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Biochar soil amendments affect mycorrhizal colonization, root nodulation and dry matter accumulation in cowpeas (*Vigna unguiculata* (L.) Walp.)

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Abstract. There is increasing demand for renewable inputs to improve soil productivity to satisfy the ever-growing human population. Biochar as a renewable resource has attracted attention over the years as soil amendment, notable for climate change mitigation and crop improvement. This study assessed the impact of pine wood biochar soil amendments on mycorrhizal root colonization, root nodulation, growth and yield of cowpeas in 2017 and 2018. The experimental design was a randomized complete block design with three replications. Biochar was applied with or without inorganic fertilizer ($NPK_{15:15:15}$), cattle dung and nutrient source control two weeks before planting cowpea. Data on growth parameters, number of nodules, and plant dry matter accumulation were collected. Mycorrhizal colonization and spore counts associated with root nodules were determined under laboratory conditions. The results revealed that biochar amendments significantly affected nodulation, vine length, root length and weight. Percentage improvement was as follows: vine length by 8.8%, number of nodules by 14%, root length by 9.5%, root weight by 5.2% and dry matter accumulation by 92%. Application of NPK improved root length and weight over the control. Stereomicroscopic visualization of the roots indicated high presence of arbuscules in the biochar treatment over the control. Biochar addition is essential for improvement of rhizosphere traits essential for plant growth enhancement.

Keywords: arbuscules, glomus species, mycorrhizal spore count, pine wood biochar, vesicles.

Introduction

Mycorrhizal colonization and root nodulation by rhizobia are important plant-microbe symbiotic interactions that benefit both plants and mycorrhizal fungi. Microorganisms in symbiotic association with plants are known for enhancing plant growth and increasing crop yield. Through mycorrhizal associations, plants obtain phosphorus from soil that is otherwise difficult for plants to reach (Igiehon and Babalola, 2017; Emmanuel *et al.*, 2021). This access to extra nutrients enhances plant performance. Symbiotic relationships between leguminous plants and rhizobia stimulate nodule formation, offering the plant an additional source of nitrogen. Beyond access to nutrients, microorganisms offer protection against pathogens while producing growth-promoting hormones (Omomowo & Babalola, 2023). Plants attract these beneficial microorganisms through the secretion of root exudates, which include sugars, amino acids, and organic acids that act as chemical signals and energy sources, thereby creating a suitable environment for microbial colonization in the rhizosphere (Uzoh & Babalola, 2020; Akanmu *et al.*, 2021). Cowpea (V*igna unguiculata*), a leguminous plant, owes its rich protein content to the microbial colonization in its rhizosphere (Agbodjato and Babalola, 2024).

Cowpea is a source of protein for rural and urban dwellers in sub-Saharan Africa, where it is widely grown (Omomowo & Babalola, 2021; Atugwu *et al.*, 2023). It is commonly cultivated in the West and Central Africa. Cowpea contains 20 - 25% protein in their dry seed (Ddamulira *et al.*, 2015). The fresh leaves and young pods are consumed as vegetables; the dry seeds are used for different food preparations, while the leaves are fed to livestock as fodder. It can tolerate drought and poor soil (Chinma *et al.*, 2008). Cowpeas are important part of agricultural production systems since nitrogen fixation makes agricultural systems sustainable. Nitrogen requirement of subsequent crop or intercrop is thereby reduced. In addition, cowpeas' canopy protects the soil from the harmful effect of winds and runoff, thus preventing erosion.se of cowpea in cropping systems has increased farmers adoption of legume-based cropping systems.

Despite the roles cowpea plays in agricultural diversification of cropping systems, cowpea yield is still below expectation (Atugwu *et al*.,2023). A contributory factor to this poor yield is the low fertility of soils of savannah and semi-arid zones where cowpeas are prominently grown (Emmanuel *et al*., 2020; Ayangbenro *et al*., 2023). The misconception that cowpea does not need soil amendments due to its nitrogen fixing potential exacerbates the low yield. The study by Atugwu *et al*. (2023) demonstrates that cowpea responds well to soil amendments. Hence, any agronomic practice that improves soil fertility can improve the yield of cowpea.

