GROWTH AND NUTRITIONAL RESPONSES OF *SESBANIA SESBAN* (L) MERR.TO ROCK PHOSPHATE, BIOFERTILIZER AND RHIZOBIAL APPLICATIONS

By

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A thesis submitted in conformity with the requirements for the degree of Master of Science in Forestry
Graduate Department of Forestry
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Growth and Nutritional Responses of *Sesbania Sesban* (L) Merr. to Rock Phosphate, Biofertilizer and Rhizobial Applications

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Abstract

Continuous crop cultivation without adequate fertilizer input has led to poor yields and soil degradation in the tropics. To restore soil fertility through agroforestry practices, N_2-fixing tree fallows are planted to produce nutrient-rich biomass that is incorporated in soils. The quantity and quality of biomass produced can be improved by phosphorus fertilization, inoculation with rhizobia or use of biofertilizers. My study examined the effects of rhizobial inoculation, biofertilizer and rock phosphate applications and their interactions on growth and nutrition of *S. sesban* planted in potted acidic soils of western Kenya. Rhizobial inoculation improved nodulation only when rock phosphate was added. Although biofertilizer failed to stimulate root nodulation, it enhanced plant nutrient absorption. Fertilization with rock phosphate enhanced growth and nutrition of *S. sesban* most and is recommended for use in agroforestry. There was a small but significant beneficial interaction between rock phosphate and biofertilizer use on *Sesbania* growth and nutrition.

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CHAPTER ONE

1.0 GENERAL INTRODUCTION

Declining soil fertility as a result of continuous cropping without adequate input of fertilizers has been identified as one of the major biophysical problems facing smallholder farmers in Sub-Saharan Africa. The result of which, is the perpetual low per-capita food availability experienced in this region (Buresh and Tian, 1998; Sanchez, 2002; Garrity, 2004). The problem of low crop yields is aggravated by the pressure on agricultural lands due to the ever increasing human population. Shifting cultivation, a familiar paradigm that includes a period in sequence referred as fallow, the resting state of an agricultural field, used to be a remedial measure for degraded farmlands (Sanchez, 1999). During fallow periods, the naturally regenerating secondary vegetation is expected to restore soil fertility by building up nutrient stocks through biological processes, retrievals from subsoil, and through nutrient capture from subsurface lateral flow.

Shifting cultivation practice can effectively restore soil nutrient status provided the natural fallow periods are long enough; this may take 3 to 15 years for most nutrients, except for nitrogen which may be shorter (Szott et al., 1999). After long periods of fallowing, burning vegetation and woody biomass may add additional nutrient reserves to the soil through ash, as well as help clear agricultural lands of weeds (Gallagher et al., 1999). The nutrients accumulated in the topsoil through litter fall and in the vegetation during fallow period represent a capital gain for farmers. However, natural fallow periods have become shorter because of agricultural demands that the vegetation no longer impact sound ecological effects on degraded farmlands (Franzel, 1998; Jaiyeoba, 2003). Consequently, restoring soil fertility of degraded farms through natural fallowing has become unattainable in densely populated regions like western Kenya, where land size per household is small and land is continuously under cultivation (Rao et al., 2002). An obvious strategy to improve crop yields would be to apply inorganic N and P based fertilizers, since these two mineral nutrients have been diagnosed as most limiting for tropical soils. Significant yield responses are often observed in trials involving inorganic fertilizer applications (Palm et al., 1997; Rao et al., 2002) The major and most serious constraints to inorganic fertilizer use by resource-poor farmers in Sub-Saharan Africa are associated with their high costs and low market availability (Sanchez, 2002). Consequently, efforts to improve crop yields in nutrient depleted soils like western Kenya and other parts of Sub-Saharan Africa by low cost and sustainable agroforestry practices such as improved fallows have been proposed (Mafongoya and Dzowela, 1999).

Improved fallow systems involve planting of selected fast growing trees/shrubs during the fallow period that are expected to not only provide readily available nutrients for the subsequent crop, but also increase soil organic matter and hence improved soil physical conditions. The strategy of the system is to plant leguminous trees that 1) accumulate nutrients in their biomass and subsequently recycle them through litter fall and green manure or mulch applications; 2) improve soil physical and chemical properties; and 3) act as a break crop to smother weeds (Gallagher et al., 1999). Such agroforestry interventions to meet the growing need for productive and environmentally friendly agricultural systems for resource-poor farmers have stimulated renewed interest in the mechanisms by which fallows restore ecosystem fertility (Szott et al., 1999). Generally, improved fallows are established to overcome constraints to crop production by enhancing soil fertility during the cropping period. Trees and shrubs in fallow systems improve soil fertility through the supply of N, P and cations; adjustment of soil pH and building up of soil organic matter; improving soil physical structure (which in turn interacts with soil fertility); and controlling soil-borne pests and weeds (Buresh and Tian , 1998).

Relay planting of leguminous trees of the genera *Crotalaria*, *Gliricidia*, *Sesbania* and *Tephrosia* with young maize crops and allowed to grow after the crop harvest as fallows during the dry seasons, can accumulate 100 to 200 kg N ha^{-1} over a period of 6 months to 2 years in sub-humid tropical regions of East and Southern Africa (Gathumbi et al., 2004). The trees accumulate N which can increase crop yields through biological N_2 fixation, retrieval of NO_3-N from deep soil layers, and N-cycling from plant residues and manures. Effectiveness of fallow trees in restoring soil fertility of degraded farms depends largely on environmental factors such as climate and soils; type of fallow species, their population and management; and the duration of the fallow period. The restoration of N is mainly through biological N_2 - fixation which can contribute up to 100 and 300 kg N ha^{-1} yr^{-1} on low and high base status soils respectively (Giller, 2001). Although plant-available phosphorus is also critically low in most acidic soils of the tropics, P cannot be added through a biological process similar to N.

The main sources of plant-available P are weathering of minerals rocks, mineralization of soil organic matter, fertilizers and organic materials. Sustainable crop production in most tropical soils requires P inputs because the soils are either formed from parent materials with low P levels, or have been depleted of plant-available P through continuous cropping with little or no artificial fertilizer inputs (Sanchez et al., 1997). In cases where soils may have large reserves of total P, the amount of plant-available P is limited to sustain crop production due to transformations of extractable P to less readily available forms (Fe and Al phosphates) in acidic soils and Ca phosphates in near neutral or alkaline soils. Most tropical soils are characterized by inherently high Fe^{3+} ions and Al^{3+} toxicities (Fardeau and Zapata, 2002; Giesler et al., 2004), and their presence may account for the majority of the apparent P losses. Fardeau and Zapata (2002) postulated that when phosphorus is added to acid soils (pH < 5), for example in the form of phosphate rock, a coating of Al and Fe compounds is formed around phosphate rock particles with time, thus reducing further dissolution. Therefore, even in areas where large proportions of soil P may be found, the plant-available P ($H_2PO_4^-$ and HPO_4^{2-}) may be limiting. The cycling of P from organic materials is normally insufficient to meet the P requirements of crops. Hence, sustainable crop production under agroforestry on P-deficient soils will typically require external P inputs (Palm, 1995). However, application of inorganic fertilizers by resource-poor and small-scale farmers in areas where they are desperately needed, like western Kenya, is not feasible because of high costs and poor market structures associated with their use and distribution (Sanchez, 2002). Rock phosphates, such as Minjingu rock phosphate from Tanzania, has been proposed as a potential source of P for improved crop production in western Kenya and many other places within the region (Waigwa et al., 2003). Although rock phosphates are slow P-releasing, they are relatively cheap compared to other inorganic P fertilizers (e.g. triple super phosphate) for small scale farmers. However, the low solubility of phosphate ions to become plant-available is one of the major concerns for its agronomic importance. Many tree species in the tropics form associations with some beneficial fungal species that enhances P uptake. Through this association, trees are able to exploit larger soil volume as a result of extensive proliferation of mycorrhizal hyphae. Mycorrhizal fungi may also contribute to the production of phosphatase enzymes and organic acids that improve availability of soil P (Radersma and Grierson, 2004). Apart from the fungal associations, there are also arrays of soil microbes that are found within the rhizosphere of plants that aid nutrients availability for plant uptake.

Phosphate solubilizing bacteria that secrete organic acids and phosphatases are common in the rhizosphere (Dodor and Tabatabai, 2003). Together with other soil micro-organisms such as rhizobia that make nutrients become available to plants, the P-solubilizing bacteria are referred to as biofertilizers. Biofertilizer, which is a contraction of the term "biological fertilizer" can be defined as a substance which contains living micro-organisms which, when applied to seeds, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant (Vessey, 2003). Whether the existence of micro-organisms increases the growth of plants by replacing soil nutrient (e.g., by biological N_2 fixation (Giller, 2001) or by making nutrient more available (e.g.

solubilization of phosphates (Rice et al., 1994) or by increasing plant access to nutrients (e.g. increasing root surface area), the substance that was applied qualifies to be a biofertilizer as long as the nutrient status of the plant is enhanced by micro-organisms. Soil nutrient availability, whether through solubilization or biological processes such as biological N_2-fixation, is critical for biomass production and nutrient accumulation by the planted tree fallows. If soils are deficient in some nutrients such as P that are crucial in N_2-fixation process, leguminous trees may fail to maximize their potentials to accumulate more carbon and fix atmospheric N_2, thus negating the intended goal of replenishing soil fertility.

My thesis study examined the growth and nutritional responses of *S. sesban* (an improved fallow species) to rhizobia, biofertilizer and rock phosphate applications and the possible interactions. The objectives of the study were to: i) determine gains in biomass production and nutrient accumulation by S. *sesban* when inoculated with rhizobia and fertilized with biofertilizer and/or rock phosphate; ii) quantify biological N_2-fixation capacity of *S. sesban* as affected by rhozobial inoculation, biofertilizer and rock phosphate applications; and iii) evaluate beneficial interactions from the various treatment combinations applied. The working hypothesis for the study was that rhizobial inoculation, biofertilizer and rock phosphate applications improve growth and nutrition as well as the biological N_2-fixation capacity of *S. sesban* grown in acidic soils of western Kenya.

To address the study objectives, this thesis has been structured into four related chapters. The current chapter is a general introduction to the study. The second chapter critically reviews the relevant literature, sets up the rationale for the study and formulates the study objectives. Chapter three describes and presents in a journal form, a specific greenhouse study conducted in order to answer some of the research questions and test the hypotheses. The concluding chapter provides a summary of the results highlighting major contributions of the research and proposes possible direction for future research in this area.

CHAPTER TWO

2.0 LITERATURE REVIEW AND STUDY APPROACH

2.1 Introduction

This chapter reviews the literature to provide the background information necessary to explain the rationale and objectives of the study. The first section describes the general concept of improved fallow as an agroforestry practice and how it is employed to address problems of low soil fertility in some tropical regions. The success of improved fallow practice depends on the amount of biomass produced and the efficiency of nutrient cycling within the system. Species that produce more biomass of high quality within a short period are preferred for integration in improved fallow practices. Therefore, a brief review of biomass production and nutrient uptake by leguminous tree species planted as improved fallows is made. Since N and P are often deficient in most tropical soils and the costs of using chemical fertilizers are too high for small-scale farmers, symbiotic N_2-fixation by leguminous trees is crucial in replenishing soil N. Factors that affect biological N_2-fixation (BNF) process such as the presence of effective *Rhizobium* and key nutrients like P are critical to enable planted fallow species build N nutrient stocks that can be recycled. Hence, factors affecting BNF and the overall contribution of N by trees are critically examined. Unlike N, there is no biological P-fixation to add P to the plant-soil system.

Moreover, plant-available P also limits crop production due to the formation of stable phosphate complexes with Ca^{2+}, Fe^{3+} and Al^{3+} in the soil. So there is always a need for exogenous supply of P through fertilizer applications. Several reports indicate that use of commercial P-fertilizers in tropical countries is not feasible due to their high costs (Sanchez, 2002). Therefore, potential use of low-cost and locally available Minjingu rock phosphate as a source of P in the region is reviewed. It is important to note that rock phosphates are slow P-releasing unlike the water soluble commercial P-fertilizers such as triple supper phosphate. Therefore, methods of enhancing the solubility of rock phosphates and other fixed P sources are critical. There are some soil microbes found within the rhizosphere of most plants that are reported to aid P-solubilization. A review of the benefits of plant growth-promoting rhizobacteria referred to hereafter as biofertilizer is made, with specific emphasis on P-solubilizing bacteria. With favourable soil conditions for planting fallow trees, the choice of the best species that would produce more biomass and accumulate high nutrient contents within a short time becomes a priority. *Sesbania sesban* has been favoured for adoption as an improved fallow in western Kenya since it is indigenous in the region and it is also a source of fodder and fuelwood.

Hence, the advantages and factors affecting the selection of *S. sesba*n as a suitable improved fallow species in western Kenya are examined. The remaining section of this chapter provide the study rationale by identifying problems associated with biomass production and nutrients uptake in *S. sesban* as an improved fallow species. A detailed greenhouse study to evaluate the response of *S. sesban,* an improved fallow species, to rock phosphate, biofertilizer and rhizobial applications is provided in the subsequent Chapter 3. Prior to the specific study, the concept of replenishing soil fertility through planting leguminous trees is reviewed.

2.2 Improved-fallows practice as an agroforestry strategy to improve soil fertility

The concept of improved-fallow as an agroforestry intervention to improve soil fertility in most tropical countries is different from what the term 'fallowing' means in temperate regions. Fallowing in temperate refers to the period when agricultural land is out of production; left uncultivated usually due to government subsidies for not growing crops or the land is left to recover its productivity mainly through accumulation of water and attrition of pathogens (Sanchez, 1999, Moret et al., 2006). The main emphasis of fallowing in temperate regions is on conservation of soil moisture by reducing water loss through evapo-transpiration in semiarid areas since land may be bare during that time. In the tropics, improved-fallow refers to deliberate planting of leguminous trees/shrubs in degraded agricultural fields with the primary objective of improving soil fertility through litter turn-over and biological processes (Sanchez, 1999). Trees or shrubs that are usually planted in either sequential or relay systems are superior to the natural secondary vegetation in rehabilitating degraded farmlands. Sequentially arranged improved tree fallow system may be preferred to alley cropping, since it reduces direct resource-competition between trees and crops (Buresh and Cooper, 1999).

In the bimodal rainfall pattern of western Kenya, fallow trees are usually planted towards the end of the long rains (March - June) when annual crops are just about to mature and left to grow during the short rains (September –November) or for a period desirable for farmers but not less than 6 months. The longer the duration of the fallow period the better the planted trees are able to confer ecological effects on the degraded lands. During fallow periods, the planted trees are expected to replenish depleted soil nutrients in degraded farms through biological N_2-fixation, nutrient capture both from subsoil pools and the subsurface soil layers through extensive root systems (Buresh and Tian 1998, Gathumbi et al., 2003). Improvements of soil chemical and physical characteristics by trees in improved-fallow systems are reported to increase crop yields (Szott et al., 1999). Yields of maize (*Zea mays* L.), a common staple food in East and Central Africa, are found to increase substantially after improved fallows compared with continuous maize or natural fallow agroforestry systems (Jama et al. 1998, Mafongoya and Dzowela 1999). Improved fallow trees produce green manures that are usually rich in most plant nutrients that when incorporated *in situ* during land preparation, or applied as mulch during cropping season, decompose to improve soil fertility. Green manure produced by fallow trees increase soil organic matter content which results in improved soil buffering capacities of the highly weathered soils in the tropics. Apart from high costs and low availability of inorganic fertilizers in most tropical countries (Sanchez, 2002), their application to annual crops is sometimes accompanied by heavy losses through leaching due to low buffering capacity of the widely distributed low activity clay soils (Tian and Kang, 1998).

When nitrates accumulate in the highly weathered soils of the tropics either from mineralization of organic matter or fertilizer applications, and water supplied via precipitation or irrigation exceeds crop demands, nitrate ions readily combine with base cations and leach through the soil profile. It is in view of these facts that improved-fallow trees play another significant role in capturing leached nutrients and recycle them through litter-fall and root turn-over to the subsurface soil layers for easy access by annual crops: a phenomenon referred to as 'nutrient pumping'(Buresh et al., 2004). High quantities of nutrients are also sequestered in the large quantities of biomass produced in fallow systems. Planted tree fallows provide other benefits such as production of stakes or fuelwood, suppression of weeds, and reduction of soil erosion on sloping lands.

2.3 Biomass production and nutrient dynamics in improved-fallow species

The benefits of planting improved fallow trees species to enhance soil fertility are achieved through: (i) accumulation of large quantities of nutrient-rich biomass that easily decompose releasing nutrients into the soil to replenish the depleted stocks, and thus increase soil organic matter contents and soil structure (Mafongoya et al., 1998); (ii) deep root systems that effectively capture mineral nutrients leached below the root zone of most annual crops, then recycled to the subsurface soil layers through litter fall consequently reducing nutrient losses (Mekonnen, et al., 1997; Buresh et al., 2004); (iii) reduced erosion and improved weed suppression by dense fallows (Gallagher et al., 1999); and (iv) enriched soil N through biological N_2-fixation that plants can use directly (Giller, 2001; Graham and Vance, 2000). Therefore, the success of planting fast-growing leguminous trees to replenish soil fertility as a strategy to improve crop yields primarily depends on the efficiency of the species to accumulate more biomass that are loaded with high concentrations of nutrients, mainly N, P and K. The amount of biomass produced by planted tree fallows depends on the tree species, the number of trees per hectare, tree age and site factors (Buresh and Cooper 1999). Nutrient concentrations in the biomass are also influenced by tree species, phenological stage, management and site factors (Palm et al., 2001). The intensity of prior land use and the extent of top soil loss or nutrient deficiencies have significant effects and can reduce biomass and nutrient accumulation of established vegetation in improved-fallow systems.

