

NUTRIENT UPTAKE AND BIOLOGICAL NITROGEN FIXATION IN COWPEA UNDER BIOCHAR-PHOSPHORUS INTERACTION

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ABSTRACT

Research exploiting biochar-phosphorus interactions on biological nitrogen fixation (BNF) and nutrient uptake in legumes is very scarce. This paper reports the short-term effect of rice husk biochar and phosphorus application on nutrient uptake, nodulation and nitrogen fixation of cowpea in both pot and field experiments in the semi-deciduous forest zone of Ghana. Phosphorus fertilizer (triple superphosphate (TSP)) was applied at 0, 30 and 60 kg ha⁻¹ designated respectively as P₀, P₃₀ and P₆₀ while biochar was applied at 0, 2.5, 5 and 7.5 t ha⁻¹ designated respectively as B₀, B_{2.5}, B₅ and B_{7.5}. Interaction between biochar and phosphorus increased nodule dry weight, number of nodules per plant and fixed nitrogen more than the sole applications. The highest number of nodules plant⁻¹ and nodule dry weight were observed under P₃₀B₅. The highest N fixed (36.15 kg ha⁻¹) was observed under P₆₀B₅ emphasizing the role of phosphorus in BNF. Increasing biochar beyond 5.0 t ha⁻¹ decreased BNF. Nitrogen, phosphorus and potassium uptake were significantly (p ≤ 0.05) improved following biochar and TSP applications under both greenhouse and field conditions. The highest N uptake of 30.79 kg ha⁻¹ was observed under P₃₀B₅ amended plots while P₆₀B_{7.5} plots produced the highest P and K uptake. Overall, the results indicate good prospect of exploiting biochar-phosphorus interactions to enhance BNF and productivity of legumes in tropical climate.

Keywords: Biochar, cowpea, nodulation, plant nutrients, triple superphosphate

INTRODUCTION

The use of mineral fertilizer in crop production is more convenient and effective but on the other hand, a more expensive way to improve soil fertility for increased crop production. Ahmad *et al.* (2014) reported that the integrated use of organic and inorganic sources of plant nutrients will moderate the sole reliance on chemical fertilizers. The added value of leguminous crops lies in their atmospheric nitrogen fixing abilities, thus reducing the costly use of nitrogen fertilizer in enhancing soil fertility. Cowpea in this manner, sustains soil fertility and reduces farmers' sole dependence on commercial inorganic fertilizers through its nitrogen fixing ability (Odion *et al.*, 2007). However, a possible strategy for improving nitrogen fixation is by ensuring adequate mineral nutrition for the legume.

Phosphorus (P) is a major nutrient for crop growth and yield and plays key role in the regulation of enzymatic activities and in energy transformation (Schulze *et al.*, 2006; Maholtra *et al.*, 2018). It is vital in metabolic processes viz. respiration, photosynthesis, carbohydrate metabolism, synthesis of nucleic acid, activation of enzymes and signalling, among others (Zhang *et al.*, 2014). Thus, crop growth is restrained in P-deficient soils (Zhang *et al.*, 2014). In addition, inadequate P supply can negatively affect carbon absorption and distribution among above- and below-

ground plant parts (Zhang *et al.*, 2014). It has been evidenced severally that P is among the most needed nutrient elements for legume production in many tropical soils. Large increases in legume yield due to addition of phosphorus fertilizers, have been reported by many authors (Uzoma and Osundeanda, 2006; Kamara *et al.*, 2007; Yakubu *et al.*, 2010). Its functional roles in legumes viz. promotion of root development, root nodulation and increasing grain yield, have been demonstrated in many reports (Uchida, 2000; Tang *et al.*, 2001; Maholtra *et al.*, 2018). As a result of its energy-demanding nature, symbiotic nitrogen fixation requires large amounts of phosphorus (Schulze *et al.*, 2006) and the energy-generating metabolism, which depends strongly on soil P availability. Low legume yields in sub-Saharan Africa emanating from low soil fertility can be partly addressed with the application of phosphorus fertilizers (Nkaa *et al.*, 2014). However, many tropical soils are P-deficient as a result of significant levels of reactive aluminium and also surfaces, which lead to a high affinity for applied P, culminating in low agronomic P use efficiency (IUSS Working Group, 2006). Integrating phosphorus application with biochar can prove a remedial measure to enhance nutrient uptake and agronomic use efficiency in crops under these conditions.

Biochar technology has been proposed to hold promise in improving edaphic properties (Herath *et al.*, 2013; Mia *et al.*, 2014) and enhancing soil biological processes (Wang *et al.*, 2015) and crop performance. In

modern agriculture, biochar is rarely employed especially at the smallholder level, and its agronomic value and soil health benefits have not yet been fully quantified (Fahad *et al.*, 2015). Studies so far show that biochar application promotes plant growth, especially when applied in combination with N or P fertilizers in impoverished soils (Blackwell *et al.*, 2009; Major *et al.*, 2010) and reduces the leaching of nutrients (Laird *et al.*, 2009). A number of studies emphasized the potential benefits of using biochar as soil amendment (Karhu *et al.*, 2011, Amendola *et al.*, 2017). It is thus being promoted for integration into soil management programmes due to its positive impacts on edaphic properties and crop productivity (Uzoma *et al.*, 2011; Bashagaluke *et al.*, 2019). It also mitigates emission of greenhouse gases (Lehmann *et al.* 2006). Despite the individual benefits of phosphorus and biochar in crop production and soil management, research exploiting their interactive effects in biological nitrogen fixation, is limitedly studied. More so, most biochar-based studies are primarily limited to Europe, USA, Australia, China, among others (Gwenzi *et al.*, 2015; Aller *et al.*, 2018) whilst its research in Africa is virtually at the preliminary stage, yet to receive the needed attention. This study therefore aimed at contributing to the understanding of biochar-phosphorus interactions on biological nitrogen fixation and nutrient uptake of cowpea in tropical climate.

