Nodulation and biological nitrogen fixation in soybean (*Glycine max* L.) as influenced by phosphorus fertilization and arbuscular mycorrhizal inoculation

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Abstract. Arbuscular mycorrhizal fungi (AMF) can be used to promote the productivity of legumes on phosphorus- (P) deficient soils. The present study investigates the inoculation effects of three AMF species (*Funneliformis mosseae*, *Rhizophagus intraradices*, and *Claroideoglomus etunicatum*) and the control (uninoculated) on nitrogen fixation efficiency and growth performance of tropical soybean cultivar (TGx1448-2E) under varying P fertilizer rates (0, 20, and 40 kg P_2O_5 ha^{-1}) in a derived savannah of Nigeria. The results showed that shoot and root dry matter, number of nodules, relative ureide abundance (RUA), nitrogen derived from atmosphere (Ndfa), total N fixed, shoot P uptake, grain, and biomass yield significantly increased with AMF inoculation, with better performance observed in plants inoculated with *Rhizophagus intraradices* and *Funneliformis mosseae* compared to *Claroideoglomus etunicatum*. Similarly, the soybean growth variables, P uptake, and nitrogen fixation activities increased with increasing P application rates. Conversely, AMF root colonization significantly reduced with increasing P rate. Interaction of AMF inoculation and P rates significantly influenced soybean growth and nitrogen fixation. *R. intraradices* inoculation with 20 kg P_2O_5 ha^{-1} resulted in the highest amount of RUA, Ndfa, N fixed, and grain yield. It could be concluded from this study that *R. intraradices* with moderate P rate could be used to enhance nodulation, nitrogen fixation, and soybean yield in P-deficient soils.

Keywords: biological nitrogen fixation, mycorrhizal fungi isolates, phosphorus uptake, ureide assay, soybean productivity

Introduction

Legumes (*Fabaceae*) are important crops in agriculture with abundant levels of proteins and oils for both human and livestock consumption. They do great service to the sustainability of most agricultural systems. Their symbiotic relationship with soil rhizobia helps them in the fixation of atmospheric dinitrogen (N_2), thus reducing the intensive application of inorganic nitrogen (N) fertilizers on agricultural soils while maintaining soil fertility for sustainable crop production. Soybean (*Glycine max* L. Merrill) is an important oil-seed legume cultivated in many parts of the world, including Nigeria [1]. However, low phosphorus (P) availability is the major factor limiting legume productivity in most tropical soils [2]. The nitrogen fixation process in legumes is highly sensitive to adverse environmental conditions, P deficiency [3]. This has become crucial because the way N_2 fixation in soybean is affected by P deficiency is not fully explored under field conditions. Therefore, a better understanding of the responses of N_2-fixation in soybean in P-deficient soils is urgently needed for promoting soybean production towards achieving food security, particularly in sub-Saharan Africa.

Phosphorus plays an important role in the regulation of many metabolic processes in plants, including photosynthesis and N_2 fixation [4, 5]. Nevertheless, P deficiency has negative implications on nodule functioning in legumes [6, 7] due to the high P demand in N_2-fixing nodules for optimal functioning and cell energy metabolism [3, 8]. In addition, the amount of carbon delivered to the nodules and the absorption of ureides in the nodules are reduced under P deficiency [9, 10]. The report of [11] showed that P application positively influenced N_2 fixation in legumes with increased N content. Hence, an adequate P availability in the nodules is essential for N_2 fixation in P-deficient soils.

The intensive use of inorganic P fertilizers has been widely promoted in most agro-ecosystems to maintain a sufficient level of P for crop productivity [12]. The recovery of P application is reported to be very low (10–30% of the fertilizer P applied) [13]. In addition, most farmers in sub-Saharan Africa are resource-limited and may not be able to afford the high P fertilizer cost. Moreover, excessive use of P fertilizer may lead to environmental pollution due to the eutrophication of underground waters and the contamination of soils with heavy metals. There is also report of the potential scarcity of P fertilizers due to the rapid depletion of phosphate rocks. In the context of sustainable agriculture and global P crisis faced by farmers, arbuscular mycorrhizal fungi (AMF) are essential components of sustainable soil-plant systems, forming symbiotic relationships with most plants, including legumes [14]. AMF stimulate the growth of the host plants through increase in soil inorganic nutrients' uptake, particularly P [14, 15]. With reduction in the application of chemical P fertilizers,

they could play an important role in meeting the food requirements of an increasing human population [16, 17]. In legumes, AMF has been reported to promote growth and yield performance in soils with low P availability [18, 19, 20]. With increased number of nodules and size, AMF have positive effect on nodulation [21]. The co-inoculation of AMF and rhizobia is reported to promote N_2 fixation in legumes [22]. Nevertheless, the symbiotic contribution of different AMF isolates on N_2 fixation in legumes has not been fully explored, particularly in a derived savannah of Nigeria with low soil phosphorus availability. The potential of AMF in promoting P uptake in host plants under low P availability could be explored within the context of enhancing nodulation and biological nitrogen fixation in soybean grown on P-deficient soils in this agro-ecology.

The present study will help in understanding how AMF inoculation could promote the symbiotic nitrogen-fixing ability of a promiscuous soybean cultivar (TGx 1448-2E) on P-deficient soils. Thus, this study hypothesized that AMF would enhance the nodulation, nitrogen fixation, and growth performance of soybean on P-deficient soils. Hence, this study aimed to investigate the effects of AMF inoculation with three AMF (*Funneliformis mosseae*, *Rhizophagus intraradices*, and *Claroideoglomus etunicatum*) isolates on the nodulation, nitrogen fixation, and yield performance of soybean plants under different soil P availabilities.

Materials and methods

Study site

The experiment was carried out at the experimental field of the Federal University of Agriculture Research Farm, Abeokuta, Southwest Nigeria (latitude 7° 15' N, longitude 3° 28' E) in the 2017 cropping season. The initial soil properties were determined using standard methods (*Table 1*). A bulk soil sample was collected before planting to determine the physico-chemical properties of the soil using standard protocols. The soil properties determined were soil pH (1:1 soil: water) using glass electrode pH meter [23], organic carbon using the Walkley–Black method as modified by [24], total nitrogen using the micro-Kjeldahl distillation method [25], available phosphorus using Bray-1 [26] and determined colorimetrically using the method of [27]. The exchangeable bases were extracted with 1N ammonium acetate (IN NH_4 OA_C), buffered at pH of 7, and soil particle size distribution was determined using the hydrometer method [28]. The initial AMF spore count was determined using the wet sieving method [29].