Biochar, a product of organic matter pyrolysis, has the ability to improve soil health and sequence carbon when applied as soil amendments (Atkinson *et al*., 2010; Lehmann and Joseph, 2015). In addition, biochar improves crop yield, and enhances soil productivity (Chibuike-Ezepue *et al*. (2019)). The use of biochar has gained increasingly acceptance especially in the tropics, where the soils are highly weathered and low in productivity.

Crop improvements as a result of biochar application to the soil are attributed mainly to the biochar as soil conditioner, nutrient addition directly from the applied biochar and/or improvement of nutrient utilization efficiency, especially when combined with other organic or inorganic amendments (Chibuike-Ezepue *et al*., 2019). Research on biochar alone or combined with other fertilizers as a soil amendment for crop yield improvement reported positive effects on crop growth (Chen *et al*., 2010; Shen *et al*., 2016; Solaiman *et al*., 2010). However, information on the relationship between changes in soil properties, particularly biological properties, after biochar addition and the subsequent effect on crop growth is still lacking (Chibuike-Ezepue *et al*., 2019). Such biological properties include symbiotic relationships between plants and microorganisms (Babalola *et al*., 2007). There is a need to decipher the effect of biochar on nitrogen-fixing organisms and mycorrhiza root colonization, which are pivotal in N and P nutrition in tropical soils. Root nodules, which harbor rhizobia that fix nitrogen, could be used as a measure of the effect of biochar on nitrogen fixation and on the rhizobia growth (Xin *et al*., 2022).

Despite the growing interest in sustainable agriculture, a significant gap remains in our understanding of the effects of biochar on microbial symbioses, particularly in relation to root nodules, mycorrhizal colonization, and spore count. This study aims to address this gap by investigating the impact of biochar, both alone and in combination with NPK (15:15:15) or cattle dung, on cowpea. By exploring these interactions, we can enhance our knowledge of biochar's role in promoting plant health and productivity, which is crucial for developing more effective agricultural practices.

Materials and methods

Site description for the field experiment

The fieldwork was done in Faculty of Natural and Agricultural Science Farmland in North-West University, Mafikeng campus (longitude 25° 48' S, latitude 25° 38' E; 1218 m asl) in North West Province, South Africa. Mafikeng has a typical semi-arid tropical savanna climate and receives summer annual mean rainfall of 571 mm. The surface (0-20 cm) soil at the site is brown to dark reddish brown sandy loam classified as Hutton form according to the South African soil classification system (Soil Classification Working Group, 1991).

Experimental design and treatments

The experimental design used in this study was a randomized complete block design with three replications. Land area measuring 210 m^2 (15 m x 14 m) was mechanically ploughed and harrowed before being demarcated into 18 plots (6 treatments by 3 replications/blocks). The plot size was 3.75 m x 1.25 m with intra- and inter-block spacing of 1 m. Three cowpea seeds were planted per hole at a spacing of 0.75 m x 0.25 m, which were thinned down to one per hole three weeks after germination. The final plant population was 20 plants per plot. The experimental plots were manually kept weed free, while irrigation was applied using the sprinkler method.

The six treatments evaluated in this study are (i) sole application of biochar produced from pine wood at the rate of 5 tonnes per hectare, (ii) sole application of cattle dung at the rate of 5 tonnes per hectare, (iii) sole application of mineral fertilizer $NPK_{15:15:15,}$ at the rate of 60 kg per hectare (iv) combined application of biochar and cattle dung at half the rate of the sole applications, (v) combined application of biochar and cattle dung at half the rate of the sole applications, and (vi) control (no soil amendment).

The cattle dung was applied two weeks before planting to allow for mineralization, while the biochar and NPK were applied at planting. The experiment was established in October 2017 and terminated in February 2018. To assess the residual impact of the treatments on soil fertility, cowpeas were planted on the plots without applying other treatments. The second field planting was established in February 2018 after harvesting the initial planting was harvested and was terminated in June 2018.

