



Article

Combined Application of Inoculant, Phosphorus and Potassium Enhances Cowpea Yield in Savanna Soils

Obianuju Chiamaka Emmanuel ^{1,2}, Olayiwola Akin Akintola ² , Francis Marthy Tetteh ³
and Olubukola Oluranti Babalola ^{1,*} 

¹ Food Security and Safety Niche Area, Faculty of Natural and Agricultural Sciences, North-West University, Mmabatho 2735, South Africa; ujuojimadu@yahoo.co.uk

² Farming Systems Programme, National Horticultural Research Institute, Ibadan 200272, Nigeria; akinbolanle97@yahoo.co.uk

³ CSIR, Soil Research Institute, Kwadaso, Kumasi 039-5028, Ghana; fmarthy2002@yahoo.co.uk

* Correspondence: olubukola.babalola@nwu.ac.za

Abstract: Low soil phosphorus levels in savanna soils of Ghana limit cowpea response to inoculation. A two-year experiment was carried out on 2 soil types of the Guinea and Sudan savanna zones of Ghana based on the hypothesis that *Bradyrhizobia* inoculant (BR3267) in combination with phosphorus and potassium fertilizer will significantly increase cowpea root nodulation, growth and yield. The study aimed to determine the effect of phosphorus and potassium fertilizer on cowpea response to *Bradyrhizobia* inoculant. The treatments were laid out in Randomized Complete Block Design, replicated four times. The plot size was 8 × 3 m, with the sowing distance of 60 × 20 cm. The treatment comprises of commercial *Bradyrhizobia* inoculant, phosphorus (0, 30, 40 kg P₂O₅ ha⁻¹) and potassium (0, 10, 20, 30 K₂O ha⁻¹). Application of *Bradyrhizobia* inoculant with 30 kg P₂O₅ ha⁻¹ and 20 kg K₂O ha⁻¹ gave the highest grain yield (1.68 and 1.86 tons ha⁻¹) at both soils which did not differ from the yield obtained from BR-40-30 kg ha⁻¹ P₂O₅ and K₂O ha⁻¹ on the Ferric Lixisol and BR-40-20 kg ha⁻¹ P₂O₅ and K₂O on the Ferric Luvisol. The same treatment also gave the highest nodule number and nodule dry weight. The results of this study have shown that the application of *Bradyrhizobia* inoculant followed by P and K fertilizer was effective for cowpea growth in field conditions.

Keywords: biological nitrogen fixation; fertilizer; grain yield; inoculation; root nodules



Citation: Emmanuel, O.C.; Akintola, O.A.; Tetteh, F.M.; Babalola, O.O. Combined Application of Inoculant, Phosphorus and Potassium Enhances Cowpea Yield in Savanna Soils. *Agronomy* **2021**, *11*, 15. <https://dx.doi.org/10.3390/agronomy11010015>

Received: 27 November 2020
Accepted: 21 December 2020
Published: 23 December 2020

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Cowpea is a major staple food consumed in Ghana as it contributes to the protein, vitamins and minerals intake of households. Its cultivation in Ghana is concentrated in the savanna and transition zones with an average yield of < 0.45 tons ha⁻¹ [1]. Constraints to cowpea production in Ghana are related more to its management than suitable variety [2]. This is evident as there are improved cowpea varieties appropriate for different agro-ecological zones of Ghana [3]. Some of these varieties have been estimated to have a potential yield of about 3 tons ha⁻¹ for high rainfall regions and about 2 tons ha⁻¹ for drier regions [4]. Unfortunately, these improved varieties did not live up to expectation, in terms of yield, in the investigated area. Sustainable cowpea production requires an adequate supply of nitrogen (N), phosphorus (P) and potassium (K) as well as micronutrients [5]. It was reported to require less nitrogen fertilizer application (between 15–30 kg ha⁻¹) as it can fix nitrogen through a symbiotic association between its root nodules and *Bradyrhizobia* [6]. High nitrogen fertilizer doses have been blamed for luxuriant biomass growth in cowpea at the expense of grain yield and nodulation [1,7].

Biological Nitrogen Fixation (BNF) contributes to the nitrogen nutrition of cowpea with an estimate of 16 to 23 kg ha⁻¹ in the Upper West Region of Ghana depending on the variety, environment and management [8]. The nitrogen-fixing capacity of cowpea

is significantly influenced by phosphorus availability, rhizobia population and effectiveness [9,10]. Potassium can enhance nodulation and nitrogen fixation on deficient soils [11] and also assist cowpea in overcoming moisture stress [12]. Nitrogen and phosphorus have been identified as the most limiting nutrients on smallholder farms across Africa with a high depletion rate [13]. However, Potassium removed from the soil through continuous crop harvest requires replacement to enhance optimum production and soil health. External input of fertilizers is essential for plant growth but may be difficult for smallholder farmers to afford due to their high cost. The average fertilizer application rate in Ghana is about 8 kg ha⁻¹ [14], notwithstanding the Abuja summit of 2006 where African countries were encouraged to increase fertilizer use to about 50 kg ha⁻¹. Nitrogen fertilizer is increasingly getting beyond the reach of average farmers in developing countries due to seasonal scarcity and its ever-increasing cost. There is, therefore, the need to look inward for alternatives if food production must be sustained. *Bradyrhizobia* inoculant could be a cheaper substitute for N fertilizer for grain legumes. Fening et al. [15] suggested that the inoculation of cowpea with effective strains of *Bradyrhizobia* is a good strategy to increase N supply to cowpea since it showed a significant response to increasing N fertilization. However, the assumption is that the indigenous *Bradyrhizobia* species are capable of nodulating cowpea due to its promiscuous nature, thereby making inoculation with effective strains unnecessary [16]. Recent studies have however disproved this, as reported in Brazil [17,18], Ghana [1,19]; Mozambique [20]; Nigeria [21] and Tanzania [22]. Inoculating cowpea with effective *Bradyrhizobia* strain could be beneficial, environmentally safe and reduces N fertilizer application due to improved nodulation and N fixation [19]. However, other growth limiting factors like phosphorus and potassium availability have to be addressed to get the maximum benefit from inoculation.