Yield response of crops following successful improved fallow periods normally depends on the amount of biomass and N accumulated during the fallows (Szott et al., 1999). In most herbaceous leguminous fallows including a variety of annual cover crops, biomass accumulation peaks at about one year. On the other hand, woody fallows can be more effective beyond one-year duration as they are capable of larger biomass accumulation. Fallows of only six-month duration typically accumulate insufficient N to produce a yield response beyond one subsequent crop. Longer duration fallows (of 2–3 years) may accumulate larger quantities of N and may provide a residual yield effect to two or three subsequent crops (Szott et al., 1999). Increased maize (*Z. mays*) crop yields in Zimbabwe following *Sesbania* fallows were reported to be directly related to high biomass accumulation realized at the end of the fallow period. As such, *S. sesban* in a one-year fallow period produced approximately 10 Mg ha^{-1} aboveground biomass yielding about 120 kg N ha^{-1} (Mafongoya and Dzowela, 1999). The high N contents in the biomass of *Sesbania* are contributions mainly from biological N_2-fixation and nitrates absorbed from deeper soil horizons. Mekonnen et al. (1997) reported that nitrate build up in a 15-month *Sesbania* fallow at 4-m depth was 51 kg N ha^{-1} compared to the 199 kg N ha^{-1} for unfertilized maize. The maximum rooting depth was 1.2 m for maize, whereas roots in a 15-month-old *S. sesban* fallow extended below 4 m. Theses findings exemplify the advantage of using deep rooted trees/shrubs to capture and recycle leached nutrients.

Both nitrogen and phosphorus have been identified as the major limiting nutrients in the acidic and highly weathered soils of the sub-humid and humid tropics. It is important to note that planted fallows may meet the short-term N requirements of crops but they cannot overcome P deficiency, which still need to be corrected through applications from external sources. In severely degraded soils, P replenishment can be made either by regular annual additions of P fertilizers or, as a recapitalization strategy, by a single large application (Sanchez et al., 1997). However, unavailability of inorganic fertilizers is one of the major constraints for crop production in small-holder farms. Low plaint-available P in soils has also been reported in various studies to adversely affect symbiotic nitrogen fixation (Sanginga et al., 1989; Leidi and Rodriguez-Navarro, 2000). Therefore, the interaction of P and N can significantly affect the contributions of leguminous trees in building nitrogen stocks in degraded lands as expected in agroforestry systems such as improved fallows.

2. 4 Contributions of N through biological N_2-fixation

The contribution of N by leguminous trees through biological N_2 fixation (BNF) is well recognized for replenishing soil N (Sanginga et al., 1995; Graham and Vance, 2000). Although it is important to note that not all legumes fix atmospheric nitrogen. There are non-nodulating legumes such as *Senna siamea* and *S. spectabilis* that have been widely tested in some agroforestry systems and found to extract more soil N which they accumulate in their biomass (Young, 1997). Quantification of N_2-fixation, particularly for older trees, has proven difficult due to constraints in methodologies for measuring N_2-fixed (Chalk and Ladha, 1999). Nonetheless, high variability is sometimes observed among provenances or isolines of tree species in percentages of total plant N derived from the atmosphere - %Ndfa (Sanginga et al., 1990). The ranking of provenances for %Ndfa is also dependent on growth stage, and differences observed in short duration pot studies may not reflect the long-term N_2-fixation in the field.

Therefore, estimates of N_2-fixation by legume tree species are, to a large extent, site specific. For instance deficiency of available P, which is a characteristic of many tropical soils, can limit N_2-fixation and growth of legume (Sanginga et al., 1995). So how well a species is adapted to a given site/environmental conditions may determine the quantity of atmospheric N_2-fixed. Efficient absorption and utilization of P can be a critical factor for legumes growing in low P soils, lest their productivity and BNF potentials are adversely affected. Thus, increases in N_2-fixation by trees can be achieved through correct selection of tree germplasm and proper fertilization, particularly with phosphorus.

High phosphorus requirement for nodulation is considered to be responsible, at least in part, for the interactions between mycorrhiza and many legume species. Mycorrhizal infection of roots increases the acquisition of phosphorus in plants grown in low phosphorus soils (Rajendran and Devaraj, 2004) and, thus increased root nodulation is realized. Inoculating leguminous trees with the correct *Rhizobium* strain is another critical management requirement, especially for non-promiscuous species (tree species that nodulate with only specific strains of *Rhizobium*). Exogenous introduction of *Rhizobium* ensures that the correct strains are present in desirable populations to cause significant root nodulation (Turk et al., 1992). Although adoption of rhozobial inoculation requirements has remained low world-wide, despite several years of research that underlines its importance, it has a major influence on BNF (Giller, 2001). In view of this fact, the best strategy is to plant legumes in their native environment where there are high chances for the existence of compatible rhizobia that would effectively nodulate the planted trees to cause high N_2-fixation.

Leguminous trees species; *Crotalaria grahamiana, Gliricidia sepium, S. sesban* and *Tephrosia vogelii* have been found to fix 36-80%, 30-55%, 78-86% and 58-73% of atmospheric N_2 respectively (Giller, 2001). The variations in %Ndfa recorded with these species were mainly due to differences in stages of growth, management and site quality, especially the presence of effective *Rhizobium* bacteria and soil P-availability required for nodulation. Therefore, contribution of legume species such as *S. sesban* in integrated soil fertility management strategies such as improved fallow agroforestry practice is important, especially in addressing soil N deficiencies. Planted tree fallows may build up N stocks through symbiotic N_2-fixation; there is no similar biological process that they can add P to the ecosystem. Thus, P can only be added to the system from exogenous sources such as commercial inorganic P-fertilizers or rock phosphates. But the high costs associated with the use of commercial P-fertilizers leaves locally available and low cost rock phosphates as the most viable option for small-scale farmers in densely populated areas such as western Kenya.

2.5 Rock phosphate fertilizer as an agronomic source of P

Low plant-available P is always reported in most nutrient-depleted acid Oxisol and Ultisol (deep, red, and highly weathered) soils in the tropics (Waigwa et al., 2003). To overcome the problem of P nutrient deficiencies in these soils external input of phosphorus is always necessary to ensure improved plant/crop production. The most available P sources are the processed phosphate rocks (commercial inorganic P-fertilizers) and the finely ground phosphate rocks. Applications of commercial P-fertilizers are often not cost effective on most tropical soils as the phosphate ions are easily 'sorbed' by aluminium and iron oxide minerals (Fardeau and Zapata, 1999). High P-fixation associated with tropical soils demands for frequent application of P-fertilizers to ensure better crop yields. The other source of P that is locally available in most tropical regions is the rock phosphates. Although rock phosphates are slow P-releasing compared to the readily water soluble phosphorus fertilizers like triple super phosphate, they are locally available and cheaper than other commercial P-fertilizers in tropical regions (Xiong et al., 2002). Minjingu rock phosphate (MRP) from Tanzania that contain about 30% of the agronomically active phosphorus pentoxide (P_2O_5) is locally available in many parts of East Africa and can be used to replenish P in smallholder farms within the region. Wider application of MRP in P-depleted farms like in western Kenya is only limited by its low solubility (Waigwa et al., 2003)

Therefore, if the solubility of phosphate rocks could be improved, resource-poor smallholder farmers would have an affordable source of phosphorus for improved crop productions. Recent study by Savini et al., (2006) focused on ways to improve the solubility of Minjingu rock phosphate through alterations of soil pH and calcium contents and applications of organic materials. It is important to note that the factors considered in the above study affects the activities of soil micro-organisms such as P-solubilizing bacteria that are found within the rhizosphere of most plants. Phosphate solubilizing microorganisms convert insoluble phosphates into soluble forms through the processes of acidification, chelation and exchange reactions (Rodríguez and Fraga, 1999; Radersma and Grierson, 2004). One of my main objectives in this thesis study was to develop mechanisms that improve the solubility of Minjingu rock phosphate through additions of exogenous P-solubilizing micro-organisms e.g. *Bacillus subtilis*, *B. licheniformis* and *B. polymyxa* supplied as a biofertilizer as suggested by Sahu and Jana (2000).

2.6 Biofertilizers enhancing nutrient uptake and general tree growth and development

Nitrogen and phosphorus are known to be essential nutrients for plant growth and development. Leguminous plants such as *S. sesban* acquire N, partly through a symbiotic relationship with some soil bacteria mainly of the genera *Rhizobium* (Evans and Macklin 1990; Odee, 1990). Although there is no similar process as N_2-fixation that plants can access P, there are some bacterial and fungal species that have been found to solubilize phosphorus fixed in the soil (Wu et al., 2005). N_2-fixing and P-solubilizing bacteria are important in plant nutrition when they increase N and P uptake and may play a significant role as plant growth-promoting rhizobacteria (PGPR). The P-solubilizing and N_2-fixing micro-organisms constitute a group of PGPR referred to as biofertilizers. By definition, a biofertilizer is a product containing living cells of different types of microorganisms, which have the ability to convert nutritionally important elements from unavailable to available form through biological processes (Vessey, 2003). This definition makes clear distinction between biofertilizers and organic fertilizers (fertilizers containing organic compounds which directly, or by their decay, increase soil fertility).

Acid soils such as the Ultisols and Oxisols that predominate in the highlands of western Kenya are low in available phosphorus, mainly as a result of P-adsorption by aluminium (Al) and iron (Fe) oxides forming stable complexes (Waigwa et al., 2003). Hence, increases in soil plant-available P without external input that may occur through agroforestry whether from decomposing litter, mycorrhizal associations or biofertilizers are only apparent resulting from recycled P within the ecosystem (Palm, 1995). When phosphorus is added to soil as a phosphate such as in rock phosphate, part of the phosphate ions are absorbed by plants and the remainder converted into insoluble fixed phosphate complexes (e.g. $CaHPO_4$, $Ca_3(PO_4)_2$, $FePO_4$ and $AlPO_4$) that become inaccessible to plants (Fardeau and Zapata, 2002). There are a number of reports on plant growth promotion by bacteria that have the ability to solubilize inorganic and/or organic P from soil after their inoculation in soil or plant seeds and leads to increase in yields (Rodriguez and Fraga 1999; Dodor and Tabatabai, 2003; Wu et al., 2005). In a study on the effects of biofertilizer application on the growth and nutrient uptake by *Casuarina equisetifolia* (a species that fixes N_2 through an association with *Frankia*), better nutrient uptake was estimated in treatments that combined inoculation of *Azospirillum* + *Phosphobacterium* + AM + Frankia as opposed to other combinations without phosphobacterium (Rajendran and Devaraj, 2004). Secretion of organic acids and phosphatases are common methods of facilitating the conversion of insoluble forms of P to plant-available forms (Rodriguez and Fraga, 1999). Many soil micro-organisms show the ability to solubilize dicalcium phosphate and tricalcium phosphate when provided as the sole P source in laboratory media. But only a few have the ability to solubilize ferric and aluminium phosphates (Halder and Chakrabartty, 1993). If the soils have adequate P and other essential nutrients and also contain the correct rhizobial strains to cause root nodulation in legumes used as fallow tree, the onus remains to select the right tree species for the area. *S. sesban* is fast growing and commonly found interspersed in the natural vegetation.

2.7 *Sesbania sesban* as a suitable species for improved-fallow practice in western Kenya

Sesbania sesban (L) Merr., a leguminous tree of the family *Papilionaceae*, is a soft-wooded, fast growing and short-lived tree found widely distributed in Kenya and many parts of East Africa (Onim and Otieno, 1991; Heering 1995). The species exhibits rapid seedling growth; conducive for improved-fallow systems as a way of accumulating high biomass within a short period and for replanting should management reduce the regeneration vigour of old plants (Evans and Macklin, 1990). *S. sesban* is an indigenous species and is commonly grown dispersed among crops. Therefore, its use in improved-fallow technology is relatively easy for farmers to adopt (Kwesiga et al., 1999; Sanchez, 2002). The species has comparative advantages over others in terms of biological nitrogen fixation, fast growth, high-quality foliage for green manure and forage, relatively better quality fuelwood and its deep rooting habit to capture leached nutrients (Buresh and Tian, 1998). Another major advantage of the genus *Sesbania* is that most of its species are tolerant to waterlogging and can grow in both saline and alkaline conditions. Although *S. sesban* is a non-promiscuous legume, the *Rhizobium* strains that nodulate its roots are fast-growing and are found in a wide range of African soils (Bala et al., 2002). The wide distribution of *S. sesban* in Sub-Saharan Africa, its high N_2-fixing potential and other benefits has encouraged the planting of the species by small-scale farmers to improve soil fertility or for other uses (Chirwa et al., 2004). Several studies have demonstrated the potential of two- or three-year *Sesbania* fallows in restoring soil fertility and increasing maize yields. Economic analyses also indicate that improved fallow systems are feasible, profitable, and acceptable to farmers especially in areas with high population densities (Jama et al., 1998; Kwesiga et al., 1999; Rao et al., 2002).

Increased maize yields following *Sesbania* fallows are primarily due to improved N input and availability from the decomposition of high quality litter incorporated at the end of the fallow period. The potential to increase maize production without applying mineral fertilizers is of great significance to resource poor farmers who cannot afford the costs of inorganic fertilizers. In one researcher-managed trial in Zambia, 6.0 Mg ha^{-1} maize yield was realized following a three-year sesbania fallow compared to 4.6 Mg ha^{-1}continuous cropped maize with fertilization (112 kg N ha^{-1}) and 1.9 Mg ha^{-1} without fertilizer (Kwesiga et al., 1999). Chikowo et al., (2004) estimated the total N_2-fixed by *S. sesban* contained in the non-woody components of the above-ground biomass plus litter to be 84 kg N ha^{-1} in a two-year fallow. The studies highlighted above exemplify the suitability of *S. sesban* as an improved fallow species to increase crop yields at the expense of the high cost chemical fertilizers. The high N_2-fixing capability of *S. sesban* coupled with its multiple uses is desirable in improved fallow agroforestry systems.

Leguminous tree planted in improved fallow systems may indeed be agronomically more efficient than traditional fallows in restoring fertility. Traditional fallow systems, however, provide a broad range of functions and products. Hence, farmers might not consider improved-fallows as 'improved' in a broader context unless they provide functions and products in addition to soil fertility restoration (Buresh and Cooper, 1999). *Sesbania sesban* has multiple uses that make it attractive for planting on farms. Apart from improving soil fertility when planted as an improved fallow, *S. sesban* is a good source of fodder for livestock (Melaku et al., 2004). The susceptibility of *S. sesban* to root-rot nematode (*Meloidogyne* spp.) has been identified as one of major problem that may limit its use on farm lands. Disease and pest studies in improved-fallow systems indicate that root-knot nematodes (*Meloidogyne javanica*) are associated with *S. sesban* and *T. vogelii* while root-lesion nematodes (*Pratylenchus* spp.) are common in *C. grahamiana* fallows. Interestingly, *Crotalaria* spp. are antagonistic to *Meloidogyne* while *S. sesban* and *T. vogelii* suppress *Pratylenchus* (Desaeger and Rao, 1999). Therefore, mixture of contrasting species in relation to pest attack and to maximize resource capture in improved fallows has been suggested as the best option to address the problems or limitations associated with single species establishments (Desaeger and Rao, 2002, Gathumbi et al., 2002). But it is important to examine and authenticate the contributions and the factors that may affect the performance of individual trees that are planted to restore soil fertility.

2.8 Study rationale

To date, most of the studies involving integration of leguminous trees in improved fallow practices take little account of the presence of effective rhizobia capable of nodulating the planted trees for optimum biological nitrogen fixation (BNF). True to the fact, biological N_2-fixation is a symbiotic relationship between some legume plants and soil bacteria, mainly *Rhizobium* spp. Without effective rhizobia strains in soils to cause root nodule formation, the process of N_2-fixation may fail altogether, which negates the objective of planting the legumes trees to improve soil fertility. Inoculation of N_2-fixing legumes with effective *Rhizobium* or *Bradyrhizobium* bacteria is crucial for the success of N_2- fixation if other factors are favourable. Since P is also limiting in most tropical soils, the need for exogenous supply of P in improved fallow practices is well recognized (Jama et al 1998; Ndufa et al., 1999).

In most studies, P is usually applied to the system at the end of the fallow periods which unfortunately excludes the fallow trees from realizing benefits of the additional P-supply. Other studies also indicate that P-deficiencies may adversely affect biological N_2-fixation process in leguminous plants. Therefore, there are two key issues related to P-applications that this study intends to address: 1) the application of P to the legume trees to improve general growth and biological N_2-fixation; and, 2) the use of low-cost and locally available Minjingu rock phosphate as a source of P since processed commercial P-fertilizers may be too expensive for most small-scale farmers. Although rock phosphates are known to be slow P-releasing, mechanisms to improve their solubility needs to be explored further and promoted to ensure efficient use by plants. For this reason, a commercial biofertilizer containing P-solubilizing micro-organisms was used with a view to increasing the solubility of P and other nutrients to improve uptake by *S. sesban*.

2.9 Study approach

In order to effectively investigate and evaluate the responses of *S. sesban* to rock phosphate, biofertilizer and rhizobial applications as well as to monitor any interaction effect, a greenhouse approach was used to eliminate other environmental factors as much as possible. Seedlings of *Sesbania sesban* and *Tithonia diversifolia*, a non-legume reference plant for estimating biological N_2-fixation, were grown in pot soils amended with rock phosphate and biofertilizer either singly or in combinations to assess interactions. The *S. sesban* seedlings were either inoculated with known effective *Rhizobium* or left uninoculated. Specific details of the study and the subsequent responses observed are as reported in the following chapter.