Sample/Data collection

The chlorophyll content, vine length and girth, leaf length and girth, number of root nodules, shoot and root fresh/dry weight and mycorrhizal colonization were determined as detailed below: The chlorophyll meter (CCM-200 plus) was used to measure chlorophyll content in cowpea leaves. The measurements were done 3 times in the first cultivation, while in residual biochar evaluation, measurements were done twice. The first measurement for each cropping period was carried out one month after treatment application and subsequently at monthly intervals. A measuring tape was used to estimate vine length, girth, and leaf length before and after the cowpea legume was uprooted. The measurements were recorded.

Root harvesting and nodule count

A day before root harvesting, the plot was irrigated to wet the soil for root harvesting so that root nodules would not be lost in the soil. After root harvesting, roots were washed in a basin under running water. The nodules from washed roots were counted.

Yield parameters

The fresh roots and shoots were air-dried inside the screen house, and their weight was obtained by weighing them on a weighing balance (RADWAG WagiElektroniczne, Poland).

Laboratory analysis

Cowpea root mycorrhizal colonization. The roots were cut into pieces and cleared in 2% (w/v) KOH for 15 min at 120°C in an autoclave. Using a fine sieve, the roots were rinsed with water three times and then covered with 2% (v/v) hydrogen chloride (HCl) for 30 min. After 30 min, the HCl was thrown away, and the roots were covered with 0.05% (w/v) trypan blue in lacto glycerol for 15 mins in an autoclave. The roots were then placed onto a petri dish with 50% (v/v) glycerol for de-staining and were viewed under a stereomicroscope.

Mycorrhiza spore extraction and characterization. Mycorrhiza spores were extracted by the sucrose floatation method. After extraction, microscopic slides were prepared, and different mycorrhiza species were identified, counted and photographed.

Statistical analysis

Data generated from this study were subjected to analysis of variance using GenStat discovery 4 edition software. The significant means were separated using Fischer's least significant difference at 5% probability level. Correlation coefficient analysis was performed to understand the relationships among the measured parameters. The GGE Biplot was used to rank the performance of the treatments.

Results

Effect of soil amendments with biochar on root nodules and root properties

The nodule number was significantly ($p<0.05$) affected by the treatments in both years (Table 1). In the first year, the highest number of nodules was obtained from roots of plants grown on biochar + cattle dung applied plots (32) and the least was from the control and NPK plots (15). Roots of plants grown on the sole biochar amended plot had 25 nodules. Combine the application of biochar with either cattle dung or NPK improved the number of nodules over the sole application. In the second cultivation, the highest nodule number was obtained from control and biochar.

Cowpea root length in the first year of cultivation was not significantly ($p<0.05$) affected by the treatments, while root weight in the second year of cultivation was not significantly ($p<0.05$) affected by treatments. Root weight in the first year of cultivation was significantly affected by the treatments, with biochar + cattle dung having the highest root weight (7.12 g); following by biochar + NPK (5.66 g). Control plots had the least root weight (3.80 g). In the second cultivation, root length was significantly ($p<0.05$) affected by the treatments. Control had the least root length (14.37 cm). NPK amended plot had the highest root length (24.20 cm), followed by biochar + NPK (23.27 cm), following by biochar + cattle dung (22.53).

The coefficient of variation (CV) ranged from low to moderate in all the parameters (1 to 50%) except root weight, which had a high CV% (55.7). This shows that the treatment effect varied most in root weight.