Kyei-Boahen et al. [20] reported that cowpea responded to rhizobia inoculation in soils having indigenous rhizobia population in Mozambique. Inoculation effect was, however, higher in soils that have an adequate supply of P. The authors reported a 56% yield increase due to P application against 25% yield increase without P application. This, therefore, suggests that the application of inoculant should be accompanied by sufficient amounts of P fertilizer for optimal response and yield. Similarly, [23] reported increased effectiveness of *Bradyrhizobia* due to phosphorus application and also significant biomass and grain yield of cowpea in the Northern region of Ghana. The *Bradyrhizobium yuamingense* strain BR3267 inoculant could improve cowpea yield. There has been reported evaluation of the inoculant in the Northern region and some parts of the Upper West region [1,23] but yet to be evaluated in our study locations.

We, therefore, hypothesized that inoculating cowpea with *Bradyrhizobia* followed by P and K fertilizer will enhance root nodulation, growth and yield of cowpea in the investigated soils. The study aimed at determining the effect of P and K fertilizer on cowpea response to *Bradyrhizobia* inoculant on 2 soil types in the Guinea and Sudan savanna zones of Ghana.

2. Materials and Methods

2.1. Study Sites

The study was conducted in 2013 and 2014 cropping season at Lawra (Sudan Savanna zone) and Nyoli (Guinea Savanna zone) both in the Upper West region of Ghana. Lawra District and lies geographically between latitudes 10°40' and 11°00' N and longitude 2°51' and 2°45' W. The soil belongs to the Dorimon series classified as Ferric Lixisol [24]. Nyoli is found in the Wa West district and lies between longitudes 9°40' and 9°46' N and Latitudes 2°30' and 2°32' W. The soil belongs to the Vairempere series and classified as Ferric Luvisol. Both locations experience a unimodal rainfall pattern with annual values of between 800–1100 mm (Lawra) and 840 and 1400 mm (Nyoli). Both fields were previously planted with maize with no history of microbial inoculation.

2.2. Field Experiment

The study was laid out in Randomized Complete Block Design replicated four times for both soil types. The plot size was 8×3 m, with the sowing distance of $60 \text{ cm} \times 20 \text{ cm}$. The treatment comprises of a commercial *Bradyrhizobia* inoculant strain BR3267 formulated for cowpea, three levels of phosphorus (0, 30, 40 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$) and four levels of potassium (0, 10, 20, 30 kg $\text{K}_2\text{O} \text{ ha}^{-1}$). Inoculation was accomplished by applying 5 g of the *Bradyrhizobia* inoculant to 1 kg cowpea seed. The inoculated seed was spread on a flat wood and left to dry at room temperature before sowing. Omondaw, early maturing (65 days) cowpea variety used. Fertilizer was applied at sowing close to the seed, excluding the control plots. The fertilizers used to supply nutrients were triple super phosphate (46% P_2O_5) and potassium chloride (60% K_2O). Biomass yield, nodule count and dry weight were determined at flowering while grain yield was determined at pod maturity. Ten random plants were carefully uprooted from each plot using a spade. The roots were detached, rinsed, and the nodules collected. The nodules were placed in an envelope and then dried in the oven at 60°C for 72 h, and the nodule dry weights recorded. At maturity, plants were harvested from the middle rows and dried in the oven at 60°C for 72 h, after which the weights were recorded.

The treatments used were as follows: 0-0-0, BR-0-0, BR-30-0, BR-40-0, BR-30-10, BR-40-10, BR-30-20, BR-40-20, BR-30-30, BR-40-30 ($\text{N-P}_2\text{O}_5\text{-K}_2\text{O} \text{ kg ha}^{-1}$). BR represents the *Bradyrhizobia* inoculant

2.3. Statistical Analysis

All the data collected were subjected to Analysis of Variance (ANOVA). The differences between treatments were compared based on the least significant difference (LSD) $p = 0.05$.

2.4. Laboratory Analysis

Composite soil samples collected from the study site were air-dried and sieved with a 2 mm sieve. Soil pH was measured with glass electrode pH meter in a 1:1 soil to distilled water (soil: water) ratio; available P was measured by the Bray 1 method [25] while organic carbon was determined with the modified Walkley and Black procedure as described by [26]. The Micro Kjeldahl method [27] was used to determine the total nitrogen and 1.0 N ammonium acetate (NH_4OAc) extract was used for exchangeable bases. Potassium chloride (1.0 N) extract [28] was used to determine the exchangeable acidity (hydrogen and aluminum). Nutrient uptake in the biomass and grain was determined by multiplying the concentration of each nutrient with the biomass and grain yield per hectare.

2.5. Soil Physical and Chemical Properties of the Study Locations

Some of the properties of the soils at both locations are presented in Table 1. Both soils were sandy loam textured.

Table 1. Initial soil physical and chemical properties of the experimental site.

Soil Parameter	Ferric Lixisol	Ferric Luvisol
Sand (%)	57	62
Silt (%)	38	34
Clay (%)	5	4
pH (1:1 H_2O)	5.78	5.90
Organic Carbon (%)	0.46	0.90
Total Nitrogen (%)	0.07	0.08
Available P (mg kg^{-1})	1.01	5.61
Exchangeable cations ($\text{cmol}_+ \text{kg}^{-1}$)		
Ca^{2+}	3.60	9.00
Mg^{2+}	0.60	3.6
K^{2+}	0.11	0.12
Na^{2+}	0.15	0.43

The total N and available *p* values were low ($N < 0.10\%$ and $p < 10 \text{ mg kg}^{-1}$). Exchangeable potassium values were also less than the critical value of $0.15 \text{ cmol kg}^{-1}$. All these values were rated according to values reported by [29]. The pH was near neutral for both soils, which are satisfactory for cowpea production.