CHAPTER THREE

3.0 GROWTH AND NUTRITIONAL RESPONSES OF *SESBANIA SESBAN* (L) MERR.TO ROCK PHOSPHATE, BIOFERTILIZER AND RHIZOBIAL APPLICATIONS

Abstract: *Sesbania sesban* (L.) Merrill is a leguminous tree currently planted in western Kenya and other regions within East and Central Africa as an improved fallow species. The trees are planted to restore soil fertility of degraded smallholder farms through production of high-quality biomass that when applied as green manure or mulch usually improves yields of food crops planted after the fallow periods. Most studies testing leguminous trees in improved fallow agroforestry practices take little account of rhizobial inoculation and P requirements of planted trees which may enhance biological nitrogen fixation (BNF). Therefore, it is important to know the extent to which the presence of effective *Rhizobium* and soil nutrient status, especially P affect biomass production, biological N_2-fixation and overall nutrient uptake by S. *sesban*. A greenhouse experiment was set up in 2 x 2 x 2 factorial design to study the effects *Rhizobium* (KFR 647) inoculations, Minjingu rock phosphate utilization, and commercial biofertilizer applications on growth and nutritional responses of *S. sesban*. Rhizobial inoculation significantly increased root nodulation by about 50% only when P was added as rock phosphate. Phosphorus fertilization increased height growth (58.3 cm vs. 29.6 cm in controls). About 90% more biomass was obtained from P fertilization alone compared to the control or sole rhizobia inoculation treatments, and 68% more compared to the commercial biofertilizer alone or when combined with rhizobia. Fertilization with rock phosphate significantly improved biological N_2-fixation process and overall nutrient uptake. Biofertilizer application enhanced plant nutrient uptake. A significant interaction between rock phosphate and biofertilizer increased growth performance of *S. sesban*.

3.1 Introduction

In western Kenya, most smallholder subsistence farmers own 0.2-0.8 ha of lands that are continuously cultivated without use of external fertilizer inputs (Rao et al., 2002). The practice usually results in soil nutrient depletion that requires inorganic fertilizer additions to increase crop yields. However, due to weak infrastructure, poor transportation and high costs, commercial inorganic fertilizers are generally inaccessible by subsistence farmers in developing countries (Sanchez, 2002). This scenario favours intercropping legumes and other species capable of fixing atmospheric N_2 as the main source to supplement N, one of the major limiting nutrient in these systems apart from phosphorus.

Fast growing nitrogen-fixing trees such as *S. sesban, C. grahamiana, G. sepium* and *Tephrosia spp.* are currently recommended for short-duration improved-fallows in agroforestry systems (Sanchez, 1999). These leguminous tree species, especially *S. sesban,* produce large quantities of biomass that may serve to replenish soil fertility when incorporated *in situ* as green manure or mulch to increase crop yields on degraded farms. Production of high-quality, N-rich green manure by improved fallow tree species is highly dependent on the availability of sufficient soil N and P. Nitrogen availability for leguminous trees and crops is critical during early stages of development before the formation of effective root nodules for the subsequent symbiotic N_2-fixation process (Leidi and Rodriguez-Navarro, 2000). Various studies have shown that leguminous trees fix more atmospheric N_2 than most of the annual grain legumes (Giller, 2001). In both cases, the amounts of N_2-fixed and the proportion of plant N derived from the fixation process vary enormously between species, genotypes of the same species and the environments in which the legumes are grown. The major critical elements of the environment that are key to symbiotic N_2.fixation are soil fertility (cation exchange capacity), soil microbial community (containing the correct rhizobia for the legume), and the soil moisture regimes. Therefore, the selection of tree species with high N_2-fixing and biomass production potentials coupled with improved crop management strategies for efficient N use are imperative for sustained future agricultural production (Bøckman, 1997). Improved-fallows can increase P in labile fractions of soil organic matter and increase crops yields on P-deficient soils (Maroko et al., 1998). However, on severely P-deficient soils, mineral P fertilization must be used in conjunction with improved leguminous fallows to overcome P constraints to crop production (Jama et al., 1998).

Sesbania sesban usually produces high biomass that is rich in most plant nutrients. It also fixes large quantities of atmospheric nitrogen (100 - 350 kg N/year) when inoculated with effective rhizobia (Odee, 1990; Sinha, 1998) or when grown in its natural range. The success of leguminous trees/plants to enhance N build up in improved-fallow systems therefore, depends on the availability of effective rhizobia bacteria that are capable of forming root nodules with the host plants to optimize their N_2-fixation potentials. Unlike N_2-fixation, there is no similar biological process that trees can add P to the soil-plant system. Although incorporation of green manure from improved-fallow tress into the soil may increase P in labile fractions of soil organic matter (Palm, 1995), this is just recycling of P rather than net addition of P to the plant-soil system.

Therefore, with respect to P requirements, the major function of the trees is to recycle and conserve nutrients rather than to cause net increases in ecosystem nutrient stocks (Buresh and Tian 1998). The recycling and conservation of nutrients are related to rates of biomass accumulation and nutrient immobilization in biomass (Palm, 1995). Many plants exhibit mechanisms that may provide direct access to less plant-available P by solubilizing the fixed P in the soils (Radersma and Grierson, 2004). Phosphate solubilizing bacteria are common in the rhizosphere and secretion of organic acids and phosphatases are common methods of facilitating the conversion of insoluble forms of P to plant-available forms (Dodor and Tabatabai 2003). In this case the P-solubilizing bacteria and other micro-organisms that are capable of making nutrient available to plants are regarded as biofertilizers. Availability of phosphorus to leguminous trees is very critical, especially in P-deficient soils such as the Ultisols soils of western Kenya. Study by Sanginga et al. (1989) clearly demonstrates the importance of P nutrient and its effects on biological nitrogen fixation (BNF). Most studies conducted with *S. sesban* and other legume trees in improved-fallow systems recommend application of phosphorus at the end of the fallow period, especially at the time of land preparation when incorporating the green manure produced from the fallows (Franzel, 1998; Jama et al., 1998). Noting that the design of most improved fallow practices are either sequential or relay, application of P at the end of fallow period mainly benefit the annual crops at the expense of the fallow trees that also require external P inputs in these P deficient soils to enhance their BNF and biomass accumulation capabilities.

The main research questions that propelled this study were; i) Does Minjingu rock phosphate application have the potential to supply P to enhance growth performance, nutrient uptake and biological nitrogen fixation in *S. sesban* grown in P-deficient soils of western Kenya? (ii) Will *Rhizobium* inoculations and biofertilizer applications enhance growth performance, nutrient availability and symbiotic N_2-fixation potentials of *S. sesban*? and; (iii) will *S. sesban* biomass production and nutrient uptake be affected by interactions between rock phosphate, biofertilizer and rhizobia inoculations? A factorial experiment (2 x 2 x 2) was designed to identify the best treatment combination that can maximize biomass production, nutrient uptake and biological N_2-fixation by *S. sesban*. These attributes are important in selecting a suitable tree species for integration in improved-fallow agroforestry practices.

3.2 Materials and Methods

3.2.1 Experimental site

A glasshouse experiment was conducted at the Kenya Forestry Research Institute near Nairobi, Kenya. The institute is located at an altitude of about 2100 meters above sea level, latitude 1° 13'S, and longitude 36°38'E. The mean day and night temperatures were 23°C and 15°C respectively. The soil used for this pot experiment was collected from a farmland that had been under natural fallow for the past five years in Nyabeda region of western Kenya. The region receives mean annual rainfall of 1000-1800 mm distributed in two seasons. The soils of the area are acidic and highly weathered, generally described as Ultisols according to USDA soil taxonomy. Top soils (0 – 20 cm depth) were collected from different spots within the farm then bulked, thoroughly mixed and air-dried. The dried soil was sieved to pass through a 2-mm mesh and then used to fill 5 kg plastic pots. Minjingu rock phosphate and commercial biofertilizer acquired from a local Agrochemical store in western Kenya were applied to the designated pots and mixed thoroughly with the soil. The basic properties of the soil used before the application of the experimental treatments were as shown (Table 3.1).

3.2.2 Seeds scarification and biofertilizer preparation

Seeds of *Sesbania sesban* were pre-treated by soaking in hot water for 12 hours to ensure uniform germination. The pre-treated seeds of *S. sesban* and those of reference plant, *Tithonia diversifolia* (Gathumbi et al., 2002), were surface sterilized with 3.5% ml/V hypochlorite solution and then rinsed in several changes of sterile distilled water before sowing in trays of sterilized washed quartz sand to germinate in an incubator set at 28°C. For rhizobial inoculations, a known effective *Rhizobium* strain KFR647 originally isolated from root nodules of *S. sesban* collected from Yala swamp in western Kenya was cultured in yeast-mannitol medium (Vincent, 1970) in rotary incubator at 28°C for five days. At the time of inoculation, the bacterial culture was estimated to contain 1x 10^9 cells per 1ml of the broth. The bacterial strain KFR647 had been screened and found to be very effective in nodulating *S. sesban* leading to high nitrogen fixation (Desaeger et al., 2005). The other micro-organisms (*Bacillus subtilis*, *B. licheniformis*, *B. polymyxa B. megaterium* and *Trichoderma harzianum*) were supplied through a commercial biofertilizer traded as ***Organica Plant Booster Plus.*** In this product, the micro organisms were embedded in a solid medium composed of feather meal, steamed bone meal and sulphate of potash (potassium sulphate). Apart from the micro-organisms, the medium also contained 8%N (NO_3^-), 2%P (P_2O_5) and 4%K (K_2O).

3.2.3 Experimental treatments and design

The commercial biofertilizer (B) (***Organica Plant Booster Plus***) and rock phosphate (P) were applied to the designated pots at a rate of 30 and 100 kg P ha^{-1} soil respectively. The soil and the applied products were thoroughly mixed before irrigating the pots with distilled water and planting the seedlings. Four-day old seedlings of *S. sesban* (legume) and those of a reference plant, *T. diversifolia* (non-legume) were pricked out and planted at a rate of two plants per pot. *Sesbania sesban* seedlings assigned inoculation treatments were supplied with 2 ml of the bacterial broth at the base (Odee, et al., 2002) immediately after transplanting. The ^{15}N isotope dilution method (Chalk and Ladha, 1999) was used in order to separate N contributions from different pools in *S. sesban*. A single application of 10% ^{15}N atom excess labelled ammonium sulphate, $(NH_4)_2SO_4$, fertiliser solution was made to supply N at a rate of 20 Kg N ha^{-1} (10mg N kg^{-1} soil) to all the pots as a tracer immediately after planting.

The experiment was a 2 x 2 x 2 factorial treatment structure with four replicates laid out in a completely randomised design in which the effects of rock phosphate, commercial biofertilizer and rhizobial applications were examined in all-additive combinations. Specific treatments and codes applied were as follows: C- Control; I- Inoculation with *Rhizobium;* IB- Inoculation with *Rhizobium* + Biofertilizer (Organica); B- Biofertilizer; IP- Inoculation with *Rhizobium* + Rock Phosphate; P- Rock phosphate; BP- Biofertilizer + Rock phosphate; BIP- Biofertilizer + Inoculation with *Rhizobium* + Rock phosphate. To control the sources of nitrogen in the test plants, pots were watered only with deionised water. The pots were randomly rearranged after every week to reduce edge effects during the 90 days the seedlings were allowed to grow.

Table 3.1 Some initial physico-chemical characteristics of the soil used in pot experiment collected from Nyabeda area in western Kenya

Characteristic	Values
Sand:Silt:Clay (%)	56:38:6
Textural class	Sandy-loam
pH (H_2O)	4.86
Organic carbon (%)	4.23
Total N (%)	0.12
NO_3^- (ppm)	82.2
Available P (ppm)	15.33
Exchangeable bases (cmol$_c$)	
K	50.00
Ca	4.83
Mg	3.81

3.2.4 Measurements of growth response parameters

Seedlings heights were measured 40 days after planting (DAP), then after every 14 days and later at an interval of 7 days. At the end of the experiment (90 DAP) root collar diameters of the seedlings were also measured to test for any correlation between these parameters and the treatments applied and the overall biological nitrogen fixation capacity of *Sesbania sesban*. Shoot, root and nodule biomass produced by *S. sesban* were also determined at the end of the experiment to evaluate the effect of the treatments on biomass production, a factor which is crucial in selection of improved-fallow species.

3.2.5 Plant sampling procedure and sample preparation

Total destructive sampling of the whole plants was carried out at 90 days after planting. The shoots were cut at the base and cleaned of any traces of soil particles that could cause contamination during ^{15}N and other nutrient analysis. The harvested shoot portions were cut into small pieces of about 2 mm and put into sampling paper bags. On the same day, the roots were washed clean of the soil medium and rinsed with distilled-deionised water. Root nodules from the fixing species were separated and put in separate sampling containers. The remaining root portions of the fixing species and those of the reference plants were also chopped into small pieces and put in separate bags. All the samples were oven-dried for 72 hours at 70°C and later weighed to determine the biomass production of the different portions of the test plants. After weighing the samples, the root nodules were mixed with the other root parts from where they were collected and then ground into fine powder as one sample. For $\%^{15}N$ atom excess, total %N and other plant nutrient analysis, the shoot and root portions were treated separately.

3.2.6 Plant tissue $\%^{15}N$ atom excess determination

For ^{15}N atom excess determination, plant samples were sent to the Joint FAO/IAEA-Agriculture and Biotechnology laboratories at Seibersdorf, Austria where the isotopic N determination was conducted using Mass Spectrometer. From the determined values of total %N and $\%^{15}N$ atom excess in the legume (F) and reference (NF) plants, the proportions of N derived from different pools i.e. percent nitrogen derived from fertilizer (%Ndff), percent nitrogen derived from soil (%Ndfs) and percent nitrogen derived from the atmosphere (% Ndfa) were calculated based on the following isotope dilution method equations as outlined (IAEA, 2001):

$$\%Ndff = \frac{atom\,\%^{15}N\ excess\,(plant)}{atom\,\%^{15}N\ excess\,(fertilizer)} x100 \ldots\ldots\ldots\ldots\ldots\ldots (1)$$

$$\frac{\%\ Ndff_{\ NF}}{\%\ Ndfs_{\ NF}} = \frac{\%\ Ndff_{\ F}}{\%\ Ndfs_{\ F}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

With equation 2 being the only assumption in this model, the contribution of N from the atmosphere through biological fixation by the legume was calculated from the equation;

$$\%\ Ndfa = \left(1 - \frac{\%\ Ndff_{\ F}}{\%\ Ndff_{\ NF}}\right) \times 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (3)$$

The equation is arrived at after substituting equation (2) in the general equation expressing the percentages of N derived from different pools by the legume expressed as,

$$\%Ndff \ + \ \%Ndfs \ + \%Ndfa \ = \ 100 \ldots\ldots\ldots\ldots\ldots\ldots (4)$$

In this model it is generally assumed that both fixing (F) and non-fixing (NF) plants take up nitrogen from the soil and fertiliser in the same ratio. For this to be true the fixing and non-fixing crops must meet some of the following conditions: a) either the fertiliser distribution is even with depth or the legume and reference crops have similar root systems and spatially similar nutrient uptake profiles, i.e. the root systems be similar, the contribution of seed N is assumed to be negligible and (b) the enrichment of substrate remains constant with time or the legume and reference crops have similar N uptake patterns (Hardarson and Danso, 1993). These conditions are easy to meet in a pot experiments where the roots of both fixing and reference plants are in a confined space and source for nutrients in all sections of the rooting medium as was observed in this experiment. The quantity of nitrogen fixed by the legumes per pot during the experimental period was calculated from the equation below:

$$N_2\ Fixed(g/pot) = \frac{\%Ndfa \times N\ Content\ in\ fixing\ tree}{100} \ldots\ldots (5)$$

3.2.7 Plant tissue and soil nutrient analysis

Comparative N, P and K nutrient analysis of shoots and roots were carried out to assess their uptake and accumulation by *Sesbania sesban* as influenced by the treatments. Sub-samples of oven-dried plant materials were digested following wet-ashing procedures, using a heated mixture of hydrogen peroxide and concentrated sulphuric acid (Lowther, 1980). The digest was analyzed for N by the phenol blue (Berthelot) reaction using a Technicon AutoAnalyzer (Schuman et al., 1973). Phosphorus (P) was determined using colorimetric method following molybdenum reaction (Allen 1974). Potassium (K) was determined using atomic absorption spectrophotometer. Soil samples were collected from each pot before the soil medium was washed off from the roots for soil nutrient analysis to determine the residual effects of the treatments. Soil pH, total %N, NO_3-N, %Org.C and other soil macronutrients (P, K, Ca, and Mg) were determined. Total soil organic carbon (TOC) was determined using the Loss-On-Ignition (LOI) method as described by De Vos et al., (2005); where

$$TOC = -0.1046Clay + 0.5936\ LOI = \ldots\ldots (6)$$

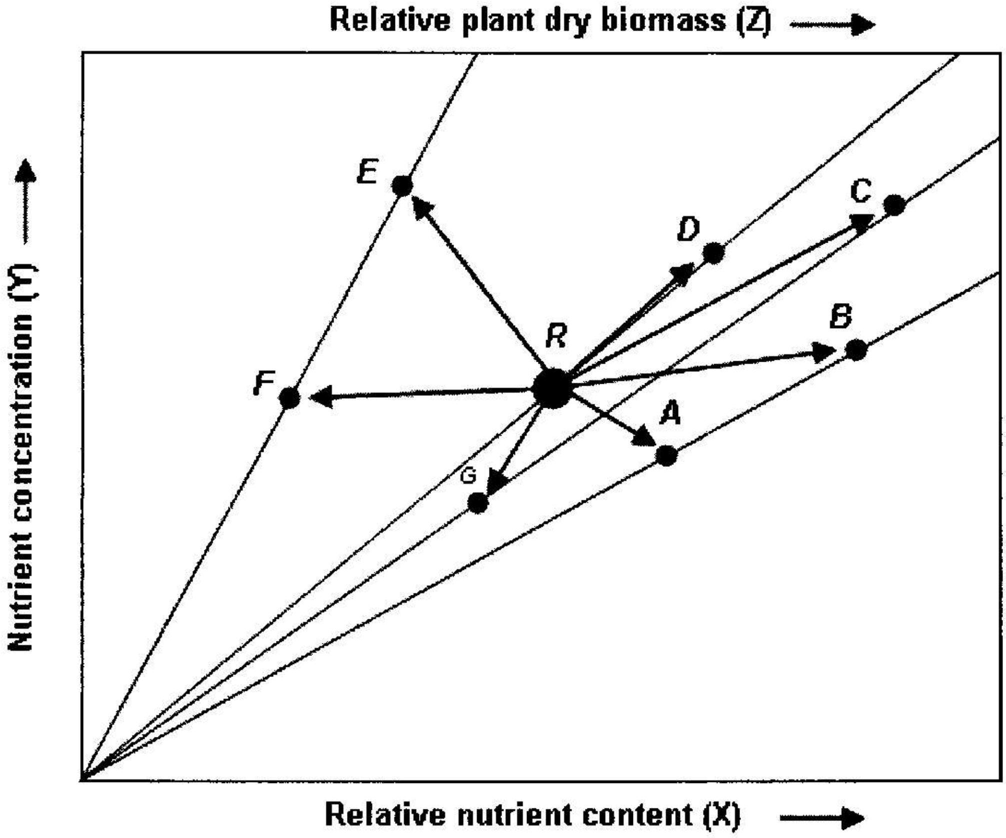
Nutrient vector diagnosis model was used to examine the growth responses of the seedlings in relation to their nutrient contents.