Table 1. Effect of soil amendment on root nodulation and root parameters

Soil treatment	First planting			Residual planting		
	Nodule number	Root length (cm)	Root weight (g)	Nodule number	Root length (cm)	Root weight (g)
Control (0 tha^{-1})	15	29.7	3.80	7	14.37	3.37
Biochar (5 tha^{-1})	25	27.7	5.46	5	16.40	5.40
NPK (60 kg ha^{-1})	15	26.0	4.31	4	24.20	5.76
Cattle dung (5 tha^{-1})	16	26.3	5.32	4	17.10	4.76
Biochar +NPK	19	34.7	5.66	4	23.27	5.50
Biochar +cattle dung	32	27.4	7.12	7	22.53	3.72
Cv (%)	36.4	19.4	30.2	27.2	12.9	55.7
F-LSD	13.61	Ns	2.99	2.77	4.59	Ns

Note: CV = coefficient of variation, ns = means not significant at 5%

Effect of biochar soil amendments, including on cowpea growth parameters and dry matter yield

Most of the growth parameters had low to moderate coefficient of variation (1 to 50%). Of all the parameters, only leaf lengths at 1MAP (month after planting) and 2MAP during the second-year planting were not significantly ($p<0.05$) affected by the treatments (Table 2). Biochar + cattle dung had the highest vine length 1MAP (38.80 cm) during the first year of planting. Sole cattle dung was recorded 33.33 cm vine length 1MAP, while biochar + NPK had the highest vine length 2MAP (63.1 cm). This was followed by biochar + cattle dung (50 cm). Among the sole applications, cattle dung produced the highest vine length 2MAP (48.7 cm) followed by biochar (47.7 cm), and the least vine length was from NPK-amended plots (39.3 cm). All the values obtained in the treatment's variants were higher than that of control. In the second-year planting, similar trend was observed.

Regarding leaf length, biochar + cattle dung had miniature leaf lengths at 1MAP and 2MAP (7.27 cm, 9.17 cm), followed by the control (7.33 cm, 9.33 cm). Amendment with biochar ensured the highest initial plant leaf length (9.50 cm), while the highest subsequent leaf length II was obtained from the NPK amended plot (11.23 cm). In the second year of cultivation, although the treatments did not significantly affect the leaf lengths, control had the least value. The highest leaf length during the second cultivation was obtained on plant cultivated on biochar + cattle dung plot (10.03 cm), followed by sole biochar (10.0 cm).

Table 2. Soil amendments, plant growth properties and dry matter yield

Soil treatment	First planting					Residual planting				
	Vine length 1MAP (cm)	Vine length 2MAP (cm)	Leaf length 1MAP (cm)	Leaf length 2MAP (cm)	Dry matter Yield (g)	Vine length 1MAP (cm)	Vine length 2MAP (cm)	Leaf length 1MAP (cm)	Leaf length 2MAP (cm)	Dry matter yield (g)
Control (0 tha^{-1})	28.3	34.7	7.33	9.33	53	23.0	31.6	6.40	7.87	3.06
Biochar (5 tha^{-1})	31.57	47.7	9.50	10.87	214	34.4	44.6	8.27	10.00	5.33
NPK (60 kg ha^{-1})	30.78	39.3	8.70	11.23	87	27.23	33.9	7.57	8.07	4.75
Cattle dung (5 tha^{-1})	33.33	48.7	9.30	10.60	125	38.07	44.4	8.7	9.07	6.67
Biochar +NPK	30.67	63.1	9.33	10.50	176	38.67	46.1	8.27	9.33	5.78
Biochar +cattle dung	38.80	50.0	7.27	9.17	123	33.47	42.2	8.53	10.03	7.07
Cv (%)	11.3	50.7	10.5	9.2	39.8	11.9	12	22.3	24.5	32.9
F-LSD	6.65	23.88	1.64	1.73	4.10	7.02	8.94	Ns	ns	3.25

Note: MAP = months after treatment application, CV = coefficient of variation, ns means not significant at 5%

The difference in dry matter yield between the first year and the second year was most likely because of winter that caught with the second cultivation, terminated the growth processes, and finally dried the plant. In the first year of planting, the highest dry matter yield was obtained from sole biochar (214 g), this result was followed by that of biochar + NPK (176 g). The least dry matter yield was obtained from control plots (53 g), followed by sole NPK amended plots (87 g). In the second year of planting, biochar + cattle dung ensured the highest dry matter yield (7.07 g) and the least was equally from the control plot (3.06 g).