3. Results

3.1. Dry Matter and Grain Yield

The results in Table 2 showed that the dry matter and grain yield were significantly different ($p < 0.05$) among the treatments for the two soil types. The combined application of *Bradyrhizobia* inoculant with 30 and 40 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ while omitting K_2O increased dry matter yield (29% more than control for the Ferric Lixisol and 25% for the Ferric Luvisol) which did not differ statistically to the weight produced by plants that received *Bradyrhizobia* inoculant only (Table 2). The values ranged from 2.31 to 2.45 tons ha^{-1} for the Ferric Lixisol and 2.45 to 2.56 tons ha^{-1} for the Ferric Luvisol. The application of *Bradyrhizobia* inoculant with 10, 20, and 30 kg $\text{K}_2\text{O ha}^{-1}$ accompanied by 30 and 40 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ in different treatments did vary significantly in dry matter production. The application of 10 kg $\text{K}_2\text{O ha}^{-1}$ with 30 and 40 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ on the Ferric Lixisol resulted in dry matter yield of 2.74 and 2.67 tons ha^{-1} respectively. Increasing the K rate to 20 kg $\text{K}_2\text{O ha}^{-1}$ with 30 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ gave 2.77 tons ha^{-1} dry matter for the Ferric Lixisol, which was 55% more than the control. Application of inoculant with 40 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ with 20 kg $\text{K}_2\text{O ha}^{-1}$ yielded a dry matter weight of 2.87 tons ha^{-1} . Dry matter yield from the Ferric Luvisol followed the same trend with values ranging from 2.85 to 3.02 tons ha^{-1} .

Table 2. Effect of inoculant and fertilizer application on cowpea dry matter and grain yield.

Treatments BR(P_2O_5 - K_2O)	Ferric Lixisol		Ferric Luvisol	
	Dry Matter (tons ha^{-1})	Grain Yield (tons ha^{-1})	Dry Matter (tons ha^{-1})	Grain Yield (tons ha^{-1})
0-0-0	1.78 a	0.52 a	1.96 a	0.78 a
BR-0-0	2.31 b	0.70 b	2.45 b	0.86 b
BR-30-0	2.45 bc	1.40 c	2.62 bc	1.48 c
BR-40-0	2.53 bcd	1.42 c	2.56 b	1.55 d
BR-30-10	2.74 de	1.49 d	2.85 d	1.68 e
BR-40-10	2.67 cde	1.45 d	2.82 cd	1.65 e
BR-30-20	2.77 e	1.68 f	3.02 d	1.86 f
BR-40-20	2.87 e	1.64 e	2.92 d	1.87 f
BR-30-30	2.77 e	1.62 e	2.86 d	1.72 g
BR-40-30	2.83 e	1.67 f	2.96 d	1.75 g

BR—*Bradyrhizobia* inoculants, P_2O_5 —Phosphorus was supplied by triple superphosphate, K_2O —potassium was supplied by muriate of potash, Means followed by the same letter(s) are not significantly different ($p < 0.05$). Values are means of two years.

Application of *Bradyrhizobia* inoculant with 30 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ and 20 kg $\text{K}_2\text{O ha}^{-1}$ gave the highest grain yield (1.68 and 1.86 tons ha^{-1}) at both soils which however did not differ from the yield obtained from BR-40-30 kg P_2O_5 and $\text{K}_2\text{O ha}^{-1}$ on the Ferric Lixisol and BR-40-20 kg P_2O_5 and $\text{K}_2\text{O ha}^{-1}$ on the Ferric Luvisol. Again, the results show that the grain yield of cowpea from both soils significantly increased when the *Bradyrhizobia* inoculant was applied together with different rates P and K fertilizer. Yield increase (34%) due to inoculant application alone was from 0.52 to 0.7 tons ha^{-1} on the Ferric Lixisol. An increase of 10% (0.78 to 0.86 tons ha^{-1}) was recorded from sole inoculation treatment on the Ferric Luvisol. Application of P (30 and 40 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$) with *Bradyrhizobia* inoculant without K significantly increased ($p < 0.05$) cowpea grain yield more than the control and sole *Bradyrhizobia* inoculation. The yield obtained from the Ferric Lixisol was 1.40 and 1.42 tons ha^{-1} for 30 and 40 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$, which did not differ from each other. On the Ferric Luvisol, the grain yield obtained from applying P at 30 and 40 kg $\text{P}_2\text{O}_5 \text{ ha}^{-1}$ was 1.48 and 1.55 tons ha^{-1} , respectively. Increasing K from 10 to 20 and 30 kg $\text{K}_2\text{O ha}^{-1}$ with a

P applied at 30 kg ha⁻¹ gave a grain yield of 1.49, 1.68, 1.62 tons ha⁻¹ on the Ferric Lixisol while 1.68, 1.68 and 1.72 tons ha⁻¹ was obtained from the Ferric Luvisol respectively.

3.2. Nodule Number and Nodule Dry Weight

Inoculation significantly increased ($p < 0.05$) the nodule number and nodule dry weight compared to that for the un-inoculated plants at both locations (Figures 1 and 2). On the Ferric Lixisol, the combined application of *Bradyrhizobia* inoculant and with 30 and 40 kg P₂O₅ ha⁻¹ resulted in nodule number that did not differ significantly with that from the treatment where inoculant was applied with the same rate of P₂O₅ and 30 kg K₂O ha⁻¹. Similarly, applying inoculant with 30 and 40 kg P₂O₅ ha⁻¹ along with 10 and 20 kg K₂O ha⁻¹ gave nodule number that was not significantly different ($p > 0.05$) from each other. A higher nodule number was generally obtained from the Ferric Luvisol. Application of inoculant with 30 kg P₂O₅ ha⁻¹ and 20 kg K₂O ha⁻¹ gave the highest nodule number, which was not statistically different from all other treatments except the control and sole inoculant application.

