



# Organic Manure Improves Soybean Response to Rhizobia Inoculant and P-Fertilizer in Northern Ghana

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Inherently low concentrations of soil nutrients and erratic rainfall pattern in sub-Saharan Africa limit soybean response to rhizobia inoculant and P-fertilizer. The study was conducted to: (i) improve soybean response to rhizobia inoculation and P-fertilizer through the addition of organic manure; (ii) enhance rain water use efficiency and (iii) determine the economic viability of combined application of the three factors in soybean cropping systems in the Northern region of Ghana. A factorial experiment with two levels of rhizobia inoculant, two levels of Phosphorus, two different kinds organic manure [fertisoil (a commercially prepared compost from urban waste, rice husks, of poultry manure and shea butter waste) and cattle manure] and a control arranged in randomized complete block design with three replications was established on farmers' fields. The combined application of rhizobia inoculant, P-fertilizer, and organic manure markedly increased nodulation, shoot biomass, haulms, harvest index (HI), P agronomic efficiency (P-AE), and rain water use efficiency (RUE) compared to the control. The combined application of rhizobia inoculant, P-fertilizer, and fertisoil increased grain yield by four-folds whereas the combined application of rhizobia inoculant, P-fertilizer, and cattle manure increased grain yield by three- folds. Harvest index, P-AE, and RUE were relatively higher with the fertisoil treatment combinations than with the cattle manure combinations and the control treatment. The application of rhizobia inoculant, P-fertilizer in combination with fertisoil was profitable with VCR of 2 as compared to the combination of cattle manure which had a VCR of 0.40. The results showed that fertisoil offers a better option of improving soybean response to rhizobia inoculant and P-fertilizer; and has the potential to enhance rain water use efficiency. However, the long term benefit must be quantified.

**Keywords:** grain yield, fertisoil, harvest index, rain water use efficiency, value cost ratio

## INTRODUCTION

Soybean is among the leguminous crops increasingly gaining considerable attention in sub-Saharan Africa (SSA), particularly Ghana. It is primarily grown for food, however, the haulm is used for feeding animals (Adjei-Nsiah et al., 2019). Additionally, the haulms, senesced nodules, and decayed roots improve the fertility of the soil for subsequent crops. Soybean is a major source of vegetable oil and feed for the poultry industry.

Despite the importance of this crop, average yields as low as  $1.0 \text{ t ha}^{-1}$  are observed on smallholder farms (Mensah, 2014). These low yields are primarily due to the inherently low concentrations of soil nutrients such as organic carbon, nitrogen, and phosphorus in the soybean growing areas of Ghana (Masso et al., 2016; Ulzen et al., 2018). There have been several attempts to improve soybean grain yield in smallholder farms but prominent among them is the use of effective rhizobia strain through inoculation and the application of P-based fertilizers (Ronner et al., 2016; Thuita et al., 2018; Raji et al., 2019). Grain yield increases in these studies were attributed to the addition of P-based fertilizers, although, the rhizobia strains used were effective. This is mainly due to the fact that P availability increases the amount of nitrogen derived from the atmosphere (Sanginga et al., 1996). In addition, P deficiencies limit nodulation, nodule function, and consequently biological nitrogen fixation (Danso, 1992; O'Hara, 2001; Sulieman et al., 2013).

In spite of the importance of P to rhizobia inoculation in soybean cropping system, the increases in yield are usually less than the potential yield of the crop, which is  $2.5 \text{ t ha}^{-1}$  (Dugje et al., 2009; Adjei-Nsiah et al., 2018, 2019). Some reports, however, indicate lack of response to rhizobia inoculation and P-fertilizer even in areas where N and P are low (Aziz et al., 2016; Rurangwa et al., 2018; Ulzen et al., 2018). This is attributed to the low organic carbon concentrations in the soil (NSFMAP, 1998; Aziz et al., 2016; Masso et al., 2016; Chekanai et al., 2018; Ulzen et al., 2018) and inefficient utilization of rain water. Deficiencies of soil organic C limits the effectiveness of other nutrients in the soil (Gruhn et al., 2000), hence its sustenance is of greater importance for maintaining soil fertility (Lakaria et al., 2012). Improving the C content of the soil through the addition of quality organic manure has the tendency to improve the response of soybean to rhizobia inoculation and P-fertilizer. Legumes can benefit from other essential nutrients aside from N and P through the application of manure (Zingore et al., 2008). Furthermore, the addition of organic manure stimulates microbial activity that results in organic acids production leading to the availability of adsorbed P in the soil (Nziguheba et al., 2016; Nosratabad et al., 2017).

Recently, there has been an erratic rainfall distribution pattern in terms of the on-set and amount in the northern part of Ghana consequently affecting grain yield in smallholder farms. Cumulative rainfall had a negative effect on soybean growth in northern Ghana (Ulzen et al., 2018). Soil management practices that make effective and efficient use of rain water in addition to supplying the required nutrients is likely to enhance grain yield of soybean. Application of manure is known to enhance

crop growth as it improves the soil's ability to hold water (Vanlauwe, 2004).

