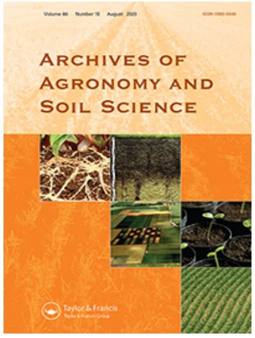

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Combined application of inoculant, phosphorus and organic manure improves grain yield of cowpea

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ABSTRACT

Low concentrations of P and organic manure in savanna soils limit cowpea response to rhizobia. The study was conducted to determine the combined effect of P and organic manure on cowpea response to rhizobia in a factorial experiment arranged in randomized complete block design with three replications on smallholder farmers' fields in northern Ghana in 2015. The factors were two levels of *Bradyrhizobium* inoculant, two levels of P fertilizer, three treatments of manure (fertisoil, cattle manure, and no manure). Addition of *Bradyrhizobium* inoculant to P and fertisoil significantly increased shoot biomass yield from 1677 kg ha^{-1} in the plots without *Bradyrhizobium* inoculation to 1913 kg ha^{-1}. Likewise, the addition of *Bradyrhizobium* inoculant to P and cattle manure significantly increased shoot biomass from 1437 kg ha^{-1} to 1813 kg ha^{-1}. Grain yield increases of 1427 and 1278 kg ha^{-1} were obtained over the control when either fertisoil or cattle manure and P, respectively, were added to *Bradyrhizobium* inoculant. The value cost ratio for adding *Bradyrhizobium* inoculant to phosphorus and fertisoil was two indicating that it could be attractive to risk-averse smallholder farmers. The study demonstrated the potential of the combined application of organic matter and P to improve cowpea response to *Bradyrhizobium* inoculation.

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Bradyrhizobium; fertisoil; nutrient release; agronomic P use efficiency

Introduction

Low inherent soil fertility limits the production of grain legumes especially cowpea on smallholder farms in sub-Saharan Africa (SSA). In Ghana, there is spatial variability in soil nitrogen (N) and phosphorus (P) contents within smallholder farms ranging from low to very low amounts (Buri et al. 2010; Masso et al. 2016), indicating the deficiency of N and P on most farms. Consequently, the observable yield on farmers' field is less than 1 t ha^{-1} (Mensah 2014). This has led to a yield gap of about 1.5 t ha^{-1} in smallholder cowpea cropping systems in northern Ghana. Therefore, a substantial increase in food production in northern Ghana essentially depends on the use of external inputs such as N and P (Chivenge et al. 2011). Nevertheless, applying adequate amounts of mineral fertilizers to meet the nutrient requirement of grain legumes is beyond the financial

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Supplemental data for the article can be accessed here.

capacity of smallholder farmers (Jansa et al. 2011). Therefore, to minimize cost while increasing production, good agronomic and soil fertility management practices should be adopted.

Bradyrhizobium inoculant is an alternative cheaper source of N for grain legumes due to nitrogen fixation from the atmosphere through legume-rhizobium symbiosis. However, due to the promiscuous nature of cowpea, it has been largely assumed not to respond to inoculation. Awonaike et al. (1990) and Mathu et al. (2012) confirmed this assumption in their work when they obtained no significant increase in growth after inoculating cowpea with rhizobia. However, recent field reports suggest that cowpea responds to rhizobia inoculation when strains with superior symbiotic effectiveness are used (Ulzen et al. 2016; Boddey et al. 2017; Kyei-Boahen et al. 2017). The grain yields of cowpea reported by Ulzen et al. (2016) as a result of rhizobia inoculation was 46% of the potential yield (2.5 t ha^{-1}), which suggests that yields of cowpea could further be improved if other limiting factors such as soil organic matter and phosphorus availability are addressed.

Phosphorus plays a major role in rhizobium symbiosis through the supply of energy in the form of ATP (O'Hara 2001). However, its deficiency in soils is widespread (Nziguheba et al. 2016) across the legume growing areas in northern Ghana (Masso et al. 2016). Recent dissemination trials have shown significant increases in soybean yields in most areas where P was added to rhizobia inoculant (Masso et al. 2016) emphasizing the importance of P in rhizobia – legume symbiosis and establishing its deficiency in legume growing areas in northern Ghana.

Kyei-Boahen et al. (2017) reported a 56% yield increase in cowpea when inoculant was applied together with P as compared with 25% yield increase in cowpea when inoculant alone was applied. These results suggest that P improves legume response to inoculation in P deficient soils.

Most soils in Ghana contain as low as 1.0% organic matter or even less (NSFMAP 1998). A recent study revealed that the organic carbon content in some locations in northern Ghana were very low with an average of 0.04% (Ulzen et al. 2016). However, organic manure enhances the survival of rhizobium through the provision of organic carbon and improves crop response to inoculation (Zengeni et al. 2006). Organic manure can also improve soil characteristics, such as water holding capacity, leading to better plant growth and efficiency of rhizobia inoculant (Vanlauwe and Sanginga 2004). In addition, organic manure can cause the release of adsorbed P on the surface of soil particles, thus enhancing the efficiency of applied P (Nziguheba et al. 2016). With increasing human population resulting in loss of agricultural lands due to infrastructural development and climate change effect, soil fertility management options that improve food production with minimal effects on the environment are important. The study is significant towards global attempt to bridge the yield gap observed by cowpea smallholder farmers and increase their livelihood. The objective of the study is to evaluate the combined effects of P and organic manure on cowpea response to *Bradyrhizobium* inoculation.

Materials and methods

Site description and soil characterization

The experiments were located at Kpalga (latitude 09°26.447′ N and longitude 000°57.575′ W with an elevation of 167 m above mean sea level) and Tunayilli (latitude 09°20.398′ N and longitude 000°59.154′ W with an elevation of 177 m above mean sea level) in the Northern region of Ghana and at Busa (09°59.186′ N and longitude 002°20.370′ W with an elevation of 345 m above mean sea level) in the Upper West region of Ghana. The soil types at the study locations were Acrisols (Kpalga and Tunayilli) and Lixisols (Busa). The experiment was conducted between July and October 2015. The cumulative rainfall for the study period was 417 mm (Kpalga), 411 mm (Tunayilli) and 542.5 mm (Busa). Five soil core samples were taken from each plot, thoroughly mixed and composite samples taken into Ziploc bags and kept in the refrigerator at 4°C prior to laboratory analyses. The soil analyses were done following standard procedures as described by Anderson and Ingram (1994). Indigenous rhizobia population in the different locations was enumerated using the most probable number count (MPN) method (Vincent 1970).