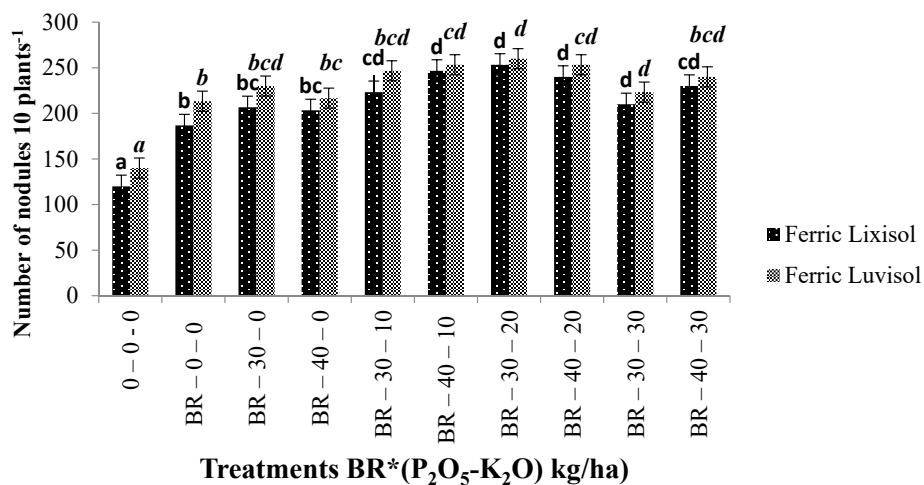


Figure 1. Number of nodules as influenced by inoculant and fertilizer treatments. BR*—*Bradyrhizobia* inoculant, P₂O₅—Phosphorus was supplied by triple superphosphate, K₂O—potassium was supplied by muriate of potash, error bars denotes the standard error of the means). Bars designated with the same letter(s) are not significantly different ($p < 0.05$). Values are means of two years.

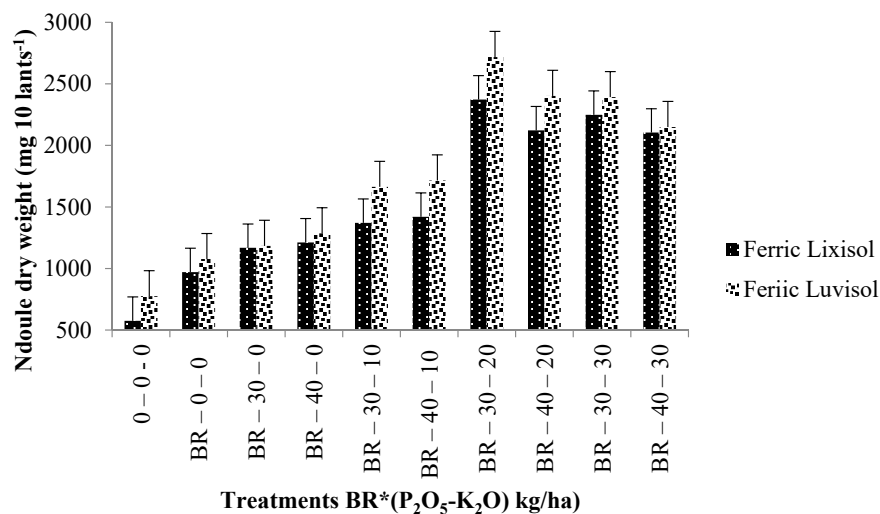


Figure 2. Nodule dry weight as influenced by inoculant and fertilizer treatments. BR—*Bradyrhizobia* inoculant, P₂O₅—Phosphorus supplied by triple superphosphate, K₂O—potassium was supplied by muriate of potash, error bars denotes the standard error of the means). Values are means of two years.

The nodule dry weight for all the treatments differed significantly from that of the control at both soils. Applying the inoculant with 30 kg P₂O₅ ha⁻¹ and 20 kg K₂O ha⁻¹ resulted in the highest nodule weight at both soils. There were no significant differences with the nodule dry weight obtained from treatment BR-30-0 and BR-30-10 kg P₂O₅ and K₂O ha⁻¹ for both soils. The same was also the case for the nodule weight obtained from treatment BR-30-10 and BR-40-10 kg P₂O₅ and K₂O ha⁻¹ for both soils.

3.3. Grain and Biomass N, P and K Uptake

The data obtained, as presented in Table 3, indicated that the grain N, P and K contents differed significantly among all the treatments. The highest grain N uptake on the Ferric Lixisol was attained by treatment BR-40-30 kg P₂O₅ and K₂O ha⁻¹, which compared to that obtained from treatments BR-30-20, BR-40-20 and BR-30-30 kg P₂O₅ and K₂O ha⁻¹. On the however, the highest grain N uptake of 6.55 kg ha⁻¹ was obtained from treatment BR-40-20 kg P₂O₅ and K₂O ha⁻¹, which did not differ statistically from that obtained from BR-30-20 kg P₂O₅ and K₂O ha⁻¹.

Table 3. Effect of inoculant and fertilizer application on cowpea grain N, P and K uptake.