3.2.8. Vector diagnosis

Vector diagnosis as described by Imo and Timmer, (1997); Salifu and Timmer (2003) is an explicit method used to analyze plants nutrient status and corroborate the effects of treatments applied on the occurrence of growth dilution, nutrients sufficiency, luxury consumption and antagonism. In this model, plants growth in terms of biomass and nutrient accumulation as affected by different treatments are simultaneously examined in a single nomogram in which nutrient content (x), nutrient concentration (y), and dry biomass (z) satisfy the function $x = f(y, z)$ (Haase and Rose, 1995). Plant nutrient concentrations are plotted on the vertical axis, while nutrient contents and dry mass are on the lower and upper horizontal axes respectively. Treatment values are normalized to a reference point (R) equivalent to 100 units of the control to facilitate relative comparisons. The lines stemming from the origin represent lines of equal biomass. Thus, points on the same line represent change in nutrient concentrations and contents without change in biomass as is the case of luxury consumption or nutrient loading when compared with control set as the R treatment (Figure 3.1). Changes in nutrient availability affecting the three parameters are integrated into vectors (*A, B, C* etc.) defined by orientation and magnitude. The length of the vector represents the magnitude of the response. The box beneath Figure 3.1 provides the diagnostic interpretation of the vector direction and possible nutrient status of the plant or crop.

3.2.9 Data analysis

The data generated were analysed using SAS 8.2 (SAS Institute Inc.1999) testing the hypothesis (at $P < 0.05$ significant level) that rhizobial inoculation, rock phosphate and biofertilizer applications affect growth and nutritional status as well as biological N_2-fixation of *S. sesban*. A one-way analysis of variance (ANOVA) was performed on each data set and where there were significant differences means were separated by Tukey's highly significant difference test at $P < 0.05$. For the main effects of rock phosphate (P), biofertilizer (B) applications and rhizobial inoculation (I) and their interactions, the data was analyzed using procedures for a split-plot design with a 2 x 2 x 2 factorial treatment structure, replicated four times. The main treatments were tested at two levels: with or without their applications. Growth and nutrient parameters were evaluated by the vector diagnosis as described above.



Vector shift	Change in			Interpretation	Possible diagnostic
	Z	*Y*	*X*		
A	+	-	+	Dilution	Growth dilution
B	+	0	+	Sufficiency	Steady state
C	+	+	+	Deficiency	Limiting
D	0	+	+	Luxury	Accumulation
E	-	++	±	consumption	Toxic
F	-	-	-	Excess	Antagonistic
G	0,	-	-	Excess	Re-translocation
	+			Depletion	

Figure 3.1 The general vector interpretations of directional changes in relative nutrient contents (*x*), nutrient concentrations (*y*) and relative dry biomass (*z*) of plants grown in contrasting soils (Source: Imo and Timmer, 1997).

3.3. Results

3.3.1 Growth responses in height and root collar diameters

Height measurements taken at different times during the growth period and final root collar diameters differed significantly ($P< 0.05$) as per the treatments applied (Figures 3.2 & 3.3). Superior growth performances were achieved in treatments that received rock phosphate, whether alone or in combination with other treatments (Plate 3.1, Figure 3.2). Inoculations with sole rhizobia did not significantly improve height growth or the root collar diameter increments. However, when combined with the commercial biofertilizer, seedlings height and root collar diameter increased by about 10 units in each case at the later stages of growth. Combined rhizobial inoculation and P supplementation resulted in rapid growth in early stages (48 and 62 DAP) than the combined biofertilier and rock phosphate treatments. In later the stages, combination of rhizobial inoculation, biofertilizer and rock application resulted in a much faster growth than the rest of the treatments (Figure 3.2).

3.3.2 Plant biomass accumulation and partitioning

Dry weights of shoots, roots and nodules varied significantly depending on the treatment applied (Table 3.3). Generally more biomass was accumulated in the shoots than in the roots of seedlings. Carbon allocation to roots was reduced by about 15% as soil nutrient conditions were improved. The highest biomass yields were realized in treatments that contained rock phosphate fertilizer. These were about 90% higher than those obtained in either control or sole rhizobia inoculation treatments, and 68% higher than those produced with commercial biofertilizer only or a combination of the biofertilizer and rhizobia. The application of commercial biofertilizer had a negative effect on root nodulation where virtually all treatments supplemented with this product failed to form nodules; hence no nodule biomass was recorded in those treatments. Inoculating *S. sesban* seedlings with known effective rhizobia significantly ($P<0.05$) increased root nodule biomass production, especially with application of rock phosphate fertilizer.

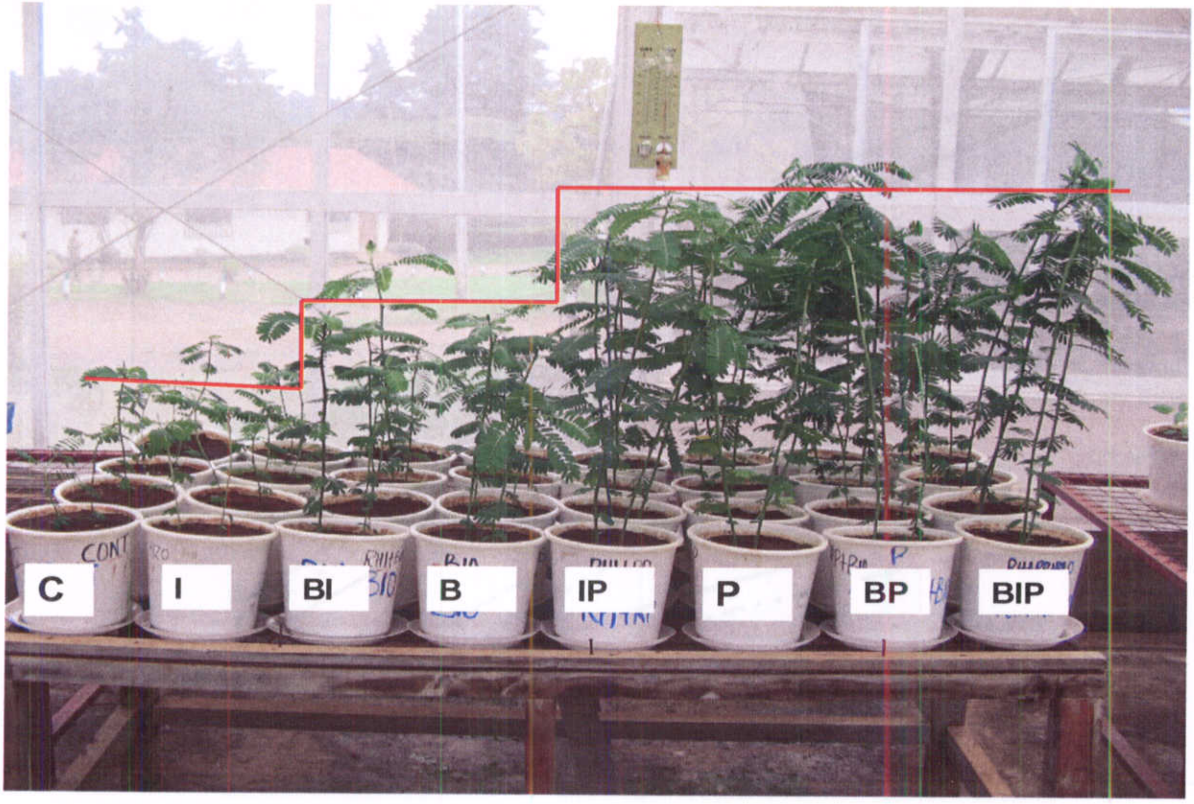


Plate 3.1 Variations in height growth of *Sesbania sesban* seedling as affected Rhizobium, rock phosphate and biofertilizer additions. Where application of rock phosphate fertilizer resulted improved growth of seedlings. Treatments: C-Control, I- Inoculation with *Rhizobium,* B-Commercial biofertilizer, P- Rock phosphate.

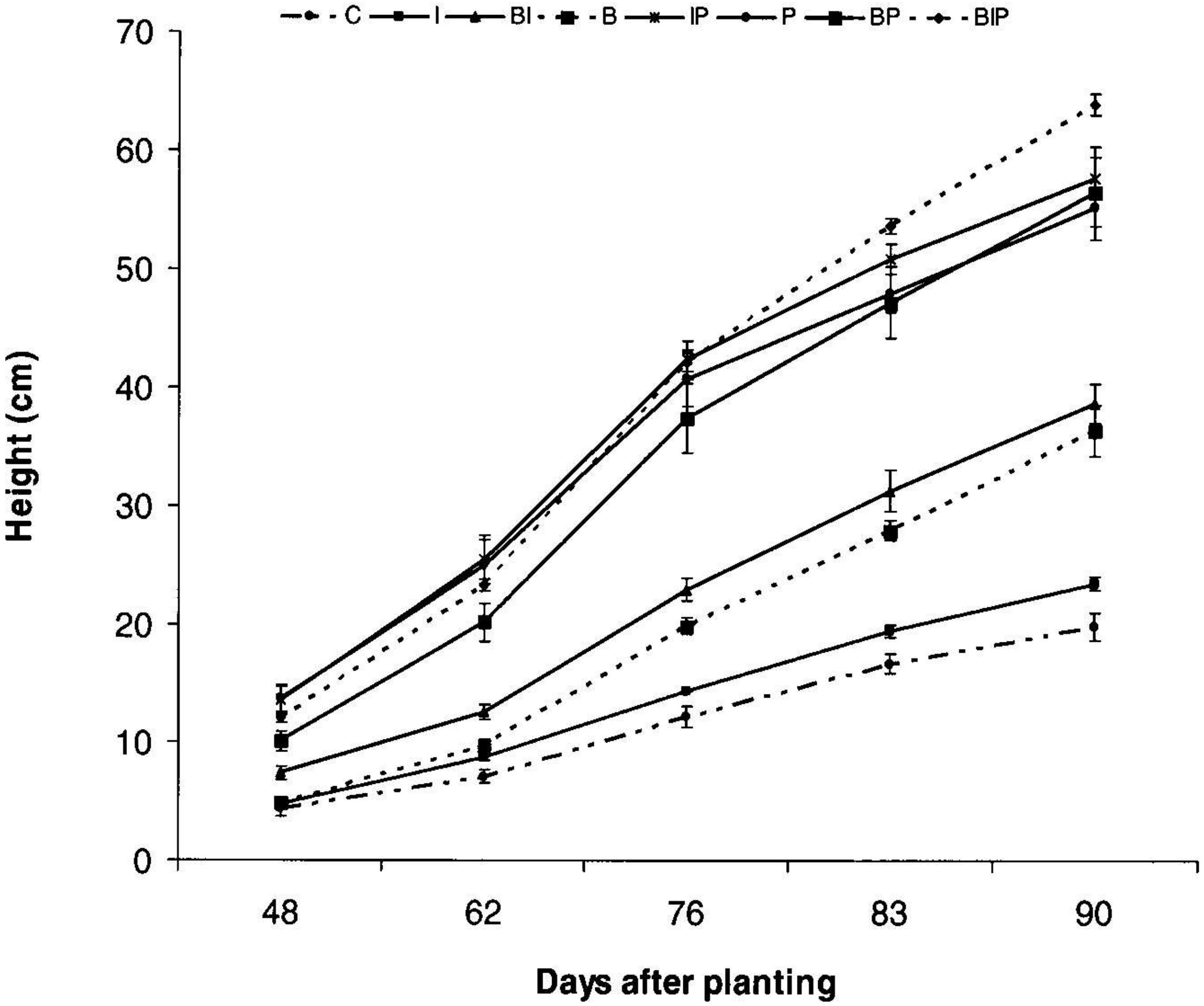


Figure 3.2 *Sesbania sesban* seedling height developments (cm) after planting in a greenhouse as influenced by; rhizobial inoculation (I), biofertilizer (B) and rock phosphate (P) treatment.

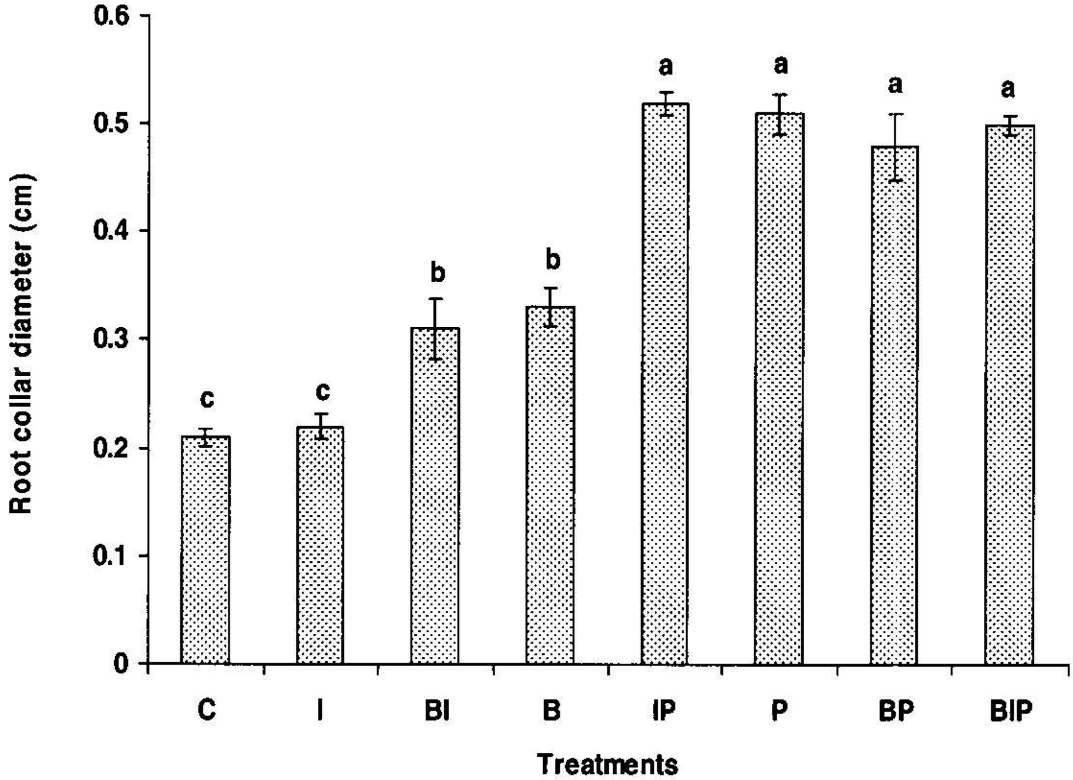


Figure 3.3 Root collar diameters of *S. sesban* seedlings 90 days after planting as influenced by rhizobial inoculation, biofertilizer and rock phosphate applications. Bars with the same letter are not significantly different at the 0.05 level of probability.

Table 3.2 Dry matter yields from different portions, shoot-to-root ratios and root collar diameters of *Sesbania sesban* as affected by rhizobial inoculation, biofertilizer and rock phosphate fertilizer applications under greenhouse conditions

Treatments	Nodules (mg pot^{-1})	Root biomass (g pot^{-1})	Shoot biomass (g pot^{-1})	Total biomass (g pot^{-1})	Shoot:Root ratio
C	0.2 c	0.43 b	0.81 b	1.25b	1.88dc
I	0.2 c	0.61 b	1.00 b	1.56b	1.62d
BI	0	0.88 b	2.32 b	3.20b	2.64bc
B	0	0.78 b	2.28 b	3.06b	2.92b
IP	48.0 a	1.92 a	6.05 a	8.02a	3.14ba
P	23.0 b	2.14 a	7.16 a	9.31a	3.34ba
BP	0	1.95 a	6.28 a	8.23a	3.22ba
BIP	0	1.72 a	6.76 a	8.48a	3.92a
LSD 5%	0.8	0.49	1.67	2.12	0.91

C-Control, I- *Rhizobium,* B-Commercial biofertilizer, P- Rock phosphate. Values within a column followed by the same letter are not significantly different at the 0.05 level of probability.