Experimental treatments and design

The study consisted of four levels of AMF inoculation (non-AMF (control), *Funneliformis mosseae*, *Rhizophagus intraradices*, and *Claroideoglomus etunicatum*) and three P fertilizer rates (0, 20, and 40 kg P_2O_5 ha^{-1}) laid in a split-plot design with three replications. The plot size was 4 m × 4 m.

Source of experimental materials, sowing, and AMF inoculation

Pure AMF inocula (*F. mosseae, R. intraradices,* and *C. etunicatum*) were obtained from the International Institute of Tropical Agriculture, Ibadan, Nigeria. The inocula were multiplied in sterilized sand, using single-plant culture of maize (*Zea mays* L.) for four months consisting of spores, hyphae, and colonized root pieces. Soybean seeds (TGx 1448-2E) were obtained from the Institute of Agricultural Research and Training, Ibadan, Nigeria.

The field was prepared by ploughing followed by harrowing. The plots were marked out with ropes and pegs and then labelled. The seeds were sown at a depth of 2–5 cm and a spacing of 50 cm × 10 cm on 20 July 2017. AMF inoculum (10 g) was applied to the planting hole before sowing. Phosphorus was supplied through single superphosphate at the time of sowing.

Data collection

Dry matter accumulation and nodulation

Ten plants were randomly harvested from the net plot at the early pod filling stage and separated into leaf, stem + petiole, root, and nodules. The samples were oven-dried at 70 ℃ for 72 hrs to determine the dry weights using a precision measuring scale. The number of nodules was counted, and the average number of nodules per plant was determined.

Estimation of relative ureide abundance and biological nitrogen fixation

The N_2 fixation in the soybean cultivars was measured during the early pod-filling stage using the xylem ureide assay method [30]. Blended subsamples of stem + petiole (0.5 g) were used to extract the xylem solutes in boiling water, and the ureide and nitrate concentration were measured according to the procedures of [31]) and [32]. The blended plant samples were digested in concentrated hot acid (H_2SO_4), and the total N content was determined colorimetrically using automated analysis. The relative ureide-N of the sample was calculated using the equation:

$$\text{Relative ureide} - \text{N} \,(\%) = \frac{4 \times \text{ureide}}{[(4 \times \text{ureide}) + \text{nitrate}]} \times 100$$

The standard curve relating the proportion of N derived from N_2 fixation (Ndfa%) to RU-N for soybean during pod filling was obtained using the equation below:

$$x = 10.7 + 0.50P + 0.0034P^2,$$

where P is N derived from atmosphere (% Ndfa), and x is the ureide-N.
The shoot N (kg ha^{-1}) was calculated using the equation below:

Shoot N (kg ha^{-1}) = [shoot N concentration (%) × shoot dry matter (kg/ha)]

Total N was calculated by multiplying with a factor of 1.5 [33].

$$\text{Crop N (kg ha}^{-1}) = (\text{Shoot N} \times 1.5)$$

The amount of N_2 fixed was calculated using the equation below:

$$\text{Amount of N}_2 \text{ fixed (kg ha}^{-1}) = (\%\text{Ndfa} \times \text{Crop N})$$

Estimation of AMF root colonization

Root colonization by AMF was measured according to [34]. The preserved root samples were cleared in hot KOH solution (10% w/v, at 90 °C) for 1 hour and stained with trypan blue lacto-glycerol (1:1:1:0.5g) at 90 °C for 30 minutes. The AMF colonization (RLC) was determined as the percentage of root length colonized by AMF was calculated [20].

$$RLC = \frac{Number\ of\ colonized\ roots}{Total\ number\ of\ roots} \times 100$$

Grain yield

At harvest maturity, the plants were harvested from the plots, threshed, and air-dried to estimate the grain yield per hectare.

Statistical analysis

The data collected were subjected to two-way analysis of variance (ANOVA) with Genstat Release 12.1 (Copyright 2009, VSN International Ltd). Duncan's multiple range test was used to separate treatment means at 5% probability level. Data expressed as percentages and relative to counts were arcsine-square-root and log $(\times + 1)$ transformed.

Table 1. Soil physical and chemical properties of the experimental site

Soil Property	Value
Texture	Sandy loam
Sand (g kg^{-1})	707.5
Silt (g kg^{-1})	127.5
Clay (g kg^{-1})	165.0
pH (H$_2$O)	5.70
Organic Matter (%)	1.78
Nitrogen (%)	0.09
Available Phosphorus (mg kg^{-1})	6.13
Potassium (cmol kg^{-1})	0.61
Calcium (cmol kg^{-1})	6.68
Magnesium (cmol kg^{-1})	1.47
Sodium (cmol kg^{-1})	0.29
Total Exchangeable Acidity (cmol kg^{-1})	0.11
Cation Exchange Capacity (cmol kg^{-1})	9.17

Results

Dry matter accumulation

Phosphorus application and AMF inoculation had significant effect on the shoot and root dry matter of the soybean (*Table 2*). The shoot and root dry matter increased with P fertilizer application compared to the control (no P fertilizer added); however, no significant differences were recorded between the application of 20 and 40 kg P$_2$O$_5$ ha^{-1} (*Table 3*). AMF inoculation significantly increased the shoot and root dry matter over non-AMF treatment (*Table 3*). The increase in shoot and root dry matter induced by *F. mosseae* (20.6 and 16.9%) and *R. intraradices* (23.9 and 15%) over non-AMF treatment was significantly greater than that obtained with *C. etunicatum* (9.3 and 5.2%). There was a

significant interaction effect of P application and AMF inoculation on the shoot and root dry matter of the soybean plant (*Table 2*). Soybean inoculated with *R. intraradices* with 20 kg P_2O_5 ha^{-1} fertilizer rate significantly had the highest shoot and root dry matter, followed by inoculation with *F. mosseae* at 20 kg P_2O_5 ha^{-1} application rate and then 40 kg P_2O_5 ha^{-1} fertilizer application alone (figures 1a and b). With high P fertilizer application (40 kg P_2O_5 ha^{-1}), the shoot and root dry matter decreased, but no significant difference was observed between the AMF isolates and non-AMF treatments (figures 1a and b).