Treatments BR(P ₂ O ₅ - K ₂ O)	N Uptake		P Uptake		K Uptake	
	Ferric Lixisol	Ferric Luvisol	Ferric Lixisol	Ferric Luvisol	Ferric Lixisol	Ferric Luvisol
0-0-0	1.71 a	2.87 a	0.08 a	0.20 a	0.16 a	1.59 a
BR-0-0	2.34 b	3.03 b	0.17 b	0.21 a	0.42 b	1.65 a
BR-30-0	3.45 c	4.12 c	0.20 cd	0.44 c	0.47 bc	2.82 b
BR-40-0	4.66 d	4.89 d	0.20 cd	0.48 de	0.46 bc	2.83 b
BR-30-10	4.89 de	5.29 e	0.19 c	0.40 b	0.43 b	2.91 b
BR-40-10	5.32 ef	6.32 h	0.20 cd	0.51 ef	0.54 d	2.92 b
BR-30-20	5.68 fg	6.51 i	0.23 e	0.57 g	1.26 e	3.72 c
BR-40-20	5.56 fg	6.55 j	0.20 cd	0.45 cd	1.22 e	3.92 d
BR-30-30	5.67 fg	5.42 f	0.21 cd	0.43 bc	1.24 e	3.67 c
BR-40-30	5.89 h	5.97 g	0.21 d	0.53 f	1.25 e	3.63 c

BR—Bradyrhizobia inoculants, P₂O₅—Phosphorus by triple superphosphate, K₂O—potassium was supplied by muriate of potash, Means followed by the same letter(s) are not significantly different ($p < 0.05$). Values are means of two years.

The data also revealed that the uppermost grain P uptake of 0.23 and 0.57 kg P ha⁻¹ was obtained from the combined application of inoculant with 30 kg P₂O₅ ha⁻¹ and 20 kg K₂O ha⁻¹ at both soils respectively. Grain P uptake obtained from other treatments apart from sole inoculation and control did not differ significantly from each other. Grain P uptake on the Ferric Luvisol obtained from the control, and sole *Bradyrhizobia* inoculant did not vary significantly; however, other treatments differed significantly. Grain K uptake from applying the *Bradyrhizobia* inoculant with 30 and 40 kg P₂O₅ ha⁻¹ with 20 and 30 kg K₂O ha⁻¹ was not significantly different on the Ferric Lixisol. The values ranged from 1.22 to 1.26 tons ha⁻¹. On the Ferric Luvisol, the application of *Bradyrhizobia* inoculant with 30 and 40 kg P₂O₅ ha⁻¹ with 0 and 10 kg K₂O ha⁻¹ was not significantly different. The highest grain k uptake of 3.92 tons ha⁻¹ was obtained from treatment BR-40-20 kg P₂O₅ and K₂O ha⁻¹ on the Ferric Luvisol.

The biomass nutrient (N, P, K) content obtained from both soils is presented in Table 4. The biomass nutrient uptake obtained increased with increased application of fertilizer at both soils. The highest biomass N uptake (12.54 and 12.86 tons ha⁻¹) for both soils was obtained from treatment BR-40-20 kg P₂O₅ and K₂O ha⁻¹. For biomass P uptake, the highest rate (1.55 and 1.33 tons ha⁻¹) was attained by applying the *Bradyrhizobia* inoculant with 40 kg P₂O₅ ha⁻¹ and 20 kg K₂O ha⁻¹. Same treatment (BR-40-20 kg P₂O₅ and K₂O ha⁻¹) gave the highest K biomass uptake of 6.96 and 6.99 tons ha⁻¹ for the Ferric Lixisol and Ferric Luvisol, respectively.

Table 4. Effect of inoculant and fertilizer application on cowpea biomass N, P and K uptake.

Treatments BR(P ₂ O ₅ - K ₂ O)	N Uptake (tons ha ⁻¹)		P Uptake (tons ha ⁻¹)		K Uptake (tons ha ⁻¹)	
	Ferric Lixisol	Ferric Luvisol	Ferric Lixisol	Ferric Luvisol	Ferric Lixisol	Ferric Luvisol
0-0-0	5.61 a	5.09 a	0.86 a	0.79 a	3.08 a	3.10 a
BR-0-0	9.64 d	9.02 b	0.96 b	0.88 b	4.21 b	4.06 b
BR-30-0	11.33 c	11.45 c	1.20 c	1.23 cd	4.57 c	4.86 c
BR-40-0	11.56 c	11.84 d	1.22 c	1.30 f	4.32 b	4.99 c
BR-30-10	12.41 d	12.24 e	1.33 d	1.20 c	5.77 e	5.74 d
BR-40-10	12.43 d	12.28 f	1.32 d	1.2 cd	5.98 e	5.85 d
BR-30-20	12.51 e	12.83 g	1.43 e	1.25 de	6.58 g	6.96 g
BR-40-20	12.54 e	12.86 g	1.55 f	1.33 f	6.96 h	6.99 g
BR-30-30	12.50 e	12.53 h	1.42 e	1.21 c	6.53 fg	6.70 f
BR-40-30	12.54 e	12.55 h	1.51 f	1.30 f	6.46 f	6.74 f

BR—Bradyrhizobia inoculants, P₂O₅—Phosphorus supplied by triple superphosphate, K₂O—potassium was supplied by muriate of potash, Means followed by the same letter(s) are not significantly different ($p < 0.05$). Values are means of two years.

