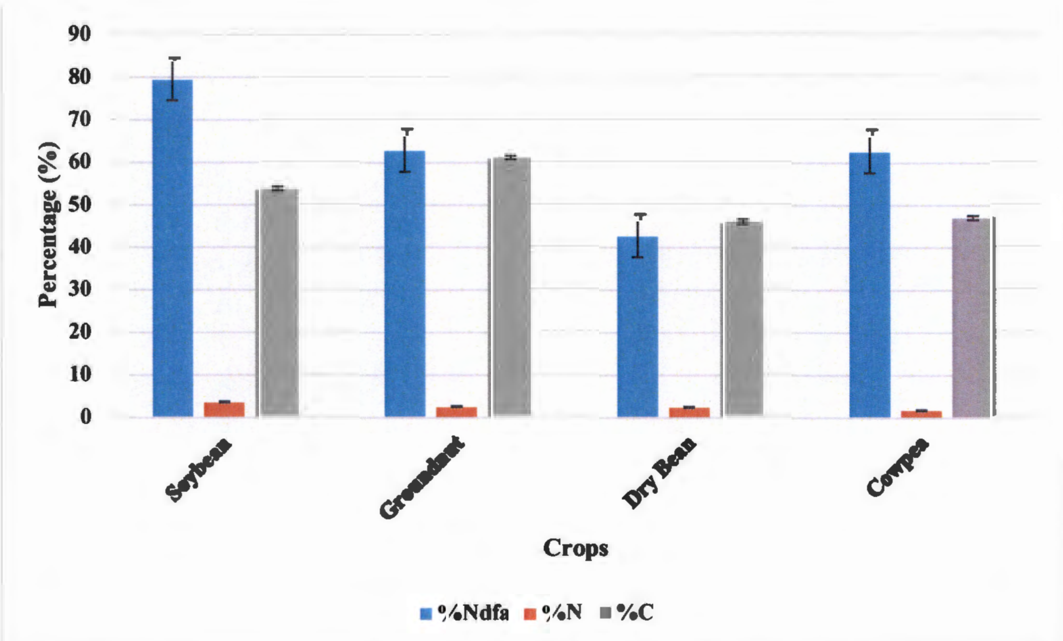

Figure 4.17: Crop effects on. %Ndfa, %N and %C at physiological maturity under field conditions

4.2.3.6 Crop effect on total amount of N and total amount of N Fixed from shoots and roots

Across the sampling intervals, crop effects showed significant differences on total N and total N fixed in shoots and roots (Figures 4.18 – 4.20). At 100% flowering, higher amounts of N and total N fixed were observed in cowpeas and lower amounts in dry beans. During full pod formation, higher amounts of N and total amounts of N fixed were observed from soya beans and lower amounts with dry beans. While at physiological maturity, higher amounts of N and total N fixed per crop were observed from soya beans while lower values were observed from dry beans.

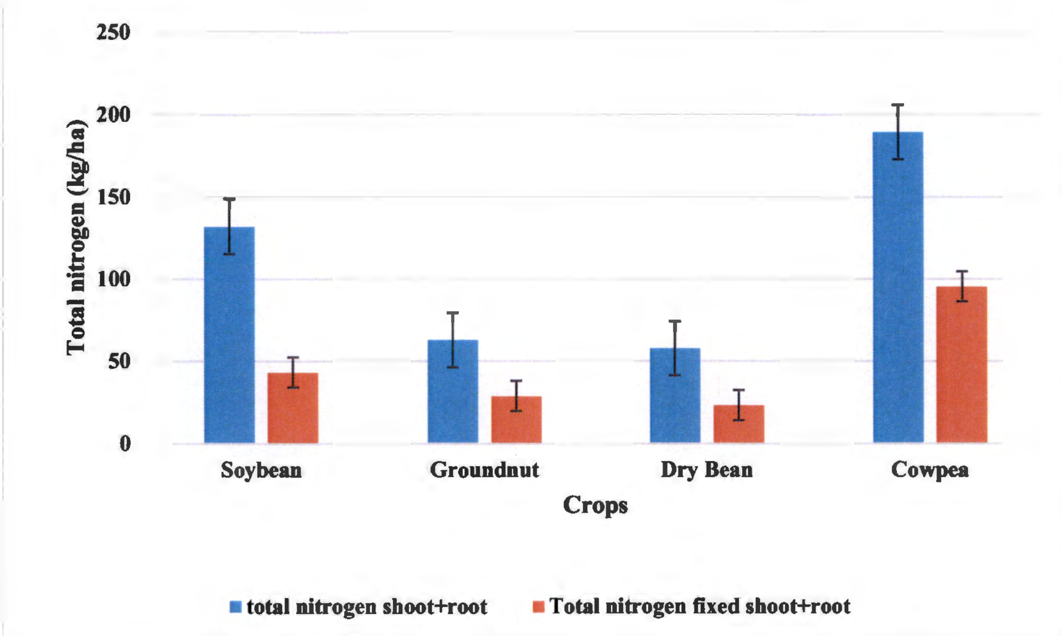

Figure 4.18: Crop effects on total amount of N and total amount of N fixed in shoots + roots at 100% flowering under field conditions

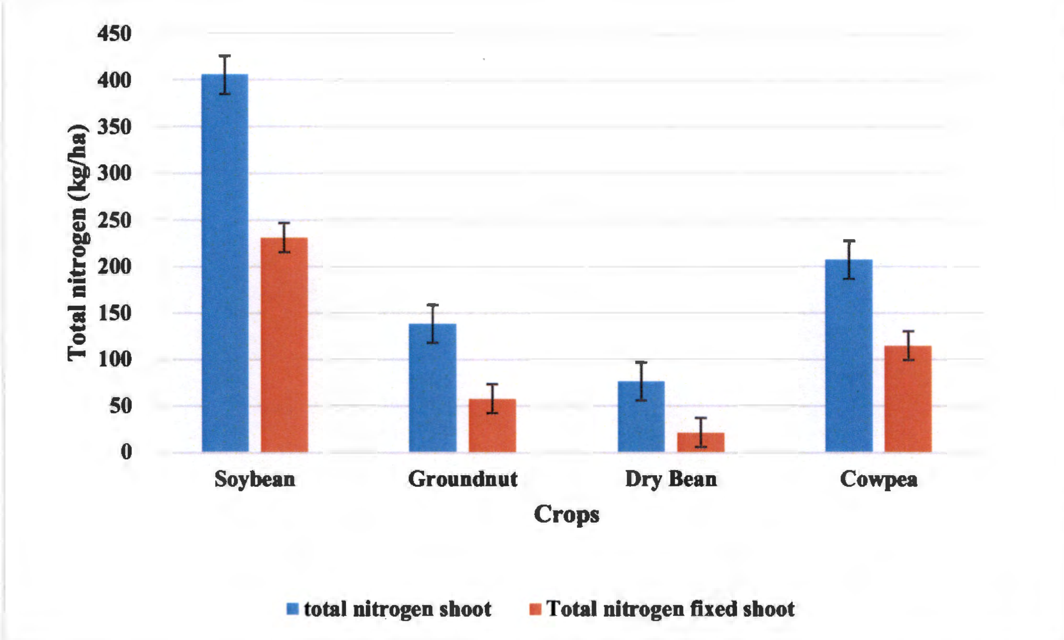

Figure 4.19: Crop effects on total amount of N and total amount of N fixed in shoots and roots at 100% pod formation under field conditions

