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ENHANCING PHOSPHORUS DEFICIENT SOILS FOR MAIZE PRODUCTION: THE ROLE OF BIOLOGICAL NITROGEN FIXATION IN DOUBLED UP LEGUME SYSTEMS

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ABSTRACT

Legumes improve soil fertility through biological nitrogen fixation (BNF). However, their performance is also dependant on the initial fertility status of the soil. Phosphorus is one of the key nutrients for legume performance. Most of the soils in Africa, including Malawi, are deficient in phosphorus. A study was conducted on twenty smallholder farms in Malawi in 2019/20 and 2020/21 rainy seasons to determine how BNF in groundnut (*Arachis hypogaea*) and pigeon pea (*Cajanus cajan*) can be improved in phosphorus deficient soils for subsequent maize production. The study was done in two sites namely Kasungu (Site 1) and Mzimba (Site 2). Site 1 is a mid-altitude while site 2 is in high altitude agroecological zone. Soils in both sites are slightly acidic (pH 4.9 – 5.0 in water), with low organic matter (<1.5%) and deficient in phosphorus and nitrogen. The study had three factors cropping system, Rhizobia inoculation of groundnut seed and P fertiliser application. There were ten treatments [Sole groundnut (GN), no inoculation, no P application; Sole pigeon pea (PP), no P application; Sole groundnut, no inoculation, 20kg P ha^{-1}; Sole groundnut, inoculated, no P application; Sole groundnut, inoculated, 20kg P ha^{-1}; Sole pigeon pea, 20 kg P ha^{-1}; Intercropped groundnut & pigeon pea (GNPP), Inoculated, no P application; Intercropped groundnut & pigeon pea, inoculated 20 kg P ha^{-1}; Intercropped groundnut & pigeon pea, no inoculation, no P application; Intercropped groundnut & pigeon pea, no inoculation, 20 kg P ha^{-1}]. Maize was used as a reference crop and had two plots (Maize without P application and Maize applied with 20 kg P ha^{-1}). The data were subjected to ANOVA using GenStat 18th edition and means were separated using Fisher's protected least significance difference at 5% level of significance. Phosphorus application increased nodule mass by 51.2% in groundnut and 57.4% in pigeon pea. Number of nodules and nodule efficiency also increased with phosphorus application. On area basis, BNF in GNPP was higher than in GN and PP (46.8 kg ha^{-1} in GNPP vs 29.4kg ha^{-1} in GN and 24.1 kg ha^{-1} in PP in 2019/20; and 84.6 kg ha^{-1} in GNPP vs 41.8kg ha^{-1} in GN and 43.9kg ha^{-1} in PP in 2020/21). The study concluded that intercropping groundnut and pigeon pea coupled with application of small doses of P fertiliser enhances BNF in the two crops.

Key words: Nitrogen fixation, yield, groundnut, pigeon pea, Doubled-up, phosphorus, rhizobia, inoculation

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INTRODUCTION

Low soil fertility and imbalanced plant nutrition are some of the major constraints to crop production. This is mainly due to soil degradation and low inherent soil fertility [1]. Among the limited essential nutrients in most of the soils are phosphorus and nitrogen. Farmers apply alot of inorganic fertilisers to supply phosphorus and nitrogen. However, in most of the developing countries, such fertilisers are not available or are unaffordable by the smallholder farmers. Legumes in association with rhizobia do fix atmospheric nitrogen to usable inorganic nitrogen forms in a process called biological nitrogen fixation (BNF). In this biochemical process, nitrogen gas (N_2) is converted into ammonia (NH_3) by some prokaryotes that produce an enzyme called nitrogenase in association with plants of the leguminoceae family [2]. Biological nitrogen fixation provides a sustainable and cheap source of nitrogen in agroecosystems. Integrated soil fertility management practices such as incorporation of legumes in cropping systems are some of the viable nitrogen sources for smallholder farming systems.