MATERIALS AND METHODS

Experimental structure: Pot and field experiments were carried out in the major cropping season of 2015 at the research field and greenhouse facility of the Faculty of Agriculture, Kwame Nkrumah University of Science and Technology, Ghana. The study site (06.68348° N, 1.55029° W) is situated in the semi-deciduous forest agro-ecological zone of Ghana. It has a bimodal rainfall regime with average annual amount of 1500 mm. The major rainy season commences in mid-March to July while the minor rainy season spans September to November. The maximum temperature during the cropping season was 30 °C while minimum temperature was 21.7 °C with average humidity of 75%. The research was carried out on a sandy loam soil classified as orthi-Ferric Acrisol (FAO, 1988).

The experimental field was manually cleared of vegetation, ploughed and harrowed and 48 plots demarcated, each measuring 3 m × 4 m. The field experiment was a factorial arranged in a Randomized Complete Block Design while the greenhouse pots filled with 9 kg soil from the experimental site were arranged in a Completely Randomized Design. The pots had diameter and height of 18 cm and 12.3 cm, respectively. Treatments used for both field and pot experiments were each replicated four times. Rice husk charred at a temperature of 450 °C was obtained from the Soil

Research Institute of the Council for Scientific and Industrial Research (CSIR), Kwadaso and applied two weeks before planting to minimize nutrient immobilization and the transient adverse impacts of biochar volatiles on crop growth (Deenik *et al.*, 2011). The test crop (“Asontem”) which is an early maturing (64 days) cowpea variety was obtained from the Legumes Division of the CSIR-Crops Research Institute, Fumesua and planted at three seeds hill⁻¹ at 60 cm x 20 cm. Two weeks after germination, seedlings were thinned to two per hill. At 80 x 40 cm spacing, three maize seeds were sown per hill and the germinated seedlings later thinned to two per hill. All cultural practices were undertaken during the growing season as and when necessary. The treatments used for both greenhouse and field experiments were:

P₀B₀ = Control (No biochar, no phosphorus fertilizer)

P₀B_{2.5} = 2.5 t ha⁻¹ biochar

P₀B₅ = 5.0 t ha⁻¹ biochar

P₀B_{7.5} = 7.5 t ha⁻¹ biochar

P₃₀B₀ = 30 kg ha⁻¹ P₂O₅

P₃₀B_{2.5} = 30 kg ha⁻¹ P₂O₅ + 2.5 t ha⁻¹ biochar

P₃₀B₅ = 30 kg ha⁻¹ P₂O₅ + 5 t ha⁻¹ biochar

P₃₀B_{7.5} = 30 kg ha⁻¹ P₂O₅ + 7.5 t ha⁻¹ biochar

P₆₀B₀ = 60 kg ha⁻¹ P₂O₅ + 0 t ha⁻¹ biochar

P₆₀B_{2.5} = 60 kg ha⁻¹ P₂O₅ + 2.5 t ha⁻¹ biochar

P₆₀B₅ = 60 kg ha⁻¹ P₂O₅ + 5 t ha⁻¹ biochar

P₆₀B_{7.5} = 60 kg ha⁻¹ P₂O₅ + 7.5 t ha⁻¹ biochar

Data collected for both pot and field experiment were separately analyzed.

Nodulation assessment: Number of nodules plant⁻¹ and nodule dry weight of the cowpea crop were determined at 50% flowering under both greenhouse and field conditions. For the greenhouse, the treatment pots were well watered at harvest to uproot plants with minimum disturbance of roots to ensure intact nodules. However, under field conditions, a spade was used to cut around the selected plant a block of soil with roughly 15 cm in diameter and 20 cm in depth. The block was pulled and gently immersed in water and the soil allowed to fall away after which nodules were collected. The fresh nodules were put in envelopes and transported to the laboratory, washed thoroughly over a 1 mm mesh sieve under tap water and counted. The nodules per plot were then put into envelopes and oven dried at 60 °C for 72 h for dry weight determination.

Shoot dry weight: At 50% flowering, ten plants were cut at the base (ca. 5 cm above ground) from the middle rows of each plot. These were oven dried for 72 h at 60 °C and the weight for each treatment plot recorded. Samples of the dry shoots were milled for each treatment and subjected to laboratory analysis.

Cowpea grain nutrient concentration and uptake: Grain samples were then oven dried at 70 °C for 72 h and

weighed. These were then finely ground and 20 g sub-samples of the ground plant material transferred into pre-marked glass containers.

Total nitrogen concentration in grain sub-samples was estimated using the macro Kjeldahl method (AOAC, 1995). Phosphorus and potassium concentrations of the grain sub-samples were determined using the calorimetric and flame-photometry methods, respectively. The nutrients uptake in kg ha⁻¹ were determined quantitatively as follows:

$$NU = \frac{\text{Grain nutrient conc.} \times \text{grain dry weight}}{100}$$

NU = Nutrients (N, P or K) uptake

Determination of nitrogen fixation: The N-difference method (Unkovich *et al.*, 2008) was used to estimate nitrogen fixation in cowpea. Samples of both the nitrogen fixing plant (cowpea) and reference crop (maize) were harvested at physiological maturity. The plant tissues were oven dried at 105 °C for 24 h and ground into 0.25 mm size. The plant N was determined using the Kjeldahl digestion method (Soil Laboratory Staff, 1984). The total amount of biologically fixed nitrogen was then calculated as;

N fixed = Total N (legume) - Total N (reference crop)

Data analysis: Data was analysed in two-way ANOVA using GenStat statistical package and means separated using the LSD (Least Significant Difference) method at 5%.

RESULTS

Initial characterization of soil and biochar: The result of the initial soil analysis is presented in Table 1. The soil was moderately acidic (pH = 5.70) with very low organic carbon content (0.49%) and moderate levels of nitrogen (0.12%) and available P (10.74 mg kg⁻¹). It was a sandy loam with bulk density of 1.37 gcm⁻³ which accords with the normal range for non – compacted mineral soils.

