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Growth and nutritional response of *Sesbania Sesban* (L) Merr. to rock phosphate, biofertilizer and rhizobial applications

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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. This species is 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 or 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 of three treatments on growth and nutritional responses of *S. sesban* to *Rhizobium* (KFR 647) inoculant, Minjingu rock phosphate, and commercial biofertilizer. Rhizobial inoculation significantly increased root nodulation by about 50% exclusively when P was added as rock phosphate. Phosphorus fertilization increased plant height (58.3 cm vs. 29.6 cm in the control). Approximately 90% more biomass was obtained with 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 rates and overall nutrient uptake, while biofertilizer application enhanced plant nutrient uptake. However, a significant interaction between rock phosphate and biofertilizer increased growth performance of *S. sesban*.

Key words: Biofertilizer, Inoculation, N_2-fixation, Phosphate rock, Sesbania

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, 1999; Vitousek et al., 2009). 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, Crotalaria. grahamiana, Gliricidia sepium* and *Tephrosia candida* are currently recommended for short-duration improved-fallows in agroforestry systems (Gathumbi et al. 2004, Jama et al. 1998, 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 (Jama et al. 1998). 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 (Mafongoya et al. 1999). 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. Critical parameters 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.

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.

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). 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 P 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) and Hogh-Jensen et al. 2002 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 question is what is the direct or interactive effect of rock phosphate application, *Rhizobium* inoculation and biofertilizer application on growth performance, nutrient availability and symbiotic N_2-fixation potentials of *S. sesban* grown in P-deficient soils of western Kenya? We employed a glasshouse-based factorial experiment to identify treatment combinations that 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.

Materials and Methods

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 the experiment was collected from a farmland that had been under natural fallow for the past five years in Nyabeda region of western Kenya. 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 1) below:

Table 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 C (%)	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

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).

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, *Tithonia 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.

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.

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.

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 isotope dilution method equations as outlined in (IAEA, 2001).

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 s 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:

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

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;

$TOC = -0.1046Clay + 0.5936\ LOI$

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.

Results

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. Superior growth performances were achieved in treatments that received rock phosphate, whether alone or in combination with other treatments. 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.

Plant biomass accumulation and partitioning

Dry weights of shoots, roots and nodules varied significantly depending on the treatment applied (Table 2). 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.

Shoot-to-root ratio was higher with P addition indicating more carbon allocation above ground than below ground (Table 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.

Table 2 Dry matter yields from different portions, shoot-to-root ratios 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	0.2c	0.43b	0.81b	1.25b	1.88dc
I	0.2c	0.61b	1.00b	1.56b	1.62d
BI	0	0.88b	2.32b	3.20b	2.64bc
B	0	0.78b	2.28b	3.06b	2.92b
IP	48.0a	1.92a	6.05a	8.02a	3.14ba
P	23.0b	2.14a	7.16a	9.31a	3.34ba
BP	0	1.95a	6.28a	8.23a	3.22ba
BIP	0	1.72a	6.76a	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.

Table 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)						
					Biomass			
Source of variation	DF	Root collar diameter	Height	Nodule	Root	Shoot	Total	Shoot: Root
Main effects								
I	1	0.7869	0.0751	0.0357	0.6401	0.7073	0.5898	0.9871
B	1	0.0540	0.0071	0.0022	0.5393	0.0801	0.0504	0.1518
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

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 (Figure1), 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). Commercial biofertilizer failed to stimulate N_2-fixation, but significantly enhanced N content in shoots by about 150% compared to the control (Figure1). 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 (Figure1)). 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 shoot N with sole biofertilizer application, biofertilizer addition improved P and K allocation to roots (Figure 1).