Field preparation and experimental design

The field at Kpalga was planted on July 29, those of Tunayilli on July 7, and Busa on 3 August 2015. The fields were ploughed and harrowed to a depth of 15 cm and divided into treatment plots before planting. Each plot measured 6 × 3 m with an alley of 1 m between plots and 2 m between blocks. The Cowpea cultivar, Songotra, was used as the test crop. Songotra is a high yielding cultivar and resistant to striga and aphids. Its maturity period is 77 days.

Five grams of the *Bradyrhizobium* inoculant was added to 1 kg of cowpea seeds using the two-step method, which involved adding a sticker to the seeds before applying the inoculant. Gum Arabic was used as the sticker at a ratio of 1.5 g to 15 ml clean lukewarm water. Inoculated cowpea seeds were air-dried for 30 min and manually sown at a spacing of 75 cm × 20 cm.

The study was a 2 × 2 × 3 factorial experiment arranged in randomized complete block design with three replications. The treatments were two levels of *Bradyrhizobium* inoculant containing *B. yuamingense* strain BR 3267 [inoculated (Rh+) and uninoculated (Rh-)], two levels of phosphorus [30 kg P ha^{-1} (P+) and 0 kg P ha^{-1} (P-)], three treatments of manure [fertisoil, cattle manure (CTM) and no manure]. The treatment combination of RH-, P- and No manure hereinafter refers to control. Phosphorus was applied in the form of triple superphosphate through band placement 2 weeks after germination. Fertisoil and cattle manure were applied at a rate of 5 tons ha^{-1}. Fertisoil is a commercially produced compost (DeCo, Ghana). Fertisoil is produced from rice husks, poultry manure, shea butter waste, charcoal particles and urban waste (fruits and kitchen leftovers). Decomposed cattle manure were sampled from kraal of a particular farmer at Kpalga. This was done to prevent the possible variability in the quality and nutrient contents of cattle manure from the different farmers whose field the study was conducted. The fertisoil and cattle manure were broadcasted and incorporated into the respective treatment plots 1 week before planting.

Harvesting and data collection

Nodulation and shoot biomass yield were assessed at the R3 stage (50% flowering) (Fehr et al. 1971). The plants were cut at about 5 cm above the soil level and the roots of the plants were carefully dug out, collected into polythene bags, together with detached nodules, and transported to the laboratory. The roots were put in a 1-mm mesh sieve and washed under running tap water to remove adhered soil. The nodules were gently removed, washed and counted. Shoot and nodules were ovendried at 60°C for 72 h. Plants were harvested from the inner rows (4.5 m × 2.8 m) excluding the border rows and ovendried at 60°C until constant weight (approximately 72 h).

Decomposition and nutrient release patterns of fertisoil and cattle manure

The decomposition and nutrient release pattern of fertisoil and cattle manure were determined using the litterbag approach (Anderson and Ingram 1994; Tetteh 2004). Equations of Gnankambary et al. (2008) were used to compute the percentage dry weight, nutrient release and decomposition rate of cattle manure and fertisoil at each sampling time.

Agronomic P use efficiency and added benefit

Agronomic use efficiency following the formula of (Vanlauwe et al. 2011) was calculated as

$$P - AE = \frac{(Y_F - Y_C)}{F_{appl}} \quad (1)$$

where YF and YC refer to grain yields (kg ha^{-1}) in the treatment where fertilizer P has been applied and in the control plot, respectively, Fappl is the amount of fertilizer P applied and P-AE is the phosphorus agronomic efficiency.

Added benefits (AB) were calculated using the formula of Vanlauwe et al. (2002) as follows (Equation 2)

$$AB = Y_{comb} - ((Y_{ino} - Y_{con}) + (Y_{om} - Y_{con}) + (Y_{phos} - Y_{con}) + Y_{con}) \quad (2)$$

where,

AB denotes 'added benefit'; Y_{comb}, Y_{con}, Y_{ino}, Y_{om}, and Y_{phos} are the grain yields obtained in the combined application of all the inputs, control treatment, rhizobia inoculant alone, organic manure alone, and phosphorus alone, respectively.

Economic analysis

The profitability of investing in the treatments; rhizobia inoculant, fertisoil, cattle manure and P were determined using the value cost ratio (VCR) equation (Roy et al. 2006).

$$VCR = \frac{\text{Value of extra crop produced due to treatment } (\$\ ha^{-1})}{\text{Cost of treatment}(\$\ ha^{-1})} \quad (3)$$

Rhizobia inoculant, fertisoil, cattle manure and phosphorus were procured at the cost of 6 US$ ha^{-1}, 4 US$ ha^{-1}, 4 US$ ha^{-1}, and 26 US$ ha^{-1}, respectively. The cost of sampling for 50 kg cattle manure was estimated as the cost price as it was not on commercial sales. Cowpea was procured at 0.6 US$ per kilogram from the open market. The dollar to the cedi exchange rate as at the time of this study was USD $ 1 to GH¢3.60. A VCR value ≥2 was considered profitable (Roy et al. 2006).

Statistical analysis

The data obtained from each experimental site were pooled together because preliminary analysis showed that treatment x location interaction was not significant. The data were transferred to SISVAR software version 5.6 for analysis of variance (ANOVA) (Ferreira 2008). Treatment means were separated with Scott Knott at 5% probability. Stepwise regression was performed with Minitab version 17 to determine the growth parameters contributing significantly to grain yield. The p-value threshold for a parameter to enter or leave the stepwise regression model was set at 0.05. Two-sample t-test was used to compare the nutrient released by fertisoil and cattle manure at week 10.

Results

Physical and chemical characteristics of soil and manure used and most probable number count of rhizobia at the study locations

The physical and chemical characteristics of the study locations are presented in Table 1. The concentrations of major nutrients (N, C, and P) were very low according to the ratings of Landon (2014). The locations had moderate levels of exchangeable K. The pH of the soil at three locations was classified as medium. The soil contains a high proportion of sand (64–70%) at the three locations and the texture classified as a sandy loam (Table 1).

The properties of the added fertisoil and cattle manure are illustrated in Table 2. Fertisoil had nitrogen content >2.5 percent with that of cattle manure being <2.5 percent. The carbon content of cattle manure was relatively higher than that of fertisoil. The two manure types had C: N ratios less than 30 (Table 2). The total calcium and magnesium of the fertisoil were relatively higher than that of cattle manure. The lignin and polyphenol contents were less than 15% and 4%, respectively.

Table 1. Physical and chemical properties of the soils at Kpalga, Tunayilli and Busa in northern Ghana.