Doubled-up legume system is a cropping system in which two legumes with complementary growth habits are intercropped. Although doubled up legumes improve soil fertility due to the increased biomass yield and nitrogen fixed per unit area, there is variation in amount of nitrogen fixed in the system [3-6]. Biological nitrogen fixation is influenced by a number of factors including phosphorus availability and rhizobia strains inherently available in the rhizosphere. Legume plants actively fixing nitrogen require more phosphorus than the non-fixing ones [7]. Some studies have also reported an increased performance of doubled up legume systems in response to phosphorus availability [4, 5]. Poor nodule development and low plant vigour has also been reported in legumes grown on fields where extractable phosphorus is deficient despite inoculating the seed with a right strain of rhizobia [8]. Though rhizobia are tolerant to low levels of phosphorus in the soil, nodulation and BNF are very sensitive to its limitation. Successful nodulation and BNF have been reported to be highly correlated with availability of phosphorus in the soil. Deficiency of phosphorus in soil has an adverse impact on legume production as it is required for energy transformation in nodules and enhanced nitrogen fixation [9].

Some legumes nodulate with specific strains of rhizobia while others are promiscuous, nodulating with many different rhizobia [10]. Only rhizobia that are specifically compatible with a particular species of legume can stimulate nodulation. Even among rhizobia species that can nodulate the same legume plant, there are genetically distinct strains. Some fix nitrogen more efficiently than others, resulting in superior plant growth. These efficient strains may or may not be available in the soil. Seed inoculation is done to ensure availability of a right strain of rhizobia in the root zone of the emerging seedling. Inoculants that contain effective rhizobia isolated from plant nodules and cultured in the laboratory are produced commercially and available on the market in different brands.

Low crop productivity is common in degraded soils that are deficient from phosphorus and nitrogen. Incorporation of legumes such as groundnut and pigeon pea into cropping systems dominated by cereals such as maize fails to reverse the situation since the legumes also perform poorly and the is very low biological nitrogen fixation due to lack of P. This calls for techniques on how to improve legume performance for an increased BNF in P deficient soils. An on-farm study was conducted over two rainy seasons (2019/20 and 2020/21) in phosphorus deficient soils of Kasungu and Mzimba districts in Malawi to assess the effect of phosphorus fertiliser application and/or rhizobia inoculation on BNF in groundnut-pigeon pea doubled up system.

MATERIALS AND METHODS

The study involved multi-environmental on-farm trials in Kasungu district, Kaluluma EPA (Site 1: Latitude -12.7610445, longitude 33.4232891) in central Malawi and Mzimba district, Bwengu EPA (Site 2: Latitude - 11.1233823, longitude 33.9280798) in northern Malawi in the 2019/20 and 2020/21 rainy seasons. Site 1 has average temperatures of about 20.8^{0}C while annual rainfall averages at 680 mm. Site 2 has average temperatures of 20.1^{0}C while annual rainfall averages at 915 mm. In both sites the soils are generally Lixisols with low organic matter, nitrogen and phosphorus (Table 1). The study was conducted on 20 farms (10 in each site) that served as replicates. The experiment had three factors namely, cropping system (sole cropped groundnut or pigeon pea and intercrop of groundnut and pigeon pea), rhizobia inoculation (seed inoculated and not inoculated) and phosphorus application (0 kg P ha^{-1} or 9 kg P ha^{-1} thus 20 kg P_2O_5 ha^{-1}). Single super phosphate fertiliser (19 % P_2O_5) was used as a source of phosphorus applied at planting by drilling method. Maize was used as a reference crop to help in determination of BNF. Therefore, two more plots of maize with and without phosphorus fertiliser application were grown. Table 2 shows the treatments used in the experiment. The treatments were randomly assigned to the plots, replicated ten (10) times and laid out in a randomized complete block design (RCBD). The ten farms served as the replicates in each of the two sites. Gross plot size was 8 m x 4.5 m (6 ridges of 8 m long spaced at 0.75 m) and the net plot was 6 m x 3 m (4 ridges of 6 m long). Soil samples were collected from each field before land preparation at 0 – 15cm and 15–30cm depths using an auger to monitor the effect of the treatments at different soil levels. Soil texture was determined using Hydrometer method [11]. Soil pH in distilled water was measured using a pH meter. Total soil nitrogen was determined using Kjeldahl method [12]. Available phosphorus was determined using Mehlich III method while Organic carbon was determined using Walkley-Black Wet Oxidation Method [13].