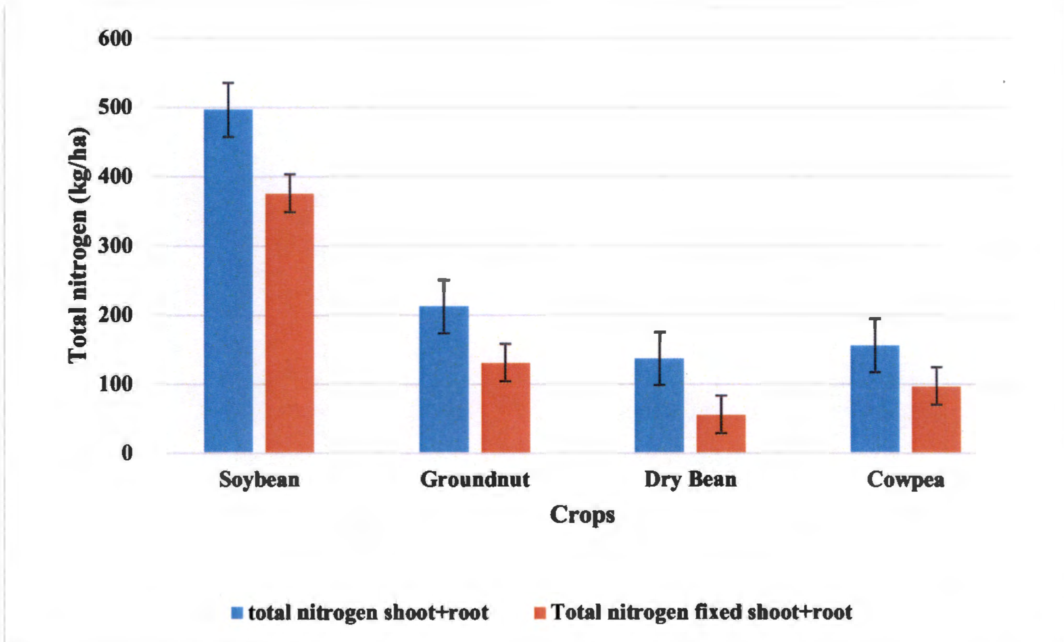

Figure 4.20: Crop effects on total amount of N and total amount of N fixed at physiological maturity under field conditions

4.2.3.7 Crop effect on C:N ratio

The ratio C/N differed significantly ($p < 0.05$) between crops at all sampling stages under field conditions. As shown in Table 4.23, cowpeas consistently showed higher C/N ratios and consistently lower values from soya beans across the sampling intervals.

Table 4.23: Treatment effects on C/N ratio at three sampling intervals under field conditions

	C/N		
Crops	100% flowering	100% pod formation	Physiological maturity
Cowpea	19.2ª	23.4a	27.5ª
Dry bean	17.1b	24.0a	18.5c
Groundnut	12.7c	19.1b	24.3b
Soya bean	14.8d	14.2c	15.1d
Mean	16.0	20.2	21.4
CV(%)	10.5	14.0	16.2
$LSD_{T\ (0.05)}$	1.2079	2.01	1.89

Means within the column followed by the same letter are not significantly different at 5% probability level.

4.2.3.8 Correlation between BNF assessment parameters

The overall results showed positive correlations between nodulation and the BNF components (Table 4.24). As shown in Table 4.24, it was observed that in all sampling intervals, the higher number of nodules (total nodules and viable nodules) increased with the percentage of N from atmosphere and the amount of N fixed by plants. There was a negative correlation between non-viable nodules and percentage of N from the atmosphere as well as the amount of N fixed in the plants.

Table 4.24: Correlation coefficients between parameters of biological N fixation under field conditions

100% flowering	Total N	N fixed	%Ndfa
TNN	0.37	0.40	0.13
VN	0.22	0.28	0.12
NVN	-0.61	-0.54	-0.03
Physiological maturity	**Total N**	**N fixed**	**%Ndfa**
TNN	0.47	0.43	0.26
VN	0.56	0.56	0.51
NVN	0.05	-0.02	-0.05

TNN = Total number of nodules

VN = Total number of Viable nodules

NVN = Non-viable nodules

%Ndfa percentage of nitrogen from atmosphere

CHAPTER 5 : DISCUSSION

The highest number of nodules and viable nodule per plant was produced when all different leguminous crop types were inoculated. This was due to the rhizobium strains that were used as inoculum that led to increased nodulation in the various legumes. Venturini *et al.*, (2005), also reported that the highest number of active nodules on inoculated plants was observed on the response of dry beans and soya beans to rhizobium inoculation. Furthermore, the study indicated that the activity of the nodules in the plants of inoculated treatment had the highest number of active nodules than the non-inoculated plants. Treatments that received N application fertilisers consistently gave lower numbers of total nodules relative to when treated with inoculant alone and/or in combinations. The N probably had negative effect on nodulation. A high concentration of mineral N has been reported by several workers to have had negative effects on weight and number of nodules and nitrogenase activity in various species of legumes (Chalk, 2000; Sinclair *et al.*, 2001; Serraj and Sinclair, 2003).

Hungria *et al.* (1997) and Novo *et al.* (1999) also reported that nodulation of the roots supplied the needs of legume plants, and recommended that one should avoid N fertiliser because it inhibits the formation of nodules, affecting BNF and biomass production. They highlighted that the use of mineral nitrogen fertilisers on legumes releases ammonia which ends up being reduced to nitrate. Both the ammonium and nitrate forms are readily absorbed and are easily taken up by leguminous plant and under such conditions this may result in partial or total inhibition of symbiotic fixation system which has high energy demand (Câmara, 2000). Numerous authors reported that minimal amount of N fertiliser is able to suppress the nodulation and activity of nodules in leguminous plants (Ferreira *et al.*, 2000; Carvalho, 2002; Venturini *et al.*, 2005; Romanini *et al.*, 2007). In this study, it was observed that the treatments that were not inoculated nodulated comparatively fairly well probably due to the presence of native strains of the soil capable of forming nodules on legumes. A similar study conducted in Brazil showed that nodulation of the uninoculated legume was due to the presence of the native population of rhizobium in the soil (Grange and Hungria, 2004; Vieira *et al.*, 2005; Graham, 2008).

The study demonstrated that the number of nodules collected on cowpea at different stages were consistently lower than other three legumes in the field and glasshouse settings. Similar results were obtained by Ferreira *et al.* (2011) for cowpeas, in a greenhouse study. Another report by Gualter *et al.* (2008) showed a decrease in the number and mass of nodules on cowpeas in the second collection to the 50 days after emergence (DAE) compared with the first sampling at 35 DAE, probably due to the beginning of nodule senescence. The high number of nodules found in dry beans during physiological maturity under field experiments may be explained as the occurrence of a secondary population of nodules after flowering. Often, a secondary population of nodules on dry beans may occur after flowering which contributes to the supply of N to the pods (Franco *et al.*, 1978).