Phosphorus and potassium contents of the rice husk biochar used were 1.30 % and 2.77% respectively (Table 2). The biochar had low nitrogen content and a C:N ratio of 43.64, with the ash content being 42.80 %.

Influence of rice husk biochar and phosphorus fertilizer on nodulation and nitrogen fixation: The effect of phosphorus fertilizer on nodule dry weight (NDW), nodule number (NN) and nitrogen fixed in the field experiment are presented in Table 3. It was evident that P₆₀ and P₃₀ treatments significantly increased nodule dry weight over P₀ by 28% and 51%, respectively. A somewhat similar trend in NDW was observed in the greenhouse experiment (Table 4). Under field conditions, number of nodules plant⁻¹ increased significantly with

increasing phosphorus levels (Table 3). In the greenhouse experiment, P₃₀ and P₆₀ had statistically similar NN which were significantly different from that of P₀ (Table 4).

Table 1. Initial edaphic characteristics of the study site.

| Soil properties | Mean value |
|--|------------|
| pH (1:2.5) (Soil:H ₂ O) | 5.70 |
| Organic carbon (%) | 0.49 |
| Total nitrogen (%) | 0.12 |
| Available phosphorus (mg kg ⁻¹) | 10.74 |
| Exchangeable cations (cmol₍₊₎kg⁻¹ soil) | |
| Potassium | 0.02 |
| Sodium | 0.27 |
| Calcium | 1.79 |
| Magnesium | 0.86 |
| Particle size distribution | |
| % Sand | 78.40 |
| % Silt | 2.96 |
| % Clay | 18.64 |
| Bulk density (gcm ⁻³) | 1.37 |

† Values represent means of triplicate samples

Table 2. Chemical characteristics of the biochar used in the study.

| Parameters | Mean value |
|-----------------------------------|------------|
| pH water (1:2.5) (Biochar: water) | 9.26 |
| Organic carbon (%) | 33.6 |
| Ash content (%) | 42.80 |
| Total nutrients (%) | |
| Nitrogen | 0.77 |
| Phosphorus | 1.30 |
| Potassium | 2.77 |
| Calcium | 0.27 |
| Magnesium | 0.42 |
| Sodium | 1.04 |
| C/N ratio | 43.64 |

† Values represent means of triplicate samples

The P₆₀ and B₅ interacted significantly to increase the amount of nitrogen fixed more than all other treatments in the field experiment (Table 3). The NDW under biochar amended plots followed the order: B₀(56.54) ≤ B_{2.5} (59.10) ≤ B_{5.0}(64.41) B_{7.5}(66.19) and B₀ (38.14) ≤ B_{7.5} (40.12) ≤ B_{2.5}(41.90) ≤ B_{5.0}(44.56) under field (Table 3) and greenhouse conditions (Table 4), respectively. Biochar applied at 2.5 t/ha produced the highest NN with B_{7.5} being the least (Table 3). However, B_{5.0} gave the highest N-fixed while B_{7.5} produced the least. Similar trends in NDW and N fixed were observed.

The interaction results (Tables 3 and 4) showed that P₃₀B₅ and P₆₀B_{2.5} produced the highest NDW

respectively in field and pot experiments while P₀B₅ gave the least. Generally, biological nitrogen fixation parameters decreased when biochar increased beyond 5t ha⁻¹. Greenhouse results showed no significant differences among P treatments in terms of N fixed (p>0.05).

Table 3. Effect of rice husk biochar and P fertilizer on cowpea nodulation and nitrogen fixation at 50% flowering (Field experiment).

| Treatments | NDW (mg) | NN plant ⁻¹ | N fixed (kg ha ⁻¹) |
|--|----------------------|------------------------|--------------------------------|
| Phosphorus (P) levels (kg P₂O₅ ha⁻¹) | | | |
| P ₀ | 48.80 ^c | 39.11 ^c | 28.51 ^a |
| P ₃₀ | 73.50 ^a | 60.10 ^b | 26.51 ^{bc} |
| P ₆₀ | 62.37 ^b | 67.56 ^a | 27.48 ^{ab} |
| F Pr. | ≤.001 | ≤.001 | 0.51 |
| Biochar (B) rates (t ha⁻¹) | | | |
| B ₀ | 56.54 ^b | 55.25 ^{bc} | 26.94 ^{bc} |
| B _{2.5} | 59.10 ^b | 63.44 ^a | 28.00 ^{ab} |
| B ₅ | 64.41 ^{ab} | 57.35 ^b | 31.77 ^a |
| B _{7.5} | 66.19 ^a | 46.33 ^d | 23.28 ^d |
| F Pr. | 0.01 | ≤.001 | 0.01 |
| P * B interaction | | | |
| P ₀ B ₀ | 41.57 ^f | 39.80 ^{ef} | 32.67 ^{ab} |
| P ₀ B _{2.5} | 61.48 ^{cde} | 37.55 ^f | 27.06 ^{abcd} |
| P ₀ B ₅ | 18.51 ^g | 31.30 ^f | 32.62 ^{ab} |
| P ₀ B _{7.5} | 73.65 ^{bc} | 47.80 ^{de} | 21.70 ^{bcd} |
| P ₃₀ B ₀ | 70.39 ^{bcd} | 48.52 ^{de} | 30.14 ^{abcd} |
| P ₃₀ B _{2.5} | 67.44 ^{bcd} | 74.97 ^b | 30.74 ^{abc} |
| P ₃₀ B ₅ | 116.45 ^a | 77.80 ^{ab} | 26.55 ^{abcd} |
| P ₃₀ B _{7.5} | 39.73 ^f | 32.13 ^f | 18.61 ^{cd} |
| P ₆₀ B ₀ | 57.66 ^{de} | 77.43 ^{ab} | 18.01 ^d |
| P ₆₀ B _{2.5} | 48.37 ^{ef} | 84.80 ^a | 26.21 ^{abcd} |
| P ₆₀ B ₅ | 63.61 ^{cde} | 55.95 ^{cd} | 36.15 ^a |
| P ₆₀ B _{7.5} | 79.84 ^b | 59.05 ^c | 29.53 ^{abcd} |
| F Pr. | ≤.001 | ≤.001 | ≤.001 |
| CV (%) | 10.2 | 6.7 | 15.1 |