4. Discussion

4.1. Bradyrhizobia Inoculation, P and K Fertilizer Effect on Grain and Dry Matter Yield at Lawra and Nyoli

This study evaluated the effect of inoculating cowpea with *Bradyrhizobia* inoculant together with P and K fertilizer at 2 soils of the Savanna zones of Ghana (Table 2). Both soils showed a similar pattern in response to treatment application. Our results indicated that applying the *Bradyrhizobia* inoculant together with P and K fertilizer could increase cowpea grain yield more than 200%, with about 50% increase of dry matter yield. It can be deduced that each of the added nutrients played a complementary role with the inoculant. This is demonstrated by the initial low soil P and K levels in investigated areas. Phosphorus is known to supply the required energy for the rhizobial strain to convert atmospheric N to NH₄ (O'hara 2001). Applying P fertilizer to legumes where the soil is deficient is known to increase N fixation and yield. Kyei-Boahen et al. [20] reported a higher yield of cowpea that was inoculated and applied 40 kg P ha⁻¹ than the ones that did not receive P application. However, the authors did not apply K due to its adequate content in the study locations. Similarly, [1] reported that grain yield of cowpea in the Northern region of Ghana doubled following inoculant application with 26 kg P ha⁻¹. Even though the application of the inoculant with P increased both the dry matter and grain yield at both soil types, greater yield response was obtained when K was added at both locations. Potassium could have contributed to the general growth of cowpea and enhanced nodulation. Improved plant growth contributes to the optimization of biological nitrogen fixation which is a symbiotic association [23]. Singh. and Kataria [30] reported that K aided in maintaining N fixation rates and N partitioning to meet the requirement of the reproductive parts and the nodules. As has been previously stated, increased nodulation following K application increases N fixation, thereby ensuring improved plant growth and yield.

Luvisols and Lixisols are known to have high base saturation with greater amounts of K⁺, however, continuous cropping without adequate K replacement may limit the contribution of K to plant growth. The addition of K was to eliminate any nutrient-related stress to the plant to ensure optimal growth. It was observed that the addition of K₂O beyond 20 kg ha⁻¹ was not necessary as no significant yield response was recorded. This could indicate that yield increase was not only due to P addition, but balanced nutrition provided by the supplied K fertilizer. Ferreira et al. [17] observed a low cowpea grain yield of 582.83 kg ha⁻¹ following inoculation with same *B. yuamingense* strain. This low yield could be attributed to the fact that the authors did not apply P and K fertilizer along with the inoculant. The grain yield obtained from the Ferric Luvisol was more than that obtained from the Ferric Lixisol, which could be due to better rainfall distribution at Nyoli.

Even though both soils are responsive to fertilizer application, the initial nutrient content of Nyoli was slightly higher than that of Lawra.

4.2. *Bradyrhizobia* Inoculation, P and K Fertilizer Effect on Nodule Number and Dry Weight at Lawra and Nyoli

Cowpea is known to be nodulated by *Bradyrhizobium* spp, which exists in tropical soils [31]. Cowpea's response to inoculation mostly occurs when the indigenous rhizobia population is low or ineffective. Also, the effectiveness, infectiveness, and ability of the inoculated bacterial strain to outcompete the native strains determine the response to inoculation [32]. From our study, the inoculation of cowpea with *Bradyrhizobia* inoculant increased the nodule number and dry weight of cowpea at both study locations (Figures 1 and 2). Nodulation observed in the control treatment, and inoculated treatments suggest that the indigenous rhizobial strains were effective in nodule formation, however, the inoculated strain performed better. This result agrees with the findings of [10] whose report indicated that some strains of *B. yuamingense* are beneficial as inoculates for cowpea in Sub-Saharan Africa. Fening and Danso [32] reported that most cowpea *Bradyrhizobia* strains indigenous to the Guinea Savanna soils of Ghana were moderately effective. This was corroborated by the report of [15], which recommended the inoculation of cowpea with effective rhizobial strains in savanna zones of Ghana since cowpea showed a significant response to increasing N fertilizer addition. Ulzen et al. [19] reported that the indigenous rhizobia population of the Nyankpala soil of Ghanaian Guinea savanna was low (<10 cells g^{-1}) and most likely ineffective. Our data also indicated that the absence of added P and K fertilizer in the control and sole rhizobia inoculation limited the extent of the nodulation by the inoculant strain and the native strain (Figures 1 and 2). The nodule number and dry weight significantly increased with the addition of fertilizer; however, the addition of K enhanced both parameters. Kyei-Boahen et al. [20,23] also reported increased nodule number and weight of cowpea following inoculation and application of 30 and 40 kg P_2O_5 ha^{-1} . Boddey et al. [1] also reported a higher nodule weight of more than 300 mg $plant^{-1}$ in Northern Ghana due to the application of *Bradyrhizobia* inoculant with 26 kg P ha^{-1} . From our results, the addition of K further increased the nodule number and nodule weight. This could be attributed to the better nutrition of the plant after the addition of K. Potassium application on legumes also affects the activity of glutamine synthetase (enzyme in the nodules) positively [33]. Glutamine synthetase is involved in nitrogen assimilation and the biosynthesis of glutamine [34]. Nodulation of a legume is usually positively correlated with the nitrogen fixed by the plant.

4.3. *Bradyrhizobia* Inoculation, P and K Fertilizer Effect on Cowpea Nutrient Uptake at Lawra and Nyoli

Cowpea residue is usually incorporated into the soil after harvest at both study locations. This practice ensures that nutrients in the biomass returned to the soil. This could confer huge benefits to subsequent crops (especially cereals) grown in that same location [35]. Uzoh et al. [36] reported that rotating legume with cereal could considerably increase soil N, K and Mg when the legume is incorporated into the soil. The improved nutrient uptake by plants that were inoculated with *Bradyrhizobia* inoculant alongside P and K fertilizer, instead of sole inoculant alone, could be explained by the ability of the added nutrient to complement the inoculant in ensuring optimal plant growth and nutrient accumulation. Comparable results were obtained by [20], where P application increased shoot content of N and P. Ferreira et al., 2013 observed that *Bradyrhizobia* inoculant led to high concentrations of nitrogen, phosphorus, potassium, magnesium and calcium without the addition of complementary phosphorus and potassium.

5. Conclusions

Our results have shown that the application of *Bradyrhizobia* inoculant (3267) followed by P and K fertilizer enhance the growth of cowpea in field conditions in the study areas. The growth and yield increase due to inoculation was more pronounced in soils that

received 30 kg P₂O₅ ha⁻¹ and 20 kg K₂O ha⁻¹. Using inoculant along with the adequate rate of P and K fertilizer could be a means of increasing cowpea yield and the nutritional quantity of smallholder farmers in the regions. The residual nutrient in the biomass could also contribute substantially to the nutrition of the subsequent crop in the field. Further research on the inoculant in other soil types in agro-ecologies is required to capture variations in soil properties.