Nodulation was determined at 50% flowering stage. Number of nodules per plant, nodule mass and internal nodule colour were determined by destructive sampling. Six plants of groundnut and two for pigeon pea were uprooted from each of the two border ridges representing a 1m length on each ridge. Number of nodules was recorded and ten nodules were randomly removed from each plant for weighing. The ten nodules were then opened to check internal colour using a colour chart. Nodules with reddish internal colour (red or pink) were considered actively fixing nitrogen, green or white nodules were considered inactive while black ones were considered dead [14].

Biologically fixed nitrogen was calculated using the Modified Nitrogen difference method [15] as presented in the equation below:

$$Q = (N\ yield_L - N\ yield_R) + (N\ soil_L - N\ soil_R)$$

Where:
Q= Amount of biologically fixed N (kg ha^{-1})
N $yield_L$ = N yield in legume crop plant samples
N $yield_R$ = N yield in reference crop plant samples
N $soil_L$ = Post harvest soil N in legume crop plot
N $soil_R$ = Post harvest soil N in reference crop plot

The data were tested for normality using graphical method before parametric testing. Data on nodule numbers per plant were transformed to logarithms [$\log_{10}(x+1)$] before analysis. The data were then subjected to analysis of variance (ANOVA) using Genstat 18th edition statistical package. Treatment means were separated using Fisher's protected least significant difference (LSD) at 5% level of significance.

RESULTS AND DISCUSSION

Groundnut and pigeon pea nodule mass

Tables 3 and 4 show mass of nodules in groundnut and pigeon pea respectively in different cropping systems with and without phosphorus fertiliser application and rhizobia inoculation. Highly significant ($P<0.001$) differences in nodule mass for both groundnut and pigeon pea were observed between plots applied with phosphorus fertiliser and those not applied in different cropping systems. Rhizhobia inoculation had no significant effect on nodule mass. Nodule mass increased by an average of 51.2 % in groundnut and 57.4 % in pigeon pea with phosphorus application.

Phosphorus is essential in most metabolic processes such as energy generation, nucleic acid synthesis, respiration, mitochondrial and symbiosome membrane synthesis during nodule development [16]. Phosphorus availability enhances nodule growth and development and hence the significant differences in nodule mass [17]. An increase in nodule mass with phosphorus application has been reported in soybean and cowpea [1, 7, 13]

The fields used in the study were not newly opened but have been under cultivation of different crops such as maize, soyabean, ground nut and beans. Soils under cultivation contain more native rhizobia populations than uncultivated soils [17]. The insignificant differences between inoculated and uninoculated groundnut suggest natural availability of rhizobia species that can nodulate groundnut in most of the fields rendering rhizobia inoculation not necessary. This explains presence of nodules in non-inoculated plots.

In 2020 planting season, site 1 received high rainfall of 1285 mm which was almost twice more than the average annual rainfall for the area. This might have caused leaching of some nutrients especially nitrogen and other anions leading to competition for nutrients. The lower nodule mass in the intercropped groundnut in site 1 can be attributed to competition for growth resources both underground and above ground. Availability of resources such as soil nutrients may determine the extent of the negative effects of competition on growth and development of the plant including nodule mass. Where soil nutrients are deficient, the effects of interspecific competition become more pronounced than where there are sufficient nutrients [18].

Number and effectiveness of nodules
Number of nodules per plant significantly increased ($P < 0.001$) with phosphorus application in both seasons and sites (figure 1 and plate 1). However, seasonal variations in number of nodules were also observed with 2019/20 season registering lower average as compared to 2020/21 season regardless of cropping system and phosphorus application (35 vs 82 nodules per plant). A higher positive response to phosphorus application was also observed in 2020/21 season (91% increase) than in 2019/20 season (42% increase). Proportion of effective nodules also significantly increased with phosphorus application in both groundnut and pigeon pea (Table 5). In plots where phosphorus was applied, a higher proportion of nodules (72-76%) were actively fixing nitrogen as compared to those plots where phosphorus was not applied (41-51%).