Soil parameters	Kpalga	Tunayilli	Busa
pH(1:2.5) (H_2O)	6.4 ± 0.5*	6.6 ± 0.02	6.4 ± 0.5
Total N (%)	0.043 ± 0.02	0.052 ± 0.022	0.033 ± 0.003
Available P (mg kg^{-1})	1.69 ± 0.23	1.53 ± 0.22	1.20 ± 0.18
Exchangeable K ($cmol_{(+)}$ kg^{-1})	1.21 ± 0.09	1.06 ± 0.1	1.11 ± 0.05
Organic C (%)	0.42 ± 0.02	0.74 ± 0.05	0.49 ± 0.01
Exchangeable Ca ($cmol_{(+)}$ kg^{-1})	3.15 ± 0.11	4.41 ± 0.65	2.93 ± 0.09
Exchangeable Mg ($cmol_{(+)}$ kg^{-1})	0.38 ± 0.02	0.60 ± 0.52	0.62 ± 0.08
Sand (%)	64.42 ± 1.50	70.08 ± 7.04	68.52 ± 1.60
Silt (%)	27.74 ± 1.54	24.08 ± 0.96	24.64 ± 1.64
Clay (%)	7.84 ± 1.54	5.84 ± 6.08	6.84 ± 0.04
Texture	Sandy loam	Sandy loam	Sandy loam

* Represents standard error of the means.

Table 2. Selected chemical characteristics of fertisoil and cattle manure.

Parameter	Fertisoil	Cattle manure
Carbon (%)	11.7	21.15
Total Nitrogen (%)	4.5	2.43
Total Phosphorus (%)	0.37	0.24
Total Potassium (%)	0.41	0.3
Lignin (%)	10.5	5.5
Polyphenol (%)	0.08	0.02
Total calcium (%)	1.3	0.65
Total magnesium (%)	0.92	0.41
Dry matter content (%)	50.10	54.02
C:N Ratio	2.6	8.7

The indigenous rhizobia population of the study locations were below 50 rhizobia cells g^{-1} soil. The native rhizobia population at Tunayilli (40.4 rhizobia cells g^{-1} soil; Confidence Interval (CI) = 14.0–116.5) and Busa (43.6 rhizobia cells g^{-1} soil; CI = 15.1 – 125.6) was relatively higher than that of Kpalga (32.8 rhizobia cells g^{-1} soil; CI = 11.4–94.6) but there was no significant difference between counts from the locations.

Bradyrhizobium inoculation, P and organic manure effect on nodule number and nodule drymatter of cowpea cultivar Songotra

Unlike the other parameters, only the combined application of inoculant and compost were significantly different (p = 0.001) for nodule number under the two-way interaction (Figure 1(b)). Combined application of *Bradyrhizobium* inoculant and fertisoil resulted in a significantly higher nodule number than the combined application of *Bradyrhizobium* inoculant and cattle manure, and control treatments (Figure 1(b)). The three-way interaction involving *Bradyrhizobium*, phosphorus and organic manure was significant (p = 0.00001) for nodule number (Figure 1(d)). Combined application of *Bradyrhizobium* inoculant, P and fertisoil increased nodule number by 32% over that of combined application of *Bradyrhizobium* inoculant, phosphorus and cattle manure, and 165% over the control (Figure 1(d)). The addition of *Bradyrhizobium* inoculant, P and cattle manure increased nodule number by 100% over that of the control (Figure 1(d)).

Except the interaction between inoculation and phosphorus (p = 0.015) (Figure 2(a)), no other interaction was significant (p > 0.05) for nodule dry weight (Figure 2(b-d)). Combined application of *Bradyrhizobium* inoculant and P increased nodule dry weight from 672 mg (10 $plant)^{-1}$ to 1077 mg (10 $plant)^{-1}$ (Figure 2(a)), which corresponds to about 126% of that of the control treatment.

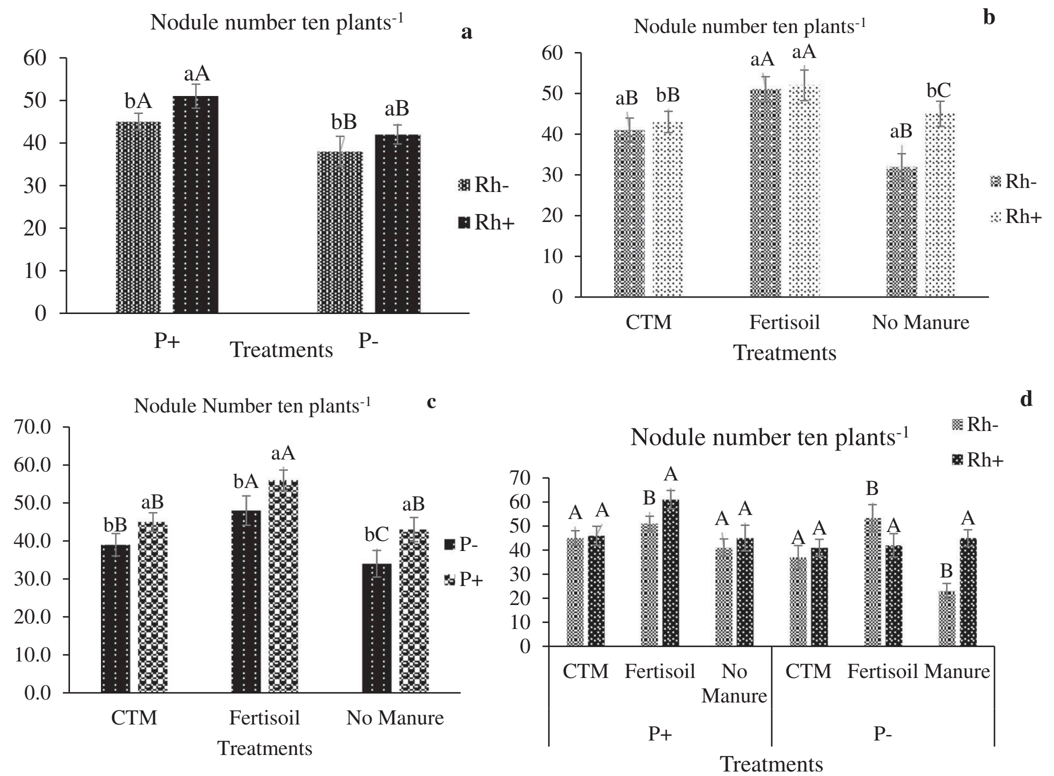

Figure 1. *Bradyrhizobium* inoculation, P and organic manure effect on nodule number of cowpea cultivar Songotra. Bars denote standard error of means. Means followed by distinct letters comparing Rh+ and Rh- within different levels of P (P+ and P-) (a), organic amendments (CTM, Fertisoil, No Manure) (b) and within each P and different types of organic amendment (d) and distinct letters comparing P (P+ and P-) (c) within different organic amendments are different based on Scott Knot test at 5% probability. Values are means of three locations.