‡NDW= Nodule dry weight, NN= Nodule number, P₀: P₂O₅ at 0 kg/ha, P₃₀: P₂O₅ applied at 30 kg/ha, P₆₀: P applied at 60 kg/ha; B₀: No biochar applied, B_{2.5}: Biochar applied at 2.5 t/ha, B₅: Biochar applied at 5 t/ha, B_{7.5}: Biochar at 7.5 t/ha

Influence of biochar and phosphorus on grain nutrient uptake: The effect of rice husk biochar and phosphorus on N, P and K uptake into cowpea grain under field conditions is as presented in Fig. 1. There was no significant difference in N uptake between the three phosphorus rates. Similarly, no significant differences were observed in P uptake into cowpea grain under the different levels of P. The highest K uptake was observed on plots that received 60 kg ha⁻¹ P₂O₅. On biochar-amended plots, the highest N uptake (P ≤ 0.05) into grains was observed under B_{2.5} (Fig. 1). On the other

hand, lower P and K uptake values were observed in B₀ (5.05 kg ha⁻¹) and B₅ (7.54 kg ha⁻¹) respectively. The interaction of phosphorus and rice husk biochar was significant with respect to N, P and K partitioned into cowpea grain. The highest N uptake was observed on P₃₀B₅ treated plot, which was statistically at par (p>0.05) with uptake values on P₀B_{2.5} and P₆₀B_{2.5} plots (Results not shown).

Table 4. Effect of rice husk biochar and P fertilizer on cowpea nodulation and nitrogen fixation at 50% flowering (Greenhouse experiment).

| Treatments | NDW (mg) | NN plant ⁻¹ | N fixed (kg ha ⁻¹) |
|--|---------------------|------------------------|--------------------------------|
| Phosphorus (P) levels (kg P₂O₅ ha⁻¹) | | | |
| P ₀ | 35.46 ^c | 25.77 ^b | 27.48 ^a |
| P ₃₀ | 41.85 ^b | 43.35 ^a | 27.16 ^a |
| P ₆₀ | 46.24 ^a | 41.02 ^a | 27.25 ^a |
| F Pr. | ≤.001 | ≤.001 | 0.41 |
| Biochar (B) rates (t ha⁻¹) | | | |
| B ₀ | 38.14 ^{bc} | 36.25 ^b | 27.65 ^b |
| B _{2.5} | 41.90 ^a | 43.08 ^a | 29.35 ^a |
| B ₅ | 44.56 ^a | 36.53 ^b | 27.81 ^b |
| B _{7.5} | 40.12 ^{ab} | 31.00 ^c | 24.38 ^c |
| F Pr. | 0.01 | ≤.001 | ≤.001 |
| P * B interaction | | | |
| P ₀ B ₀ | 34.27 ^{cd} | 26.33 ^e | 28.15 ^{bc} |
| P ₀ B _{2.5} | 40.42 ^{bc} | 24.83 ^{de} | 29.85 ^{ab} |
| P ₀ B ₅ | 25.79 ^d | 18.92 ^e | 26.86 ^{cd} |
| P ₀ B _{7.5} | 41.35 ^{bc} | 33.00 ^{cd} | 25.08 ^{ef} |
| P ₃₀ B ₀ | 41.33 ^{bc} | 34.17 ^{cd} | 26.05 ^{de} |
| P ₃₀ B _{2.5} | 46.75 ^b | 52.75 ^a | 28.59 ^{bc} |
| P ₃₀ B ₅ | 38.52 ^{bc} | 56.33 ^a | 30.64 ^a |
| P ₃₀ B _{7.5} | 34.44 ^{cd} | 20.83 ^e | 23.36 ^f |
| P ₆₀ B ₀ | 38.81 ^{bc} | 48.25 ^{ab} | 28.76 ^b |
| P ₆₀ B _{2.5} | 62.42 ^a | 51.67 ^a | 29.60 ^{ab} |
| P ₆₀ B ₅ | 45.48 ^{bc} | 34.33 ^{cd} | 25.94 ^{de} |
| P ₆₀ B _{7.5} | 44.58 ^{bc} | 39.17 ^{bc} | 24.68 ^{ef} |
| F Pr. | ≤.001 | ≤.001 | ≤.001 |
| CV (%) | 11.3 | 11.5 | 2.2 |

‡Values represent means of triplicate samples. †NN=Nodule number, NDW= Nodule dry weight,

Under greenhouse conditions, P₆₀ produced the highest grain N uptake, which was significantly more than that of P₀ and P₃₀ (Fig. 2). Appreciable differences in N uptake were noticed under the biochar-amended plots (Fig. 2). The P and K uptake under the rice husk treatments followed a similar trend as N uptake. Under combined effects, P₆₀B_{2.5} treatment had the highest N uptake whilst the highest K uptake was recorded on P₆₀B_{7.5} plots (Interaction results not shown).