For groundnuts, the presence of nodules in almost all the sampling intervals tended to increase with the plant growth period. Tajima *et al.* (2006) also evaluated the distribution of nodules on the roots of two groundnut cultivars and found the presence of nodules throughout the crop cycle. The percentage of active and non-active nodules in groundnuts under the field experiment was 72% and 27% at the flowering stage and 40.8% and 59.2% at physiological maturity. Under glasshouse experimental conditions, the active and the non-active nodules were 78.6% and 21.4% during flowering stage, 57.9% and 42.1% during the pod stage and 44% and 56% during physiological maturity. This observation shows a characteristic that inherently occurs in this leguminous species. There is therefore a need to carry out further investigations on the relationship between groundnuts and different strains of rhizobium spp. under various agro-ecological regions. The number of nodules observed throughout the cycle of groundnuts can also be explained by the fact that this crop is considered promiscuous due to their ability to establish symbiosis with Rhizobium that can infect a diverse group of legumes (Alwi *et al.*, 1989).

In a long-term study of polyphasic characterization of isolated groundnut, Yang *et al.* (2005) concluded, based on phenotypic test data, using molecular biology techniques the group of isolates of rhizobium strains from different regions of China, they are very similar species of *Bradyrhizobiumjaponicum* or *Bradyrhizobuim* spp. The authors indicated further that the group of isolates exhibited great variability even though they belonged to the same genus. All isolates of groundnuts were able to infect *Arachishypogaeae* and *Phaseolus vulgaris*, but they did not show ability to fix N with *Phaseolus vulgaris* and some of the isolates were able to nodulate and fix N when in association with soya beans (*Glycine max).*

Chueiri *et al.* (2005) and Merchant (2005) also argued that N in the mineral form, applied at sowing or at any stage of crop development, has been shown to be unnecessary due to the fact that it did not contribute to significantly increase productivity of soya beans, in addition to affecting nodulation and BNF processes and it increases production costs. A similar report by Campos (1999) using different doses of inoculants, with fertilisation of 200 kg N ha^{-1} and control showed that the control did not differ statistically significantly from the other treatments on seed production, and N inhibits the formation of nodules, affecting BNF. This can be explained by that these legumes are independent of the effect of N mineral fertiliser. (Chueiri *et al.* 2005)

Significant differences on biomass were observed between the different crop types with cowpea presenting high values across all sampling stages. Similar results with respect to the biomass of cowpeas were found by Soares *et al.* (2006), Melo *et al.* (2009) Zilli *et al.* (2009) and Nascimento *et al.* (2010), both under greenhouse and field conditions. Generally, high biomass values were observed irrespective of the legume species from the inoculated plants. This was most probably due tothe efficiency of nitrogen fixation. The nutrient more directly related to increased plant biomass is N, thus efficient BNF provides more nitrogen to the plants increasing plant biomass, as observed for soya beans (Souza *et al.*, 2008) and in cowpea (Xavier *et al.*, 2008).

This study clearly showed that among the crops investigated, dry beans during the season showed low percentage of N derived from the atmosphere, as well as low value of total amount of N fixed. Similar results were found by other authors that the BNF capacity of dry bean seems to not be effective as compared to cowpeas (Martins *et al.*, 2003; Xavier *et al.*, 2007) and soya beans (Hungria *et al.*, 1991; Mendes *et al.*, 2008). This can be attributed to factors related to the individual crop N fixing potential. The successful formation of a functional symbiosis for efficient biological fixation of N is dependent on many factors, such as physical, environmental, nutritional and biological influences (Hungria *et al.*, 1991), and also to factors related to crop cultivar and rhizobia strain as well as the interaction of these two variables (Hungria and Vargas, 1997; Smith *et al.*, 2006). The response of variability in nodulation among crops or cultivars has been reported to be common by various other workers (Soares *et al.*, 2006; (Xavier *et al.*, 2006). In Brazil, Franco *et al.* (1978) estimated that the BFN in soya bean varied from 40 to 206 kg Nha^{-1}. However various authors have reported different values varying from the earlier reports, for example, Boddey *et al.* (1990) found rates of BNF to be 109-250 kg N ha^{-1} and Zotarelli (2000) of up to 294 kg N ha^{-1}, compared to groundnuts which fixed between 72-124 kg Nha^{-1}, Grain-Chickpeas (50-103 kg N ha^{-1}), the dry beans (2.7 -110 kg N ha^{-1}) and peas (52-77 kg N ha^{-1} (Siqueira and Franco, 1988).

All treatments had sufficient capacity to form nodules necessary to guarantee a successful BNF process. However, for cowpeas, there is no conclusive information on the minimum numbers of nodules necessary to guarantee good performance of the BNF, as observed with other crops such as soya beans, which it is recognized as sufficient when there are 15-20 nodules on the main crown of the root (Hungria and Bohrer, 2000). Dry beans, though having a considerable number of nodules, was the crop which presented the lowest amount of N fixed during the three stages of sampling. This can be explained by the fact that not all the rhizobium which form nodules in symbiosis with this crop show efficiency in the biological process of fixing N. However, Carvalho (2002) points out that not always the highest number of nodules implies a better utilization of the BNF by the plant, suggesting that the high nodulation results in the formation of smaller nodules, with lower relative efficiency. Cowpeas and soya beans were the crops that exhibited greater Ndfa and N fixation. In general, inoculated treatments exhibited higher percentage of N derived from atmosphere and a higher amount of fixed N irrespective of the legume. This can be explained by the effect of Rhizobia inoculation which resulted in more nodules and contributing to the increase of BNF per plant. Similar results were reported by Romanini Junior *et al.*, (2007),

During the three sampling intervals for total N in the plants under both glasshouse and field settings, although there were no significant differences between treatments within crop types, treatments with inoculants did not show significant difference in N total, confirming the high efficiency of the strains to fix N. The lack of differences between control and inoculated treatments on percentage of N and total N content in the plant proves the capacity of native populations in establishing symbiosis with legumes, their adaptation to the soil and climatic conditions of the site (Zilli *et al.*, 2006). However, between the crops, significant differences were observed for total N in the plants where soya beans showed higher amount of total N in the field, and cowpeas in the glasshouse, while groundnuts showed higher amount of total N than dry beans and soya beans, although dry beans presented least amounts of total N in the plant under both field and glasshouse conditions.

The results showed positive correlations (0.60) between nodulation and biological N fixation under both field and glasshouse conditions components. In all sampling intervals, a higher number of nodules (total nodules and viable nodules) increased the percentage of N from atmosphere and the amount of N fixed by the plants. There was also a negative correlation between non-viable nodules and percentage of N from the atmosphere as well as the amount of N fixed by the plants. These results indicate that BNF was satisfactorily and was proportional to the nodulation, since there was a positive correlation between these parameters

(Silveira *et al.*, 2003; Didonet *et al.*, 2005). The results from this study also showed positive correlations between nodulation and grain yield within crops. These results agree with (Câmara, 2000) who emphasized that the increase in the number of nodules results in increased dry mass of nodules and providing better nodulation efficiency and productivity of leguminous crops.