Author Contributions: All the authors contributed significantly to this work. O.C.E. conducted the research; F.M.T. supervised the work while O.A.A. made a significant contribution to the discussion. O.O.B. supervised the write-up including its organization. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Alliance for Green Revolution in Africa (AGRA), Soil Health Programme).

Acknowledgments: Alliance for Green Revolution Soil Health Project is acknowledged for the Ph.D. scholarship granted to OCE. Food Security and Safety Niche Area, Faculty of Natural and Agricultural Sciences, North-West University is also acknowledged for the Post-Doctoral fellowship granted to OCE. OOB expresses her gratitude to the National Research Foundation, South Africa, for grants (Unique Grant No: 123634) that have supported research in her laboratory.

Conflicts of Interest: The authors declare no competing interest.

References

- Boddey, R.M.; Fosu, M.; Atakora, W.K.; Miranda, C.H.; Boddey, L.H.; Guimaraes, A.P.; Ahiabor, B.D. Cowpea (*Vigna unguiculata*) crops in Africa can respond to inoculation with rhizobium. *Exp. Agric.* **2017**, *53*, 578–587. [CrossRef]
- Egbadzor, K.; Yeboah, M.; Offei, S.; Ofori, K.; Danquah, E. Farmers key production constraints and traits desired in cowpea in Ghana. *J. Agric. Ext. Rural Dev.* **2013**, *5*, 14–20.
- Agyeman, K.; Berchie, A.; Bonsu, I.; Nartey, T.; Fordjour, J. Growth and yield performance of improved cowpea (*Vigna unguiculata* L.) varieties in Ghana. *Agric. Sci.* **2014**, *2*, 44–52. [CrossRef]
- CSIR; CRI. Annual Report, Council for Scientific and Industrial Research—Crops Research Institute, Ghana. 2010. Available online: <https://csir.org.gh/index.php/publications/technical-reports/annual-reports/item/395-annual-report-2010> (accessed on 15 November 2014).
- Singh, A.K.; Bhatt, B.; Sundaram, P.; Kumar, S.; Bahrati, R.; Chandra, N.; Rai, M. Study of site specific nutrients management of cowpea seed production and their effect on soil nutrient status. *J. Agric. Sci.* **2012**, *4*, 191–198. [CrossRef]
- Karikari, B.; Arkorful, E.; Addy, S. Growth, nodulation and yield response of cowpea to phosphorus fertilizer application in Ghana. *J. Agron.* **2015**, *14*, 234–240. [CrossRef]
- Daramy, M.; Sarkodie-Addo, J.; Dumbuya, G. The effects of nitrogen and phosphorus fertilizer application on crude protein, nutrient concentration and nodulation of cowpea in Ghana. *ARPN J. Agric. Biol. Sci.* **2016**, *11*, 470–480.
- Naab, J.B.; Chimphango, S.M.; Dakora, F.D. N₂ fixation in cowpea plants grown in farmers' fields in the Upper West Region of Ghana, measured using 15 N natural abundance. *Symbiosis* **2009**, *48*, 37–46. [CrossRef]
- Nziguheba, G.; Zingore, S.; Kihara, J.; Merckx, R.; Njoroge, S.; Otinga, A.; Vandamme, E.; Vanlauwe, B. Phosphorus in smallholder farming systems of sub-Saharan Africa: Implications for agricultural intensification. *Nutr. Cycl. Agroecosystems* **2016**, *104*, 321–340. [CrossRef]
- Bongo, A.; Pietr, S. Biodiversity of nitrogen fixing indigenous Rhizobia nodulating common bean (*Phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* (L.) Walp.), Soybean (*Glycine max* L. Merry), And Others Pulses Isolated from Sub-Saharan Africa soils. A review. *Electron. J. Pol. Agric. Univ.* **2019**, *22*, 3–14. [CrossRef]
- Giller, K.E.; Wilson, K.J. *Nitrogen Fixation in Tropical Cropping Systems*, 3rd ed.; CAB International: Nairobi, Kenya, 2011; p. 423.
- Sangakkara, U.; Frehner, M.; Nösberger, J. Influence of soil moisture and fertilizer potassium on the vegetative growth of mungbean (*Vigna radiata* L. Wilczek) and cowpea (*Vigna unguiculata* L. Walp.). *J. Agron. Crop Sci.* **2001**, *186*, 73–81. [CrossRef]
- Kiboi, M.N.; Ngetich, F.K.; Mugendi, D.N. Nitrogen budgets and flows in African smallholder farming systems. *AIMS Agric. Food* **2019**, *4*, 429–446. [CrossRef]
- Martey, E.; Wiredu, A.N.; Etwire, P.M.; Fosu, M.; Buah, S.; Bidzakin, J.; Ahiabor, B.D.; Kusi, F. Fertilizer adoption and use intensity among smallholder farmers in Northern Ghana: A case study of the AGRA soil health project. *Sustain. Agric. Res.* **2014**, *3*, 24–36. [CrossRef]
- Fening, J.; Dogbe, W.; Danso, S. Assessment of the potential to improve N fixation by cowpea (*Vigna unguiculata* (L.) Walp.) in Ghanaian soils. *Am. J. Altern. Agric.* **2001**, *16*, 57–65. [CrossRef]
- Kimiti, J.M.; Odee, D.W. Integrated soil fertility management enhances population and effectiveness of indigenous cowpea rhizobia in semi-arid eastern Kenya. *Appl. Soil Ecol.* **2010**, *45*, 304–309. [CrossRef]