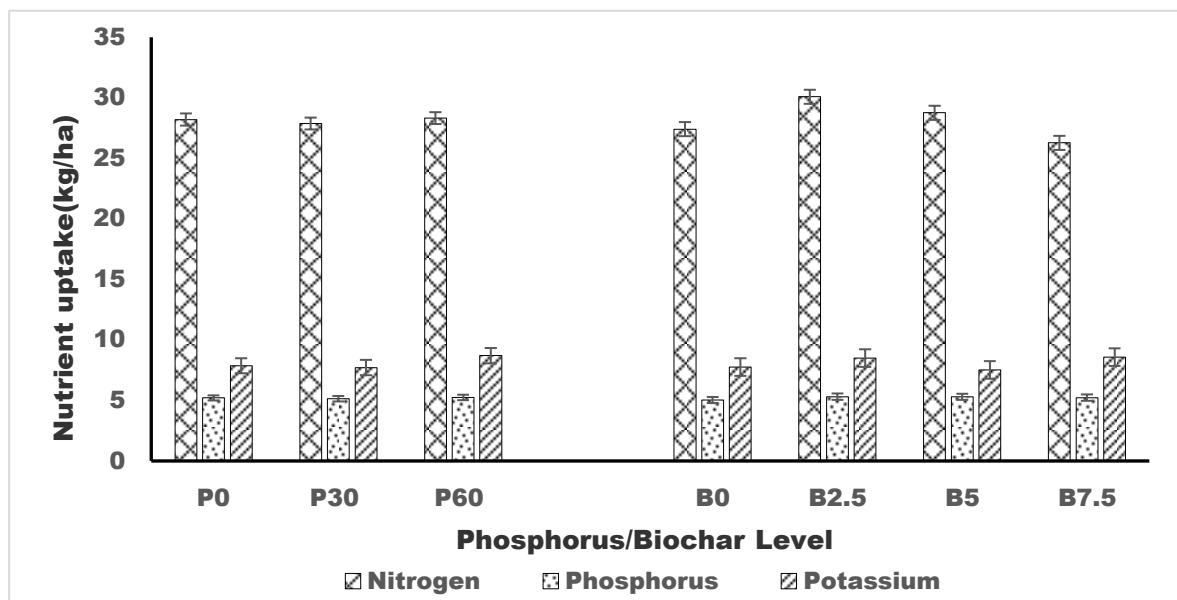


Fig. 1. Effect of rice husk biochar and phosphorus application on cowpea grain N, P and K uptake in field experiment; P0 = 0 kg ha⁻¹ P₂O₅, P30= 30 kg ha⁻¹ P₂O₅, P60 = 60 kg ha⁻¹ P₂O₅; B0= Biochar at 0 t ha⁻¹, B2.5 = biochar at 2.5t ha⁻¹, B5= biochar at 5t ha⁻¹, B7.5= biochar at 7.5 t ha⁻¹; bars are LSD values.

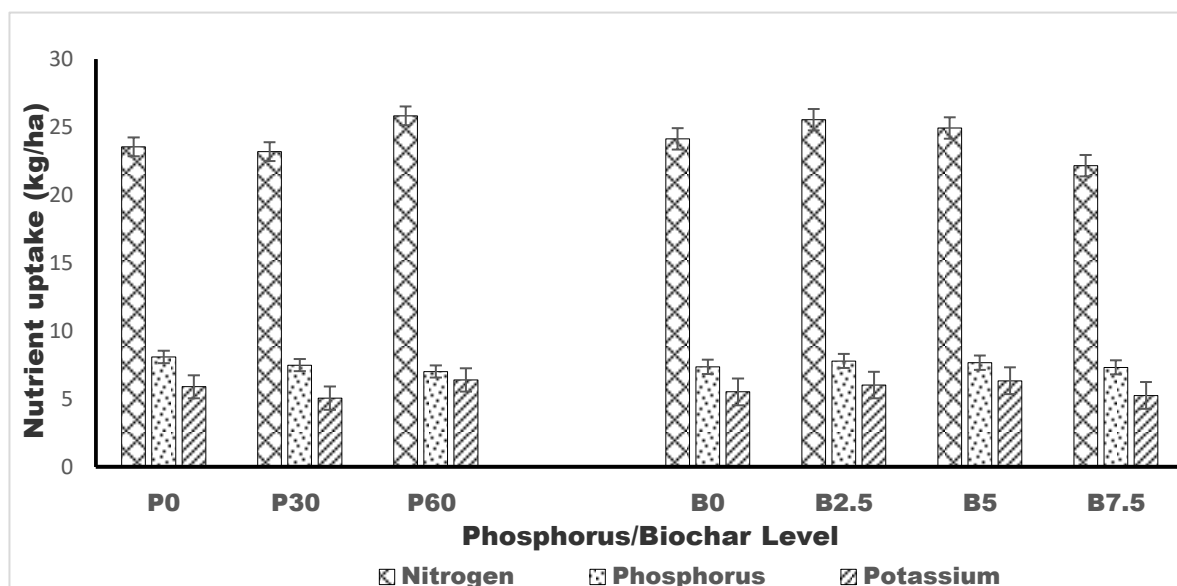


Fig. 2. Effect of rice husk biochar and phosphorus application on cowpea grain N, P and K uptake in pot experiment; P0 = 0 kg ha⁻¹ P₂O₅, P30= 30 kg ha⁻¹ P₂O₅, P60 = 60 kg ha⁻¹ P₂O₅; B0= Biochar at 0 t ha⁻¹, B2.5 = biochar at 2.5t ha⁻¹, B5= biochar at 5t ha⁻¹, B7.5= biochar at 7.5 t ha⁻¹; bars are LSD values.

Effect of mineral phosphorus and biochar application on cowpea biomass and grain yields: Mineral phosphorus fertilizer and biochar application generally influenced cowpea biomass and grain yields in both pot and field experiments (Tables 5 and 6). Among the P levels in the field experiment (Table 5), the highest

above ground biomass of 594.23 kg ha⁻¹ was recorded under P₃₀. However, the highest grain yield was recorded under P₆₀ in the field experiment (Table 5). There were significant differences in grain yield between the biochar rates in both experiments. Combined application of phosphorus and biochar led to significant differences in

grain yield. In the field experiment, the interaction effect on the above ground biomass was highest and lowest under P₆₀B₅ and P₆₀B₀, respectively. Pod weight was

Table 5. Effect of mineral phosphorus and biochar on cowpea yield, pod weight, 100 seed weight, biomass yield and grain yield (field experiment).