CHAPTER 6 : CONCLUSIONS

The study showed that:

The effect of rhizobium inoculant application exhibited significant improvement on the numbers of nodules, percentage of N from atmosphere and total amount of N fixed irrespective of the legume.

The effect of inoculation on groundnuts showed higher total number of nodules and viable nodules across the growth period under both field and glasshouse conditions.

The effect of inoculating dry beans under field conditions showed high total number of nodules and viable nodules at physiological maturity stage in comparison to treatments with N application, control and crops. While cowpeas showed lower number of total nodules and viable nodules across the cycle under both field and glasshouse conditions.

Despite the poor performance with respect to the other parameters measured on cowpeas, it gave nodules that showed greater efficiency on BNF relative to the other legumes studied. This was indicative of the higher percentage of N from atmosphere and total amount of N fixed at 100% flowering and 100% pod formation. Dry beans exhibited lower percentage of N from atmosphere and total amount of N fixed across the cycle under field conditions as well as under glasshouse conditions. Groundnuts exhibited higher percentage of N from atmosphere and total amount of N fixed across the cycle under glasshouse conditions. Fertiliser application and bacterial inoculation as well as their interaction did not exert statistically significant effect on the performance of all the studied crops.

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APPENDICES/ANNEXURES

Appendix 1:ANOVA for nodules at 100% flowering under field conditions

		Total nodules		Viable nodules		Non-viable nodules	
Source	DF	MS	p	MS	P	MS	p
Rep	3	68.33668	0.4134	20.23769	0.8000	14.805568	0.2390
Treatment	3	439.86328	0.0012	292.92815	0.0052	33.974152	0.0273
Crop	3	6087.78839	<.0001	4522.11245	<.0001	352.003952	<.0001
CropxTreatment	9	68.32949	0.4740	65.43973	0.3935	26.984756	0.0147
Error	45	70.16041		60.40161		10.163322	
Total	63						

Appendix2: ANOVA for dry matter at 100% flowering under field condition

		Shoot biomass		Shoot dry matter		Root biomass		Root dry matter	
Source	DF	MS	P	MS	P	MS	p	MS	P
Rep	3	222835604	0.1335	8577165	0.133	344957.59	0.2738	19308.304	0.1160
Treatment	3	28086516	0.4949	3545239	0.494	429321.73	0.1878	5874.947	0.5973
Crop	3	3907867385	<.0001	1667236	<.0001	6345891.05	<.0001	692789.696	<.0001
CropxTreatment	9	44401851	0.8431	2329936.9	0.8431	245612.38	0.4905	12101.130	0.2613
Error	45	73977982		4375539.4		257741.26		9275.371	
Total	63								

Appendix 3:ANOVA for dry matter at 100% pod formation under field conditions

		Shoot biomass		Shoot Dry Matter	
Source	DF	MS	p	MS	p
Rep	3	28150535	0.8745	3223455.2	0.5838
Treatment	3	152937870	0.3017	9757140.2	0.1299
Crop	3	7055888535	<.0001	320090121.1	<.0001
Crop x Treatment	9	135900316	0.3729	7787672.9	0.1494
Error	45	122011347		4918691	
Total Corrected	63				

Appendix 4: ANOVA for nodule at physiological maturity under field conditions

		Total nodules		Viable nodules		Non-viable nodules	
Source	DF	MS	p	MS	P	MS	P
Rep	3	404.80254	0.1910	18.29250	0.8501	321.34778	0.1990
Treatment	3	2981.52640	<.0001	815.69153	<.0001	847.01828	0.0099
Crop	3	18630.89952	<.0001	3360.34491	<.0001	7197.38520	<.0001
CropxTreatment	9	1175.51773	0.0002	236.86619	0.0027	728.57433	0.0017
Error	45	245.16423		68.96541		198.87060	
Total	63						

Appendix 5:ANOVA for dry matter at physiological maturity under field conditions

		Shoot biomass		Shoot dry matter		Root biomass		Root dry matter	
Source	DF	MS	P	MS	p	MS	p	MS	p
Rep	3	104617081	0.2090	24561975.6	0.1477	711025.93	0.1109	84680.431	0.0955
Treatment	3	108239487	0.1962	25807984.1	0.1323	305480.22	0.4433	33556.018	0.4529
Crop	3	136131272	<.0001	306355444.1	<.0001	24745244.53	<.0001	3094370.590	<.0001
CropxTreatment	9	57048680	0.5684	10974259.1	0.5864	446716.86	0.2479	52599.967	0.2180
Error	45	66492938		13113238		335390.21		37644.32	
Total	63								

Appendix 6: ANOVA for nodule at 100% flowering under glasshouse conditions

		Total nodules		Viable nodules		Non-viable nodules	
Source	DF	MS	p	MS	P	MS	P
Rep	3	153.640625	0.7226	186.875000	0.2707	142.937500	0.2372
Treatment	3	2670.765625	0.0003	1112.166667	0.0002	231.104167	0.0835
Crop	3	653.390625	0.1450	322.041667	0.0877	565.604167	0.0020
CropxTreatment	9	225.210069	0.7474	71.652778	0.8545	185.090278	0.0773
Error	45	15564.82813		138.59722		97.681944	
Total	63						

Appendix 7:ANOVA for dry matter at 100% flowering time under glasshouse conditions

		Shoot biomass		Shoot dry matter		Root biomass		Root dry matter	
Source	DF	MS	p	MS	p	MS	p	MS	p
Rep	3	4906554865.2	0.2076	12294850	0.8265	6009994.1	0.3770	364027.10	0.1525
Treatment	3	2712214589.4	0.4623	21750381	0.6656	12112576.6	0.1097	152078.08	0.5163
Crop	3	51070599281	<.0001	809582141	<.0001	59723861.7	<.0001	3954501.96	<.0001
CropxTreatment	9	2010842392.4	0.7509	15908377	0.9358	7761467.5	0.2326	105446.18	0.8415
Error	45	3107644548.4		41227289		5688246.3		197248.88	
Total	63								

Appendix 8: ANOVA for nodule at 100% pod formation under glasshouse conditions

		Total nodules		Viable nodules		Non-viable nodules	
Source	DF	MS	p	MS	P	MS	p
Rep	3	84.85417	0.9121	70.625000	0.8199	150.041667	0.6448
Treatment	3	2926.68750	0.0015	1566.875000	0.0007	351.125000	0.2834
Crop	3	5379.56250	<.0001	2168.750000	<.0001	1941.958333	0.0005
CropxTreatment	9	844.52083	0.1050	202.097222	0.5502	616.416667	0.0322
Error	45	482.14306		10338.12500		268.39722	
Total	63						

Appendix 9: ANOVA for dry matter at 100% pod formation under glasshouse conditions