While studies on the combined application of rhizobia inoculant and P fertilization abound in the legume cropping systems in Ghana and SSA, few studies have assessed the combined application of rhizobia inoculation, P-fertilizer and organic manure [e.g., Rurangwa et al. (2018)] without considering rain water use efficiency. In this study, we hypothesize that the addition of organic manure will improve soybean response to rhizobia inoculant and P-fertilizer and enhance rain water use efficiency. The objectives of the study are (i) to enhance soybean response to rhizobia inoculation and P-fertilizer through the addition of organic manure. (ii) to determine the rain water use efficiency due to the application of organic manure; and (iii) to determine the economic viability of the combined application of rhizobia inoculation, P-fertilizer, and organic manure in soybean cropping systems in the northern part of Ghana.

## MATERIALS AND METHODS

### Description of Experimental Sites

The trials were set up on farmers' field at Tunayilli with geo-reference position  $09^{\circ}20' \text{ N}$ ,  $000^{\circ}59' \text{ W}$  and Kpalga with geo-reference position  $09^{\circ}26' \text{ N}$ ,  $000^{\circ}57' \text{ W}$  in the Tolon-Kumbungu district of the Northern region of Ghana. Tunayilli and Kpalga are 177 and 167 m above sea level, respectively. The dominant soil type at both sites was Acrisols. The field work was conducted during the 2015 cropping season (between July and October). The rainfall distribution pattern during the experimental period is presented as **Figure S1**.

### Characterization of Soil at Experimental Sites

Prior to the establishment of the trial, about seven soil cores were sampled from each site. The sampled soil was thoroughly mixed and sub-samples taken into Ziplock bags. The sub-samples were kept in  $4^{\circ}\text{C}$  refrigerator for laboratory analyses. The soil and organic manure characterization was carried out in the Soil Chemistry laboratory, KNUST. Soil organic carbon and total nitrogen analyses was performed following the procedures of Walkley and Black (Nelson and Sommers, 1996) and Kjeldahl method (Bremner and Mulvaney, 1982). The available soil phosphorus analysis was carried out as described in Bray's method (Olsen et al., 1982). The other soil analysis included particle size (hydrometer method), soil pH in water (1:2.5) and exchangeable potassium, calcium, and magnesium extracted using ammonium acetate ( $\text{NH}_4\text{OAc}$ ) (Black, 1965). Lignin and polyphenol contents of the organic manures were determined by Acid-detergent fiber (ADF) and Folin-Denis method, respectively (Anderson and Ingram, 1994). Soils taken from Kpalga had a pH (1:2.5  $\text{H}_2\text{O}$ ) of 6.5, total N (%), 0.042; organic C (%), 0.41; available P ( $\text{mg kg}^{-1}$ ), 1.27; and exchangeable K, Ca and Mg;  $1.14 \text{ (cmol}_{(+)}\text{ kg}^{-1})$ ,  $3.10 \text{ (cmol}_{(+)}\text{ kg}^{-1})$ , and  $0.37 \text{ (cmol}_{(+)}\text{ kg}^{-1})$  respectively. Those from (soil) Tunayilli had pH (1:2.5  $\text{H}_2\text{O}$ ) of 6.6, total N (%), 0.05; organic C (%), 0.71; available P ( $\text{mg kg}^{-1}$ ), 1.4 and exchangeable K,

Ca and Mg; 1.01 (cmol<sub>(+)</sub> kg<sup>-1</sup>), 4.54 (cmol<sub>(+)</sub> kg<sup>-1</sup>), and 0.70 (cmol<sub>(+)</sub> kg<sup>-1</sup>), respectively. The soil texture was described as sandy loam for both Kpalga and Tunayilli. The population of rhizobia in the soils at the experimental sites was estimated using the Most Probable Number (MPN) count method (Vincent, 1970). The MPNES software was used to assign population estimates (Woomer et al., 1990). The native rhizobia population at Tunayilli and Kpalga were 43.6 and 42.2 rhizobia cells g soil<sup>-1</sup>, respectively.

## Field Preparation and Planting

The experimental fields were plowed, harrowed to a depth of 15 cm and divided into treatment plots. The treatment plot measured 6 × 3 m with an alley of 1 m between plots and 2 m between blocks. Three seeds per hill were manually sown and thinned to two, a week after planting. The planting was done at a spacing of 75 × 10 cm resulting in 360 plants plot<sup>-1</sup> (200,000 plants ha<sup>-1</sup>). The experiments were established on July 4 and July 7, 2015, at Tunayilli and Kpalga, respectively. The soybean cultivar, Jenguma, was used as the test crop as it is the most preferred soybean cultivar in the study locations. Jenguma is a non-shattering striga resistant cultivar with a maturity period between 110–115 days and a potential yield of 2.5 t ha<sup>-1</sup> (Dugje et al., 2009).

## Treatment and Experimental Layout

The experimental design was a cross factorial (2 × 2 × 3) arranged in randomized complete blocks. The treatments were replicated three times. The treatments consisted of 2 levels of rhizobia inoculant designated as Rh+ (inoculated) and Rh- (uninoculated), 2 levels of P-fertilizer; P+ (30 kg P ha<sup>-1</sup>), and P- (0 kg P ha<sup>-1</sup>), 2 sources of manure; fertisoil, cattle manure (CM), and a control. In all, there were 12 treatment combinations and 36 plots at each location. Rhizobia inoculant containing *Bradyrhizobium* strain USDA 110 (10<sup>9</sup> cells g<sup>-1</sup> peat) was used to inoculate soybean seeds at a rate of 5 g kg<sup>-1</sup> seed. The P-fertilizer in the form of triple super phosphate was applied using the band placement method, two weeks after planting. Fertisoil and decomposed cattle manure were applied at a rate of 5 t ha<sup>-1</sup> each. Fertisoil is produced from rice husk, poultry manure, shea butter, and urban wastes. The analysis prior to the study showed that fertisoil had a nutritional content of C (%), 11.7; total N (%), 4.5; total P (%), 0.37; total K (%), 0.41; total Ca (%), 1.3; total Mg (%), 0.92; lignin (%), 10.5; and polyphenol

through broadcasting by using hoe to manually incorporate it into the soil.