Nodulation

The results showed that P fertilizer application rates and AMF inoculation had significant effect on the number of nodules of soybean but not on the nodule dry weight (*Table 2*). The number of nodules increased with the application of 20 and 40 kg P_2O_5 ha^{-1} rates (58.6 and 60.2% respectively), but no significant difference was observed between the two rates (*Table 3*). Soybean inoculated with *R. intraradices* significantly had the highest number of nodules compared to other AMF isolates and non-AMF treatment (*Table 3*). A significant interaction between P fertilizer application rates and AMF inoculation was observed for the number of nodules of soybean (*Table 2*). The lowest number of nodules was recorded in non-AMF soybean plants and no P fertilizer application, while the highest number of nodules was recorded in plants inoculated with *R. intraradices* with 20 kg P_2O_5 ha^{-1} rate (*Figure 2*).

Table 2. Mean squares for the effect of P fertilizer application (P) and mycorrhizal inoculation (M) and their interactions (P × M) on dry matter accumulation and nodulation in soybean

|	df	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Number of nodules (plant^{-1})	Nodule dry weight (g plant^{-1})
Reps	1	11.8	0.016	102.1	0.029
P rates (P)	2	20.1**	0.33**	674.6**	0.061^{ns}
Error a	2	0.09	0.005	7.72	0.014
AMF (M)	3	10.2***	0.12***	190.9***	0.027^{ns}
P × M	6	7.32**	0.091***	96.8***	0.019^{ns}
Error b	9	0.09	0.003	2.84	0.011
Total	23				

Notes: *, **, *** indicate significance at 0.05, 0.01, and 0.001 probability level respectively; ns indicates not significant.

Table 3. Effects of P fertilizer application (P) and mycorrhizal inoculation (M) on dry matter accumulation and nodulation in soybean

	Shoot dry weight (g plant^{-1})	Root dry weight (g plant^{-1})	Number of nodules (plant^{-1})	Nodule dry weight (g plant^{-1})
P rates (P)				
0 kg ha^{-1}	8.75	1.39	10.9	0.19
20 kg ha^{-1}	11.6	1.73	26.3	0.35
40 kg ha^{-1}	11.4	1.76	27.4	0.32
Lsd (p < 0.05)	0.67	0.15	5.97	ns
AMF inoculation				
Non-AMF	9.05	1.47	15.0	0.22
F. mosseae	11.4	1.77	24.6	0.37
R. intraradices	11.9	1.73	27.6	0.31
C. etunicatum	9.98	1.55	18.9	0.24
Lsd (p < 0.05)	0.39	0.07	2.20	ns

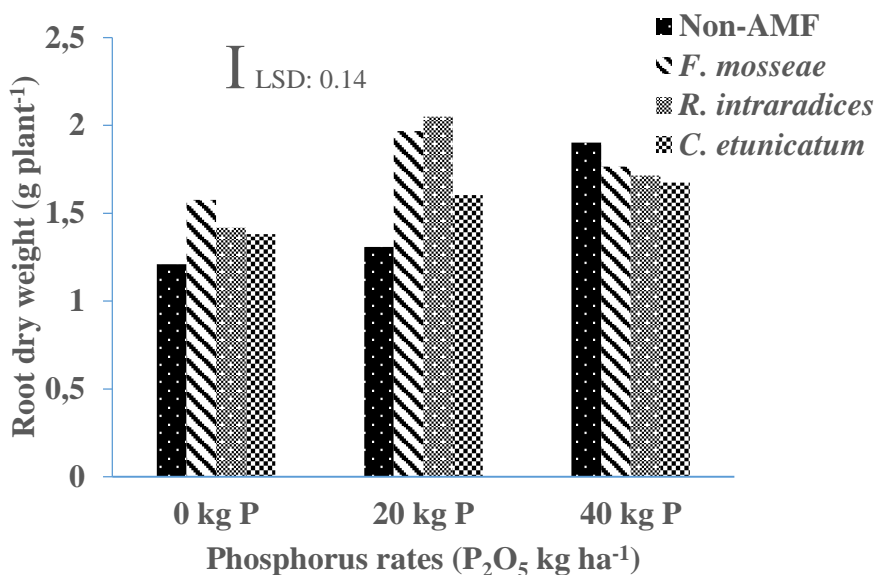Note: ns indicates non-significant differences at p value < 0.05.

a.
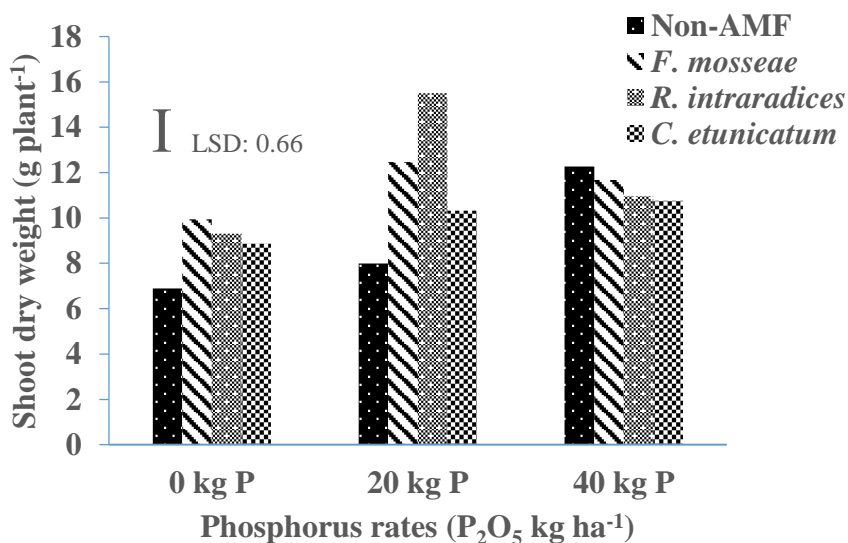
b.