Phosphorus promotes root growth and development [19]. The robust root system in groundnut plants applied with phosphorus fertiliser accommodated more nodules on their surface. Phosphorus also serves as energy source for the rhizobia thereby leading to increased nodule formation and their effectiveness in BNF. The role of phosphorus in energy transformation and nucleic acid production influences nodulation in legumes. An increase in number of nodules per plant and root length

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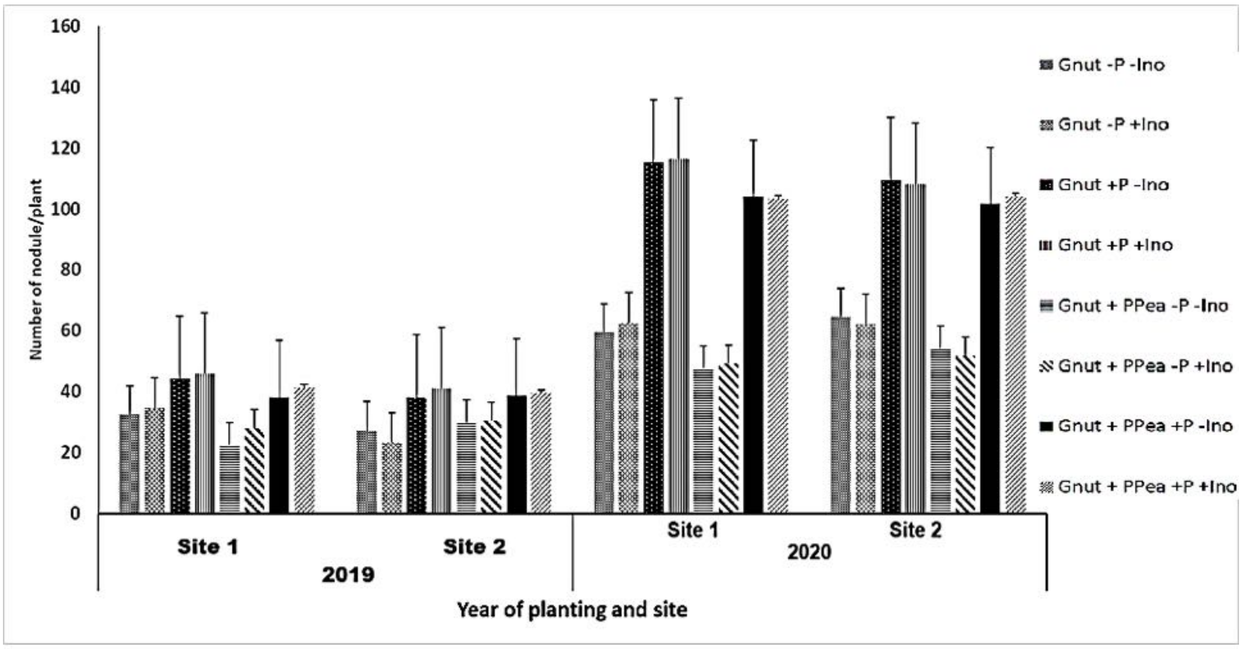
in response to phosphorus fertiliser application in groundnut has also been reported in other crops [20]. Increasing level of phosphorus applied to groundnut increases number of nodules per plant by a range of 56% to 144% depending on variety [21].

Insignificant effect of rhizobia inoculation can be attributed to natural presence of rhizobia strains in the soil. As seen in section 3.1, most of the fields were deficient in key essential nutrients such as nitrogen and phosphorus. Effectiveness of rhizobia inoculation is affected by inherent soil fertility as it affects the ability of the host plant to support the rhizobia with photosynthates [22]. Nutrient deficiency reduces the ability of the inoculated rhizobia isolates to compete with native strains that are used to the poor soils. The insignificant differences between sole and intercropped groundnut indicate low competition between the two legume species.

Low number of nodules per plant in the 2019/20 season may be attributed to the dry spells experienced during the season when flowering was about to start in groundnut. This indicates that return on phosphorus investment may vary from one season to another due to climatic conditions such as rainfall.