Shoot-to-root ratio was higher with P addition indicating more carbon allocation above ground than below ground (Table 3.2). Apparently, phosphorus addition was the principal factor that affected biomass production of the *S. sesban* seedlings. Considering main effects, only P application resulted in positive responses in most growth and nutrition parameters considered. Biofertilizer had a significant positive effect on height growth (by about 25%) but negatively affected root nodulation (Table 3.3 and Table 3.4) Positive significant interactions occurred mainly between rock phosphate and biofertilizer (Table 3.4).

3.3.3 Plant nutrient status and partitioning

Inoculation with rhizobia alone without supplementation with either rock phosphate or the commercial biofertilizer did not significantly increase N contents in *S. sesban* seedlings (Figure 3.2(i)), even though the strain (KFR647) is known to be effective in nodulating the species and increasing N_2-fixation and acquisition (Desaeger et al., 2005). Overall, inoculation only improved root nodulation (Table 3.3, Table 3.4). Commercial biofertilizer failed to stimulate N_2-fixation, but significantly enhanced N content in shoots by about 150% compared to the control (Figure 3.4(i)). Much higher N uptake was induced with rock phosphate additions (350%) compared to those in control or sole rhizobia inoculation (C or I). The trend was almost similar with the other nutrients, P and K especially in shoots (Figure 3. 4 (ii) and (iii)). Proportionally more nutrients were allocated to shoots than roots, although the roots also retained substantial quantities of phosphorus (P) and potassium (K) ranging from 22 to 44% and 22 to 36%, respectively. Plants supplied with a combination of commercial biofertilizer and rock phosphate significantly absorbed more N than sole rock phosphate treatments. Commercial biofertilizer alone raised N uptake by more than one-half in the shoots compared to the control and sole rhizobial inoculation. Although changes in shoot P and K contents were not as great as N with sole biofertilizer application, biofertilizer addition improved P and K allocation to roots (Figure 3.4 (ii) and (iii)).

Table 3.3 Analysis of variation for the effects of *Rhizobium* (I), rock phosphate (P) and biofertilizer (B) applications on growth responses of *S. sesban* seedlings

		Significance level (Pr > F)						
Source of variation	DF	Root collar diameter	Height	Nodule biomass	Root biomass	Shoot biomass	Total biomass	Shoot: Root ratio
Main effects								
Inoculation (I)	1	0.7869	0.0751	0.0357	0.6401	0.7073	0.5898	0.9871
Biofertilizer (B)	1	0.0540	0.0071	0.0022	0.5393	0.0801	0.0504	0.1518
Rock Phosphate (P)	1	0.0005	0.0003	0.0034	0.0006	0.0003	<0.0001	0.0516
Interactions								
I * B	1	0.7293	0.5753	0.0357	0.8068	0.2433	0.2085	0.5625
I * P	1	0.6740	0.5468	0.0377	0.1072	0.4525	0.1787	0.4701
B * P	1	0.0203	0.0267	0.0034	0.0504	0.0599	0.0116	0.3767
I * B * P	1	0.5715	0.3525	0.0377	0.8289	0.1826	0.1359	0.6460

Table 3.4 Mean height (cm), nodule, root, shoot, and total biomass (g) of *S. sesban* as influenced by the main treatment effects

	Inoculation (I)		Biofertilizer (B)		Rock Phosphate (P)	
Variables	-I	+I	-B	+B	-P	+P
Height	42.0 (4.0)	46.0 (4.2)	39.1 (4.6)	48.9 (3.2)	29.6 (2.2)	58.3 (1.4)
Nodules biomass	0.005 (0.002)	0.011 (0.005)	0.016 (0.005)	0 (0)	0.001 (0.0003)	0.015(0.005)
Roots biomass	1.32 (0.20)	1.28 (0.17)	1.28 (0.21)	1.33(0.15)	0.68 (0.07)	1.93 (0.09)
Shoot biomass	4.13(0.72)	4.03 (0.68)	3.75 (0.78)	4.41(0.61)	1.60 (0.23)	6.56 (0.34)
Total biomass	5.46(0.92)	5.32 (0.86)	5.05(1.01)	5.74(0.74)	2.28 (0.29)	8.51 (0.42)

Values in parentheses are standard errors of the mean.

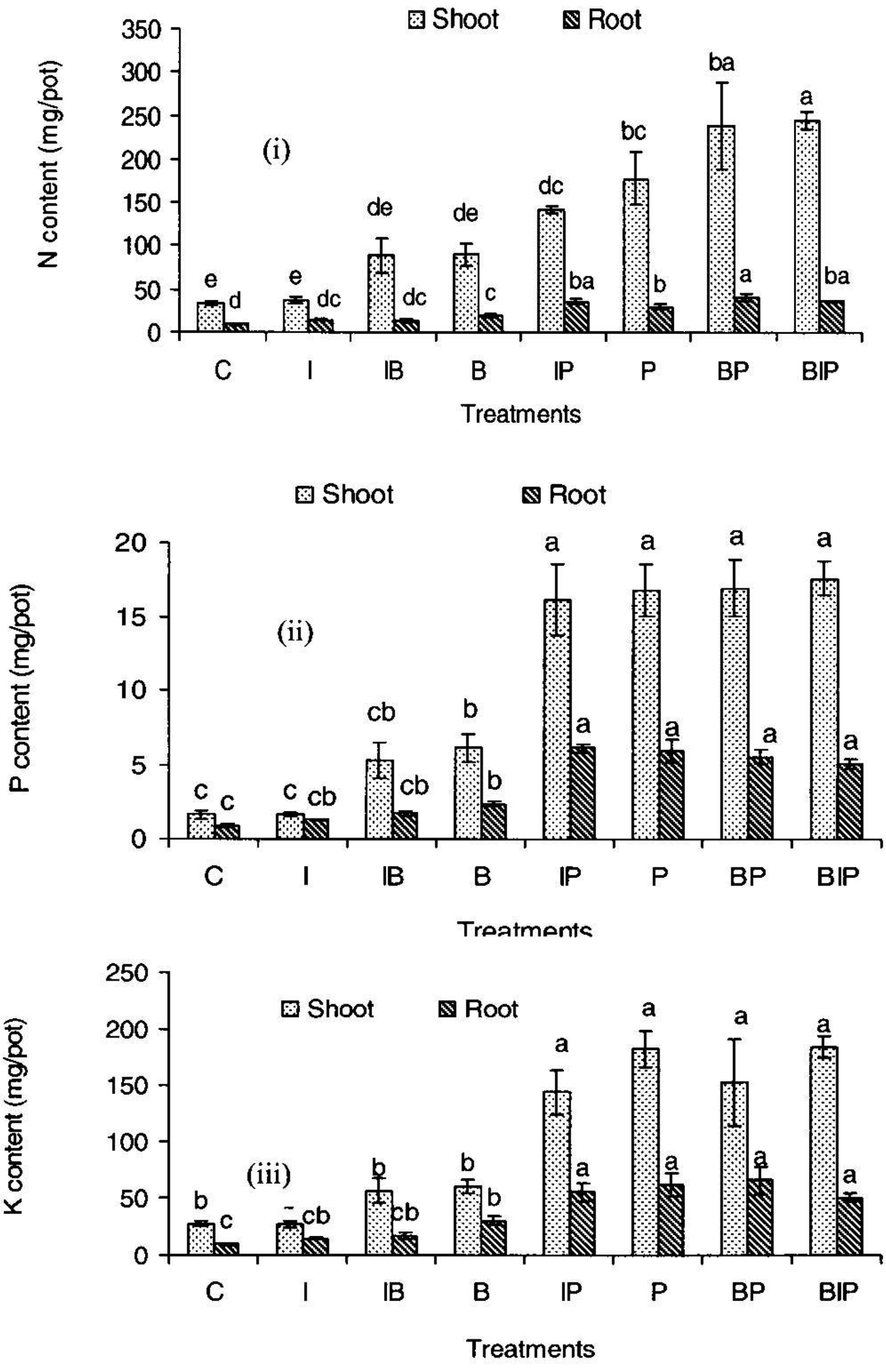


Figure 3. 4 Nutrient accumulation in the shoots and roots of *S. sesban*. C-Control, I-Inoculation with *Rhizobium*, B-Commercial biofertilizer, P- Rock phosphate. Bars of particular plant portions with the same letters did not differ significantly at the 0.05 level of probability.

3.3.4 Biological N_2-fixation

Root nodules (a physical indication of biological N_2-fixation) were formed on both inoculated and non-inoculated seedlings, but only in treatments without biofertilizer (Plate 3.2 and Appendix I). The presence of nodules in non-inoculated seedlings indicated that the test soil contained indigenous rhozobia capable of nodulating *S. sesban*. This was expected as the soil was collected from an area with *S. sesban* trees, and the potting soil was not sterilized before planting. However, there were fewer nodules formed in the control and sole-rhizobial inoculations without phosphorus added (Table 3.2 and Plate 3.2). Addition of rock phosphate stimulated profuse root nodulation in both inoculated and non-inoculated seedlings (Plate 3.2), resulting in high nodule biomass production in both treatments (Table 3.2). Evidently, inoculated treatments had about twice as many nodules as there were in non-inoculated. Estimating the amount of N_2-fixed by the ^{15}N isotope dilution method (using *Tithonia diversifolia* as the non-fixing the reference plant) showed that *Sesbania sesban* can fix large quantities of atmospheric nitrogen (50 kg/ha in within three months). This is depicted by the high proportion of N derived from the atmosphere (%Ndfa) with adequate soil P supplementation (Figure 3.5) and the absolute amount of N fixed within the 90 days of growth after planting (Figure 3.6). About three times more ^{15}N dilution (low $\%^{15}N$ atom excess) was detected in *S. sesban* plants that received P application without the biofertilizer than in either the control or inoculation with rhizobia (data not shown). The high ^{15}N dilution observed in rock phosphate treatments without biofertilizer is attributed to high atmospheric N_2-fixation. Conversely, the ^{15}N dilutions detected in the shoots of the seedlings that received commercial biofertilizer could not be attributed to atmospheric N_2-fixation alone since the %Ndfa in those treatments were significantly lower compared to sole rock phosphate or a combination of rock phosphate and rhizobia treatments that had similar dilution magnitudes.

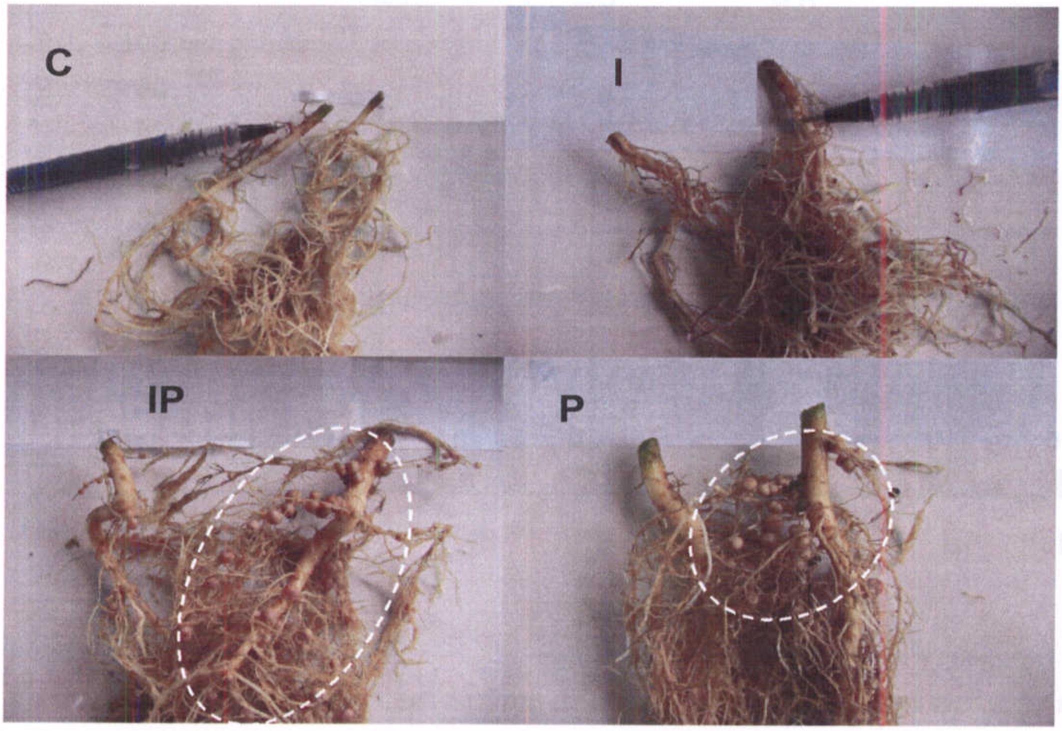


Plate 3.2: Comparisons of *Sesbania sesban* root nodule formation with and without rock phosphate fertilizer application. More nodules were formed with application of rock phosphate (IP & P) than control without (C) or inoculation with *Rhizobium* alone (I). The hatched lines denote prominent root nodules.

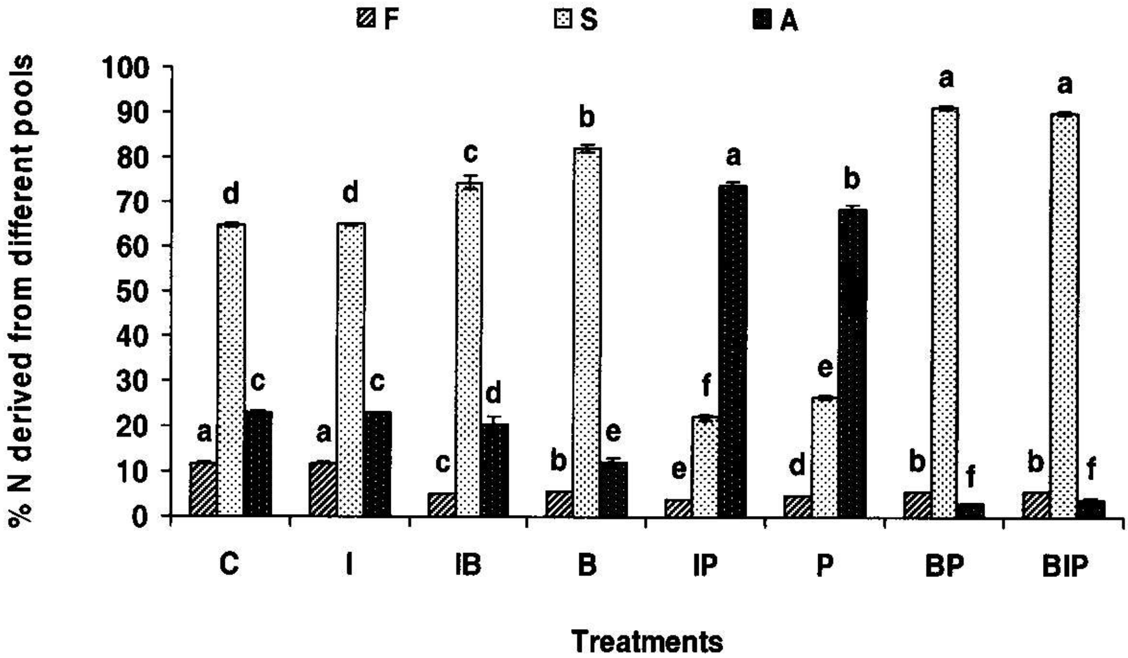


Figure 3.5 Percent nitrogen derived from different pools by *S. sesban* seedlings as influenced by rhizobial inoculations, biofertilizer and rock phosphate applications. Where F-nitrogen derived from tracer fertilizer [$(^{15}NH_4)_2SO_4$], S-nitrogen derived from the mixture of soil and biofertilizer and A-nitrogen derived from the atmosphere. The treatments were defined as: C-Control, I- Inoculation with *Rhizobium*, B-Commercial biofertilizer, P- Rock phosphate. Bars for a given N pool with the same letters are not significantly different ($P < 0.05$).