Bradyrhizobium inoculation, P and organic manure effect on shoot biomass of cowpea cultivar Songotra

The results showed significant ($p < 0.05$) differences in shoot biomass for the two and three treatment combinations (Figure 3(a-d)). The addition of P to *Bradyrhizobium* inoculation increased shoot biomass yield from 1482 to 1792 kg ha^{-1} (Figure 3(a)). The addition of fertisoil to *Bradyrhizobium* inoculation increased the dry matter yield from 1509 to 1753 kg ha^{-1} while the addition of cattle manure increased biomass yield from 1283 to 1432 kg ha^{-1} (Figure 3(b)). The combined application of *Bradyrhizobium* inoculant and fertisoil significantly increased shoot biomass yield over that of combined application of *Bradyrhizobium* inoculant and cattle manure by 320 kg ha^{-1} (Figure 3(b)). Similarly, the addition of fertisoil or cattle manure to P treatment increased shoot biomass yield from 1625 to 1795 kg ha^{-1}, and 1090 kg ha^{-1} to 1467 kg ha^{-1} respectively (Figure 3(c)). The shoot biomass yield of combined application of P and fertisoil was significantly higher than that of the combined application of cattle manure and P (Figure 3(c)). Shoot biomass increased from 856 kg ha^{-1} in the control treatment to 1057 kg ha^{-1} in the combined treatment of *Bradyrhizobium* inoculant, P and fertisoil (Figure 3(d)). Similarly, the combined application of *Bradyrhizobium* inoculant P and cattle manure increased shoot biomass by 957 kg ha^{-1} (Figure 3(d)).

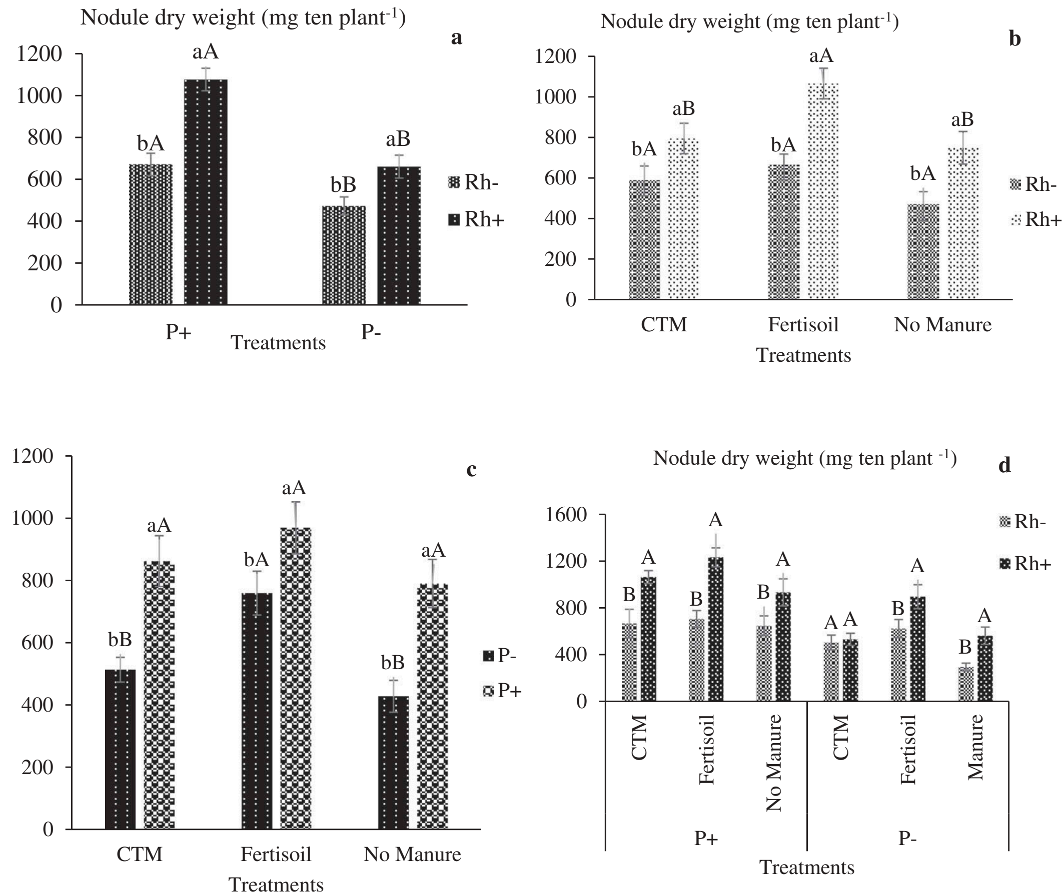

Figure 2. *Bradyrhizobium* inoculation, P and organic manure effect on nodule dry weight of cowpea cultivar Songotra. Bars denote standard error of means. Means followed by distinct letters comparing Rh+ and Rh- within different levels of P (P+ and P-) (a), organic amendments (CTM, Fertisoil, No Manure) (b) and within each P and different types of organic amendment (d) and distinct letters comparing P (P+ and P-) (c) within different organic amendments are different based on Scott Knot test at 5% probability. Values are means of three locations.

Bradyrhizobium inoculation, P and organic manure effect on grain yield of cowpea cultivar Songotra

The results showed significant ($p < 0.05$) differences in grain yield for the two- and three-way interactions (Figure 4(a-d)). A grain yield of 1055 kg ha^{-1} was obtained when P was applied without *Bradyrhizobium* inoculation but the yield significantly increased to 1602 kg ha^{-1} with *Bradyrhizobium* inoculation (Figure 4(a)). Similarly, the application of fertisoil and cattle manure without *Bradyrhizobium* inoculation, resulted in grain yields of 995 and 977 kg ha^{-1}, respectively. The addition of *Bradyrhizobium* inoculant to fertisoil and cattle manure resulted in additional grain yield of 495 and 295 kg ha^{-1}, respectively (Figure 4(b)). The grain yield for the combined application fertisoil and *Bradyrhizobium* inoculant treatment was significantly higher than the grain yield of combined application of cattle manure and *Bradyrhizobium* inoculant (Figure 4(b)). Combined application of phosphorus, fertisoil or and cattle manure significantly increased grain yield from 943 and 842 kg ha^{-1} to 1543 and 1480 kg ha^{-1}, respectively (Figure 4(c)). Applying P to fertisoil significantly increased grain yield by 135 kg ha^{-1} over that of combined application of P and cattle manure (Figure 4(c)).