| Treatments | Pod weight | AGB | Grain yield | 100 SW (g) |
|--|----------------------|----------------------|----------------------|--------------------|
| kg ha⁻¹ | | | | |
| Phosphorus (P) levels (kg P₂O₅ ha⁻¹) | | | | |
| P ₀ | 619.61 ^a | 583.75 ^a | 408.09 ^a | 14.94 ^a |
| P ₃₀ | 500.44 ^b | 594.23 ^a | 360.86 ^b | 15.51 ^a |
| P ₆₀ | 613.31 ^a | 469.69 ^b | 438.91 ^a | 15.10 ^a |
| F Pr. | ≤.001 | ≤.001 | 0.01 | 0.40 |
| Biochar (B) rates (t ha⁻¹) | | | | |
| B ₀ | 514.94 ^c | 528.39 ^{bc} | 386.39 ^b | 15.49 ^a |
| B _{2.5} | 584.02 ^b | 546.80 ^b | 411.29 ^{ab} | 14.88 ^a |
| B ₅ | 675.82 ^a | 633.88 ^a | 439.41 ^a | 15.02 ^a |
| B _{7.5} | 536.39 ^c | 487.82 ^c | 373.38 ^b | 15.34 ^a |
| F Pr. | ≤.001 | ≤.001 | 0.04 | 0.60 |
| P * B interaction | | | | |
| P ₀ B ₀ | 623.10 ^c | 633.24 ^a | 436.32 ^a | 14.94 ^a |
| P ₀ B _{2.5} | 520.74 ^{de} | 569.14 ^{ab} | 392.35 ^b | 14.84 ^a |
| P ₀ B ₅ | 775.57 ^a | 631.13 ^a | 448.04 ^a | 14.71 ^a |
| P ₀ B _{7.5} | 559.04 ^d | 501.47 ^b | 355.65 ^b | 15.27 ^a |
| P ₃₀ B ₀ | 554.67 ^d | 654.24 ^a | 418.03 ^b | 16.06 ^a |
| P ₃₀ B _{2.5} | 595.47 ^c | 611.75 ^a | 391.77 ^b | 14.87 ^a |
| P ₃₀ B ₅ | 474.48 ^e | 615.16 ^a | 357.45 ^b | 15.04 ^a |
| P ₃₀ B _{7.5} | 377.13 ^f | 495.78 ^b | 276.17 ^c | 16.08 ^a |
| P ₆₀ B ₀ | 367.04 ^f | 297.69 ^c | 304.82 ^c | 15.47 ^a |
| P ₆₀ B _{2.5} | 635.84 ^b | 459.51 ^b | 449.75 ^{ab} | 14.95 ^a |
| P ₆₀ B ₅ | 777.39 ^a | 655.33 ^a | 512.73 ^a | 15.30 ^a |
| P ₆₀ B _{7.5} | 672.98 ^b | 466.22 ^b | 488.32 ^a | 14.68 ^a |
| F Pr. | ≤.001 | ≤.001 | ≤.001 | 0.81 |
| CV (%) | 5.6 | 12.9 | 15.8 | 8 |

Values are means of four replicates; SW: seed weight, AGB: above ground biomass; P₀: P₂O₅ at 0 kg/ha, P₃₀: P₂O₅ applied at 30 kg/ha, P₆₀: P applied at 60 kg/ha; B₀: No biochar applied, B_{2.5}: Biochar applied at 2.5 t/ha, B₅: Biochar applied at 5 t/ha, B_{7.5}: Biochar at 7.5 t/ha

DISCUSSION

Cowpea nodulation and nitrogen fixation: Application of phosphorus increased nodulation in cowpea (Table 3). This is attributable to the role of phosphorus in the growth and development of plants, as it is needed in their molecular structure to facilitate transformation of energy and regulation of several enzymatic activities (Schulze *et al.*, 2006, Maholtra *et al.*, 2018). Rotaru and Sinclair (2009) reported phosphorus as essential in energy transformations in nodules of legumes. Phosphorus deficiency in soybean has been found to have a more

significant among the various treatment combinations whilst the interaction effect on 100 grain weight was not significant ($p > 0.05$) in the pot experiment (Table 6).

Table 6. Effect of biochar and phosphorus application on yield components of cowpea (Pot experiment),

| Treatments | PW | AGB | Grain yield | 100 SW (g) |
|---|---------------------|----------------------|----------------------|--------------------|
| kg/ha⁻¹ | | | | |
| Phosphorus (P0) levels (kg P₂O₅ ha⁻¹) | | | | |
| P ₀ | 550.99 ^a | 276.80 ^b | 429.82 ^a | 14.78 ^a |
| P ₃₀ | 548.22 ^a | 289.06 ^a | 419.33 ^b | 14.43 ^a |
| P ₆₀ | 560.12 ^a | 270.64 ^b | 428.62 ^{ab} | 15.82 ^a |
| F Pr. | 0.47 | ≤.001 | 0.09 | 0.22 |
| Biochar (B) rates (t ha⁻¹) | | | | |
| B ₀ | 508.02 ^c | 325.65 ^a | 386.52 ^d | 15.22 ^a |
| B _{2.5} | 618.13 ^a | 288.84 ^b | 475.28 ^a | 15.26 ^a |
| B ₅ | 559.31 ^b | 262.35 ^c | 429.87 ^b | 14.83 ^a |
| B _{7.5} | 526.98 ^c | 238.5 ^d | 412.04 ^c | 14.75 ^a |
| F Pr. | ≤.001 | ≤.001 | ≤.001 | 0.93 |
| P * B application | | | | |
| P ₀ B ₀ | 510.87 ⁱ | 333.77 ^a | 394.40 ⁱ | 15.15 ^a |
| P ₀ B _{2.5} | 596.39 ^d | 261.70 ^b | 461.79 ^e | 15.50 ^a |
| P ₀ B ₅ | 534.11 ^g | 281.68 ^b | 419.93 ^g | 13.57 ^a |
| P ₀ B _{7.5} | 562.57 ^f | 230.07 ^c | 443.18 ^d | 14.90 ^a |
| P ₃₀ B ₀ | 498.49 ^j | 317.69 ^a | 374.90 ^k | 15.42 ^a |
| P ₃₀ B _{2.5} | 618.27 ^c | 306.87 ^a | 477.63 ^b | 14.10 ^a |
| P ₃₀ B ₅ | 624.56 ^b | 289.67 ^b | 466.86 ^c | 15.25 ^a |
| P ₃₀ B _{7.5} | 451.56 ^k | 242.01 ^b | 357.94 ⁱ | 12.95 ^a |
| P ₆₀ B ₀ | 514.72 ⁱ | 325.49 ^a | 390.25 ^j | 15.07 ^a |
| P ₆₀ B _{2.5} | 639.72 ^a | 297.95 ^{ab} | 486.40 ^a | 16.18 ^a |
| P ₆₀ B ₅ | 519.25 ^h | 215.69 ^c | 402.83 ^h | 15.65 ^a |
| P ₆₀ B _{7.5} | 566.81 ^e | 243.42 ^c | 434.99 ^f | 16.40 ^a |
| F Pr. | ≤.001 | 0.01 | ≤.001 | 0.573 |
| CV (%) | 5.1 | 5.5 | 3.4 | 15.5 |