## Measurements of Grain Yield and Yield Parameters

Nodule number, nodule dry weight, and shoot biomass were harvested at the R<sub>2</sub> stage (50% flowering) (Fehr et al., 1971). Soybean plants were carefully dug out with a shovel and shoots separated from the roots. The roots were washed with clean water to remove adhering soil. The nodules were carefully detached from the roots and counted. Shoot biomass and nodules were oven dried at 60°C until constant weight was attained. At the R<sub>3</sub> stage (Fehr et al., 1971), the soybean plants were harvested from the inner rows excluding the border rows and oven dried at 60°C until constant weight was attained for grain yield estimation. The whole plant (shoots and seeds) was weighed after harvesting just before threshing to determine the haulm weight. The difference between the seed weight and the whole plant was considered as the haulm weight and estimated on kilogram per hectare basis.

## Rain Water Use Efficiency (RUE)

In this study, RUE was used as a proxy for water use efficiency (Issoufa et al., 2019). It was calculated by dividing the grain yield by the total amount of rainfall during the cropping season (i.e., from planting to harvest) (Wang et al., 2016; Issoufa et al., 2019):

$$\text{Rain water use efficiency} = \frac{\text{Grain yield (kg ha}^{-1}\text{)}}{\text{Total amount of rainfall (mm)}} \quad (1)$$

## Phosphorus Agronomic Efficiency

Phosphorus Agronomic efficiency (P-AE) was estimated as follows (Vanlauwe et al., 2011):

$$\text{P - AE} = \frac{(\text{Grain yield of P-fertilized plants} - \text{Grain yield of control plants})}{\text{Amount of P-fertilizer applied}} \quad (2)$$

## Value Cost Ratio Analysis

The value cost ratio (VCR) was used as the index of profitability of the applied treatments. The estimated VCR was based on the adopted equation from Masso et al. (2016).

$$\text{VCR} = \frac{(\text{Grain yield of Treated plots} - \text{Grain yield of Control plots}) \times \text{Unit Price of Grain yield} (\$ \text{ ha}^{-1})}{\text{Cost of treatment} (\$ \text{ ha}^{-1})} \quad (3)$$

of 0.08 (%). Decomposed cattle manure used in this study was sampled from the kraal of the farmer at Kpalga site. The cattle manure was sampled from only one farmer to eliminate variation in nutritional quality and ensure uniformity. Cattle manure had nutritional content of C (%), 21.15; total N (%), 2.43; total P (%), 0.24; total K (%), 0.3; total Ca (%), 0.65; total Mg (%), 0.41; lignin (%), 5.5; and polyphenol of 0.02 (%). Fertisoil and cattle manure had C: N ratios of 2.6:1 and 8.7:1, respectively. The fertisoil and cattle manure were applied one week before planting

The unit cost of rhizobia inoculant, fertisoil, cattle manure, and P-fertilizer were 6 US\$ ha<sup>-1</sup>, 4 US\$ ha<sup>-1</sup>, 4 US\$ ha<sup>-1</sup>, and 26 US\$ ha<sup>-1</sup>, respectively (Ulzen et al., 2019). Cattle manure is not on commercial sales; therefore, the estimated cost of sampling 50 kg cattle manure was used as the cost price (Ulzen et al., 2019). Soybean was sold at 0.44 US\$ per kg from the open market. The dollar to cedi exchange rate as at the time of this study was USD \$ 1 to GH3.60 as indicated by Ulzen et al. (2019). A VCR value ≥ 2 was considered

profitable, while  $VCR \leq 1$  was considered to be non profitable (Roy et al., 2006).

### Statistical Analysis

The data were checked for normality using Shapiro–Wilk’s test. The data from both study sites were combined as preliminary analysis showed no interaction effect. Data on the various parameters were subjected to analysis of variance (ANOVA) using the factorial platform in STATISTIX software version 10 (Analytical Software 2105 Miller Landing Rd Tallahassee, FL 32312, USA). Treatment means were separated with Tukey HSD test at 5% probability. Principal Component Analysis (PCA) was used to determine the yield index which contributed most to grain yield. The grain yield used for the PCA analysis was on dry weight basis.

## RESULTS

### Rhizobia, P-Fertilizer, and Organic Manure Effect on Soybean Agronomic and Economic Benefits

Soybean grain yields increased markedly with the combined application of inoculant, P-fertilizer, and organic manure

compared to control plots (Table 1). In general, soybean yields were higher in plots with three-way treatment combinations than plots with two-way treatment combinations. The combined application of inoculant, P-fertilizer, and fertisoil increased grain yield by four-folds over the control whereas the combined application of inoculant, P-fertilizer, and cattle manure increased yield by three-folds over the control (Table 1). All the treatment combinations showed significant effect except the three-way. However, a contrast analysis revealed that the grain yield recorded by plots that received combined application of inoculant, P-fertilizer, and fertisoil was significantly different from plots that received the combined application of inoculant, P-fertilizer, and cattle manure (Table 3).