The combined application of *Bradyrhizobium* inoculant, P and fertisoil resulted in a significant increase of grain yield of 1427 kg ha^{-1} over that of the control treatment (Figure 4(d)). Similarly, the

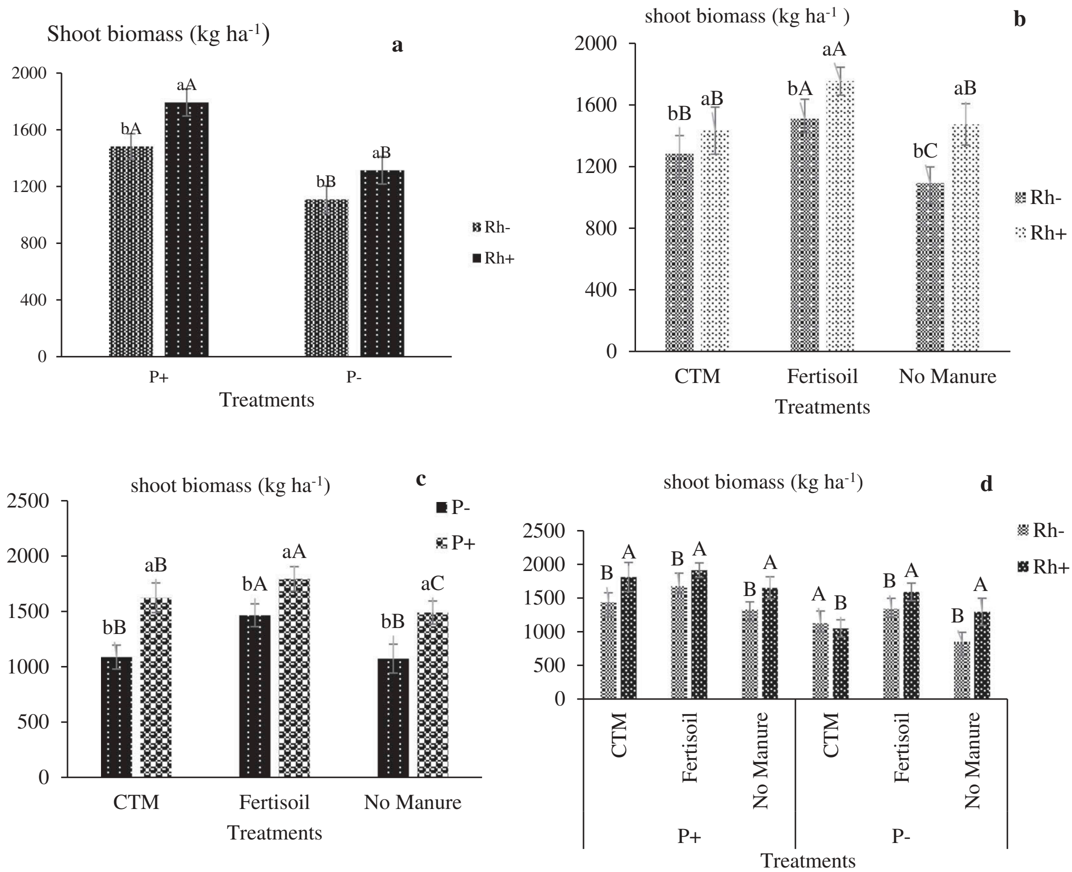

Figure 3. *Bradyrhizobium* inoculation, P and organic manure effect on shoot biomass of cowpea cultivar Songotra. Bars denote standard error of means. Means followed by distinct letters comparing Rh+ and Rh- within different levels of P (P+ and P-) (a), organic amendments (CTM, Fertisoil, No Manure) (b) and within each P and different types of organic amendment (d) and distinct letters comparing P (P+ and P-) (c) within different organic amendments are different based on Scott Knot test at 5% probability. Values are means of three locations.

combined application *Bradyrhizobium* inoculant, P and cattle manure elicited an increase in grain yield of 1278 kg ha^{-1} over that of the control treatment (Figure 4(d)). Combined treatment of *Bradyrhizobium* inoculant, P and fertisoil resulted in an increased grain yield of 148 kg ha^{-1} over that of the combined treatment of *Bradyrhizobium* inoculant, P and cattle manure (Figure 4(d)). An increase in grain yield of 634 and 485 kg ha^{-1} resulted from the application of P, fertisoil and or cattle manure with *Bradyrhizobium* inoculant over the amendment without *Bradyrhizobium* inoculant (Figure 4(d)).

The application of *Bradyrhizobium* inoculant, phosphorus and organic manure produced a positive added benefit in grain yield of cowpea. The combined application of *Bradyrhizobium* inoculant, phosphorus and fertisoil resulted in the added benefit of 85 kg ha^{-1} grains of cowpea whiles the combined application of *Bradyrhizobium* inoculant, phosphorus and cattle manure had added grain yield benefit of 56 kg ha^{-1}.

Bradyrhizobium inoculation, P and organic manure effect on agronomic P use efficiency of cowpea cultivar Songotra

Significant (p = 0.005) interactions between *Bradyrhizobium* inoculant and organic manure (Figure 5(b)), and phosphorus and organic manure (Figure 5(c)) were observed. Addition of fertisoil or cattle manure to