Figure 1. Interactive effect of phosphorus rates and AMF inoculation on (a) shoot dry weight and (b) root dry weight of soybean. Bar denotes Lsd value at 5% probability level.
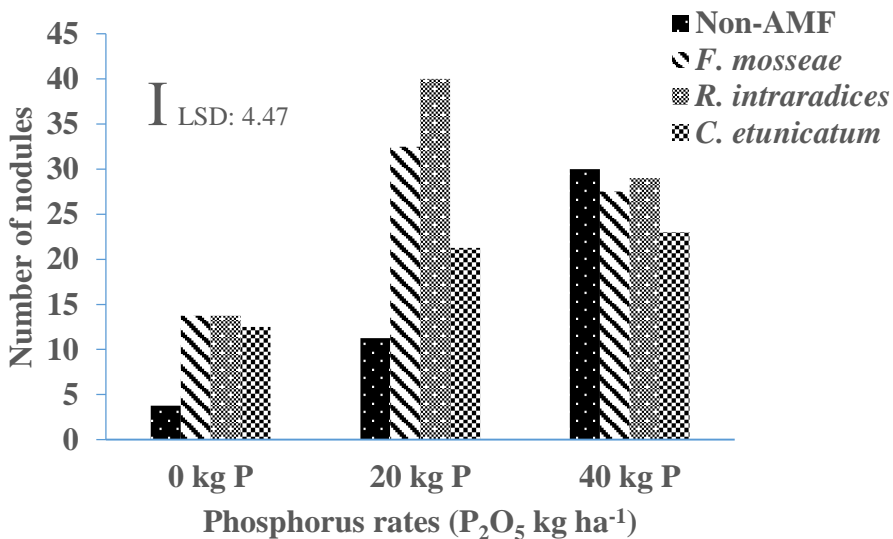

Figure 2. Interactive effect of phosphorus rates and AMF inoculation on the number of nodules of soybean. Bar denotes Lsd value at 5% probability level.

Nitrogen fixation

The P fertilizer application had significant effect on the relative abundance of ureide-N and the amount of nitrogen fixed by the soybean plant but not on concentrations of nitrate-N (NO_3-N) and ureide-N and the proportion of N derived from atmosphere (Ndfa) (*Table 4*). The relative ureide-N and the amount of N fixed increased with increase in P rates (*Table 5*). However, there was no significant difference between the application of 20 and 40 kg P_2O_5 ha^{-1} rates (*Table 5*). The concentration of nitrate-N was recorded to decrease with increase in P rates, while ureide-N and Ndfa increased with increase in P rates; however, no significant difference was observed among the P rates (*Table 5*).

The concentrations of nitrate-N (NO_3-N) and ureide-N, the relative abundance of ureide-N, the proportion of N derived from atmosphere (Ndfa), and the amount of N fixed in the soybean plant were significantly influenced by AMF inoculation (*Table 4*). Significant differences were observed among the AMF isolates and the non-AMF treatments regarding these parameters (*Table 5*). The *Funneliformis mosseae* and *R. intraradices* inoculation effects were significantly higher than in the case of *C. etunicatum*. Soybean inoculated with *F. mosseae* had the highest concentrations of nitrate-N and ureide-N in the soybean, while those inoculated with *R. intraradices* had the highest relative ureide-N, Ndfa, and the amount of N fixed in soybean (*Table 5*).

Table 4. Mean squares for the effect of P fertilizer application (P) and mycorrhizal inoculation (M) and their interactions (P × M) on the concentration of nitrate, ureide, relative ureide-N, the proportion of N derived from atmosphere, and the amount of N fixed in soybean

	Df	Nitrate N (μmol)	Ureide-N (μmol)	Relative ureide-N (%)	Ndfa (%)	Amount of N fixed (kg ha^{-1})
Reps	1	0.002	2.91	5.33	5.12	30.01
P rates (P)	2	0.029[ns]	1.07[ns]	441.3[ns]	789.9[ns]	560.3**
Error a	2	0.006	7.26	70.9	110.6	3.80
AMF (M)	3	0.006***	1.03**	211.1**	432.6**	900.1***
P × M	6	0.013**	1.24[ns]	88.2*	170.5*	253.3**
Error b	9	0.002	8.51	26.0	44.1	30.5
Total	23					

Notes: *, **, ***indicates significant at 0.05, 0.01, and 0.001 probability level respectively; ns indicates not significant.

Table 5. Effects of P fertilizer application (P) and mycorrhizal inoculation (M) on the concentration of nitrate, ureide, relative ureide-N, the proportion of N derived from atmosphere, and the amount of N fixed in soybean

	Nitrate-N (µmol)	Ureide-N (µmol)	Relative ureide-N (%)	Ndfa (%)	Amount of N fixed (kg ha^{-1})
P rates (P)					
0 kg ha^{-1}	0.32	0.028	27.3	27.4	18.7
20 kg ha^{-1}	0.26	0.033	35.3	38.1	32.2
40 kg ha^{-1}	0.20	0.036	42.2	47.3	34.0
Lsd (p < 0.05)	ns	Ns	18.1	ns	4.19
AMF inoculation					
Non-AMF	0.37	0.029	26.9	25.8	12.6
F. mosseae	0.24	0.037	38.9	43.2	35.7
R. intraradices	0.22	0.035	39.9	44.2	40.0
C. etunicatum	0.23	0.029	34.1	37.1	24.8
Lsd (p < 0.05)	0.07	0.004	6.66	8.67	7.21

Note: ns indicates non-significant differences at p value < 0.05.

There were interactive effects of P fertilizer rates and AMF inoculation on the concentration of nitrate-N, relative ureide-N, Ndfa, and nitrogen fixed in the soybean plants (*Table 4*). The concentration of nitrate-N was observed to significantly reduce with AMF inoculation under zero P (0 kg P_2O_5 ha^{-1}) and low P fertilizer (20 kg P_2O_5 ha^{-1}) application, but no significant difference was observed at high P rate (*Figure 3*). The highest concentration of nitrate-N was observed in uninoculated soybean plants under zero P application (0.49 µmol) and 20 kg P_2O_5 ha^{-1} (0.44 µmol) (*Figure 3*).