Gnut= Groundnut, Ppea = Pigeon pea, P = Phosphorus fertiliser, Ino = Rhizobia Inoculant

Figure 1: Number of nodules per plant as influenced by phosphorus fertiliser application, cropping system and rhizobia inoculation in 2019/20 and 2020/21 at two sites

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Plate 1: Variation in root growth and number of nodules in groundnut with (+P) and without (-P) Phosphorus fertiliser application

Biological nitrogen fixation per unit area

Figures 2 and 3 show total nitrogen fixed (kg/ha) in groundnut and pigeon pea for the two seasons and sites. Biological nitrogen fixation (BNF) increased significantly ($P < 0.001$) with phosphorus fertiliser application in both seasons and sites. Cropping system also significantly influenced BNF. Total BNF in doubled up system was higher than in sole crops. Highest BNF was recorded in plots where groundnut was intercropped with pigeon pea with phosphorus fertiliser application. Biological nitrogen fixation is an energy demanding process. Phosphorus is required for energy transformation during biological nitrogen fixation as Adenosine Triphosphate (ATP) [23]. Legumes that are actively fixing nitrogen have also been reported to require more phosphorus than non-symbiotic plants that rely on mineral nitrogen from the soil only [23]. Adverse impact of phosphorus deficiency in the soil on legume production has been reported in different studies [9].

Intercropping groundnut and pigeon pea increased leafy biomass produced per unit area since it was a totality of the two crops thereby contributing to an increased nitrogen contribution from the two crops to the system. Quantities of biologically fixed nitrogen in plots where groundnut was intercropped with pigeon pea are totals for the two crops while those for the sole crop plots are from a single crop since computation was on area basis. Similar results have also been reported in other studies [5, 28, 26].

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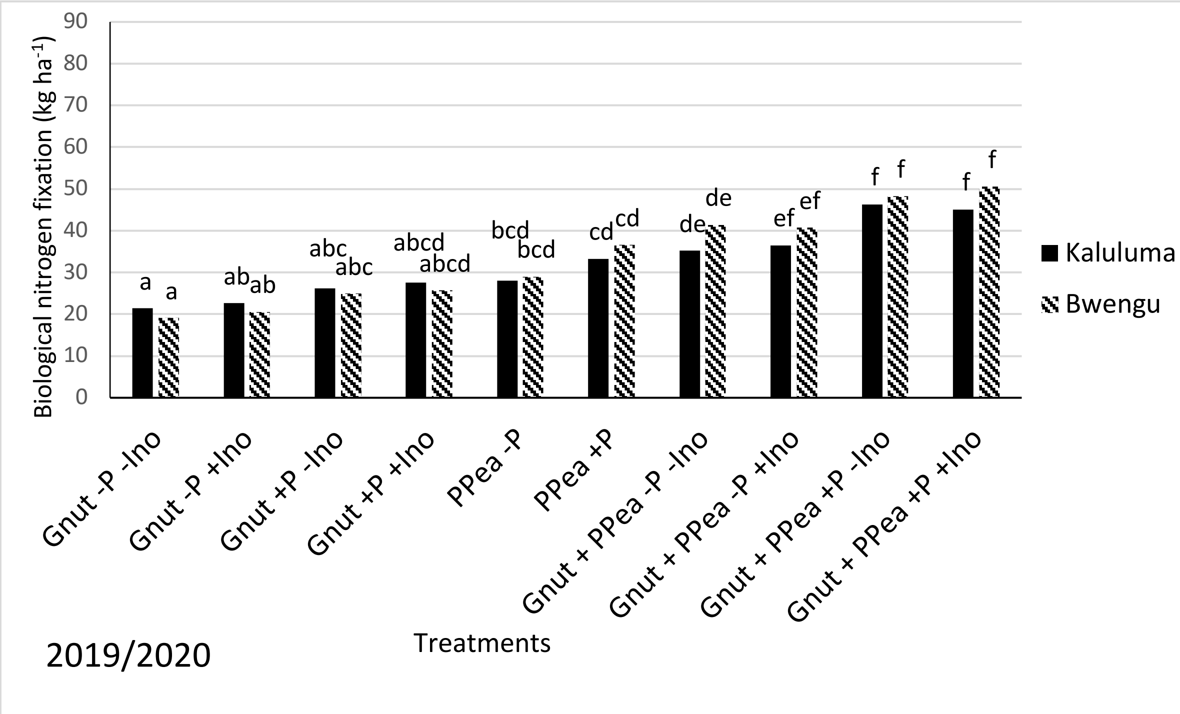