The results showed that the combined application of inoculant, P-fertilizer, and fertisoil had the highest harvest index (163%), however, neither the *F*-test nor the contrast analysis, revealed significant differences over the combined application of inoculant, P-fertilizer, and cattle manure, which had harvest index of 100% (Table 1). Only the interaction between inoculant and organic manure was significant for the two-way interactions (Table 1).

Comparatively, the combined application of inoculant, P-fertilizer, and fertisoil had higher P-AE than the combined

**TABLE 1 |** Soybean grain yield, Harvest Index, Agronomic, and Rain water use efficiencies as affected by the application of rhizobia, P-fertilizer, and organic manure.

Treatment			Grain yield (kg ha <sup>-1</sup> )	Harvest Index (HI)	P-AE (kg grain <sup>-1</sup> P)	RUE (kg ha <sup>-1</sup> mm <sup>-1</sup> rainfall)
Inoculation (I)	P-fertilizer (P)	Organic manure (O)				
+RH	P	Cattle manure	1,830.6 ± 58.27 <sup>†</sup>	1.10 ± 0.22	31.47 ± 1.40	2.78 ± 0.11
		Fertisoil	2,338.5 ± 101.42	1.63 ± 0.34	40.23 ± 1.90	3.56 ± 0.20
		No manure	1,288.6 ± 79.59	0.81 ± 0.18	23.48 ± 2.73	1.96 ± 0.13
+RH	-P	Cattle manure	865.8 ± 54.36	0.70 ± 0.11	29.32 ± 6.32	1.32 ± 0.09
		Fertisoil	1,361.7 ± 66.89	1.04 ± 0.17	57.16 ± 5.17	2.10 ± 0.12
		No manure	768.7 ± 59.52	0.67 ± 0.09	0.00 ± 0.00	1.17 ± 0.11
-RH	P	Cattle manure	1,583.3 ± 223.38	1.28 ± 0.36	25.23 ± 4.68	2.43 ± 0.38
		Fertisoil	1,577.8 ± 110.84	1.16 ± 0.30	22.79 ± 1.69	2.41 ± 0.20
		No manure	920.1 ± 49.95	0.92 ± 0.24	11.19 ± 0.72	1.40 ± 0.10
-RH	-P	Cattle manure	841.0 ± 71.05	0.86 ± 0.19	26.73 ± 3.59	1.29 ± 0.13
		Fertisoil	1,064.1 ± 134.67	0.84 ± 0.24	35.28 ± 7.59	1.63 ± 0.23
		No manure	584.4 ± 40.84	0.79 ± 0.09	0.00 ± 0.00	0.89 ± 0.08
<b>P-values (0.05)</b>						
I			<0.001	0.8002	<0.001	<0.001
P			<0.001	<0.001	0.6517	<0.001
O			<0.001	0.0001	<0.001	<0.001
I × P			0.0025	0.476	0.3801	0.0036
I × O			0.0034	0.0036	0.0106	0.0042
P × O			0.0011	0.095	<0.001	0.0012
I × P × O			0.408	0.634	0.2972	0.3986
Tukey HSD value			382.47	0.54	18.11	0.59
CV (%)			15.51	27.77	36.44	15.90

<sup>†</sup> Represents standard error of mean.



application of inoculant, P-fertilizer, and cattle manure but the difference was not significant ( $P = 0.2972$ ). Except the interaction between inoculant and P-fertilizer, all the other two-way interactions were significant (**Table 1**).

The treatments had RUE of more than one except the control plots (**Table 1**). In general, the RUE was higher in plots that received combined application of inoculant, P-fertilizer, and organic manure (**Table 1**). The combined application of inoculant, P-fertilizer, and fertisoil increased RUE over the control by 300%, while the combined application of inoculant, P-fertilizer, and cattle manure increased RUE over the control by 212% (**Table 1**). The contrast analysis indicated significant ( $P = 0.0027$ ) differences between the RUE of the combined application of inoculant, P-fertilizer, and fertisoil and that of inoculant, P-fertilizer, and cattle manure combination (**Table 3**).