The relative ureide-N of AMF-inoculated soybean was observed to significantly increase under zero P and low P application rates compared to non-AMF soybean plants; however, no difference was observed at high P rate (*Figure 4*). Soybean plants inoculated with *F. mosseae* had the highest relative ureide-N at zero P rate, while those inoculated with *R. intraradices* had the highest relative ureide-N at 20 kg P_2O_5 ha^{-1} and 40 kg P_2O_5 ha^{-1} application rates (*Figure 4*).
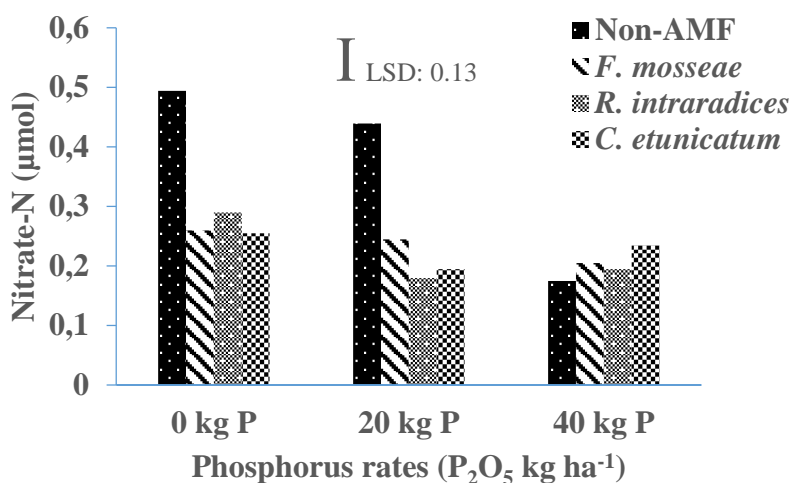
Figure 3. Interactive effect of phosphorus rates and AMF inoculation on nitrate-N of soybean. Bar denotes Lsd value at 5% probability level.
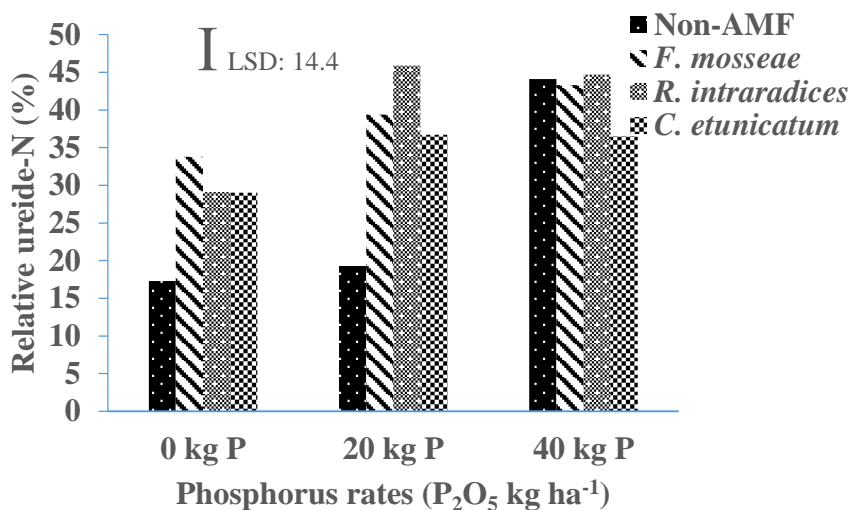

Figure 4. Interactive effect of phosphorus rates and AMF inoculation on relative ureide-N of soybean. Bar denotes Lsd value at 5% probability level.

The Ndfa was recorded to increase with the inoculation of the three AMF isolates under zero P and low P application rates compared to non-AMF soybean plants, while under high P rate no significant difference was observed among the AMF and non-AMF treatments (*Figure 5*). The highest Ndfa was observed in soybean inoculated with *R. intraradices* fertilized with 20 kg P_2O_5 ha^{-1}.
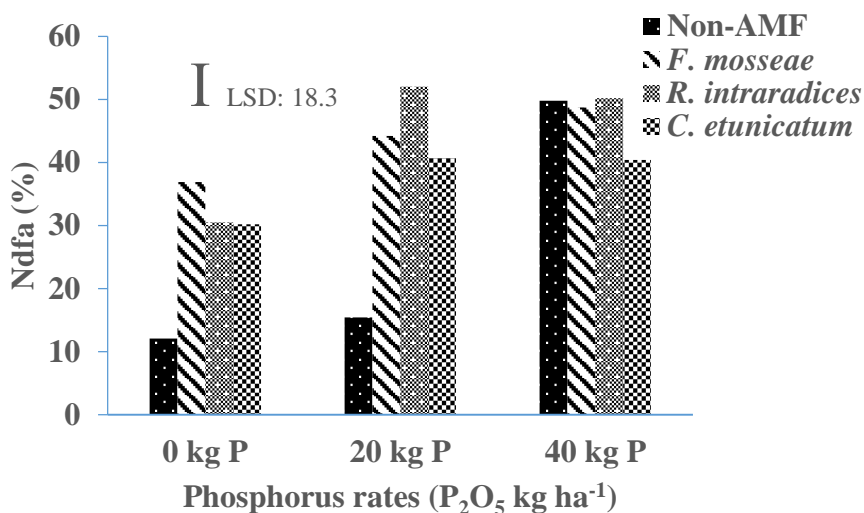
Figure 5. Interactive effect of phosphorus rates and AMF inoculation on N derived from atmosphere (% Ndfa) of soybean. Bar denotes Lsd value at 5% probability level.