Gnut= Groundnut, Ppea = Pigeon pea, P = Phosphorus fertiliser, Ino = Rhizobia Inoculant

Figure 2: Total Biological nitrogen fixation (kg ha^{-1}) in groundnut and pigeon pea as influenced by rhizobia inoculation and phosphorus application at two study sites in 2019/2020 season

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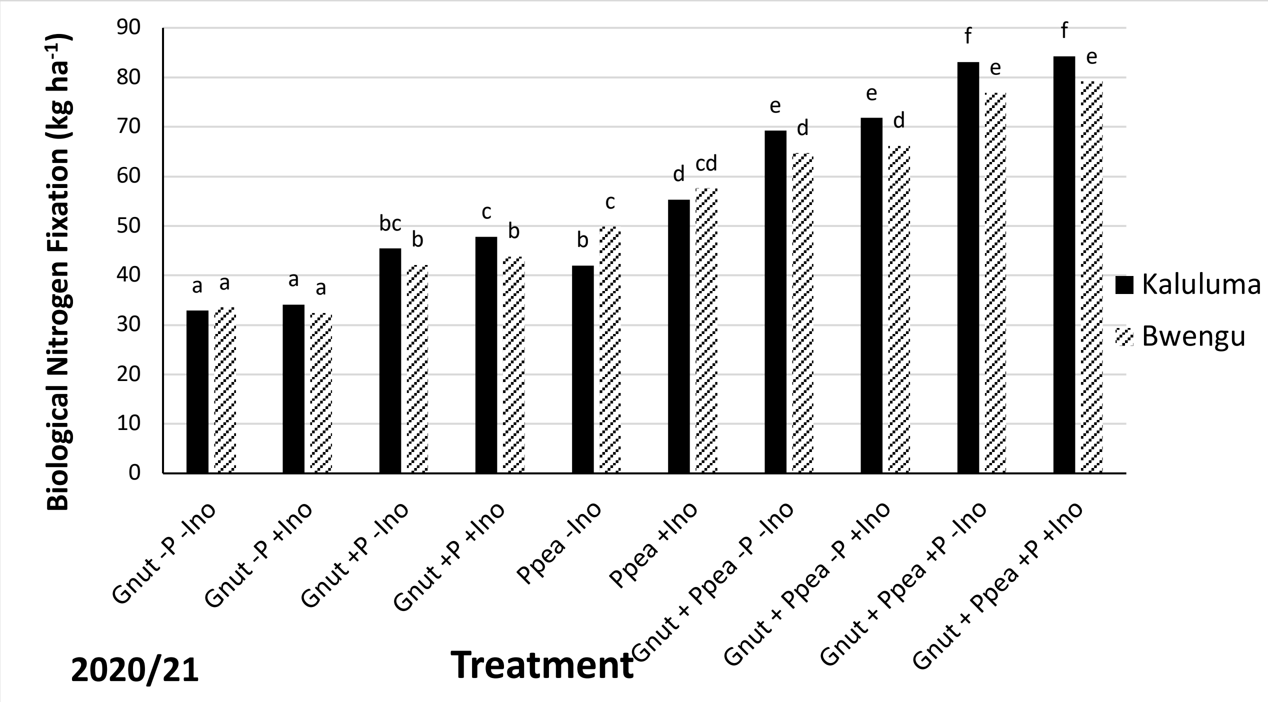

Gnut= Groundnut, Ppea = Pigeon pea, P = Phosphorus fertiliser, Ino = Rhizobia Inoculant

Figure 3: Total Biological nitrogen fixation (kg ha^{-1}) in groundnut and pigeon pea as influenced by rhizobia inoculation and phosphorus application at two study sites in 2020/2021 season

CONCLUSION AND RECOMMENDATIONS FOR DEVELOPMENT

Phosphorus application increases biological nitrogen fixation in groundnut and pigeon pea in phosphorus deficient soils. Intercropping ground nut and pigeon pea also increases biological nitrogen fixation on area basis. In this study, rhizobia inoculation did not influence nodulation and biological nitrogen fixation. It is therefore recommended that for phosphorus deficient soils, application of inorganic phosphorus fertilisers will improve biological nitrogen fixation in groundnut and pigeon pea. Intercropping compatible legumes such as groundnut and pigeon pea should be encouraged to improve soil fertility through increased biological nitrogen fixation. Future studies should focus on determining phosphorus application rates and establishing native rhizobia strains available in different soils to ascertain the need for inoculation.