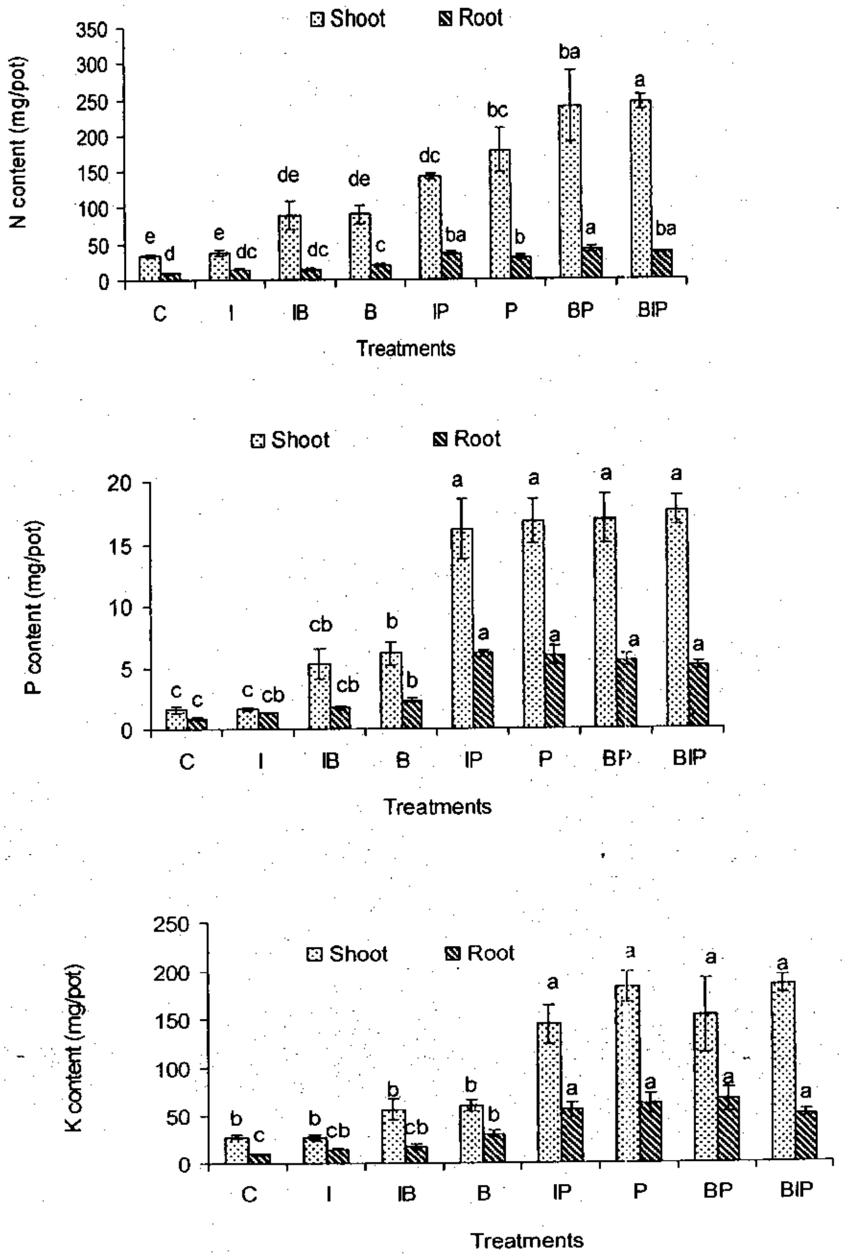

Figure 1: Nutrient accumulations in the shoots and roots of *S. sesban*. C-Control, I-Inoculation with *Rhizobium*, B-Commercial biofertilizer, P- Rock phosphate. Bars of particular plant portions with the same letters did not differ significantly at the 0.05 level of probability.

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. 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 P application (Table 2). Addition of rock phosphate stimulated profuse root nodulation in both inoculated and non-inoculated seedlings, resulting in high nodule biomass production in both treatments (Table 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 2 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.

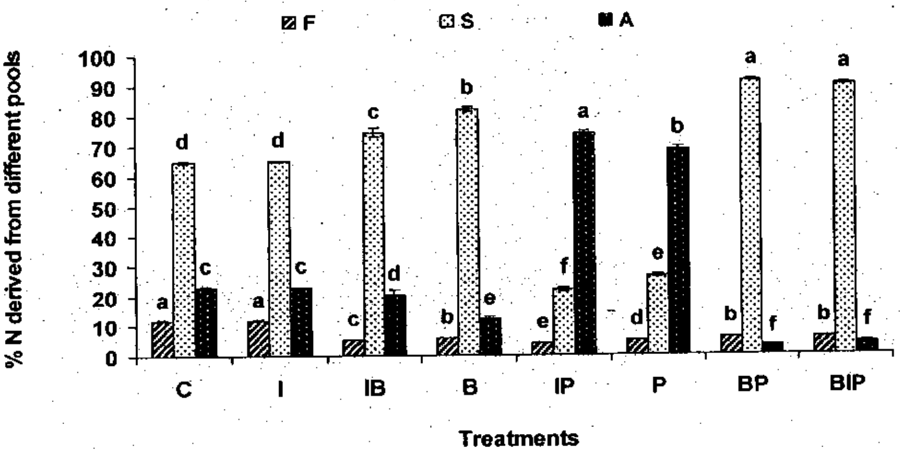

Figure 2. Percent nitrogen derived from different pools by *S. sesban* seedlings as influenced by rhizobial inoculations, biofertilizer and rock phosphate applications. Where F-nitrogen derived from tracer fertilizer [$(^{15}NH_4)_2SO_4$], S-nitrogen derived from the mixture of soil and biofertilizer 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 ($P < 0.05$).

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 root nodule biomass production (Table 2). 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 2). 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 2). Nutrient uptake and N_2-fixed were increased with P-application (Fig.1). 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. 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) (Figure2).

Discussion

Plants growth and biomass production

This study clearly demonstrates that P and N are critically deficient in some soils of western Kenya and can adversely affect the performance of *S. sesban* (Table 2) when supply is limited. Phosphorus was the most limiting nutrient probably due to low P availability detected in the potting soil (Table 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). Our 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 our finding that without exogenous supply of P, the potential of *S. sesban* to produce high biomass is limited (Table.2).

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.

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. 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 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. 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, data indicated high K contents in the roots associated with external P input.

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.

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).

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 4) 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 a combination of 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 2).

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 4), 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.

Table 4 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.

Conclusions

The capability of *S. sesban* to produce large quantities of biomass and accumulate nutrients under varied soil treatments was tested in a controlled greenhouse environment. 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. *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. Fertilization with Minjingu rock phosphate improved BNF with or without inoculation suggesting its promotion in agroforestry systems because it is locally - available and is inexpensive compared to other P sources.

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