		Shoot biomass		Shoot dry matter		Root biomass		Root dry matter	
Source	DF	MS	p	MS	p	MS	p	MS	p
Rep	3	1440972660	0.4120	83432522	0.4262	402712.8	0.9430	214031.256	0.5439
Treatment	3	45282796	0.8202	8910162	0.9590	3929596.3	0.3035	132835.406	0.7197
Crop	3	24722438770	<.0001	1047811502	<.0001	51391187.3	<.0001	2898645.160	<.0001
CropxTreatment	9	1870538485	0.2803	36564885	0.9205	1417579.2	0.8995	57621.050	0.9937
Error	45	1475022395.1		88157205				296289.48	
Total	63								

Appendix 10: ANOVA for nodules at physiological maturity under glasshouse conditions

		Total nodules		Viable nodules		Non-viable nodules	
Source	DF	MS	p	MS	p	MS	p
Rep	3	118.64063	0.4711	18.854167	0.6575	120.854167	0.3646
Treatment	3	141.84896	0.3915	191.104167	0.0027	19.229167	0.9142
Crop	3	7428.30729	<.0001	1753.729167	<.0001	2042.854167	<.0001
CropxTreatment	9	228.33507	0.1310	18.604167	0.8430	233.743056	0.0494
Error	45	138.68507		34.931944		111.25417	
Total	63						

Appendix 11: ANOVA for dry matter at physiological maturity under glasshouse conditions

		Shoot biomass		Shoot dry matter		Root biomass		Root dry matter	
Source	DF	MS	p	MS	p	MS	p	MS	p
Rep	3	2876433733	0.0945	3036489	0.9707	9084324.4	0.0475	226902.630	0.3418
Treatment	3	618315853	0.6941	22684902	0.6216	4220513.7	0.2775	124184.426	0.6020
Crop	3	15775982598	<.0001	1025995397	<.0001	67767833.0	<.0001	2365827.121	<.0001
CropxTreatment	9	930336964	0.6788	51639826	0.2373	5664142.8	0.0987	202662.145	0.4380
Error	45	1273655769.4		38132740		3180956.4		198423.69	
Total	63								

Appendix 12: ANOVA for ^{15}N, %Ndfa and %N at 100% flowering stage under field conditions

		^{15}N		%Ndfa		%N	
Source	DF	MS	p	MS	P	MS	P
Rep	3	1.38104167	0.0610	510.642917	0.0674	0.21937500	0.2487
Treatment	3	0.89354167	0.1791	351.592917	0.1690	0.31354167	0.1228
Crop	3	4.65437500	<.0001	1480.416250	0.0004	6.78562500	<.0001
CropxTreatment	9	0.64784722	0.2972	224.290833	0.3683	0.06854167	0.9033
Error	45	0.52359722		200.12525		0.15426389	
Total	63						

Appendix 13: ANOVA for %C total N in the shoot and total N in the root at 100% flowering under field conditions

		%C		Total N in the shoot		Total N in the root	
Source	DF	MS	p	MS	P	MS	p
Rep	3	4.5472917	0.3453	5277.7652	0.0717	2.5780729	0.2383
Treatment	3	5.4335417	0.2685	2813.1177	0.2760	0.4676563	0.8504
Crop	3	85.7272917	<.0001	59082.6060	<.0001	49.1330729	<.0001
CropxTreatment	9	4.1796528	0.4223	1463.4270	0.7117	0.9600174	0.8348
Error	45	4.0085139		2112.6689		1.7665174	
Total	63						

Appendix 14: ANOVA for total N (shoot + root), total N fixed in the shoot and total N fixed in the root at full flowering under field conditions

		Total N shoot + root		Total N fixed in the shoot		Total N fixed in the root	
Source	DF	MS	p	MS	P	MS	p
Rep	3	5500.7558	0.0704	458.90057	0.4475	0.41103073	0.5445
Treatment	3	2890.7796	0.2793	413.89557	0.4930	3.56724323	0.0012
Crop	3	62486.6804	<.0001	14354.65057	<.0001	4.99388490	0.0001
CropxTreatment	9	1460.5892	0.7335	550.56724	0.3941	0.82512517	0.1969
Error	45	2188.2753		508.58224		0.56982295	
Total	63						

Appendix 15: ANOVA for total N fixed (shoot + root), and C/N ratio at 100% flowering under field conditions

		Total N fixed (shoot + root)		C/N ratio	
Source	DF	MS	p	MS	p
Rep	3	688.05459	0.3844	3.5721974	0.1804
Treatment	3	665.79592	0.3991	3.9541641	0.1461
Crop	3	17343.63507	<.0001	82.4101266	<.0001
CropxTreatment	9	523.01739	0.6269	1.7029641	0.6089
Error	45	662.05887		2.1007163	
Total	63				

Appendix 16: ANOVA for ^{15}N, %Ndfa, %N and C/N ratio at 100% pod formation stage under field conditions

		^{15}N		%Ndfa		%N		C/N	
Source	DF	MS	p	MS	p	MS	p	MS	p
Rep	3	1.32916667	0.0669	465.495156	0.0806	0.12375000	0.4319	8.8630729	0.2251
Treatment	3	1.98041667	0.0162	770.114323	0.0137	0.06375000	0.6968	3.8839063	0.5803
Crop	3	6.17625000	<.0001	3231.049323	<.0001	7.37541667	<.0001	241.0730729	<.0001
CropxTreatment	9	1.54194444	0.0074	614.798351	0.0048	0.29472222	0.0376	4.6943229	0.6189
Error	45	0.51961111		194.22460		0.13241667		5.874184	
Total	63								

Appendix17: ANOVA for %C total N in the shoot and total N fixed in the shoot at 100% pod formation stage under field conditions

		%C		Total N in the shoot		Total N fixed in the shoot	
Source	DF	MS	p	MS	P	MS	P
Rep	3	5.2718229	0.4406	5205.9829	0.2100	3363.1102	0.1700
Treatment	3	3.7930729	0.5813	5802.1571	0.1706	6069.8935	0.0336
Crop	3	32.4501563	0.0023	326701.6733	<.0001	134653.6843	<.0001
CropxTreatment	9	16.2707118	0.0100	3219.9776	0.4766	7598.5714	0.0009
Error	45	5.7520451		3317.736		1919.8884	
Total	63						

Appendix 18: ANOVA for ^{15}N, %Ndfa and %N at physiological maturity stage under field conditions

		^{15}N		%Ndfa		%N	
Source	DF	MS	p	MS	P	MS	p
Rep	3	0.77557292	0.1428	348.96307	0.1543	0.07041667	0.5723
Treatment	3	0.64932292	0.2043	325.06932	0.1785	0.09375000	0.4497
Crop	3	2.73598958	0.0008	3621.60807	<.0001	9.49041667	<.0001
CropxTreatment	9	0.84418403	0.0528	431.13793	0.0343	0.08611111	0.5968
Error	45	0.40768403		190.13074		0.10441667	
Total	63						

Appendix19:ANOVA for %C, Total N in the shoot and Total N in the root at physiological maturity stage under field conditions