Soybean plant inoculated with *R. intraradices* fertilized with 20 kg P_2O_5 ha^{-1} had the highest amount of N fixed (59.4 kg ha^{-1}), while the lowest (2.4 and 3.8 kg ha^{-1}) was recorded in zero P and low P fertilizer application (*Figure 6*).
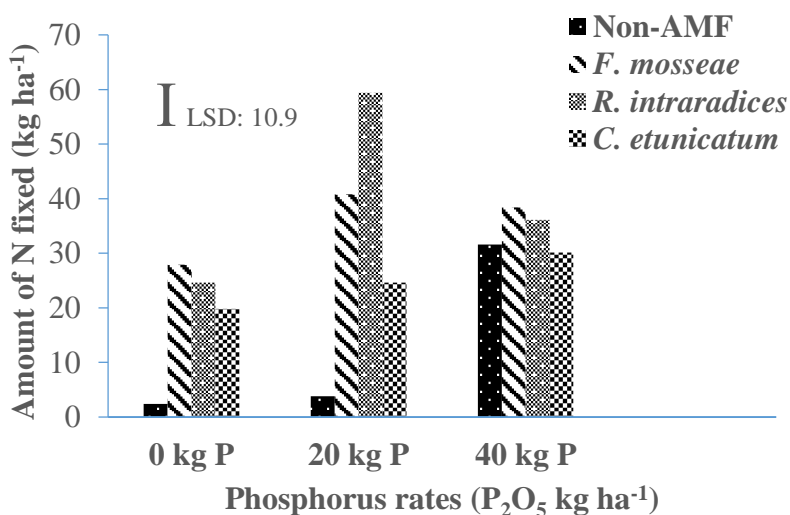

Figure 6. Interactive effect of phosphorus rates and AMF inoculation on the amount of N fixed by soybean. Bar denotes Lsd value at 5% probability level.

AMF root colonization

AMF root colonization was significantly affected by P fertilizer rates, AMF inoculation, and the interactive effect between the treatments (*Table 6*). There was a decrease in AMF root colonization with increase in P rates. The application of 40 kg P_2O_5 ha^{-1} rate significantly reduced the AMF root colonization by 55.1% compared to 20 kg P_2O_5 ha^{-1} (10%) relative to the zero P fertilizer rate; however, no significant difference was observed in the AMF root colonization of the soybean between zero P and 20 kg P_2O_5 ha^{-1} fertilizer rate (*Table 7*). Significant difference was observed among the AMF isolates and non-AMF treatments on the root colonization of the soybean (*Table 6*). The lowest root colonization (15.3%) was observed in non-AMF treatment, while soybean inoculated with *R. intraradices* had the highest root colonization (62.7%), followed by *F. mosseae* (58.7%) and *C. etunicatum* (48%) (*Table 7*).

Table 6. Mean squares for the effect of P fertilizer application (P) and mycorrhizal inoculation (M) and their interactions (P × M) on AMF root colonization, shoot N and P content, and grain and biomass yield of soybean

	Df	AMF root colonization (%)	Shoot N uptake (kg ha^{-1})	Shoot P uptake (kg ha^{-1})	Grain yield (kg ha^{-1})	Biomass yield (kg ha^{-1})
Reps	1	294.0	141.1	206.9	135219	106719
P rates (P)	2	2392.7*	179.8^{ns}	799.8*	2769766*	3421473*
Error a	2	114.0	128.1	17.6	73360	125881
AMF (M)	3	2765.1***	1525.5***	107.7***	998672***	1994264***
P × M	6	165.1***	290.4^{ns}	48.2**	495792***	687066***
Error b	9	14.0	109.0	8.08	14303	63831
Total	23					

Notes: *, **, ***indicates significant at 0.05, 0.01, and 0.001 probability level, respectively; ns indicates not significant.

Regardless of the AMF treatments, the soybean root colonization was reduced at 40 kg P_2O_5 ha^{-1} fertilizer rate (*Figure 7*).
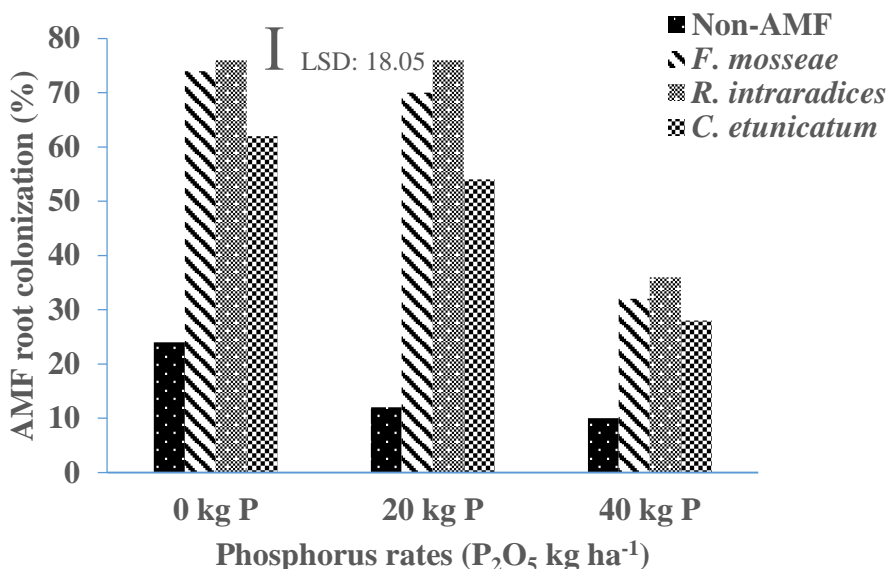
Figure 7. Interactive effect of phosphorus rates and AMF inoculation on AMF root colonization of soybean. Bar denotes Lsd value at 5% probability level.

Shoot N and P content

The soybean shoot N content was significantly influenced by AMF inoculation (*Table 6*). Significant difference was observed among the AMF isolates (*Table 7*). Inoculation with *R. intraradices*, *F. mosseae*, and *C. etunicatum* increased the soybean shoot N content by 59.7, 56.4, and 46.7%, respectively, compared to non-AMF treatment. The shoot P content was significantly influenced by P fertilizer rates, AMF inoculation, and the interactive effect between the treatments (*Table 6*). Shoot P content increased with increase in P rates. Application of 20 and 40 kg P_2O_5 ha^{-1} rates increased shoot P content by 33.2 and 57.6%, respectively, compared to zero P fertilizer rate (*Table 7*). Inoculation with *R. intraradices*, *F. mosseae*, and *C. etunicatum* increased the soybean shoot P content by 32.7, 35.4, and 27.9%, respectively, compared to the non-AMF treatment, but no significant difference was observed among the AMF isolates (*Table 7*). With zero P and 20 kg P_2O_5 ha^{-1} fertilizer rates, the three AMF isolates increased the shoot P content of the soybean compared to the non-AMF treatment, while no difference was observed among the AMF treatments at 40 kg P_2O_5 ha^{-1} fertilizer rate (*Figure 8*).