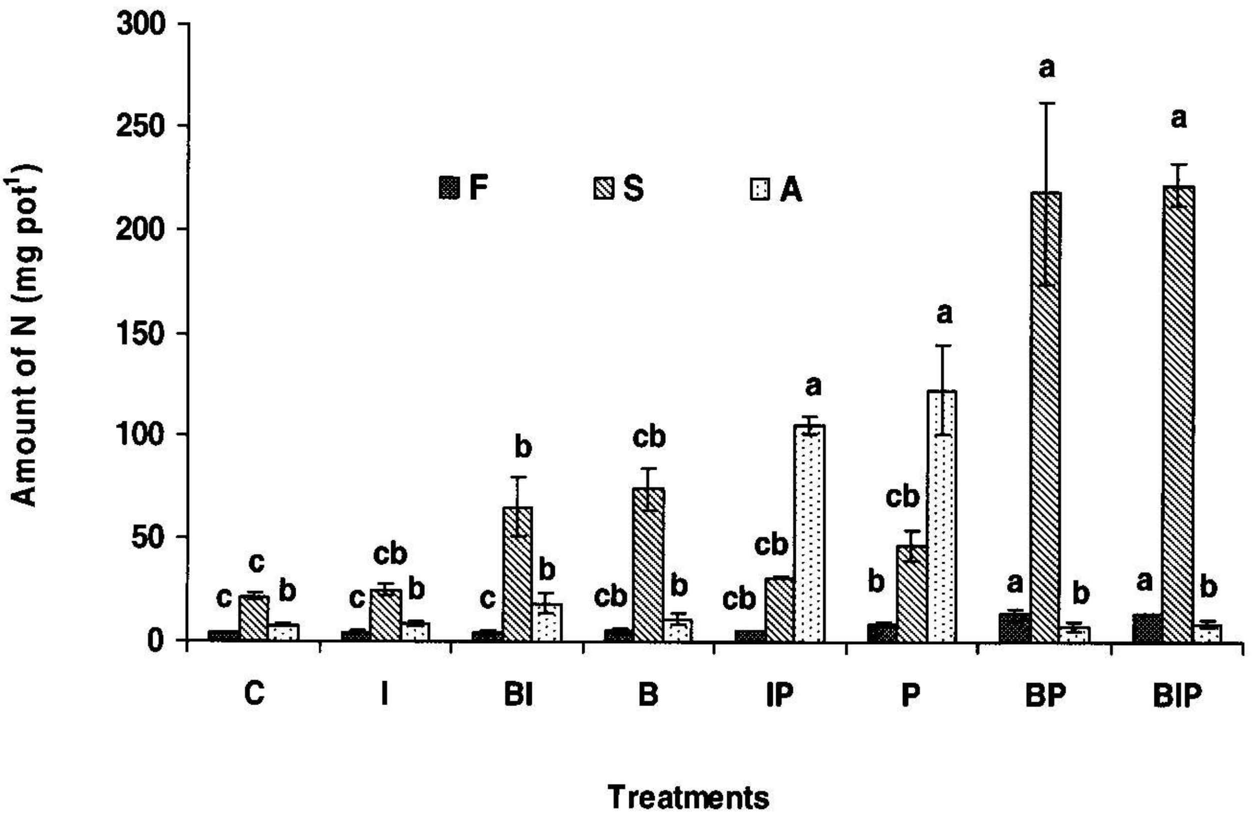


Figure 3.6 Amounts of nitrogen derived from different pools by *S. sesban* plants within 90 days of growth as influenced by *Rhizobium*, rock phosphate and biofertilizer applications. Where F-nitrogen derived from tracer fertilizer [$(^{15}NH_4)_2SO_4$], S-nitrogen derived from the mixture of soil and biofertilizer and A-nitrogen derived from the atmosphere. Treatments: C-Control, I- *Rhizobium,* B-Commercial biofertilizer, P- Rock phosphate. Bars for a given pool with the same letters are not significantly different ($P < 0.05$).

3.3.5 Main treatments and interactions effects on growth and nutritional responses

Overall comparison of main effects on growth responses revealed that inoculation with rhizobia significantly increased only on root nodule biomass production (Table 3.3), more than doubling formation (Table 3.4). Biofertilizer addition had a positive significant effect on seedlings height, and a significant negative effect on root nodulation. Overall, seedlings failed to form root nodules with biofertilizer additions (Table 3.4). In contrast to the other two main effects, rock phosphate application induced significant positive responses on all the growth parameters considered, except for shoot:root ratio (Table 3.3). Nutrient uptake and N_2-fixed were increased with P-application (Figure 3.7). Rhizobial inoculation and biofertilizer application induced negative interaction effect that suppressed root nodulation. While the interaction between rhizobial inoculation and P supply enhanced root nodulation (Table 3.3 and Table 3.4).

In terms of plant nutrition, inoculation with rhizobia significantly affected the proportions of nitrogen derived from fertilizer, soil and atmosphere. (i.e. %Ndff, %Ndfs and %Ndfa respectively). However, there was no significant effect on absolute N content of the plants as a result of rhizobial inoculation. Biofertilizer and rock phosphate had significant effects on all plant nutrient status considered (Table 3.5). All the responses resulting from P additions were positive while responses as a result of biofertilizer were either positive or negative. Generally, nutritional interaction effects were associated mainly with biofertilizer and rock phosphate applications.

Table 3.5 Analysis of variation for the effects of *Rhizobium* (I), rock phosphate (P) and biofertilizer (B) applications on nutritional responses of *Sesbania sesban* plants

		Significance level (Pr > F)								
Source of variation	DF	%Ndff	%Ndfs	%Ndfa	F	S	A	N- uptake	P- uptake	K- uptake
Main effects										
Inoculation (I)	1	0.0011	0.0038	0.0033	0.4772	0.7666	0.7085	0.6504	0.1747	0.0009
Biofertilizer (B)	1	<0.0001	<0.0001	<0.0001	0.0200	0.0033	0.0017	0.0159	0.0003	0.0006
Rock Phosphate (P)	1	<0.0001	<0.0001	<0.0001	0.0054	0.0081	0.0017	0.0021	<0.0001	0.0001
Interactions										
I * B	1	0.1354	0.0692	0.0862	0.5967	0.8993	0.2708	0.5785	0.5405	0.0003
I * P	1	0.0430	0.2165	0.3018	0.5010	0.9199	0.2891	0.5993	0.1584	0.0002
B * P	1	<0.0001	<0.0001	<0.0001	0.0334	0.0146	0.0012	0.3811	0.0010	0.0003
I * B * P	1	0.0013	0.0046	0.0039	0.2851	0.5979	0.5438	0.4453	0.0257	0.1160

Where; DF-degree of freedom, %Ndff – percent N derived from tracer fertilizer, %Ndfs- percent N derived from biofertilizer and soil, % %Ndfa- percent N derived from atmosphere. While F, S and A are absolute N content derived from tracer fertilizer, soil and s atmosphere respectively.

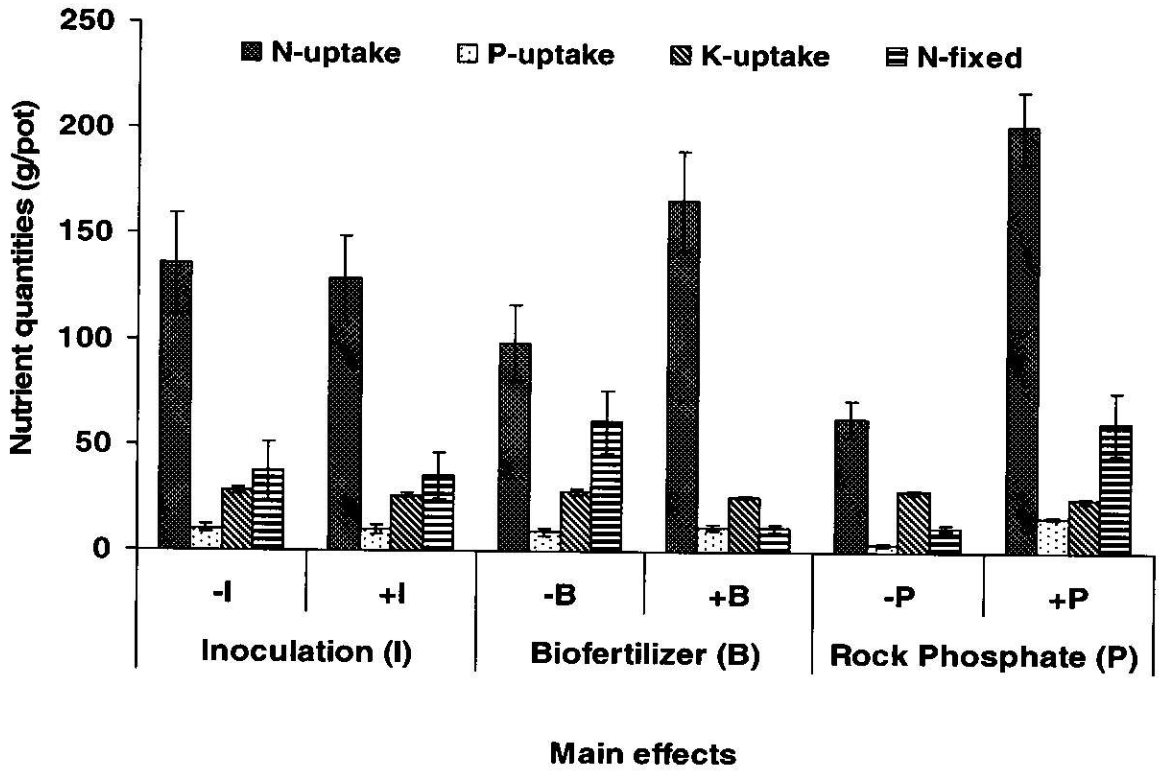


Figure 3.7 Nutrient N, P, K uptake and N-fixed by *S. sesban* as influenced by the main treatment (inoculation with *Rhizobium*, biofertilizer and rock phosphate) effects. The – and + signs denotes without and with the main treatment.

3.4. Discussion

3.4.1. Plants growth and biomass production

According to vector diagnosis of relative biomass production and nutrient accumulation, this study clearly demonstrates that phosphorus and nitrogen are critically deficient in some soils of western Kenya (as depicted by magnitude or vector length of the two nutrients) (Figure 3.8) and can adversely affect the performance of *S. sesban* (Figure 3.2, Table 3.2) when supply is limited. The shift and length of the P vectors were greater than those of N and K; indicating that P was the most limiting nutrient probably due to low P availability in the potting soil (Table 3.1). Such low levels of plant-available P have also been noted by other workers in western Kenya (Ndufa, et al., 1999, Waigwa et al., 2003). Application of Minjingu rock phosphate resulted in rapid increase in seedling height, root collar diameter and the overall biomass production. The responses due to P supplementation indicate that Minjingu rock phosphate is agronomically effective and is a potential source of P. High quality biomass that can be incorporated into soil to improve nutrient concentrations and organic matter content, together with ability to recycle nutrients from deeper soil horizons, is a desired attribute of an improved fallow species such as *S. sesban* (Gathumbi et al., 2003). My finding of high biomass yield of *S. sesban* as a result of P application agrees with reports by Rao et al., (2002), that *S. sesban* fertilized with P in the field recorded 58% more biomass than unfertilized treatments. It is also evident from my finding that without exogenous supply of P, the potential of *S. sesban* to produce high biomass is limited (Table 3.2).

Relative plant dry biomass increased about sevenfold after phosphate fertilization (Figure 3.6.). Evidently, the original soil, an Ultisol from western Kenya, was low in plant-available P and N. The high responses to added P found in this study indicate that phosphorus was more limiting for *S. sesban* growth than nitrogen. Without the external P input, *S. sesban* seedlings performance was markedly reduced - a situation that compromises the potential use of the species for in improved fallow agroforestry systems. Conversely, high biomass yields realized with adequate P fertilization is an important factor for improved fallow practices, since one of the principal objectives of planting such trees is for the production of green manure to incorporate *in situ* during cropping season. Production of more biomass within a short period is one criteria used in selecting improved fallow species. Therefore factors that encourage high biomass production by specific trees for fallow practices should be promoted to ensure the success of the practice.

3.4.2. Plant nutrients accumulation

Rock phosphate fertilizer supplementation increased not only total biomass but also tissue N, P and other nutrient concentrations in the plant tissues. Phosphorus uptake by seedlings fertilized with rock phosphate increased by about 3 and 8 fold relative to sole rhizobial inoculation and commercial biofertilizer treatments respectively (Figure 3.8). Uptake of other nutrients (N and K) was also improved through rock phosphate application. These findings are consistent with other studies where nutrient build up in some improved-fallow species increased with supplies of external sources of P in the P-deficient soils (Ndufa et al., 1999, Rao et al., 2002).

The responses to plant nutrient uptake were elucidated by vector nutrient diagnosis (Figures 3.8 and 3.9). Vector lengths and orientation (*A, B, C*) associated with each nutrient (P, N and K) showed that in the order given, P was the most limiting for growth of *S. sesban* plants. The quality of green biomass produced in terms of nutrient content may be another important aspect in improved-fallow cultivation systems and other agroforestry practices, since leaves provide fodder and green manure. Green manure rich in mineral nutrients produced is beneficial for plants in subsequent cropping season. Rock phosphate fertilization enhanced seedlings nutrient acquisition, especially N, P and K in both above and below ground biomass, which could benefit annual crops planted after the fallow period.

A marked increase in K demand and accumulation in the roots was realized when P was applied in the form of rock phosphate (Figure 3.9). Sas et al., (2001) found that legume plants on P-deficient soils tend to form clustered roots with few root hairs. With adequate plant-available P, plants increase root hair production that enhances K absorption. In another study, Shena et al., (2005) reported that K in roots was significantly lower at 1 mmol compared to 25 mmol P m^{-3} availability, although the K concentration in shoots was not affected by P supply. Decreased K concentration in roots under P deficiency was attributed to more root clusters occurring at low P status, which apparently reduced K uptake. It was speculated that more of the absorbed K was effluxed from the cluster roots than non-cluster roots, or that K was not taken up at the same extent by the two root types. Since root clusters were identified as the main site for H^+ and organic anions release under P deficiency, it was assumed that the release of organic anions was accompanied with K^+ efflux (co-transport). Similarly, vector diagnosis of root nutrients indicated high K contents in the roots associated with external P input (Figure 3.9).

Increased K uptake along with other nutrients in the roots of *S. sesban* after rock phosphate (P) and biofertilizer (B) treatments is of significance in western Kenya conditions because, apart from N and P, potassium (K) is the next most limiting nutrient. The cations K^+, Ca^{2+}, and Mg^{2+}, and anions like NO_3^- and SO_4^{2-} are mobile within the soil profile, hence are susceptible to leaching below rooting depths of most annual crops (Buresh, et al., 2004). When nutrients are captured by the roots of improved-fallow trees as observed in this study, they are sequestered in the plant biomass whether translocated to the shoots or retained in the roots. It was noted here that the commercial biofertilizer (***Organica's Plant Booster Plus***) encouraged luxury consumption of nutrients (i.e. nutrient accumulation in the tissues without change in biomass). However, how this was achieved is not clear from this study, it can only be speculated that plant growth-promoting rhizobecteria (PGPR) that were present in the biofertilizer may have increased nutrient availability. Likely, the rhizobacteria enhanced conversion of nutrients to more labile forms easy for absorption by the plants rather than improved root development. Studies indicate that the major source of plant-available P in tropical soils is from mineralization of organic matter and from recent inputs of organic materials, thus few soil rhizobacteria play a key role in biochemical transformation processes of organic P to inorganic forms available for plant uptake (de Freitas, 1997; Mafongoya et al., 2000). The significant point is that nutrient loaded foliage produced with biofertilizer treatments can be incorporated into the soil to benefit companion crops when the nutrients are released through microbial decomposition. The contributions of belowground biomass and nutrients of leguminous trees in building up soil organic matter have long been recognised.

3.4.3 Potential contribution of N through biological nitrogen fixation

The potentials of *Sesbania sesban* to fix atmospheric nitrogen was evaluated under different soil conditions using the ^{15}N isotope dilution, an integrated method of estimating biological nitrogen fixation in legumes (Chalk and Ladha, 1999). The results in this study clearly indicate that the supply of adequate P to legumes is critical for the success of biological nitrogen fixation (BNF). Evidently, inoculation with effective *Rhizobium* bacteria alone without P amendments still compromised the effectiveness of the BNF process. While with P addition, regardless of whether the plants were inoculated or not, N_2 fixation in the shoots was increased more than 10 times, especially without commercial biofertilizer applications. Therefore, adequate plant-available P is necessary to enable legumes fix atmospheric nitrogen. Even though inoculation increased nodule biomass, indigenous rhizobia were apparently more effective for N_2-fixation than the applied strain (KFR 647).