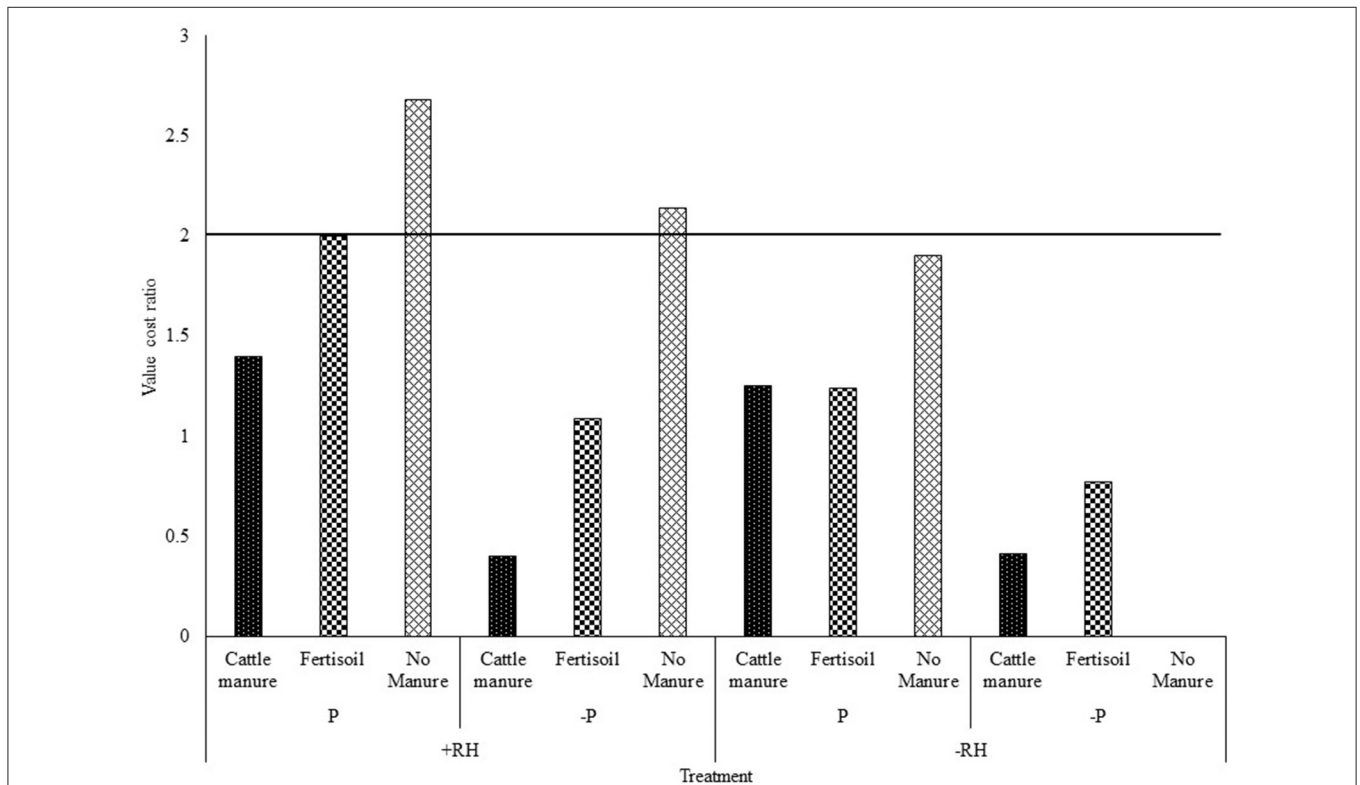
The VCR varied from 0.40 in plots that received combined application of rhizobia inoculation and cattle manure to 2.68 in the plots that received a combined application of inoculant, P-fertilizer with no manure (**Figure 1**). The VCR of the combined application of inoculant, P-fertilizer, and fertisoil was 2, which was significantly higher than the VCR (1.40) of the combined application of inoculant, P-fertilizer, and cattle manure (**Figure 1**).

Soybean shoot biomass increased markedly with the combined application of inoculant, P-fertilizer, and organic

manure compared to control plots (**Table 2**). The three-way interaction was not significant ( $P = 0.97$ ), however, the combined application of inoculant, P-fertilizer, and cattle manure had higher shoot biomass than the combined application of inoculant, P-fertilizer, and fertisoil. Except for the interaction between inoculant and P-fertilizer, all the other two-way interactions had a significant effect on shoot biomass (**Table 2**).

The effect of the treatment on haulm yields was similar to that of the shoot biomass. However, the combined application of inoculant, P-fertilizer, and fertisoil had higher haulm yield but was not significantly different ( $P = 0.29$ ) (**Table 2**) from the haulm yield recorded by the combined application of inoculant, P-fertilizer, and cattle manure. Only the interaction between P-fertilizer and organic manure had significant ( $P = <0.001$ ) effect on haulm yield. The other two-way interactions showed no significant effect on haulm yield (**Table 2**).

Nodule number ranged from 28 in the control plot to 85 in the treated plots (**Table 2**). Increases in nodule number of 141, 129, and 204% over the control treatment were recorded by the combined application of inoculant, P-fertilizer, and cattle manure, the combined application of inoculant, P-fertilizer, and fertisoil and the combined application of inoculant, P-fertilizer with no manure, respectively (**Table 2**). All the two-way



**FIGURE 1** | Value cost ratio of soybean grain yield as affected by combined application of rhizobia, inoculant and organic manure.

**TABLE 2** | Soybean shoot biomass, Haulm, and nodulation as affected by the application of rhizobia, P-fertilizer, and organic manure.