Table 7. Effects of P fertilizer application (P) and mycorrhizal inoculation (M) on AMF root colonization, shoot N and P content, and grain and biomass yield of soybean

	AMF root colonization (%)	Shoot N uptake (kg ha⁻¹)	Shoot P uptake (kg ha⁻¹)	Grain yield (kg ha⁻¹)	Biomass yield (kg ha⁻¹)
P rates (P)					
0 kg ha^{-1}	59.0	40.8	14.5	851	1899
20 kg ha^{-1}	53.0	48.8	21.7	1698	2779
40 kg ha^{-1}	26.5	49.4	34.2	1982	3177
Lsd (p < 0.05)	22.9	Ns	9.03	582.7	763.3
AMF inoculation					
Non-AMF	15.3	24.2	17.3	991	1800
F. mosseae	58.7	55.5	25.7	1858	2974
R. intraradices	62.7	60.0	26.8	1813	3067
C. etunicatum	48.0	45.4	24.0	1782	2632
Lsd (p < 0.05)	4.89	13.6	3.71	156.2	330.0

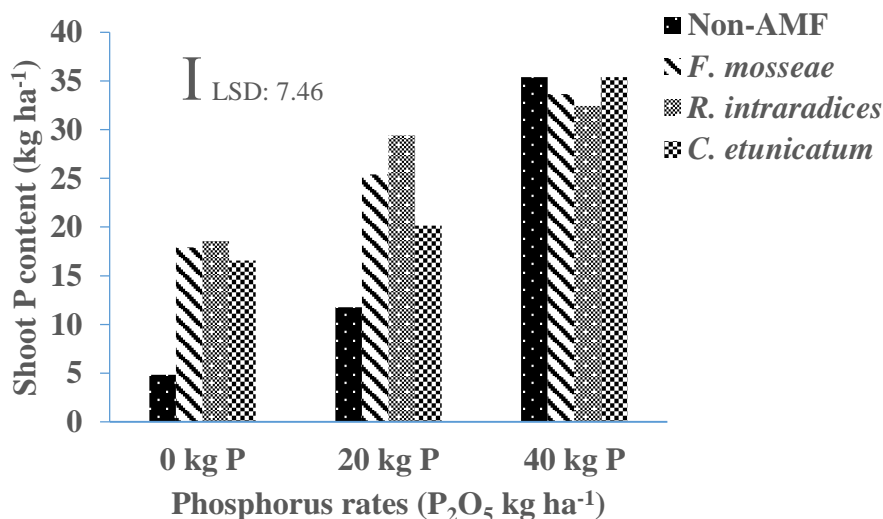Note: ns indicates non-significant differences at p value < 0.05.

Figure 8. Interactive effect of phosphorus rates and AMF inoculation on the shoot phosphorus content of soybean. Bar denotes Lsd value at 5% probability level.

Grain and biomass yields

The results showed that the grain and biomass yield of the soybean were significantly influenced by P rates and AMF inoculation (*Table 6*). The grain and biomass yields increased with P rates (*Table 7*). Application of 40 kg P_2O_5 ha^{-1} rate increased the grain and biomass yields by 57.1 and 40.2%, respectively, followed by 20 kg P_2O_5 ha^{-1} rate (49.9 and 31.7%) compared to zero P fertilizer rate. Inoculation with *R. intraradices*, *F. mosseae*, and *C. etunicatum* increased the soybean grain yield by 46.7, 45.3, and 44.4%, respectively, compared to the non-AMF treatment, but no significant difference was observed among the AMF isolates (*Table 7*). A similar pattern was observed with the biomass yield (*Table 7*). Significant interactions between P fertilizer rates and AMF inoculation were observed for grain and biomass yield (*Table 6*). The highest grain yield was obtained in soybean inoculated with *R. intraradices* under 20 kg P_2O_5 ha^{-1} application rate, followed by *F. mosseae* under the same P treatment, while the lowest grain yield was recorded in the control plot as shown in *Figure 9*. For the biomass yield, soybean inoculated with *R. intraradices* and *F. mosseae* under 20 kg P_2O_5 ha^{-1} rate application gave the highest biomass yields, with the lowest one observed in the control plot (*Figure 10*).
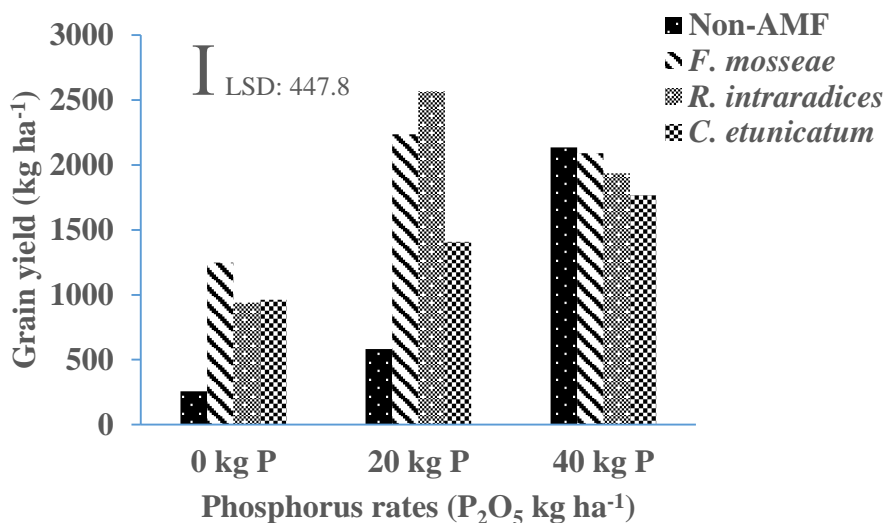