PW: Pod weight, AGB: Above ground biomass, SW: Seed weight, GY: Grain yield; P₀: P₂O₅ at 0 kg/ha, P₃₀: P₂O₅ applied at 30 kg/ha, P₆₀: P applied at 60 kg/ha; B₀: No biochar applied, B_{2.5}: Biochar applied at 2.5 t/ha, B₅: Biochar applied at 5 t/ha, B_{7.5}: Biochar at 7.5 t/ha.

direct impact on nodule functioning (Israel, 1993; Vardien, 2015). Moreover, specific nodule activity presents higher ATP requirements for functioning of nitrogenase (Ribet and Drevon, 1996). The direct relationship between biological nitrogen fixation and phosphorus content shows its importance in legume productivity (Rotaru and Sinclair, 2009; Cabeza *et al.*, 2014). In their study, Hernandez *et al.* (2009) reported that the growth of N-fixing legumes under inadequate phosphorus supply was adversely affected because nitrogen fixation on bacteroids, ammonium absorption of amino acids and ureides in the plant cell of nodules were

not enough to support plant growth. These processes require more phosphorus in the transfer of energy for nodule functioning (Rotaru *et al.*, 2014) as depicted by higher nodule dry weight on P amended plots in our study (Tables 3 & 4).

Though biochar has received research attention widely, its interaction with phosphorus on biological nitrogen fixation is scarcely studied. In this study, the highest N fixed was observed under P₆₀B₅ and P₃₀B₅, respectively in field and pot experiments (Tables 3 & 4) showing the positive interaction between biochar and P application in enhancing biological nitrogen fixation. However, the decrease in nodulation and nitrogen fixation beyond 5t ha⁻¹ biochar was possibly a result of increased C: N ratio (Quilliam *et al.*, 2012), which reduced nutrient uptake (Figs. 1 & 2) for biological nitrogen fixation. The infection process of nodule-forming bacteria, involving chemotaxis, includes complex signalling pathways; resulting in nodule formation in legumes being initiated by the release of host-derived flavonoids which are polyphenolic signalling compounds (Slattery *et al.*, 2001; Makoi and Ndakidemi, 2007). Though biochar has high affinity for the adsorption of polyphenolic compounds (Komnitsas and Zaharaki, 2016; Mubarik *et al.*, 2016), its incorporation into the soil at moderate rates (2.5-5.0 t ha⁻¹) with mineral phosphorus did not interfere with these signalling pathways but probably did so at higher rate (7.5 t ha⁻¹) resulting in decreased N fixation at this rate (Tables 3&4). Our results have thus shown that applying biochar at moderate rate (up to 5 t ha⁻¹) in combination with phosphorus can enhance biological nitrogen fixation in cowpea. Although the highest nodule dry weight was obtained under the highest biochar rate in sole application (B_{7.5}) (Table 3) in the field experiment, this was not consistent across both experiments coupled with the fact that the highest nodule dry weight observed in this treatment did not translate correspondingly into the highest N fixed. Although higher number of nodules was produced under P₆₀B_{2.5} than P₆₀B₅ (Table 3), nodule dry weight was relatively lower with lower N fixed than under P₆₀B₅. This was a result of the fact that sink demand for photosynthate was partitioned between fewer nodules (Quilliam *et al.*, 2012) under P₆₀B₅ resulting in greater biomass per nodule and higher N fixed perhaps due to more effective nodules.

Cowpea nodulation and nitrogen fixation: Application of phosphorus increased nodulation in cowpea (Table 3). This is attributable to the role of phosphorus in the growth and development of plants, as it is needed in their molecular structure to facilitate transformation of energy and regulation of several enzymatic activities (Schulze *et al.*, 2006, Maholtra *et al.*, 2018). Rotaru and Sinclair (2009) reported phosphorus as essential in energy transformations in nodules of legumes. Phosphorus deficiency in soybean has been found to have a more

direct impact on nodule functioning (Israel, 1993; Vardien, 2015). Moreover, specific nodule activity presents higher ATP requirements for functioning of nitrogenase (Ribet and Drevon, 1996). The direct relationship between biological nitrogen fixation and phosphorus content shows its importance in legume productivity (Rotaru and Sinclair, 2009; Cabeza *et al.*, 2014). In their study, Hernandez *et al.* (2009) reported that the growth of N-fixing legumes under inadequate phosphorus supply was adversely affected because nitrogen fixation on bacteroids, ammonium absorption of amino acids and ureides in the plant cell of nodules were not enough to support plant growth. These processes require more phosphorus in the transfer of energy for nodule functioning (Rotaru *et al.*, 2014) as depicted by higher nodule dry weight on P amended plots in our study (Tables 3 & 4).