		%C		Total N in the shoot		Total N in the root	
Source	DF	MS	p	MS	P	MS	p
Rep	3	0.436875	0.8749	9332.804	0.5073	0.54932292	0.2484
Treatment	3	0.235208	0.9455	5325.549	0.7191	0.35682292	0.4367
Crop	3	781.168542	<.0001	446036.509	<.0001	26.44098958	<.0001
CropxTreatment	9	1.304236	0.7164	5096.340	0.9119	0.26765625	0.7110
Error	45	1.897764		11854.842		0.3859896	
Total	63						

Appendix 20: ANOVA for total N shoot + root, total N fixed in the shoot and total N fixed in the root at physiological maturity stage under field conditions

		Total N shoot + root		Total N fixed in the shoot		Total N fixed in the root	
Source	DF	MS	p	MS	P	MS	p
Rep	3	1746080.7	0.3673	5325.0313	0.4436	0.25991042	0.2799
Treatment	3	1206393.4	0.5303	1220.8654	0.8899	0.14591875	0.5334
Crop	3	40122590.8	<.0001	332196.2979	<.0001	9.79823542	<.0001
CropxTreatment	9	1264400.4	0.6339	2346.6839	0.9279	0.16731458	0.5758
Error	45	1617045.7		5849.939		0.19701042	
Total	63						

Appendix 21:ANOVA for Total N fixed (shoot + root), C/N ratio, at physiological maturity under field conditions

		Total N fixed (shoot + root)		C/N ratio	
Source	DF	MS	p	MS	P
Rep	3	5378.5484	0.4414	6.421875	0.4428
Treatment	3	1234.3717	0.8890	10.746042	0.2207
Crop	3	331879.8688	<.0001	500.536042	<.0001
CropxTreatment	9	2353.1979	0.9284	6.178681	0.5521
Error	45	5879.801		7.042653	
Total	63				

Appendix 22:ANOVA for 15N, %Ndfa and %N at 100% flowering under glasshouse conditions

		^{15}N		%Ndfa		%N	
Source	DF	MS	p	MS	P	MS	p
Rep	3	0.5387500	0.9295	120.72938	0.9066	0.23056875	0.3215
Treatment	3	11.4029167	0.0335	1954.87229	0.0411	0.08011875	0.7422
Crop	3	42.0629167	<.0001	7068.20563	<.0001	1.82826042	<.0001
CropxTreatment	9	4.2172222	0.3372	738.49785	0.3644	0.30227292	0.1535
Error	45	3.6064167		655.4949		0.19251319	
Total	63						

Appendix 23:ANOVA for %C, Total N in the shoot and Total N in the root at 100% flowering under glasshouse conditions

		%C		Total N in the shoot		Total N in the root	
Source	DF	MS	p	MS	P	MS	p
Rep	3	1.46804720	0.6334	2065.46057	0.2881	1.62469375	0.0880
Treatment	3	5.81705648	0.0917	971.54631	0.6129	0.38621042	0.6498
Crop	3	29.03246788	<.0001	25162.64392	<.0001	8.16748542	<.0001
CropxTreatment	9	2.39290404	0.5009	1239.57688	0.6387	0.52828403	0.6580
Error	45	2.5459614		1596.5011		0.70035264	
Total	63						

Appendix 24: ANOVA for total N shoot + root, total N fixed in the shoot and Total N fixed in the root at 100% flowering under glasshouse conditions

		Total N shoot + root		Total N fixed in the shoot		Total N fixed in the root	
Source	DF	MS	p	MS	P	MS	p
Rep	3	2172.30193	0.2711	101.597681	0.5961	0.11238659	0.0036
Treatment	3	996.13452	0.6072	1012.969106	0.0011	0.02476255	0.3412
Crop	3	25965.06451	<.0001	1982.814810	<.0001	0.10906764	0.0043
CropxTreatment	9	1284.35055	0.6213	502.973686	0.0050	0.06452841	0.0072
Error	45	1612.6723		159.91318		0.02162548	
Total	63						

Appendix 25: ANOVA for total N fixed (shoot + root), C/N ratio, at 100% flowering under glasshouse conditions

		Total N fixed (shoot + root)		C/N for shoot	
Source	DF	MS	p	MS	P
Rep	3	63.116272	0.8253	6.3355729	0.3077
Treatment	3	786.454845	0.0176	0.4197396	0.9695
Crop	3	1575.041981	0.0004	46.1280729	<.0001
CropxTreatment	9	333.540523	0.1490	8.1705729	0.1461
Error	45	210.47047		5.1251285	
Total	63				

Appendix 26: ANOVA for 15N, %Ndfa and %N at 100% pod formation stage under glasshouse conditions

		^{15}N		%Ndfa		%N	
Source	DF	MS	p	MS	P	MS	p
Rep	3	2.50291667	0.1951	386.85354	0.3043	.34445324	0.1107
Treatment	3	5.36291667	0.0230	1156.15021	0.0178	0.32031149	0.1315
Crop	3	12.92708333	0.0001	4044.15938	<.0001	0.37983632	0.0860
CropxTreatment	9	4.25694444	0.0112	1009.69507	0.0040	0.08306699	0.8583
Error	45	1.5330278		310.47421		0.16233293	
Total	63						

Appendix 27: ANOVA for %C, Total N in the shoot and Total N in the root at 100% pod formation stage under glasshouse conditions

		%C		Total N in the shoot		Total N in the root	
Source	DF	MS	p	MS	P	MS	p
Rep	3	4.6084896	0.2531	197.791904	0.5726	123.44485	0.8163
Treatment	3	2.2289063	0.5686	134.591646	0.7126	344.09675	0.4633
Crop	3	38.6176563	<.0001	2161.078704	0.0004	3706.35389	<.0001
CropxTreatment	9	4.8798785	0.1809	53.644197	0.9950	156.21476	0.9310
Error	45	3.2752674		293.53488		395.19237	
Total	63						

Appendix 28: ANOVA for total N shoot + root, total N fixed in the shoot and total N fixed in the root at 100% pod formation stage under glasshouse conditions

		Total N shoot + root		Total N fixed in the shoot		Total N fixed in the root	
Source	DF	MS	p	MS	P	MS	p
Rep	3	176.210117	0.6587	186.561652	0.0359	3.15066406	0.1776
Treatment	3	159.954588	0.6921	149.149302	0.0732	7.57786406	0.0115
Crop	3	2448.053321	0.0004	726.128472	<.0001	4.44946823	0.0784
CropxTreatment	9	69.939772	0.9911	50.966507	0.5774	3.98107517	0.0429
Error	45	327.53547		60.146988		1.8383563	
Total	63						

Appendix 29: ANOVA for total N fixed (shoot + root), C/N ratio, at 100% pod formation stage under glasshouse conditions

		Total N fixed (shoot + root)		C/N ratio	
Source	DF	MS	p	MS	P
Rep	3	236.116477	0.0228	421.409583	0.0709
Treatment	3	222.539639	0.0286	348.821250	0.1168
Crop	3	746.636727	<.0001	700.541250	0.0109
CropxTreatment	9	66.063209	0.4689	131.610556	0.6328
Error	45	67.373985		168.04947	
Total	63				