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Table 1: Baseline soil characteristics in site 1 and site 2 of the field experiment

Parameter	Soil depth (cm)	Critical Values	Average		Range		Comment
			Site 1	Site 2	Site 1	Site 2	
pH	0-15		5.0±0.2	4.9±0.1	4.7-5.3	4.7-5.1	Acidic.
	15-30		4.8. ±0.1	4.6±0.2	4.2-5.1	4.3-5.0	
Total N (%)	0-15	0.1	0.04±0.02	0.10±0.04	0.02-0.08	0.04-0.14	Very low to medium
	15-30		0.12±0.01	0.17±0.03	0.06-0.16	0.06-0.21	
Available P. (ppm)	0-15	25	23.8±9.4	11.4±5.7	9.9-35.9	4.9-21.6	Low
	15-30		18.2±1.7	7.1±0.8	6.8-29.3	2.1-16.5	
SOM (%)	0-15	1.5	1.3±0.6	1.4±0.8	1.0-2.7	1.1-3.8	Low to high
	15-30		1.1±0.4	1.0±0.7	0.8-2.3	0.8-3.1	
Textural class	0-15		Loamy sand	Sandy clay loam			

Note: SOM = Soil organic matter, N=Nitrogen, P = phosphorous, ppm = Parts per million

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Table 2: Factor Level combination for the trials

Treatment Number	Cropping System	G/nut Inoculation	P_2O_5 Application
T1 (Control 1)	Sole Gnut	No inoculation	No Application
T2 (Control 2)	Sole P/Pea	NA	No Application
T3	Sole Gnut	No inoculation	20 kg ha^{-1}
T4	Sole Gnut	Inoculated	No Application
T5	Sole Gnut	Inoculated	20 kg ha^{-1}
T6	Sole PPea	NA	20 kg ha^{-1}
T7	Intercropped Gnut & PPea	Inoculated	No Application
T8	Intercropped Gnut & PPea	Inoculated	20 kg ha^{-1}
T9	Intercropped Gnut & PPea	No inoculation	No Application
T10	Intercropped Gnut & PPea	No inoculation	20 kg ha^{-1}
R1	Maize	NA	No Application
R12	Maize	NA	20 kg ha^{-1}

Gnut = Ground nut, Ppea = Pigeon pea, NA = Not Applicable, R= Reference crop

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Table 3: Average nodule mass (g) in groundnut under sole and doubled up cropping systems with or without phosphorus (P) application and rhizobia inoculation in 2019/20 and 2020/21 seasons

			2019/2020			2020/2021		
	P Application	**Cropping system**	**Inoculated**	**No Inoculation**	**Mean**	**Inoculated**	**No Inoculation**	**Mean**
Site 1	P applied	Sole crop	0.998	0.924	0.912 [b]	0.822	0.834	0.807[a]
		Doubled up	0.931	0.800		0.785	0.785	
	P not applied	Sole crop	0.634	0.539	0.508 [a]	0.601	0.580	0.571[b]
		Doubled up	0.469	0.392		0.550	0.552	
	Mean		0.761	0.663		0.690	0.688	
	F Pr	Cropping system		0.050			0.024	
		Phosphorus		<0.001			<0.001	
		Inoculation		0.133			0.908	
		Cropping system x Phosphorus		0.642			0.922	
		Cropping x Inoculation		0.867			0.869	
		Phosphorus x Inoculation		0.899			0.653	
		Cropping x Phosphorus x Inoculation		0.771			0.621	
	CV (%)		22.7				10.9	
	LSD		0.258		0.174		0.071	0.035
Site 2	P applied	Sole crop	0.882	0.905	0.867 [b]	0.828	0.796	0.809[a]
		Doubled up	0.801	0.878		0.830	0.784	
	P not applied	Sole crop	0.676	0.718		0.606	0.585	0.593[b]
		Doubled up	0.485	0.445	0.721 [a]	0.582	0.599	
	Mean		0.711	0.737		0.712	0.691	
	F Pr	Cropping system		0.126			0.961	
		Phosphorus		<0.001			<0.001	
		Inoculation		0.109			0.257	
		Cropping system x Phosphorus		0.404			0.730	
		Cropping x Inoculation		0.399			0.520	
		Phosphorus x Inoculation		0.605			0.313	
		Cropping x Phosphorus x Inoculation		0.353			0.703	
	CV (%)		19.9				11.0	
	LSD		0.244		0.174		0.072	0.036

Means with same letter within a column are not significantly different at 5% level of significance.