Treatment			Shoot biomass (kg ha <sup>-1</sup> )	Haulm (kg ha <sup>-1</sup> )	Nodule number plant <sup>-1</sup>	Nodule dry weight (mg plant)
Inoculation (I)	P-fertilizer (P)	Organic manure (O)				
+RH	P	Cattle manure	2,039.9 ± 395.78 <sup>†</sup>	3,394.9 ± 416.02	67.50 ± 4.16	0.77 ± 0.04
		Fertisoil	1,811.2 ± 347.88	3,612.5 ± 250.46	64.17 ± 2.88	0.88 ± 0.04
		No manure	2,016.2 ± 415.57	4,182.0 ± 303.68	85.00 ± 3.06	0.91 ± 0.03
+RH	-P	Cattle manure	1,390.8 ± 218.76	2,487.1 ± 344.23	55.83 ± 4.28	0.65 ± 0.02
		Fertisoil	1,477.1 ± 221.96	3,873.6 ± 562.29	78.83 ± 7.84	0.97 ± 0.02
		No manure	1,243.8 ± 156.62	1,937.2 ± 155.03	51.00 ± 2.96	0.71 ± 0.07
-RH	P	Cattle manure	1,644.5 ± 323.89	3,465.7 ± 241.41	52.33 ± 2.91	0.72 ± 0.04
		Fertisoil	1,840.1 ± 384.38	3,608.5 ± 190.36	72.00 ± 6.34	0.75 ± 0.03
		No manure	1,401.8 ± 310.76	3,285.8 ± 487.78	60.00 ± 2.73	0.64 ± 0.02
-RH	-P	Cattle manure	1,178. ± 189.06	2,021.8 ± 77.24	39.33 ± 3.06	0.56 ± 0.03
		Fertisoil	1,735.8 ± 343.67	3,181.0 ± 262.71	63.83 ± 5.31	0.71 ± 0.03
		No manure	7,59.3 ± 45.04	1,200.0 ± 152.97	28.00 ± 3.15	0.31 ± 0.03
<b>P-values (0.05)</b>						
I			0.0053	0.0002	<0.001	<0.001
P			<0.001	<0.001	<0.001	<0.001
O			0.0027	<0.001	<0.001	<0.001
I × P			0.2722	0.1300	0.1335	0.0115
I × O			0.0037	0.0831	0.0045	<0.001
P × O			0.0512	<0.001	<0.001	<0.001
I × P × O			0.9692	0.2880	0.0855	0.5363
Tukey HSD value			668.10	965.63	0.16	20.27
CV (%)			22.39	16.23	17.20	11.43

<sup>†</sup> Represents standard error of mean.

**TABLE 3** | Contrast analysis of Agronomic parameters of soybean as affected by the application of rhizobia, P-fertilizer, and organic manure.

Contrast	Grain yield (kg ha <sup>-1</sup> )	Harvest Index	RUE (kg ha <sup>-1</sup> mm <sup>-1</sup> rainfall)	Shoot biomass (kg ha <sup>-1</sup> )	Haulm (kg ha <sup>-1</sup> )	Nodule number plant <sup>-1</sup>	Nodule dry weight (mg plant)	P-AE (kg grain <sup>-1</sup> P)
<b>P-values</b>								
RH × P × Fert vs. Control	0.0007	0.10	0.0027	0.59	0.63	0.59	0.04	0.11
RH × P × CM Control	4e-18	0.0123	1e-15	0.0159	1.4e-6	2e-7	6e-16	4e-10
RH × P × CM vs. Control	2e-12	0.342	2e-10	0.0037	8.4e-6	2.4e-8	2e-12	2.4e-7

RH, Rhizobia; Fert, Fertisoil; CM, Cattle manure; P, Phosphorus.

interactions had a significant effect on soybean nodule number except the interaction between inoculant and P-fertilizer.

Nodule dry weight followed almost a similar pattern as the nodule number. There was a marked difference between the nodule dry weight of the control treatment and the combined application of inoculant, P-fertilizer, and organic manure (Table 2). The combined application of inoculant, P-fertilizer, and fertisoil increased nodule dry weight by 184%

over the control treatment whereas the combined application of inoculant, P-fertilizer, and cattle manure increased nodule dry weight by 148% over the control treatment (Table 2). The contrast analysis showed that the nodule dry weight obtained from plots that received combined application of inoculant, P-fertilizer, and fertisoil was significantly ( $P = 0.04$ ) different from the nodule dry weight from plots that received combined application of inoculant, P-fertilizer, and cattle manure (Table 3).