Appendix 30: ANOVA for ^{15}N, %Ndfa and %N at physiological maturity stage under glasshouse conditions

		^{15}N		%Ndfa		%N	
Source	DF	MS	p	MS	P	MS	p
Rep	3	0.07541667	0.9890	123.57750	0.9024	0.45237292	0.2120
Treatment	3	0.32708333	0.9121	48.26458	0.9735	0.24406042	0.4780
Crop	3	17.78708333	<.0001	8648.75542	<.0001	7.29316042	<.0001
CropxTreatment	9	5.04000000	0.0129	1956.16833	0.0067	0.26616458	0.5182
Error	45	1.8584167		649.13406		0.28979514	
Total	63						

Appendix 31: ANOVA for %C, Total N in the shoot and Total N in the root at physiological maturity stage under glasshouse conditions

		%C		Total N in the shoot		Total N in the root	
Source	DF	MS	p	MS	P	MS	p
Rep	3	6.9891667	0.0483	1290.80720	0.5599	0.09021667	0.9796
Treatment	3	1.4904167	0.6144	632.16088	0.7962	1.69372917	0.3347
Crop	3	142.4287500	<.0001	22686.28409	<.0001	5.16897083	0.0218
CropxTreatment	9	1.5525000	0.7641	1123.69529	0.7860	2.02815833	0.2205
Error	45	2.4587222		1857.0362		1.4576000	
Total	63						

Appendix 32: ANOVA for total N shoot + root, total N fixed in the shoot and total N fixed in the root physiological maturity stage under glasshouse conditions

		Total N shoot + root		Total N fixed in the shoot		Total N fixed in the root	
Source	DF	MS	p	MS	P	MS	p
Rep	3	1294.63702	0.5671	1452.83042	0.2284	0.08271643	0.5557
Treatment	3	681.30151	0.7826	1013.65625	0.3823	0.25908047	0.1012
Crop	3	23330.32155	<.0001	13739.61125	<.0001	2.12618689	<.0001
CropxTreatment	9	1144.45896	0.7871	1176.13500	0.3120	0.09497102	0.6128
Error	45	1895.7861		970.8713		0.11779874	
Total	63						

Appendix 33: ANOVA for total N fixed (shoot + root) and C/N ratio at physiological maturity stage under glasshouse conditions

		Total N fixed (shoot + root)		C/N for shoot		C/N for Root	
Source	DF	MS	p	MS	p	MS	p
Rep	3	1461.28401	0.2251	4.3720833	0.5619	405.27766	0.4601
Treatment	3	1017.59727	0.3794	3.0154167	0.6998	162.91432	0.7875
Crop	3	13994.90708	<.0001	133.2629167	<.0001	7901.15766	<.0001
CropxTreatment	9	1170.46091	0.3136	10.7713889	0.1160	1070.68057	0.0308
Error	45	968.3683		6.3200833		462.11255	
Total	63						

Appendix 34: ANOVA for height and pod number under field conditions

		Height		Pod Number	
Source	DF	MS	p	MS	P
Rep	3	88.13075	0.3981	320.19266	0.1420
Treatment	3	128.03688	0.2370	1445.83932	0.0001
Crop	3	9762.29912	<.0001	26849.78932	<.0001
CropxTreatment	9	216.41931	0.0218	2450.23266	<.0001
Error	45	87.44336		167.8921	
Total	63				

Appendix 35: ANOVA for pod length, pod diameter, seed weight and seed number under field conditions

		Pod length		Pod diameter		Seed weight		Seed Number	
Source	DF	MS	P	MS	P	MS	p	MS	P
Rep	3	0.116017	0.7623	0.01546823	0.4591	79693.38	0.8722	0.6553391	0.0108
Treatment	3	0.246187	0.4882	0.01088906	0.6064	372568.90	0.3615	0.2460849	0.2103
Crop	3	450.946842	<.0001	0.32617240	<.0001	18171591.90	<.0001	211.7051224	<.0001
CropxTreatment	9	0.632329	0.0481	0.03948767	0.0361	982813.01	0.0089	0.3034641	0.0711
Error	45	0.299200		0.01759601		340584.50		0.1569524	
Total	63								

Appendix 36:ANOVA for 30 Pods mass, Seed weight from 30 pods, 100 Seed weight under field conditions

		Pods mass		Seed weight from 30 pod		100 Seed weight	
Source	DF	MS	p	MS	p	MS	P
Rep	3	22.28131	0.5384	14.800481	0.5300	1.78322	0.8067
Treatment	3	46.73303	0.2183	35.003339	0.1672	5.47289	0.4016
Crop	3	4562.27836	<.0001	3247.030631	<.0001	4364.51031	<.0001
CropxTreatment	9	72.81408	0.0261	58.749997	0.0075	8.72614	0.1460
Error	45	30.43949		19.82121		5.47296	
Total	63						

Appendix 37: Abstract

EFFECT OF DIFFERENT INOCULANTS ON NODULATION OF TWO DIFFERENT SOYA BEAN CULTIVARS

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INTRODUCTION

Inoculation of legumes is fundamentally important, especially for soil where there is no efficient native strains of Rhizobium. Rhizobium is soil bacteria that is characterised by their ability to infect root hairs of legumes and induce nodulation, ie the formation of nodules. Inoculation of soya bean seeds with bacteria of the genus Bradyrhizobium promotes the production in per hectare basis of nodules in leguminous species, thus decrease production costs due to the ability of biological nitrogen fixation (Zill *et al.*, 2008). This advantage enables the replacement partial and/or total nitrogen through chemical fertilisers. This study aimed to evaluate the effect of different inoculants on the nodulation and performance of two soya bean cultivars.

MATERIALS AND METHODS

The experiment was conducted during 2013/14 summer-cropping season at ARC Grain Crops Research Station, Potchefstroom. The treatments consisted of two soya bean species namely, PAN1454R and PAN1729R. These cultivars were inoculated with different inoculant registered for soya bean. Unamended control plots were also were included as standard checks. The cultivar were fertilised at optimum recommended rates for phosphorus (P) and potassium (K), while no nitrogen was added. The sources of P and K were superphosphate and potassium chloride, respectively. Treatments were fitted in a randomised complete block design and replicated four times. Data collected was of total number of nodules and seed yield.

RESULTS AND DISCUSSION

The effect of inoculant showed significant differences on the number of nodules per plant, while no significant effects was found for cultivar. The higher total number of nodules per plant (44.6) were observed from plants inoculated by hicoat super + extender and minimum (22.3) by Vault Hp. The effect of inoculants did not exert statistically significant effects on soya bean seed yields, but significant for cultivar. Significantly higher seed yield was produced with PAN1729R. Generally, higher seed yield across the inoculants was achieved with soygro and vault HP with PAN1729R.

CONCLUSIONS

The study showed that hicoat super + extender promoted nodulation. Soya bean yields were not affected by the type of inoculant applied, but limited to the effects of the cultivar. Comparatively, PAN1729R produced higher yield than PAN1454R regardless of the type of inoculant used.