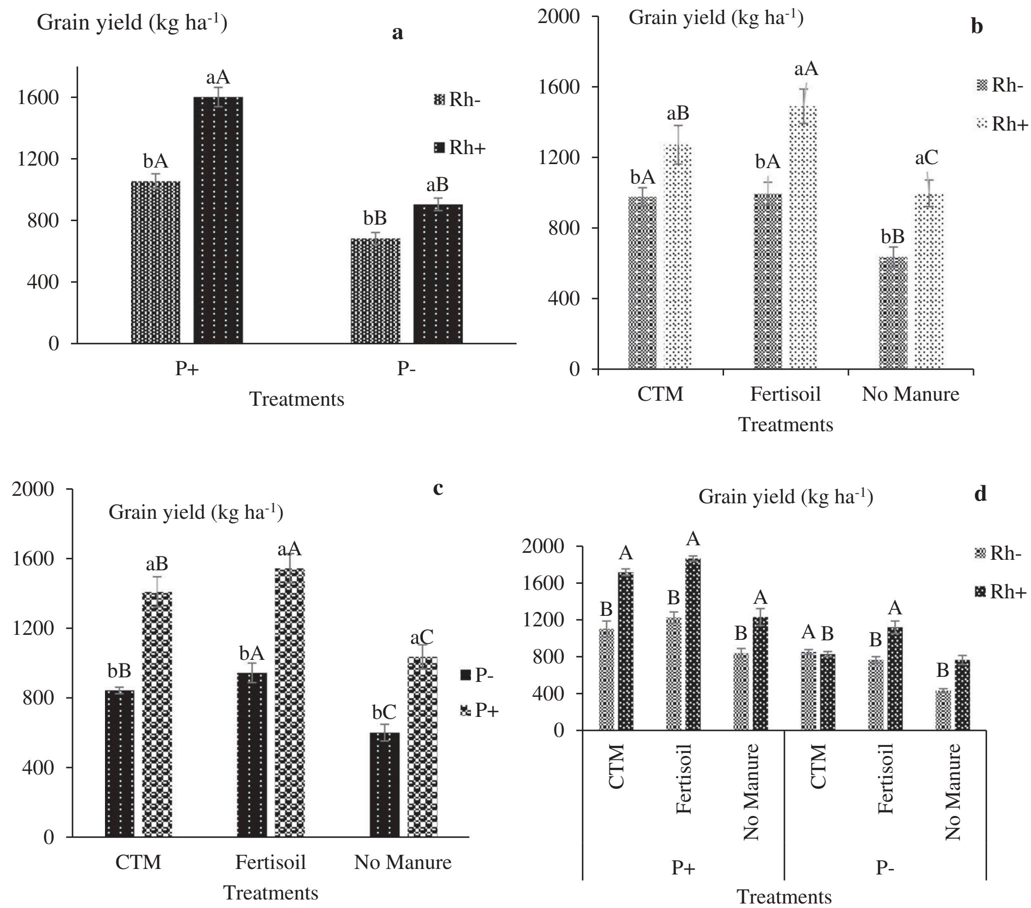

Figure 4. *Bradyrhizobium* inoculation, P and organic manure effect on grain yield of cowpea cultivar Songotra. Bars denote standard error of means. Means followed by distinct letters comparing Rh+ and Rh- within different levels of P (P+ and P-) (a), organic amendments (CTM, Fertisoil, No Manure) (b) and within each P and different types of organic amendment (d) and distinct letters comparing P (P+ and P-) (c) within different organic amendments are different based on Scott Knot test at 5% probability. Values are means of three locations.

Bradyrhizobium inoculated plots increased P use efficiency from 21 to 41 kg grain kg^{-1} P and from 32 to 33 kg grain kg^{-1} P, respectively (Figure 5(b)). Application of *Bradyrhizobium* inoculant and fertisoil resulted in an increase in P use efficiency of 24% over the application of *Bradyrhizobium* inoculant and cattle manure (Figure 5(b)). In contrast, a reduction in P use efficiency from 37 to 25 kg grain kg^{-1} P and from 38 to 26 kg grain kg^{-1} P was obtained when P was applied together with fertisoil or cattle manure, respectively (Figure 5(c)). The results showed a significant ($p = 0.0027$) three-way interaction between *Bradyrhizobium* inoculant, phosphorus and organic manure (Figure 5(d)). The combined effect of *Bradyrhizobium* inoculant, P and fertisoil resulted in P use efficiency of 33 kg grain kg^{-1} P over that of the control whereas that of the combined application of *Bradyrhizobium* inoculant, P and cattle manure resulted in an increase of 32 kg grain kg^{-1} P over that of the control (Figure 5(d)).

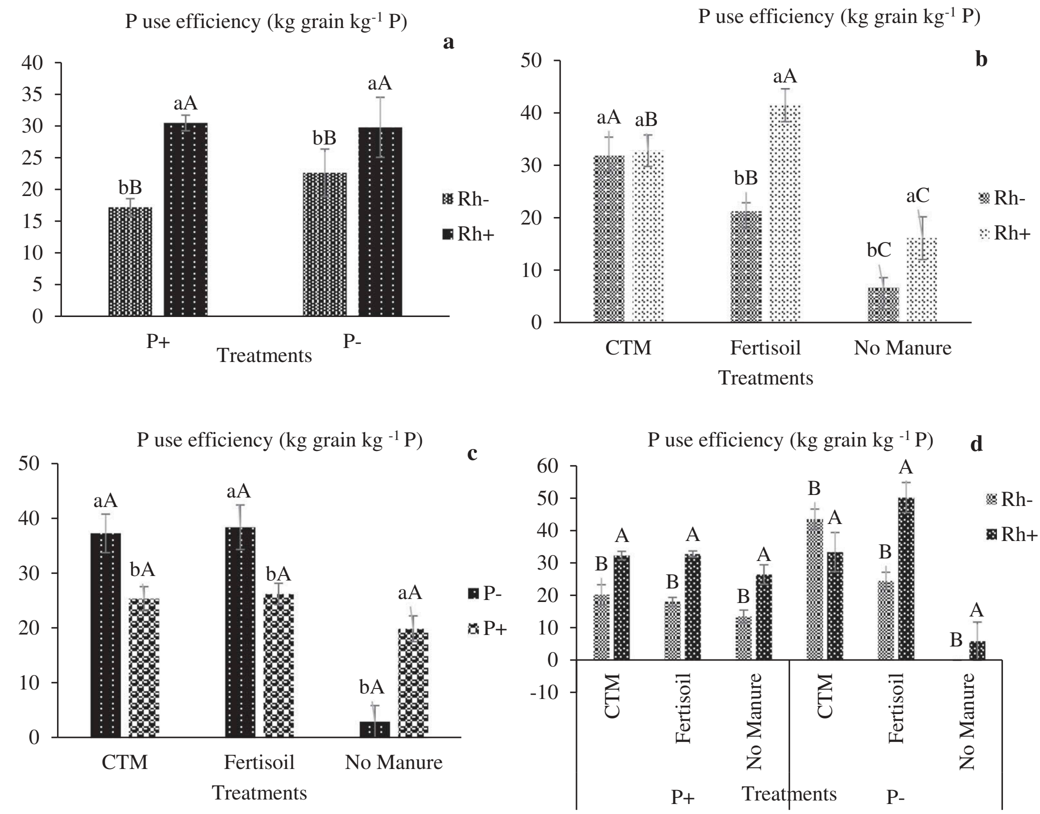

Figure 5. *Bradyrhizobium* inoculation, P and organic manure effect on agronomic P use efficiency of cowpea cultivar Songotra. Bars denote standard error of means. Means followed by distinct letters comparing Rh+ and Rh- within different levels of P (P+ and P-) (a), organic amendments (CTM, Fertisoil, No Manure) (b) and within each P and different types of organic amendment (d) and distinct letters comparing P (P+ and P-) (c) within different organic amendments are different based on Scott Knot test at 5% probability. Values are means of three locations.