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Table 4: Average nodule mass in pigeonpea under sole and doubled up cropping systems with or without P fertiliser application in 2019/20 and 2020/21 seasons

		2019/20			2020/21		
		P application			P application		Mean
	Cropping system	P applied	P not applied	Mean	P applied	P not applied	
Site 1	Sole Ppea	1.50	0.91	1.21	1.42	0.85	1.13^{a}
	Doubled up	1.14	0.79	0.97	1.31	0.82	1.07^{b}
	Mean	1.44^{A}	0.87^{B}	1.15	1.35^{A}	0.83^{B}	1.09
	FPr Cropping system			0.744		0.020	
	P Application			<0.001		<0.001	
	Cropping system*P application			0.131		0.920	
	CV (%)		14.8			11.2	
	LSD		0.114	0.093		0.115	0.067
Site 2	Sole Ppea	1.51	1.02	1.30	1.31^{a}	0.95^{b}	1.13
	Doubled up	1.59	1.13	1.36	1.34^{a}	0.83^{b}	1.09
	Mean	1.58^{A}	1.09^{B}	1.34	1.33^{A}	0.87^{B}	1.10
	FPr Cropping system			0.170		0.233	
	P Application			<0.001		<0.001	
	Cropping system*P application			0.237		0.030	
	CV (%)		10.7			11.0	
	LSD		0.145	0.084		0.099	0.070

Means with same uppercase letter within a row and lower-case letter within a column are not significantly different at 5% level of significance
Ppea = Pigeon pea

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Table 5: Proportion (%) of effective nodules as influenced by P application, cropping system and rhizobia inoculation in groundnut

			2019/2020			20/2021		
	P Application	Cropping system	Inoculated	No Inoculation	Mean	Inoculated	No Inoculation	Mean
Site 1	P applied	Sole crop	86.7	71.7	76.5[a]	69.7	79.7	72.1[a]
		Doubled up	78.6	78.9		66.1	72.8	
	P not applied	Sole crop	53.5	47.1	51.8[b]	41.9	40.6	41.0[b]
		Doubled up	54.2	52.5		39.7	41.7	
	Mean		68.2	62.6	63.6	59.4	58.7	59.0
	F Pr	Cropping system		0.954			0.434	
		Phosphorus		<0.001			<0.001	
		Inoculation		0.061			0.794	
		Cropping system x Phosphorus		0.053			0.323	
		Cropping x Inoculation		0.156			0.064	
		Phosphorus x Inoculation		0.191			0.715	
		Cropping x Phosphorus x Inoculation		0.732			0.114	
	CV (%)		16.1				19.0	
	LSD		6.83		4.83		10.58	5.29
Site 2	P applied	Sole crop	73.3	78.6	76.3[a]	76.7	67.8	72.2[a]
		Doubled up	75.4	68.3		74.2	70.0	
	P not applied	Sole crop	51.1	49.5	50.9[b]	46.7	43.1	44.9[b]
		Doubled up	49.8	47.0		46.1	44.4	
	Mean		62.4	60.9	63.9	60.8	56.3	58.5
	F Pr	Cropping system		0.207			0.923	
		Phosphorus		<0.001			<0.001	
		Inoculation		0.064			0.127	
		Cropping system x Phosphorus		0.533			0.885	
		Cropping x Inoculation		0.326			0.597	
		Phosphorus x Inoculation		0.362			0.471	
		Cropping x Phosphorus x Inoculation		0.515			0.773	
	CV (%)		14.1				20.8	
	LSD		6.47		4.54		7.75	4.06

Means with same letter within a column are not significantly different at 5% level of significance

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