Though biochar has received research attention widely, its interaction with phosphorus on biological nitrogen fixation is scarcely studied. In this study, the highest N fixed was observed under P₆₀B₅ and P₃₀B₅, respectively in field and pot experiments (Tables 3 & 4) showing the positive interaction between biochar and P application in enhancing biological nitrogen fixation. However, the decrease in nodulation and nitrogen fixation beyond 5t ha⁻¹ biochar was possibly a result of increased C: N ratio (Quilliam *et al.*, 2012), which reduced nutrient uptake (Figs. 1 & 2) for biological nitrogen fixation. The infection process of nodule-forming bacteria, involving chemotaxis, includes complex signalling pathways; resulting in nodule formation in legumes being initiated by the release of host-derived flavonoids which are polyphenolic signalling compounds (Slattery *et al.*, 2001; Makoi and Ndakidemi, 2007). Though biochar has high affinity for the adsorption of polyphenolic compounds (Komnitsas and Zaharaki, 2016; Mubarik *et al.*, 2016), its incorporation into the soil at moderate rates (2.5-5.0 t ha⁻¹) with mineral phosphorus did not interfere with these signalling pathways but probably did so at higher rate (7.5 t ha⁻¹) resulting in decreased N fixation at this rate (Tables 3&4). Our results have thus shown that applying biochar at moderate rate (up to 5 t ha⁻¹) in combination with phosphorus can enhance biological nitrogen fixation in cowpea. Although the highest nodule dry weight was obtained under the highest biochar rate in sole application (B_{7.5}) (Table 3) in the field experiment, this was not consistent across both experiments coupled with the fact that the highest nodule dry weight observed in this treatment did not translate correspondingly into the highest N fixed. Although higher number of nodules was produced under P₆₀B_{2.5} than P₆₀B₅ (Table 3), nodule dry weight was relatively lower with lower N fixed than under P₆₀B₅. This was a result of the fact that sink demand for photosynthate was partitioned between fewer nodules (Quilliam *et al.*, 2012) under P₆₀B₅ resulting in

greater biomass per nodule and higher N fixed perhaps due to more effective nodules.

Cowpea grain nutrient uptake: Crop nutrient uptake is dependent on soil fertility status and synthetic fertilizer application at various stages of crop growth. Our study shows that phosphorus and biochar applications influenced nutrient uptake in grains (Figs. 1&2). Phosphorus uptake under P₆₀ in cowpea grain was significantly higher than the control and P₃₀ signifying that uptake somewhat increased with phosphorus fertilizer application (Fig. 1) as a result of increased P concentration in soil solution (Logah *et al.*, 2013). As observed for biological N fixation, nodule dry weight, nodule numbers and nutrient (N,P, K) uptake in grains decreased for biochar rate greater than 5 t ha⁻¹ giving ample indication that biochar application up to this rate is enough to cause positive agronomic response in cowpea and perhaps other related legumes. The lower response observed at 7.5 t ha⁻¹ may be attributed to a shift in soil C: N ratio (Quilliam *et al.*, 2012) as biochar rate increased. The increased uptake of N under lower rice husk biochar rates (2.5-5.0 t ha⁻¹) is indicative of its ability to enhance fertilizer use efficiency in soils where N loss constitutes a major environmental problem (Nigussie *et al.*, 2012). Biochar is known to improve soil structure and nutrient holding capacity, thus preventing leaching losses into underground water. According to Lehmann (2007) and Brown (2009), increased nutrient adsorption capacity of biochar-amended soil reduced total fertilizer requirements.

Nutrient uptake values recorded in grains under combined applications of phosphorus and biochar was not greater than the sum of uptake values of either applications (results not shown) highlighting antagonistic interactions which also characterizes many integrated soil fertility management measures in the tropics. This, however, does not downplay the importance of integrated nutrient management in legume production but calls for efforts geared towards enhancing synergistic interactions in biochar-phosphorus applications in tropical cropping systems.

Cowpea biomass and grain yields: The increased grain yield in 2.5 and 5t ha⁻¹ biochar treatments over the control (Tables 5&6) is attributable to the generally enhanced soil nutrient levels under these application rates (Results not shown). The agronomic parameters measured viz. grain yield, the above ground biomass, 100 seed weight and the pod weight decreased when biochar exceeded 5t ha⁻¹ probably as a result of shift in C: N ratio under higher biochar rate speculated earlier. Several authors reported positive impacts on crop yield when biochar was applied as soil amendment. Purakayastha (2010) reported increase in yield of maize with biochar and NPK application in an Inceptisol. Positive effect on biomass yield following biochar application and mineral

fertilizer applications were reported by Bashagaluke *et al.* (2019) for maize in Ghana whilst Steiner *et al.* (2007) and Asai *et al.* (2009) reported positive effects for grain yield of upland rice in Brazil and Northern Laos, respectively. There were improvements in biomass yield at P₆₀B₅ (Table 5) and grain yield at P₆₀B_{2.5} (Table 6) compared to the individual effects of these amendments. Though this was not synergistic, the results pointed to the added benefits derived from the integrated use of P fertilizer and biochar as the former readily makes available nutrient for early crop growth whilst the latter improves soil conditions for enhanced growth.

Conclusion: The interaction between phosphorus and biochar increased nodule dry weight, number of nodules per plant and nitrogen fixed more than the sole applications. The study highlighted the importance of P in nodulation as more N was fixed at increasing P levels (though at diminishing returns) but reduced at increasing biochar rates. Increasing biochar rate beyond 5t ha⁻¹ decreased biological nitrogen fixation and nutrients uptake in cowpea in the short term. The use of mineral phosphorus fertilizer at 30 and 60 kg ha⁻¹ with rice husk biochar up to to 5 t ha⁻¹ generally increased the grain yield and pod weight of cowpea. The study showed the positive interaction effect of biochar and phosphorus in enhancing biological nitrogen fixation, which hitherto was lacking in most studies.

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