Economic analysis of the combined application of Bradyrhizobium inoculant, P and organic manure

The value cost ratios for the combined application of *Bradyrhizobium* inoculant, P and fertisoil was 2 while combined application *Bradyrhizobium* inoculant, P and cattle manure resulted in a VCR of 1.7. Except for the combined application of *Bradyrhizobium* and phosphorus, which was highly profitable (VCR = 4.4), the addition of *Bradyrhizobium* inoculant to fertisoil or cattle manure only resulted in break-even situation (VCR = 1). Addition of phosphorus to either fertisoil or cattle manure also resulted in VCRs of 1.5 and 1.3, respectively.

Decomposition and nutrient release of fertisoil and cattle manure in litterbags

At the end of the tenth week, 43.5% and 44.2% of the dry matter of fertisoil and cattle manure, respectively, had disappeared representing decomposition rates of 0.094 and 0.074 for fertisoil and cattle manure, respectively. The mean mass of dry matter of fertisoil remaining was 56.50 ± 9.5 and that of cattle manure was 55.83 ± 9.12.

The rate of nutrient released differed for the different nutrients and organic inputs (fertisoil and cattle manure) (Figure 6). For fertisoil, the peak mineralization of C was attained at the sixth week. The N release increased from week 2 and peaked at week 8 while phosphorus and K attained peak

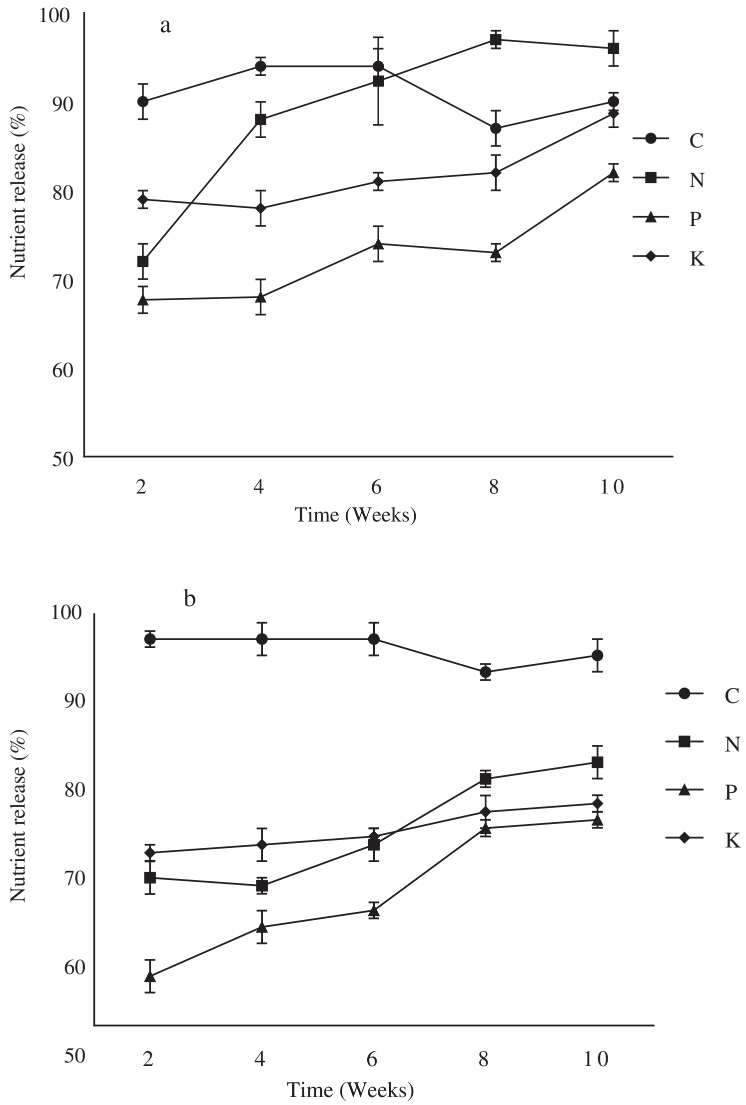

Figure 6. Carbon, nitrogen, phosphorus and potassium release patterns of fertisoil (a) and cattle manure (b) under field conditions. Bars denote standard error of mean.

mineralization on the tenth week (Figure 6(a)). Similar to the trend observed under fertisoil, peak mineralization of C from cattle manure was attained at the end of week 6. The N, P and K released from cattle manure attained peak mineralization on the tenth week (Figure 6(b)).

Except P, there were significant differences between the other nutrients released by fertisoil and cattle manure (Table S1). Cattle manure released significantly higher amounts of carbon than fertisoil. However, fertisoil released higher amounts of nitrogen and potassium than cattle manure.

Stepwise regression analysis of nodulation, shoot biomass and P use efficiency in relation to grain yield

Stepwise regression results showed that nodulation, shoot biomass and P use efficiency contributed significantly toward grain yield. Together, the growth parameters explained 67% of the variation in grain yield in this study. The general regression equation is given as grain yield (kg ha^{-1}) = – 40 + 0.27 dry matter yield + 6.94 P use eff. + 5.19 nodule number + 0.39 nodule dry weight. The stepwise regression showed that increase in dry matter yield, P use efficiency, nodule number and nodule dry weight would lead to a significant increase in cowpea grain yield.

Discussion

Bradyrhizobium inoculation, P and organic manure effect on nodulation and shoot biomass of cowpea cultivar Songotra

Bradyrhizobium, P and organic manure interacted positively to increase nodule number but not nodule dry weight. Only the *Bradyrhizobium* and P combinations had a significant effect on nodule dry weight. Compared to the threshold of 50 cells of rhizobia g^{-1} soil (Slattery et al. 2004) that can obviate significant response to nodulation, the study locations had a fewer number of rhizobia cells hence the significant increase in nodulation. Response to P was expected because the soils of the study locations had low inherent P concentrations. The high nodule biomass observed in this study is an indication of high symbiotic efficiency (Graham et al. 2004). This symbiotic efficiency coupled with the availability of P and organic manure conditioning led to significantly higher shoot biomass production. In contrast, Zengeni et al. (2006) observed no significant increase in dry matter when cattle manure with < 1% N and C: N ratio > 30 was applied. However, Ezekiel-Adewoyin (2015) reported a significant increase in soybean shoot biomass after application of fertisoil and P in the northern part of Ghana. Phosphorus plays an important role in *Bradyrhizobium* nutrition by supplying the energy required for nitrogen fixation whiles organic manure enhances its survival in the soil through the creation of conducive environment (O'Hara 2001; Zengeni et al. 2006).