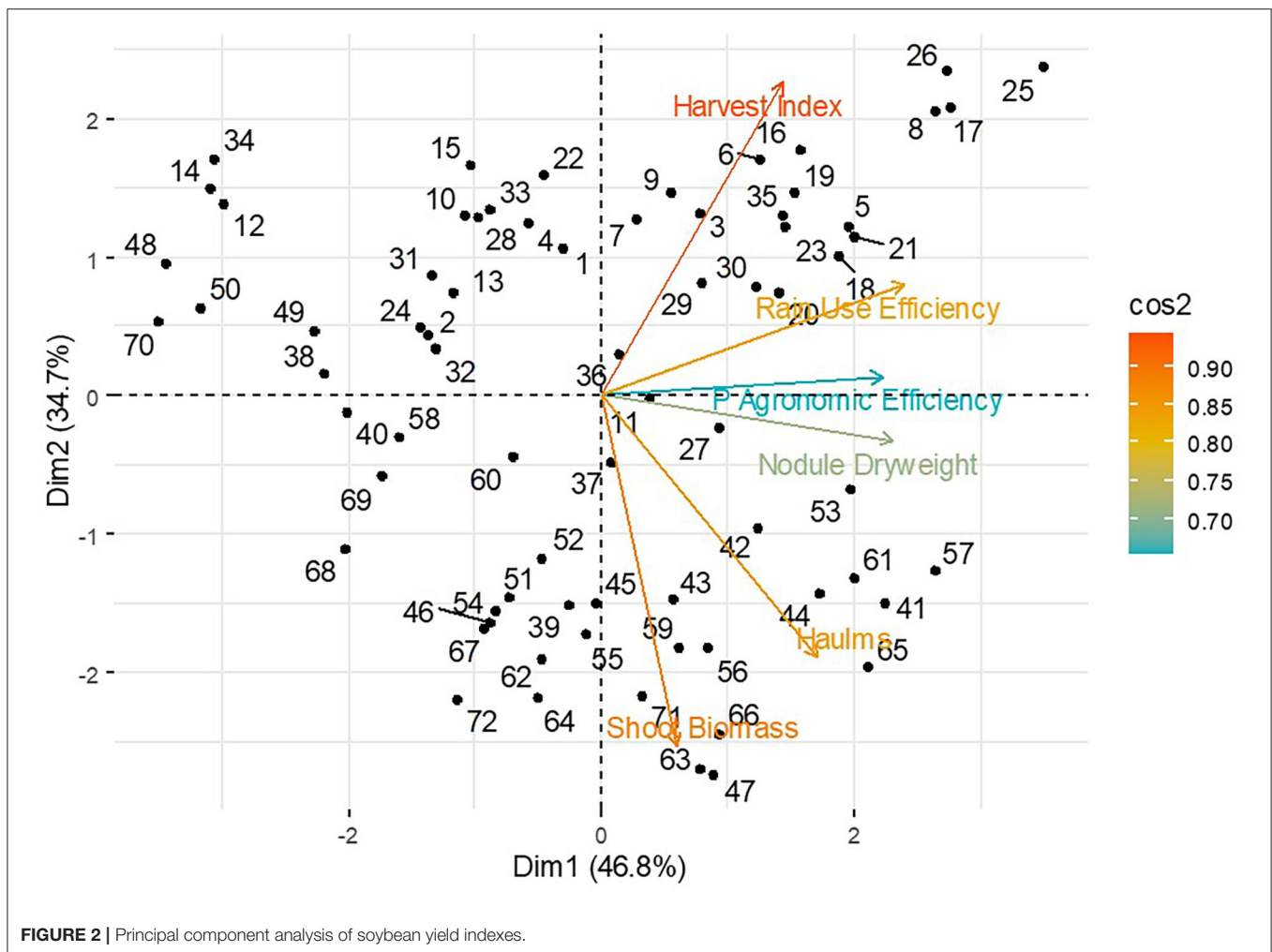
Unlike nodule number, all the two-way interactions, had significant effect on nodule dry weight.

Principal component 1 (Dim 1) explained 46.8% of the variation in grain yield while principal component 2 (Dim 2) explained 34.7% (Figure 2). The two principal component together explained 81.5% of the variability in grain yield. The main variables controlling Dim 1 were rain use efficiency, nodule dry weight and P-AE. Rain use efficiency, P-AE, and harvest index were positively correlated with treatments clustering in their direction recording high grain yields while those clustering in the opposite direction produced low grain yields. The percentage variation (34.7%) explained by Dim 1 was highly influenced by shoot biomass and harvest index but the two were negatively correlated (Figure 2).

### DISCUSSION

The combined application of rhizobia inoculant, P-fertilizer, and organic manure significantly increased soybean grain yields compared to the control treatment. The application of fertisoil

and cattle manure separately as a means to enhance soybean response to rhizobia inoculation and P-fertilizer accounted for 73 and 93%, respectively of the potential yield of soybean. The grain yield as a result of the combined application of rhizobia inoculant, P-fertilizer, and organic manure is in excess of more than 830 kg ha<sup>-1</sup> over the current yield recorded by smallholder farmers in northern Ghana. The study locations had low concentrations of the major nutrients required for plant growth, therefore, the improved response of soybean to the amendments could be due to the additive effects of rhizobia, P-fertilizer, and organic manure and more importantly other nutrients (for example, calcium and magnesium) released by the organic manure. Zingore et al. (2008) indicated that legumes may benefit from other nutrients aside from N and P when supplied with organic amendments. P-fertilizers promote rhizobia growth, nodulation, and nodule function as well as biological nitrogen fixation (Danso, 1992; Sanginga et al., 1996; O'Hara, 2001). The organic manure used in this study was of high quality as indicated by the low C: N, N: P, C: P ratios, and the N content. Sileshi et al. (2017) indicated that these ratios of nutrients in manure were determining factors in nutrient release and carbon use efficiency.



Therefore, the low ratios could have promoted microbial activity to enhance grain yield of soybean. The differences in grain yield between treatment combinations with fertisoil and cattle manure is due to the differences in nutrient concentrations. The low yield recorded under rhizobia inoculant, P-fertilizer, and cattle manure compared to rhizobia inoculant, P-fertilizer, and fertisoil suggests that the latter may have higher residual effect due to the slow release of nutrients. The increase in yield due to the combined application of rhizobia inoculant, P-fertilizer, and manure has been reported by Rurangwa et al. (2018) in Rwanda on common bean and soybean and in Ghana by Ulzen et al. (2019) on cowpea.