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Keywords: Inoculants, leguminous plants, nodulation, seed yield

Appendix 38: Abstract

EFFECT OF FERTILISER AND INOCULATION ON THE NODULATION AND PERFOMANCE OF FOUR DIFFERENT LEGUMES

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INTRODUCTION

Nodules are organ consisting mainly of infected cells of legumes with batteries rhizhobium genus that promote nitrogen fixation. Within the nodules the bacteria assume an endo-symbiotic way, being able to reduce atmospheric nitrogen to ammonia. As the ammonia is toxic to plants it is rapidly converted to amide and / or ureide that nourishes the host plant (legume) and contributing to their production (Xavier et al., 2006). This advantage enables the replacement partial and/or total nitrogen through chemical fertilisers. This study aimed to evaluate the effect of nitrogen fertilisation and inoculation on the nodulation and performance of four legumes.

MATERIALS AND METHODS

The experiment was conducted during 2013/14 summer cropping season at ARC Grain Crops Research Station, Potchefstroom. The treatments consisted of four legume species namely, cowpea, dry bean, groundnut and soya bean. These legumes were either fertilised or inoculated and/or the combination of both. Unamended control plots were also included as standard checks. The different legumes were fertilised at optimum recommended rates for P and K, while was only applied to specific treatments to determine the minimum accretion of whether nitrogen will influence on nodulation and productivity. The legumes were inoculated with the rhizobium inoculant registered for each respective crop. The sources of N, P and K were limestone ammonium nitrate, superphosphate and potassium chloride, respectively. Treatments were fitted in a randomised complete block design and replicated four times. Data collected was total number of nodules, viable and non-viable nodules, and seed yield.

RESULTS AND DISCUSSION

The treatment effect showed significant difference on the number of nodules per plant. The higher total number and viable nodules per plant were observed from inoculated plants irrespective of the legume specie. Average total number of nodules obtained from groundnut (48.5) and soya bean (42.9) at full flowering was significantly higher than from dry bean (17.4) and cowpea (8.2), while dry bean (83.2) and groundnuts (70.6) produced significantly higher total number of nodules than soya bean (41.5) and cowpea (6.4) at physiological maturity. The effect of fertiliser application and inoculation as well as their combination did not exert statistically significant effects on crop seed yields. Generally, higher seed yield across the crops was achieved with groundnut when fertilised and inoculated, while fertilised cowpea gave the lowest yield. Average seed yields were 247, 1419, 2761 and 2104 kg ha^{-1} for cowpea, dry bean, groundnut and soya bean, respectively.

CONCLUSIONS

The crops studied showed that fertiliser application had a depressive effect on nodulation but promoted when the different crops are inoculated comparable to when used in combinations. Nodulation in groundnuts was higher in both samplings, but lower with cowpea. Nonetheless, the response of dry bean and soya bean was infrequent across the sampling.

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Keywords: Fertiliser, inoculants, leguminous plants, nodulation, seed yield

Appendix 39: Groundnut in the field

Appendix 40: Cowpea in the field

Appendix 41: Dry bean in the field

Appendix 42: Soya bean in the field

Appendix 43: Field experiment

Appendix 44: Glasshouse experiment

Appendix 45:Digging groundnut plant biomass in the field

Appendix 46:Weighing plant in the field

Appendix47: Reference plant

Appendix 48: Nodules removed from the root

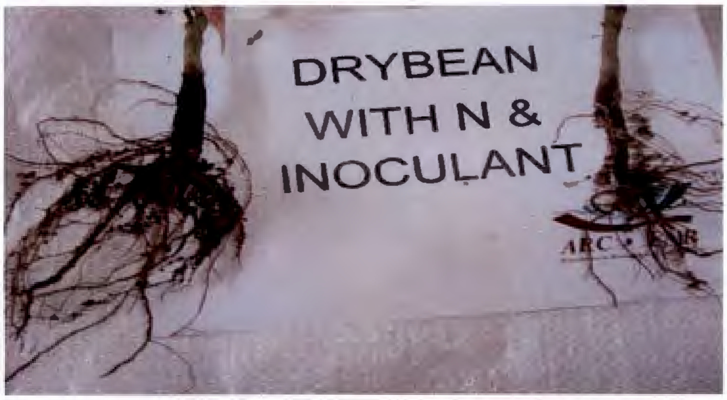
Appendix 49: Cowpea without inoculant and N fertiliser

Appendix 50: Cowpea treated withinoculation and N fertiliser

Appendix 51:Cowpea treated with N fertiliser

Appendix 52:Cowpea inoculated

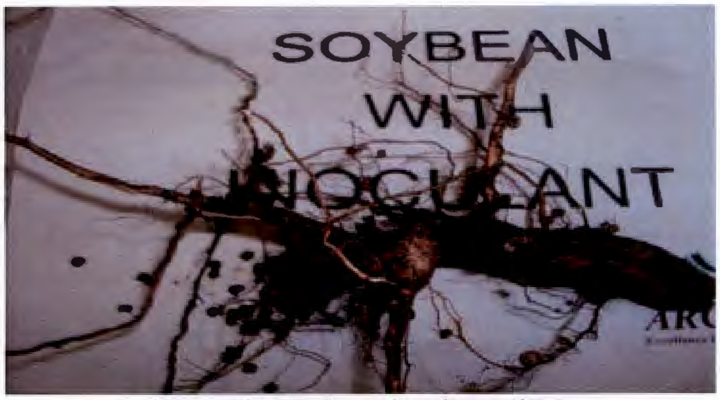
Appendix 53: Dry bean without inoculant and N fertiliser



Appendix 54: Dry bean treated with inoculation and N fertiliser

Appendix 55: Dry bean applied N fertiliser

Appendix 56: Dry bean inoculated



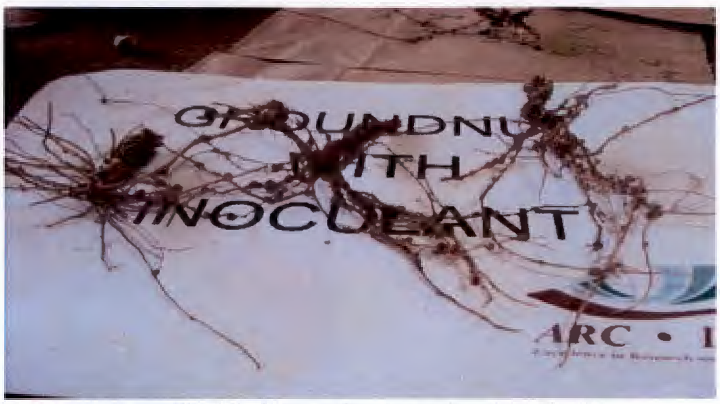
Appendix 57: Inoculated soya bean

Appendix 58: Soya bean treated with N fertiliser

Appendix 59: Groundnut without inoculant and N fertiliser

Appendix 60: Groundnut treated with inoculation and N fertiliser

Appendix 61: Inoculated groundnut



Appendix 62: Groundnut received N fertiliser

Appendix 63: Nodules removed from plants

Appendix 64: Scale, tweezers, spatula and tin capsule

Appendix 65: Weighing plant material for ^{15}N analyses