Bradyrhizobium inoculation, P and organic manure effect on grain yield, agronomic P efficiency and associated interactions

This study was designed to test the effect of organic and inorganic fertilizer use in combination with *Bradyrhizobium* inoculant on cowpea yield. The combined application of organic fertilizers and mineral fertilizer has gained extreme research interest and this practice has been adopted to increase grain yield of maize in low fertility smallholder fields in SSA (Vanlauwe et al. 2001; Chivenge et al. 2011). Our results showed that the combined application of *Bradyrhizobium* inoculant, P and organic manure could increase grain yield of cowpea up to 327%. Yield increases could be from the complementary role-played by each factor. For instance, the organic manure could have provided favorable carbon and other micronutrients to enhance the survival of the applied *Bradyrhizobium* strain and support general plant growth while P could have supplied the needed energy for nitrogen fixation and supported the overall growth of the host plant (Keyser and Li 1992; Crews 1993; O'Hara 2001). Since biological nitrogen fixation is a symbiotic association, it is optimized when the growth of the host plants is improved. Greater yield response observed in this study could also be attributed to the addition of other nutrients made available to the plants from mineralization of added organic manures, which promoted plant growth and provided the needed carbon for the biological nitrogen fixation process (Palm et al. 2001).

Zengeni et al. (2006) reported an increase in the yield of soybean due to application of *Bradyrhizobium* inoculant and manure. Enhanced survival of rhizobia due to the provision of carbon by manure and release of micronutrients to ameliorate limitations to response to the bacterial could explain the observed yield increases in cowpea. Palm et al. (1997) and Zingore et al. (2008) attributed the increase in yield due to organic and inorganic fertilizer applications to the release of micronutrients by the organic manure.

The difference in yield of treatments with fertisoil and cattle manure was due to the higher quality and amount of nutrient released by the fertisoil (N = 4.5%) compared to that of cattle manure (N = 2.4%). The difference in grain yield between the combination with fertisoil and with cattle manure could also be attributed to the added benefits of 300 kg ha^{-1} shoot biomass produced by the former as compared to the added benefit of (74 kg ha^{-1}) shoot biomass produced by the latter. In general, higher dry matter yield tends to result in higher grain yield. The application of fertisoil and cattle manure separately as added amendments accounted for 75% and 69% of the potential yield of the Songotra cultivar used in this study, respectively. The significant difference in grain yield between combined application of *Bradyrhizobium* inoculant, P and fertisoil and *Bradyrhizobium* inoculant, P and cattle manure treatment plots suggests that addition of fertisoil could better improve cowpea response to *Bradyrhizobium* inoculant than cattle manure. Grain legumes such as cowpea require a high amount of nitrogen during flowering, podding and seed filling stages for enhanced yield. The applied organic manure released up to 97% of its nitrogen to supplement that of the applied *Bradyrhizobium* during the crop cycle hence the observable yield increases in all the study locations. Addition of quality organic manure such as fertisoil (>2.5% N, with low polyphenol (0.08%) and lignin (10.5%) contents), results in fast release of nutrients, which may be assimilated by plants if the release is in synchrony with crop demand (Palm et al. 2001; Vanlauwe et al. 2001). The major nutrients required for plant growth were limiting at the study locations (Table 1). Therefore, responses to *Bradyrhizobium* inoculant, P and organic manure were expected. The addition of manure could have improved soil structure and moisture retention to create a conducive environment for the *Bradyrhizobium* survival and function, hence the observed enhanced grain yield.

The purpose of combining Bradyrhizobium inoculant, P and organic manure was to maximize agronomic P use efficiency. It was generally observed that the addition of P or fertisoil to Bradyrhizobium inoculant increased agronomic P use efficiency due to the enhanced grain yield. The addition of either fertisoil or cattle manure to P resulted in higher grain yield, but P use efficiencies were lower than those treatments without P. This indicates that yield increases were not solely due to efficient utilization of P but due to the provision of additional nutrients supplied by the fertisoil or cattle manure. This could also be due to luxury consumption by the plant due to excess P. This suggests that not all P captured by the plant was utilized for grain yield production. Cassman et al. (2002) and Ezekiel-Adewoyin (2015) reported lower agronomic efficiencies when higher P fertilizer quantities were applied. Agronomic P use efficiency is directly proportional to grain yield and inversely proportional to the amount of fertilizer applied indicating that the higher the fertilizer, the lower the P use efficiency. O'Hara (2001) has discussed the importance of phosphorus nutrition for Bradyrhizobium in legumes extensively. Chivenge et al. (2011) observed high grain yields from maize but lower agronomic N efficiency from Meta-analyses of several works on the combined application of organic manure and mineral fertilizer and attributed it to extra nitrogen from the treatments.

Chivenge et al. (2011) also reported both positive and negative interaction between organic manure and mineral fertilizer. Badu (2015) observed a positive interaction between organic manure and mineral fertilizer on maize in Ghana. In this study, positive interactions were observed between all the three factors. The positive interactions are indications that the three factors have a synergistic effect. The stepwise regression output revealed that, all measured parameters contributed significantly towards the grain yield. *Bradyrhizobium* inoculant, P and organic manure could be used by smallholder cowpea farmers to increase their grain yields and close the gap between the existing farm yields of 0.6 t ha^{-1} and the potential yield of 2.5 t ha^{-1} with subsequent increase in their income levels and livelihoods as demonstrated by the value cost ratios. It is worth noting that, not all the benefits of organic manure can be realized in the first year of application because of potential residual effects. Therefore, obtaining a 200% return (VCR of 2) above the cost of fertisoil in the first year indicates that such a treatment has the potential to increase the income of farmers in the medium to long term. Many smallholder farmers cannot afford to supply mineral fertilizers at required quantities and in addition obtain organic manures in high quantities to meet the nutrient requirement of targeted plants. Therefore, the government and other non-governmental organizations in Ghana may consider disseminating integrated soil fertility management packages (that include factors adopted in this study) to increase grain yields and improve the livelihood of the smallholder farmers.

Conclusion

The present study has shown that adding high-quality organic manure and phosphorus to effective *Bradyrhizobia* strain can increase grain yield of cowpea up to four-folds. The increase in cowpea biomass yields was considerably higher and more significant for the combined application of *Bradyrhizobium* inoculant, P and fertisoil than the cattle manure – based combination. Thus, fertisoil with *Bradyrhizobium* inoculant and P fertilizer is a better option for improving grain and biomass yield of cowpea for smallholder farmers in northern Ghana. The findings support the hypothesis that adding organic manure and P to *Bradyrhizobium* inoculant could improve the grain and biomass yield of cowpea. The findings also highlight the importance of improving organic matter content of the soils to improve the general nutrition of *Bradyrhizobium* for enhanced symbiosis.

Disclosure statement

No potential conflict of interest was reported by the authors.

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