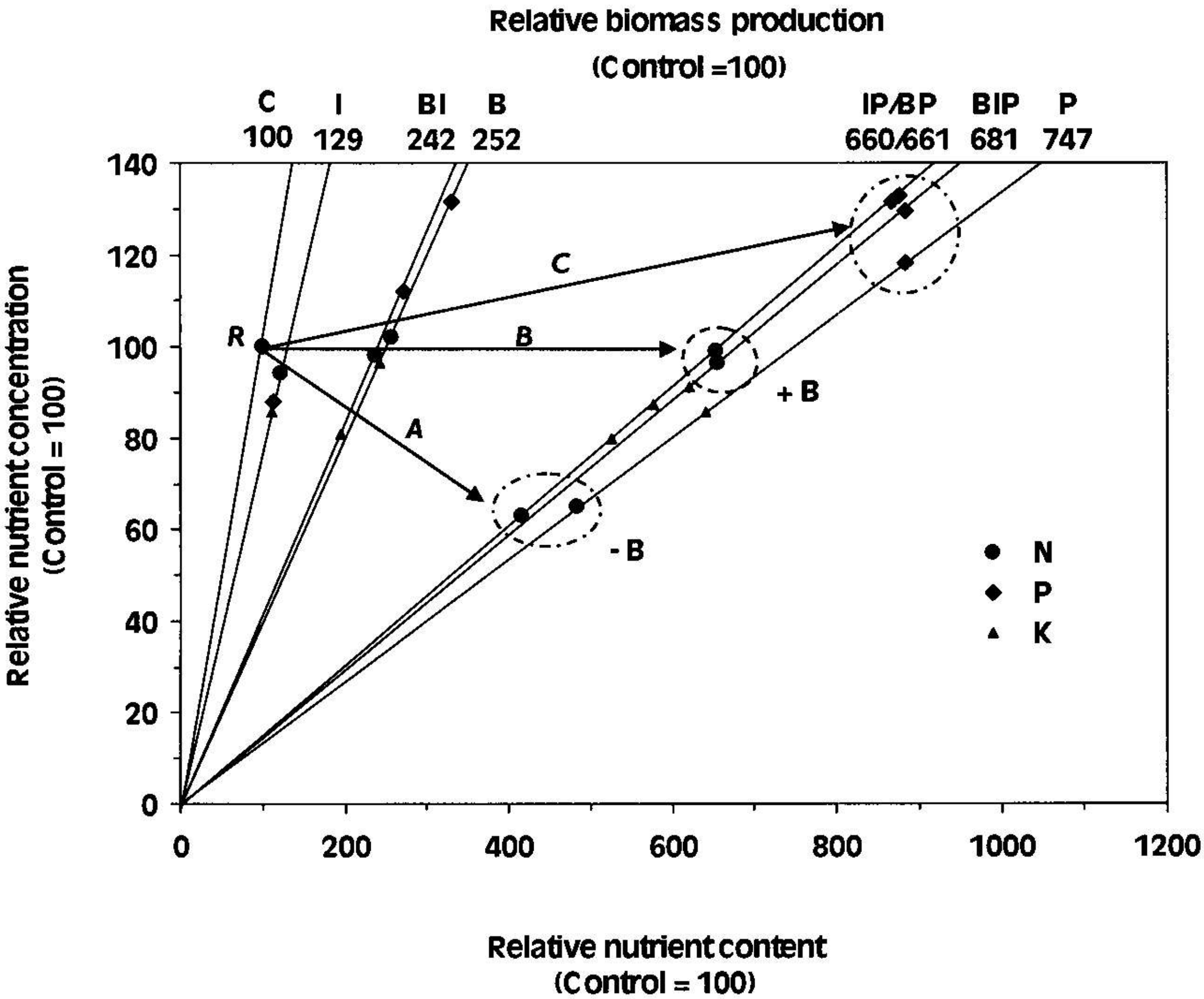


Figure 3.8 Vector nomogram showing relative variations in total plant dry biomass, N, P and K nutrient contents and concentrations as affected by *Rhizobium*, rock phosphate and biofertilizer amendments. The treatments were; Control, I- *Rhizobium,* B-Commercial biofertilizer, P- Rock phosphate. +B and -B implies with or without addition of biofertilizers respectively

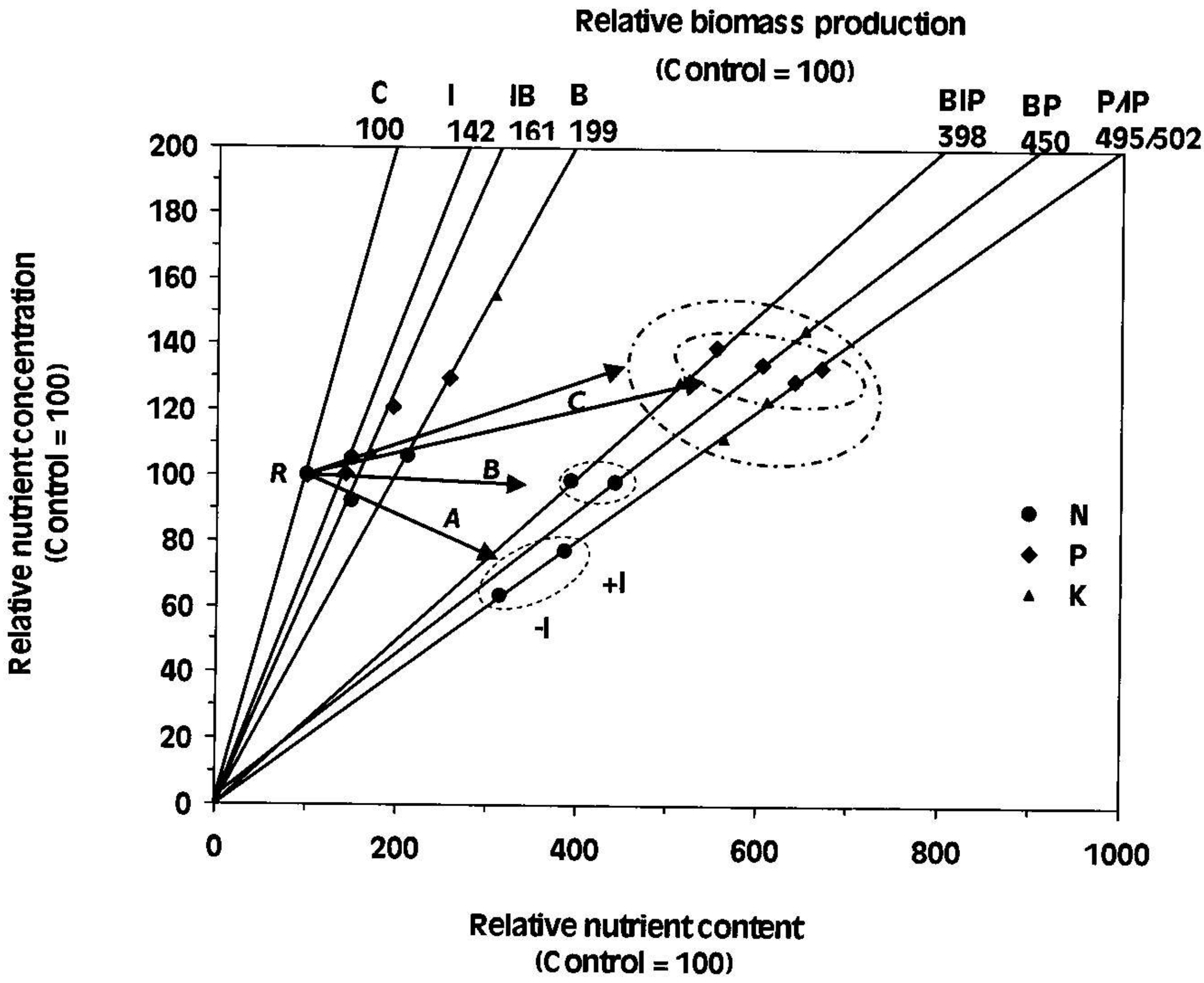


Figure 3.9 Vector nomogram showing relative variations in root dry biomass, N, P and K nutrient contents and concentrations as affected by *Rhizobium*, rock phosphate and biofertilizer additions. +I and –I means with and without rhizobial inoculation respectively

Nodule efficiency (*NE*) as described by Aronson, et al., (2002), can be expressed by the equation, $NE = \frac{ANiF}{NW}$, where *NE*- nodule efficiency, *ANiF*-absolute quantity of nitrogen fixed, and *NW*-nodules dry weight. Thus the efficiencies for indigenous nodule formers and the KFR 647 strain were 5.6 and 2.2, respectively, in the treatments where there was significant N_2-fixation (see Table 3.2 and Table 3.3 for nodule biomass and absolute N-fixed per pot). Since the experimental soil was collected from a site with *S. sesban* trees nearby, the indigenous strains were likely to have been more adapted to the soil type than the introduced strain. The inhibition of nodule formation when the soil was amended with commercial biofertilizer was probably due to high nitrate (NO_3^-) concentrations associated with those treatments. Analysis of the residual soil nutrient status after the experiment (Table 3.6) revealed that nitrate concentrations were high in the treatments that received commercial biofertilizer.

Other studies have shown that heavy fertilization of legumes with N-fertilizers inhibit nodulation, and significantly lowers symbiotic nitrogen fixation (Daimon and Yoshioka, 2001; Singh and Usha, 2003). These findings are in agreement with observations in this study that commercial biofertilizer with high nitrate content may have inhibited nodule formation on the roots of *S. sesban* seedlings even when inoculated with a known effective *Rhizobium* strain. Although the plants that were treated with biofertilizers and rock phosphate accumulated more nitrogen than those without, the build up was not apparently due to symbiotic N_2-fixation as this was minimal (Figure 3.10).

The biofertilizer failed to stimulate root nodulation but the interactions between N_2-fixing microbes and the host species resulted in small quantities of atmospheric N_2- fixation. High N_2-fixation and conspicuous root nodulation occurred only when P was supplied in the form of rock phosphate. The large positive response to P application in BNF emphasizes the critical importance of soil P-availability along with other nutrients to ensure that legumes exploit their full potentials in symbiotic N_2-fixation process if other environmental factors are favourable, as suggested in other studies (Haque et al., 1996; Leidi and Rodriguez-Navarro, 2000).

It was noted from this study that as much as *S. sesban* was capable of fixing large quantities of atmospheric N, they are also capable of depleting the soil N as was indicated by significantly ($P>0.05$) low residual soil NO_3-N (Table 3.6 and Table 3.7), especially when there was adequate P. Therefore, removing biomass produced by legumes for other uses elsewhere (such as fodder for livestock and fuelwood production) without ensuring efficient recycling would risk exporting large quantities of N from the system. There is evidence from various studies that when legume plants get access to adequate N from the soil, they might not expend resources on symbiotic N_2-fixation (Singh and Usha, 2003). Thus, in the presence of high levels of fertilizer N, as in this study with the biofertilizer addition, N_2-fixation is inhibited.

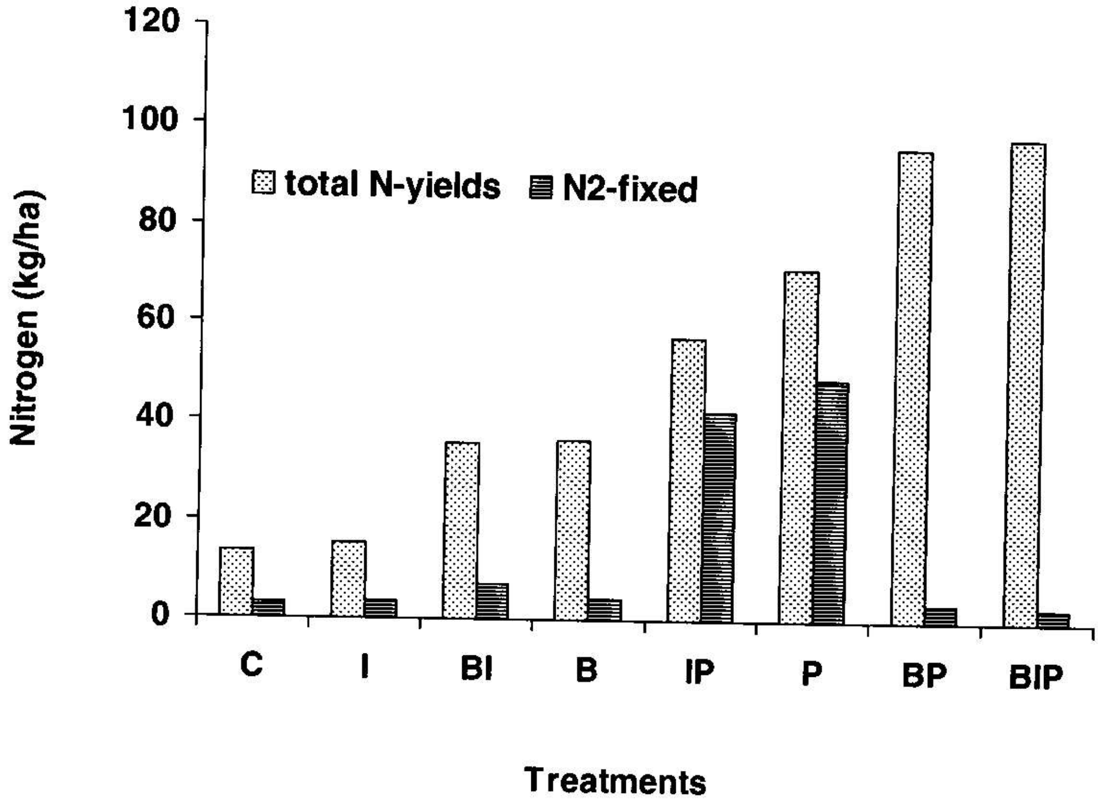


Figure 3.10 The shoot total N-yield and N_2-fixed partitioned in the shoots of *S. sesban* after 90 days of growth showing high N_2-fixation with application of rock phosphate.

Table 3.6 Residual soil chemical characteristics after growing *S. sesban* seedlings for ninety days showing effects of individual treatments.

Treatment	pH	%Org.C	%N	NO_3^- (ppm)	$NH4^+$ (ppm)	P (mg/100g soil)	K ($Cmol_c$)	Ca ($Cmol_c$)	Mg ($Cmol_c$)
C	4.85dc	3.68dc	0.094d	50.34b	74.55a	0.133c	16.72c	3.80c	1.23d
I	4.88c	3.83bc	0.122c	52.77b	74.30a	0.150c	18.13bc	4.40bc	1.30cd
BI	4.80d	4.05a	0.163a	134.70a	79.78a	0.283ba	23.91a	5.42a	1.83a
B	4.90c	4.00a	0.133cb	130.42a	41.00c	0.167c	22.81ba	4.97ba	1.53b
IP	5.10a	3.95ba	0.144b	8.60c	36.44c	0.317a	16.88c	4.87ba	1.31cd
P	5.08a	3.90ba	0.142b	6.71c	48.47b	0.217bc	16.56c	4.91ba	1.35cd
BP	5.00b	3.65d	0.141b	53.80b	38.44c	0.367a	23.75a	5.12a	1.53b
BIP	5.00b	3.65d	0.138cb	47.82b	52.29b	0.350a	23.13ba	4.86ba	1.41cb
LSD 5%	0.06	0.17	0.019	29.43	7.46	0.092	5.05	0.70	0.17

C-Control, I- *Rhizobium*, B-Commercial biofertilizer, P- Rock phosphate. Values within a column followed by the same letter are not statistically different at $P < 0.05$ level of probability.

Table 3.7 Analysis of variation for the effects of *Rhizobium* (I), rock phosphate (P) and biofertilizer (B) applications on residual soil characteristics

Source of variation	DF	Significance level (Pr > F)						
		%Org.C	pH	Total %N	NO_3-N conc.	NH_4-N conc.	Extrac.-P	K conc.
Main effects								
Inoculation (I)	1	0.2872	0.4266	0.0994	0.9451	0.0033	0.1912	0.6509
Biofertilizer (B)	1	1.0000	0.7617	0.0580	0.0057	0.0177	0.0731	0.0102
Rock Phosphate (P)	1	0.1308	0.8323	0.1134	0.0055	0.0003	0.0278	0.7933
Interactions								
I * B	1	0.4950	0.6651	0.9326	0.8738	0.0008	0.9061	0.7933
I * P	1	0.4950	0.5447	0.0941	0.7769	0.0043	0.7236	0.5655
B * P	1	0.0108	0.6579	0.0361	0.1184	0.0054	0.9061	0.7442
I * B * P	1	0.8130	0.3322	0.7864	0.7987	0.0675	0.1904	0.8953

CHAPTER FOUR

4.0 SUMMARY AND CONCLUSIONS

The capability of *S. sesban* to produce large quantities of biomass and accumulate more nutrients under varied soil treatments was tested in a controlled greenhouse environment. *Sesbania sesban* is preferred as a suitable improved fallow species in Kenya and other regions within East and Central Africa because of its ability to grow fast and produce nutrient-rich biomass. Production of more biomass that is of high quality (readily decomposable) is a key factor considered in the selection of desirable improved fallow species. The amounts of biomass produced and nutrients accumulated, especially N, by leguminous trees depend on their capabilities to fix atmospheric N_2. The process of symbiotic N_2-fixation is also affected by other environmental factors such as the presence of effective rhizobia and soil nutrient availability, especially P.

Results show that it is important to address the problem of low P-availability through exogenous supply P in order to maximise the capability of *S. sesban* to produce more biomass and fix large amounts of atmospheric nitrogen. Apparently Minjingu rock phosphates have the agronomic potentials to improve *S. sesban* growth and significantly increase BNF capacities. This source of P fertilizer can be used to supplement plant-available P requirements in western Kenya. External supply of P through addition of rock phosphate resulted in enhanced seedlings growth, nutrient uptake and high N_2-fixation, except for treatments that received biofertilizer. My findings that *S. sesban* seedlings inoculated with known effective rhizobia without P application failed to induce significant growth and BNF suggests that leguminous trees planted in improved fallows should be fertilized with P to effectively fix atmospheric N_2. The biofertilizer increased soil nitrate concentrations, but inhibited symbiotic N_2.fixation as was evident by the lack of root nodule formation in those treatments (see Appendix I). Fertilization with Minjingu rock phosphate improved BNF with or without inoculation and I would suggest its use in agroforestry systems because it is locally available and is cheap compared to other P sources.

Since the un-sterilized bioassay soil used in this study was collected from a site with nearby *S. sesban* trees, it was noted that indigenous *Rhizobium* strains were equally or even more efficient in fixing atmospheric nitrogen than the introduced strains. Although rhizobial inoculations significantly increased nodule biomass yield with P application, slightly more nitrogen was fixed in non-inoculated treatments. Apparently the indigenous strains were well adapted to this native soil type, hence were more efficient in fixing N_2 than the introduced strain. These observations emphasize the need to plant leguminous plants in their natural habitats or in areas where similar species had been grown before. In this study the interactions between the *Rhizobium* strain used to inoculate the *S. sesban* seedlings and micro-organisms in the biofertilizer could not be isolated because of the lack of information on the population dynamics of bacteria involved.

The biofertilizer additions increased nutrient availability, especially soil nitrate level as indicated by the high residual soil nitrate concentrations. The increased nutrient availability encouraged luxury nutrient consumption (+B in Figure 3.6) leading to high nutrient accumulation in the biomass, which may be beneficial in improved fallow practices. But specific mode of action of the biofertilizers needs to be evaluated further.