Figure 9. Interactive effect of phosphorus rates and AMF inoculation on the grain yield of soybean. Bar denotes Lsd value at 5% probability level.
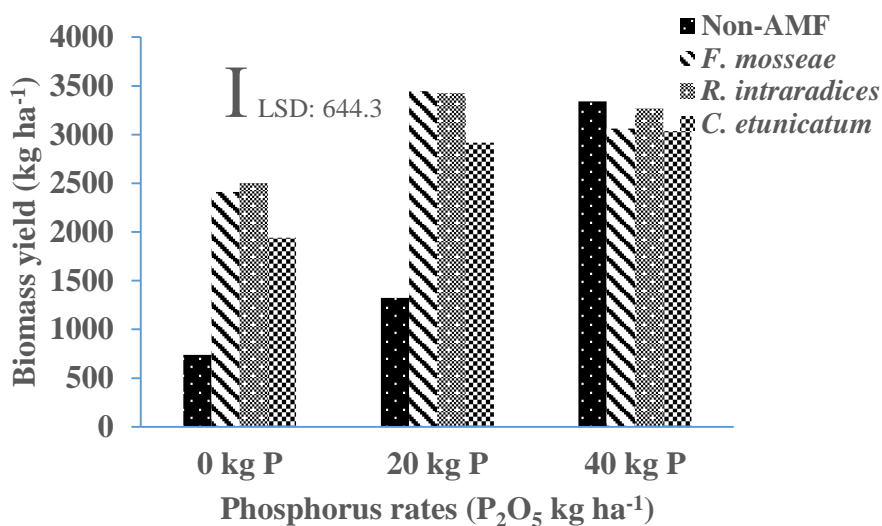
Figure 10. Interactive effect of phosphorus rates and AMF inoculation on the biomass yield of soybean. Bar denotes Lsd value at 5% probability level.

Discussions

The growth and yield performance of soybean improved with P fertilization. The increased shoot dry matter, number of nodules, and seed yield of the soybean are attributed to the increased P supply to the shoot P, which is essential for many metabolic processes such as photosynthesis in the plants. P fertilization in legumes has been reported to increase P content in the nodules and shoot, with increased dry matter accumulation and grain yield [35]. The poor nodulation in terms of number of nodules and the nodule weight of the soybean with low available P could be attributed to the inability of the plant to partition sufficient P for nodule development [36]. This confirmed the reports of [37] that P deficiency reduced the number of nodules and weight in legumes. The relative ureide-N and the amount of N fixed in the soybean plants were improved with P fertilization compared to P-deficient plants in this present study. This is attributed to the increased concentration of ureides and reduced nitrate-N with P fertilization levels. P deficiency has been reported to reduce ureide translocation in the xylem sap, thus reducing nitrogen fixation potential [38]. The increased relative ureides-N with increasing P fertilization resulted in high N_2 fixation observed in this study. Low P availability is reported to reduce nitrate reductase activity in roots, which restricts nitrate transport from roots to shoots in P-deficient soybean plants [39, 40]. Low P availability has been reported to reduce the supply of C from photosynthesis to the nodules [41]. The report of [42] showed that nodule

construction cost and growth respiration of different legume plants increased with P deficiency. Furthermore, this study demonstrated increased shoot N content with increased P fertilization levels. This corroborates the evidence from [11], who reported increased N_2 fixation with P fertilization. Based on the above evidence, the low grain and biomass yield of the soybean could be attributed to the reduced dry matter accumulation and nitrogen fixation under P deficiency conditions.

The present study showed the increased root colonization of the soybean in all the AMF isolates used. This suggests the adaptability and higher competitiveness of AMF isolates with the indigenous mycorrhizal population and the soybean cultivar. The positive effects of AMF inoculation on the dry matter and grain yield of the soybean observed in this study corroborate several other reports under field conditions [43, 20]. Several reports showed that AMF promoted plant growth through increased P uptake [44, 45], which also confirmed the result of this study with high shoot P uptake in AMF-inoculated plants compared to the non-AMF control plants. The main benefit of AMF symbiosis with plants is the enhancement of P by the extra-radicular hyphae [14]. The increased N_2 fixation in the soybean plants with AMF inoculation in this study could be attributed to the improved P uptake in mycorrhizal inoculated plants, thus enhancing the transport of ureide-N in the xylem.

The AMF isolates used for this study (*F. mosseae*, *R. intraradices*, and *C. etunicatum*) are generalist AMF widely distributed in most P-deficient soils and can colonize a wide variety of host plants, including legumes [46]. The root colonization of soybean plants by AMF isolates was reduced at high P fertilization levels. High soil P availability has been reported to reduce AMF root colonization and hyphae development [14, 47]. In contrast, the application of P has been reported to promote mycorrhizal development in P-deficient soils [48]. This confirmed the result observed upon the application of 20 kg P_2O_5 ha^{-1} in this study. Among the three AMF tested, *R. intraradices* and *F. mosseae* had the best effect on plant performance in terms of phosphorus uptake, dry matter accumulation, nodulation, nitrogen fixation, and the yield performance of the soybean. The increased growth performance and nitrogen fixation in soybean inoculated with *R. intraradices* and *F. mosseae* could be attributed to their high ability in shoot P uptake [49, 50, 51].

Conclusions

The findings demonstrated the potential of field inoculation with compatible and effective arbuscular mycorrhizal isolates in increasing phosphorus, nodulation, nitrogen fixation, and the grain yield of soybean. Among the three AMF isolates, *Rhizophagus intraradices* and *Funneliformis mosseae* gave higher

nodulation and nitrogen fixation of the soybean. Therefore, to ensure higher soybean productivity and sustainable agriculture with reduced high input of chemical P fertilizer through nitrogen fixation, the inoculation of soybean with *Rhizophagus intraradices* or *Funneliformis mosseae* (combined with 20 kg P_2O_5 ha^{-1}) could be recommended, thus ameliorating the negative effect of P deficiency on soybean productivity in the derived savannah of Nigeria. This study was limited to a single soybean variety and a year's field study in one location. It is recommended that further field studies should be undertaken in multi-location sites and different seasons, which will be a significant step towards the stable use of mycorrhizal fungi inoculation for promoting legume productivity in most agricultural soils of Nigeria.

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