The results showed that RUE was high in plots that received the combined application of inoculant, P-fertilizer, and organic manure. The result is consistent with the findings of Wang et al. (2016) and Sileshi et al. (2019) who observed a higher RUE in plots that received combined application of organic and mineral fertilizer. Manure is widely known for its water retention abilities (Chikowo, 1998; Wang et al., 2016). The difference in the RUE between plots that received rhizobia inoculant, P-fertilizer, and cattle manure and those that received rhizobia inoculant, P-fertilizer, and fertisoil suggests that fertisoil has higher water retention ability and is more efficient in the usage of water than cattle manure in grain yield production. In general, RUE increased with manure application. This could be due to improvement in biological and physical properties of soil by the manure (Sileshi et al., 2019). In recent times, where variation in climate has affected the onset and pattern of rainfall in northern Ghana, management practices such as the use of fertisoil that enhances rain water use efficiency are important for climate adaptation in smallholder farming systems.

The combined application of rhizobia inoculant, P-fertilizer, and organic manure led to higher harvest indices of more than one indicating the efficiency of these amendments in grain yield production. The PCA showed that harvest index correlated positively with soybean grain yield and negatively with shoot biomass confirming the widely known assertion that plant with higher biomass tend to have low harvest index. Harvest index is directly related to management practices that increases nutrient availability to crops and improve translocation of photosynthate from the biological part to the economic part (Yang and Zhang, 2010). It also depends on the efficient utilization of water (Yang and Zhang, 2010). The PCA showed that harvest index, rain water use efficiency, and P-AE were positively correlated indicating that the processes controlling these parameters are interlinked.

The P-AE in this study was within range of similar values reported for cowpea in the same environment (Ulzen et al., 2019). In general, the combined application of mineral and organic fertilizers leads to low P-AE as it is inversely related to the amount of fertilizer applied. Therefore, the higher the amount of fertilizer applied, the lower the P-AE. Our result is consistent with the reports of Sileshi et al. (2019), Chivenge et al. (2011), and Vanlauwe et al. (2011) which indicates that the combined application of mineral and organic manure results in

low P-AE. However, the result is in contrast to that of Issoufa et al. (2019) who reported higher values of agronomic P efficiency in millet due to the combined application of mineral and organic fertilizers. It is worth noting that very high amounts of compost and mineral fertilizer was applied in the study conducted by Issoufa et al. (2019).

The results showed that the combined application of rhizobia inoculant, P-fertilizer, and fertisoil was profitable as it had a VCR of 2 and therefore, can be used to increase the income and livelihood of farmers in the medium term. However, farmers will only break-even when they apply rhizobia, P-fertilizer, and cattle manure. Since manure has residual effects, farmers will eventually benefit in the subsequent cropping seasons due to soil fertility improvement. Sileshi et al. (2019) reported that combined application of mineral and organic fertilizer was profitable for maize farmers.

Higher shoot biomass generally tends to translate into yield as observed by Ulzen et al. (2019). However, the higher biomass observed in plots that received rhizobia inoculant, P-fertilizer, and cattle manure did not translate into grain yield. This could be due to the inability of plants receiving this treatment to translocate most of the photosynthate from the biomass to the yield, hence the lower harvest index as compared to treatments with fertisoil combinations. Source-sink relationship is known to be affected by water availability (Lemoine et al., 2013). There were intermittent dry periods between flowering to podding stages (Figure S1). Treatments with cattle manure combinations had low RUE explaining the inability to translate higher biomass into grain yield. Soybean require high amount of nitrogen during the grain filling stage, therefore, it could be that cattle manure released less nitrogen during this period, which affected the translocation process. The higher haulm yield observed in this study due to the combined application of rhizobia inoculant, P-fertilizer, and organic manure than the control provides alternate benefits for farmers as it can be used to feed animals or plowed back into the soil to improve the fertility for subsequent crops. The relatively higher nodule dry weight in the combined treatments compared to the control is an indication of the efficiency of the rhizobia strain used in this study (Graham et al., 2004).

## CONCLUSION

Our results indicate the possibility of improving soybean response to rhizobia inoculant and P-fertilizer with organic manure and offers alternative fertilization strategy within the context of soil fertility management for smallholder soybean farmers in Northern Ghana. The results of this study, showed that grain yield increased from 584 kg ha<sup>-1</sup> in the control plots to 1,830 kg ha<sup>-1</sup> in plots that received the combined application of inoculant, P-fertilizer, and cattle manure and 2,338 kg ha<sup>-1</sup> in plots that received combined application of inoculant, P-fertilizer, and fertisoil. To increase grain yield, rain water use efficiency and income of soybean farmers in Northern Ghana, fertisoil offers a better option than cattle manure. However, studies on the long



term benefits of the fertisoil in improving fertility of the soil for subsequent cropping are needed. The results of this study is important for other SSA with similar environmental conditions for improving rain water use efficiency and grain yield of soybean.

## DATA AVAILABILITY STATEMENT

All datasets generated for this study are included in the article/**Supplementary Material**.

## AUTHOR CONTRIBUTIONS

JU, RA, and CM designed the research. JU, RA, NE-M, CM, OO, and AO planned the research activities. JU, RA, and OO conducted the research. JU and OO collected and analyzed the data. JU wrote the manuscript. RA, NE-M, CM, OO, and AO fine-tuned the manuscript.

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## SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2020.00009/full#supplementary-material>

**Figure S1** | Rainfall distribution pattern during the cropping season at Kpalga (A) and Tunayilli (B).

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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