FUTURE RESEARCH

Since this study was carried out in a greenhouse set up, with controlled environmental conditions, results obtained might be difficult to extrapolate to more varied field conditions. I therefore recommend additional field studies in order to authenticate some of the large growth and nutritional responses of *S. sesban* observed. The positive interaction and residual effects of rock phosphate and biofertilizer applications needs to be investigated further in order to evaluate their agronomic potentials and economic benefits in improved fallow systems

Biofertilizer application showed a marked increase in soil nitrate (NO_3^-) concentrations and positive interactions with rock phosphate. These effects need to be investigated further. In relation to biofertilizer use, other studies indicate that some microorganisms supplied as biofertilizers have shown potential to reduce nematode infections. Although no nematode problems were encountered in this study, the disease is prevalent and attacks roots of *S. sesban* growing in the area where the bioassay soil used in this study was collected. Therefore, I would recommend further study involving use of biofertilizers to address nematode problem associated with planting of *S. sesban* trees.

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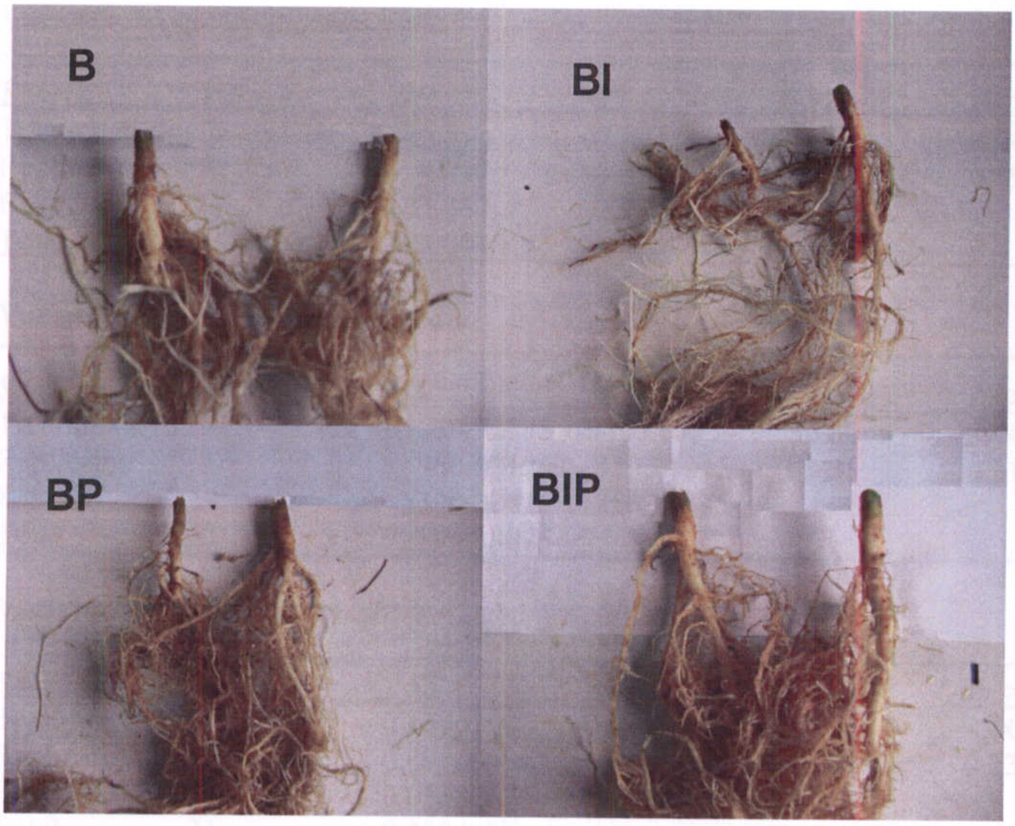
APPENDICES

Appendix I- No nodules formed on the roots of *S. sesban* as a result of biofertilizer applications



Appendix II: One-way ANOVA tables of individual treatment effects on seedlings of *S. sesban.*

Variable: Seedling height

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Treatments	7	7825.950	117.992	67.96	<.0001
Replicates	3	23.118	7.70	0.47	0.7074
Error	21	345.474	16.451		
Total	31	8194.542			

Variable: Total dry biomass yield

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Treatments	7	335.590	47.941	23.15	<.0001
Replicates	3	4.017	1.339	0.65	0.5938
Error	21	43.487	2.071		
Total	31	383.094			

Variable: Absolute nitrogen fixed

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Treatments	7	65839.72875	9405.67554	36.60	<.0001
Replicates	3	960.63125	320.21042	1.25	0.3182
Error	21	5396.02875	256.95375		
Total	31	72196.38875			

Variable: Nitrogen uptake

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Treatments	7	0.18861900	0.02694557	15.56	<.0001
Replicates	3	0.00800425	0.00266808	1.54	0.2335
Error	21	0.03637625	0.00173220		
Total	31	0.23299950			

Variable: Phosphorus uptake

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Treatments	7	4.31618788	0.61659827	4.45	0.0036
Replicates	3	0.07551837	0.02517279	0.18	0.9077
Error	21	2.91019963	0.13858093		
Total	31	7.30190588			

Variable: Potassium uptake

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Treatments	7	330.8008752	47.2572679	2.83	0.0302
Replicates	3	57.8499968	19.2833323	1.16	0.3496
Error	21	350.0546909	16.6692710		
Total	31	738.7055630			

Appendix III: ANOVA tables for the main effects and interactions of inoculation (I), biofertilizer (B) and rock phosphate (P) applications on growth and nutrition of *S. sesban*.

Dependent Variable: Root collar diameter

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	0.00015312	0.00015312	0.09	0.7869
Biofertilizer (B)	1	0.01665312	0.01665312	9.50	0.0540
Rock phosphate (P)	1	0.44415312	0.44415312	253.35	0.0005
Interactions					
I * B	1	0.00025313	0.00025313	0.14	0.7293
I * P	1	0.00037813	0.00037813	0.22	0.6740
B * P	1	0.03577813	0.03577813	20.41	0.0203
B * I * P	1	0.00070312	0.00070312	0.40	0.5715

Dependent Variable: Height

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	126.802813	126.802813	7.18	0.0751
Biofertilizer (B)	1	771.262812	771.262812	43.67	0.0071
Rock phosphate (P)	1	6598.132813	6598.132813	373.60	0.0003
Interactions					
I * B	1	6.937813	6.937813	0.39	0.5753
I * P	1	8.100313	8.100313	0.46	0.5468
B * P	1	293.425312	293.425312	16.61	0.0267
I * B* P	1	21.287813	21.287813	1.21	0.3525

Dependent Variable: Nodule dry biomass

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	0.00028203	0.00028203	13.25	0.0357
Biofertilizer (B)	1	0.00206403	0.00206403	96.99	0.0022
Rock phosphate (P)	1	0.00155403	0.00155403	73.02	0.0034
Interactions					
I * B	1	0.00028203	0.00028203	13.25	0.0357
I * P	1	0.00027028	0.00027028	12.70	0.0377
B * P	1	0.00155403	0.00155403	73.02	0.0034
I * B * P	1	0.00027028	0.00027028	12.70	0.0377

Dependent Variable: Root dry biomass

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	0.01361250	0.01361250	0.27	0.6401
Biofertilizer (B)	1	0.02420000	0.02420000	0.48	0.5393
Rock phosphate (P)	1	12.65045000	12.65045000	249.58	0.0006
Interactions					
I * B	1	0.00361250	0.00361250	0.07	0.8068
I * P	1	0.26281250	0.26281250	5.18	0.1072
B * P	1	0.51005000	0.51005000	10.06	0.0504
I * B * P	1	0.00281250	0.00281250	0.06	0.8289

Dependent Variable: Shoot dry biomass

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	0.0861125	0.0861125	0.17	0.7073
Biofertilizer (B)	1	3.4191125	3.4191125	6.78	0.0801
Rock phosphate (P)	1	196.7136125	196.7136125	389.90	0.0003
Interactions					
I * B	1	1.0585125	1.0585125	2.10	0.2433
I * P	1	0.3741125	0.3741125	0.74	0.4525
B * P	1	4.3956125	4.3956125	8.71	0.0599
I * B * P	1	1.5051125	1.5051125	2.98	0.1826

Dependent Variable: Percent nitrogen derived from tracer fertilizer (NF)

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	3.3153125	3.3153125	0.66	0.4772
Biofertilizer (B)	1	104.0403125	104.0403125	20.60	0.0200
Rock phosphate (P)	1	267.3828125	267.3828125	52.94	0.0054
Interactions					
I * B	1	1.7578125	1.7578125	0.35	0.5967
I * P	1	2.9403125	2.9403125	0.58	0.5010
B * P	1	70.5078125	70.5078125	13.96	0.0334
I * B * P	1	8.5078125	8.5078125	1.68	0.2851

Dependent Variable: Percent nitrogen derived from soil and biofertilizer (NS)

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	147.4903	47.4903	0.11	0.7666
Biofertilizer (B)	1	103956.6003	103956.6003	74.41	0.0033
Rock phosphate (P)	1	55253.1903	55253.1903	39.55	0.0081
Interactions					
I * B	1	26.4628	26.4628	0.02	0.8993
I * P	1	16.6753	16.6753	0.01	0.9199
B * P	1	36335.3403	36335.3403	26.01	0.0146
I * B* P	1	482.8278	482.8278	0.35	0.5979

Dependent Variable: Percent nitrogen derived from the atmosphere (NA)

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	29.26125	29.26125	0.17	0.7085
Biofertilizer (B)	1	19910.10125	19910.10125	115.06	0.0017
Rock phosphate (P)	1	19840.32000	19840.32000	114.65	0.0017
Interactions					
I * B	1	313.75125	313.75125	1.81	0.2708
I * P	1	285.60500	285.60500	1.65	0.2891
B * P	1	25380.04500	25380.04500	146.67	0.0012
I * B * P	1	80.64500	80.64500	0.47	0.5438

Dependent Variable: Nitrogen uptake

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	378.1250	378.1250	0.25	0.6504
Biofertilizer (B)	1	36679.8612	36679.8612	24.41	0.0159
Rock phosphate (P)	1	153901.5200	153901.5200	102.43	0.0021
Interactions					
I * B	1	579.7012	579.7012	0.39	0.5785
I * P	1	515.2050	515.2050	0.34	0.5993
B * P	1	1576.4112	1576.4112	1.05	0.3811
I * B * P	1	1154.4013	1154.4013	0.77	0.4453

Dependent Variable: Phosphorus uptake

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	0.405000	0.405000	3.14	0.1747
Biofertilizer (B)	1	47.531250	47.531250	367.98	0.0003
Rock phosphate (P)	1	1396.561250	1396.561250	10812.10	<.0001
Interactions					
I * B	1	0.061250	0.061250	0.47	0.5405
I * P	1	0.451250	0.451250	3.49	0.1584
B * P	1	21.125000	21.125000	163.55	0.0010
I * B * P	1	2.205000	2.205000	17.07	0.0257

Dependent Variable: Potassium uptake

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	25.38281250	25.38281250	176.70	0.0009
Biofertilizer (B)	1	35.49031250	35.49031250	247.07	0.0006
Rock phosphate (P)	1	96.95281250	96.95281250	674.94	0.0001
Interactions					
I * B	1	48.75781250	48.75781250	339.43	0.0003
I * P	1	75.33781250	75.33781250	524.47	0.0002
B * P	1	49.75031250	49.75031250	346.34	0.0003
I * B * P	1	0.69031250	0.69031250	4.81	0.1160

Appendix IV: Post-hoc analysis of the main effects of inoculation (I), biofertilizer (B) and rock phosphate (P) application on growth and nutrition of *S. sesban*

Inoculation (I)

t Tests (LSD) for Nodules

Least Significant Difference 0.0052

t Grouping	Mean	N	I
A	0.011000	16	1
B	0.005063	16	0

t Tests (LSD) for N-fixed

Least Significant Difference 14.801

t Grouping	Mean	N	B
A	61.325	16	0
B	11.438	16	1

t Tests (LSD) for %Ndfa

Least Significant Difference 1.279

t Grouping	Mean	N	I
A	30.2000	16	1
B	26.7250	16	0

t Tests (LSD) for N-uptake

Least Significant Difference 43.614

t Grouping	Mean	N	B
A	165.89	16	1
B	98.18	16	0

t Tests (LSD) for K-uptake

Least Significant Difference 0.4264

t Grouping	Mean	N	I
A	27.8625	16	0
B	26.0813	16	1

t Tests (LSD) for P-uptake

Least Significant Difference 0.4044

t Grouping	Mean	N	B
A	11.4938	16	1
B	9.0563	16	0

Biofertilizer (B)

t Tests (LSD) for Height

Least Significant Difference 4.7285

t Grouping	Mean	N	B
A	48.894	16	1
B	39.075	16	0

t Tests (LSD) for Nodules

Least Significant Difference 0.0052

t Grouping	Mean	N	B
A	0.016063	16	0
B	0.000000	16	1

t Tests (LSD) for %Ndfa

Least Significant Difference 1.279

t Grouping	Mean	N	B
A	47.1938	16	0
B	9.7313	16	1

Rock phosphate (P)

t Tests (LSD) for Height

Least Significant Difference 4.7285

t Grouping	Mean	N	P
A	58.344	16	1
B	29.625	16	0

t Tests (LSD) for Nodules

Least Significant Difference 0.0052

Grouping	Mean	N	P
A	0.015000	16	1
B	0.001063	16	0

t Tests (LSD) for Total biomass

Least Significant Difference 0.6314

t Grouping	Mean	N	P
A	8.5856	16	1
B	2.2650	16	0

t Tests (LSD) for N-fixed

Least Significant Difference 14.801

t Grouping	Mean	N	P
A	61.281	16	1
B	11.481	16	0

t Tests (LSD) for N-uptake

Least Significant Difference 43.614

t Grouping	Mean	N	P
A	201.39	16	1
B	62.69	16	0

t Tests (LSD) for P-uptake

Least Significant Difference 0.4044

Grouping	Mean	N	P
A	16.8813	16	1
B	3.6688	16	0

t Tests (LSD) for K-uptake

Least Significant Difference 0.4264

t Grouping	Mean	N	P
A	28.7125	16	0
B	25.2313	16	1

Appendix V: ANOVA tables for the main effects and interactions of inoculation (I), biofertilizer (B) and rock phosphate (P) applications on residual soil characteristics (after harvest)

Dependent Variable: Soil pH

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	0.08302812	0.08302812	0.84	0.4266
Biofertilizer (B)	1	0.01087812	0.01087812	0.11	0.7617
Rock phosphate (P)	1	0.00525313	0.00525313	0.05	0.8323
Interactions					
I * B	1	0.02257813	0.02257813	0.23	0.6651
I * P	1	0.04575312	0.04575312	0.46	0.5447
B * P	1	0.02365313	0.02365313	0.24	0.6579
I * B * P	1	0.13132813	0.13132813	1.33	0.3322

Dependent Variable: Total soil nitrogen (N)

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	0.00166753	0.00166753	5.57	0.0994
Biofertilizer (B)	1	0.00268278	0.00268278	8.96	0.0580
Rock phosphate (P)	1	0.00147153	0.00147153	4.91	0.1134
Interactions					
I * B	1	0.00000253	0.00000253	0.01	0.9326
I * P	1	0.00175528	0.00175528	5.86	0.0941
B * P	1	0.00393828	0.00393828	13.15	0.0361
I * B * P	1	0.00002628	0.00002628	0.09	0.7864

Dependent Variable: Soil nitrate (NO_3-N)

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	3.40605	3.40605	0.01	0.9451
Biofertilizer (B)	1	30831.41120	30831.41120	50.70	0.0057
Rock phosphate (P)	1	31579.61461	31579.61461	51.93	0.0055
Interactions					
I * B	1	18.15031	18.15031	0.03	0.8738
I * P	1	58.42805	58.42805	0.10	0.7769
B * P	1	2865.24500	2865.24500	4.71	0.1184
I * B * P	1	47.19061	47.19061	0.08	0.7987

Dependent Variable: Soil ammonium content (NH_4-N)

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	813.456112	813.456112	74.20	0.0033
Biofertilizer (B)	1	247.531250	247.531250	22.58	0.0177
Rock phosphate (P)	1	4416.590112	4416.590112	402.89	0.0003
Interactions					
I * B	1	2107.303200	2107.303200	192.23	0.0008
I * P	1	673.261513	673.261513	61.42	0.0043
B * P	1	574.266050	574.266050	52.39	0.0054
I * B * P	1	86.329800	86.329800	7.88	0.0675

Dependent Variable: Extractable phosphorus (P)

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	0.02343613	0.02343613	2.83	0.1912
Biofertilizer (B)	1	0.06090050	0.06090050	7.35	0.0731
Rock phosphate (P)	1	0.13338613	0.13338613	16.10	0.0278
Interactions					
I * B	1	0.00013612	0.00013612	0.02	0.9061
I * P	1	0.00125000	0.00125000	0.15	0.7236
B * P	1	0.00013612	0.00013612	0.02	0.9061
I * B * P	1	0.02354450	0.02354450	2.84	0.1904

Dependent Variable: Exchangeable potassium (K)

Source	*DF*	*SS*	*MS*	*F Value*	*Pr > F*
Main effects					
Inoculation (I)	1	2.3925781	2.3925781	0.25	0.6509
Biofertilizer (B)	1	320.3613281	320.3613281	33.59	0.0102
Rock phosphate (P)	1	0.7812500	0.7812500	0.08	0.7933
Interactions					
I * B	1	0.7812500	0.7812500	0.08	0.7933
I * P	1	3.9550781	3.9550781	0.41	0.5655
B * P	1	1.2207031	1.2207031	0.13	0.7442
I * B * P	1	0.1953125	0.1953125	0.02	0.8953