17. Ferreira, L.d.V.M.; Nóbrega, R.S.A.; Nóbrega, J.C.A.; de Aguiar, F.L.; de Souza Moreira, F.M.; Pacheco, L.P. Biological nitrogen fixation in production of *Vigna unguiculata* (L.) Walp, family farming in Piauí, Brazil. *J. Agric. Sci.* **2013**, *5*, 153–160. [[CrossRef](#)]
18. Guimarães, S.L.; Cardinal, M.S.; Bonfim-Silva, E.M.; Polizel, A.C. Development of cv. BRS Novaera cowpea inoculated with rhizobium recommended for pigeonpea. *Cientifica* **2015**, *43*, 149–155. [[CrossRef](#)]
19. Ulzen, J.; Abaidoo, R.C.; Mensah, N.E.; Masso, C.; AbdelGadir, A.H. Bradyrhizobium inoculants enhance grain yields of soybean and cowpea in Northern Ghana. *Front. Plant Sci.* **2016**, *7*, 1770. [[CrossRef](#)]
20. Kyei-Boahen, S.; Savala, C.E.; Chikoye, D.; Abaidoo, R. Growth and yield responses of cowpea to inoculation and phosphorus fertilization in different environments. *Front. Plant Sci.* **2017**, *8*, 646. [[CrossRef](#)]
21. Musa, M.; Tadda, S.; Mahadi, M. Growth and yield response of cowpea (*Vigna unguiculata* L. Walp.) as affected by phosphorus levels and bradyrhizobial strains in Kano State, Nigeria. *Niger. Agric. J.* **2018**, *49*, 172–180.
22. Nyoki, D.; Ndakidemi, P.A. Effects of *Bradyrhizobium japonicum* inoculation and supplementation with phosphorus on macronutrients uptake in cowpea (*Vigna unguiculata* (L.) Walp). *Am. J. Plant Sci.* **2014**, *5*, 442–451. [[CrossRef](#)]
23. Ulzen, J.; Abaidoo, R.C.; Ewusi-Mensah, N.; Masso, C. Combined application of inoculant, phosphorus and organic manure improves grain yield of cowpea. *Arch. Agron. Soil Sci.* **2020**, *66*, 1358–1372. [[CrossRef](#)]
24. World Reference Base for Soil Resources 2014: International Soil Classification System for Naming Soils and Creating Legends for Soil Maps. 2014. Available online: <http://www.fao.org/3/a-i3794en.pdf> (accessed on 13 October 2015).
25. Bray, R.H.; Kurtz, L. Determination of total, organic, and available forms of phosphorus in soils. *Soil Sci.* **1945**, *59*, 39–46. [[CrossRef](#)]
26. Nelson, D.W.; Sommers, L.E. Total Carbon, Organic Carbon, and Organic Matter. In *Methods of Soil Analysis Part 3—Chemical Methods*; Soil Science Society of America, Inc.: Madison, WI, USA, 1996; Volume 5, pp. 961–1010.
27. Miller, L.; Houghton, J.A. The microKjeldahl determination of the nitrogen content of amino acids and proteins. *J. Biol. Chem.* **1945**, *169*, 373–383.
28. Page, A.; Miller, R.; Keeney, D. Methods of soil analysis, part 2. In *Chemical and Microbiological Properties*; American Society of Agronomy, Soil Science Society of America: Madison, WI, USA, 1982; Volume 9, p. 1143.
29. Landon, J.R. *Booker Tropical Soil Manual: A Handbook for Soil Survey and Agricultural Land Evaluation in the Tropics and Subtropics*; Routledge: New York, NY, USA, 2014; p. 530.
30. Singh, N.; Kataria, N. Role of potassium fertilizer on nitrogen fixation in Chickpea (*Cicer arietinum* L.) under quantified water stress. *J. Agric. Technol.* **2012**, *8*, 377–392.
31. Abaidoo, R.C.; Keyser, H.; Singleton, P.; Dashiell, K.E.; Sanginga, N. Population size, distribution, and symbiotic characteristics of indigenous *Bradyrhizobium* spp. that nodulate TGx soybean genotypes in Africa. *Appl. Soil Ecol.* **2007**, *35*, 57–67. [[CrossRef](#)]
32. Fening, J.; Danso, S. Variation in symbiotic effectiveness of cowpea bradyrhizobia indigenous to Ghanaian soils. *Appl. Soil Ecol.* **2002**, *21*, 23–29. [[CrossRef](#)]
33. Duke, S.; Collins, M. Role of Potassium in Legume Dinitrogen Fixation. In *Potassium in Agriculture*; Munson, R., Ed.; ASA: Madison, WI, USA; CSSA: Madison, WI, USA; SSSA: Madison, WI, USA, 2015; pp. 443–465.
34. Margelis, S.; D'Souza, C.; Small, A.J.; Hynes, M.J.; Adams, T.H.; Davis, M.A. Role of glutamine synthetase in nitrogen metabolite repression in *Aspergillus nidulans*. *J. Bacteriol.* **2001**, *183*, 5826–5833. [[CrossRef](#)]
35. Adjei-Nsiah, S.; Kuyper, T.W.; Leeuwis, C.; Abekoe, M.K.; Cobbinah, J.; Sakyi-Dawson, O.; Giller, K.E. Farmers' agronomic and social evaluation of productivity, yield and N₂-fixation in different cowpea varieties and their subsequent residual N effects on a succeeding maize crop. *Nutr. Cycl. Agroecosystems* **2008**, *80*, 199–209. [[CrossRef](#)]
36. Uzoh, I.M.; Igwe, C.A.; Okebalama, C.B.; Babalola, O.O. Legume-maize rotation effect on maize productivity and soil fertility parameters under selected agronomic practices in a sandy loam soil. *Sci. Rep.* **2019**, *9*, 8539. [[CrossRef](#)]