Treatment effects on chlorophyll content of the plants

The treatments significantly affected chlorophyll content (Table 3). At one month after treatment application leaf chlorophyll content was as follows: NPK (84.7) > biochar + NPK (76.7) > cattle dung (66) >biochar (60.3) > biochar +cattle dung > control (36.6). The last measurement in the first year of planting showed that the results obtained from all treatments, were the same except biochar + cattle dung, which was significantly higher and different from all other treatments.

In the second year of planting, the chlorophyll content was generally lower than the first. And only two measurements were possible during the second year of cultivation. In the first year of planting, control had the least chlorophyll content (8.9), while in the second year, biochar + NPK had the least value (12.5). The first measurement showed that NPK amended plot had the highest chlorophyll content (45.9) > biochar + cattle dung (32.7) > biochar +NPK (32.3). In the second measurement, chlorophyll content order in plants cultivated with biochar treatment (49.7) > NPK (34.4) >cattle dung (27.8) >control (18.4) >biochar +cattle dung (15.3) >biochar +NPK (12.5).

Table 3. Effect of soil amendments on chlorophyll content of cowpea leaves

Soil treatment	First planting		Residual planting		Residual biochar application
	One Month after treatment application	Two Month after treatment application	One Month after treatment apllication	Two Month after treatment application	
Control (0 tha^{-1})	36.6	89.1	81.4	8.9	18.4
Biochar (5 tha^{-1})	60.3	105.1	122	17.3	49.7
NPK(60 kg ha^{-1})	84.7	87.8	101	45.9	34.4
Cattle dung (5 tha^{-1})	66	91.6	103.6	18.7	27.8
Biochar +NPK	76.7	107	82.4	32.3	12.5
Biochar +cattle dung	52.3	77.8	142.5	32.7	15.3
Cv	19.6	19	21.9	33.2	27.2
F-LSD	22.37	32.20 (ns)	42.04	15.95	13.02

Correlation analysis of the combination of the parameters of the two years of planting

The analysis reveals that dry matter yield (DMY) exhibits strong and significant positive correlations with several parameters, indicating its close relationship with these traits (Table 4). DMY is strongly correlated with root length (RL) ($r = 0.554$, $p < 0.01$), highlighting the importance of robust root development in contributing to biomass production. Furthermore, leaf chlorophyll content, measured at two stages (LC1MAP and LC2MAP), shows significant positive relationships with DMY, with LC2MAP having a particularly strong correlation ($r = 0.751$, $p < 0.01$). This suggests that higher chlorophyll content, especially during later growth stages, is a critical factor for achieving greater dry matter accumulation.

Leaf length at two stages (LL1MAP and LL2MAP) also contributes positively to DMY, as indicated by their significant correlations with LC2MAP ($r = 0.644$, $p < 0.01$) and RL. Interestingly, vine length at an earlier stage (PH1MAP) has a significant negative correlation with DMY ($r = -0.368$, $p < 0.05$), implying that early vigorous growth in height may not necessarily translate to higher dry matter yield, potentially due to resource allocation dynamics.

Table 4. Correlation coefficient of cowpea growth and yield parameters

	PH1MAP	PH2MAP	LL1MAP	LL2MAP	RL	NOS	WOR	NON	LC1MAP	LC2MAP	DMY
PH1MAP	1	0.246	0.212	0.024	-0.180	-0.091	-0.027	-0.232	-0.114	-.359*	-.368*
PH2MAP		1	.443**	.356*	0.152	-0.213	0.006	0.070	0.130	0.225	0.061
LL1MAP			1	.796**	0.203	0.006	0.202	-0.019	.385*	.367*	0.283
LL2MAP				1	0.201	0.072	0.244	0.097	.329*	.440**	0.300
RL					1	0.145	.573**	0.117	.508**	.613**	.554**
NOS						1	.460**	0.061	-0.007	-0.015	0.016
WOR							1	0.184	0.220	0.134	0.318
NON								1	0.142	.389*	.451**
LC1MAP									1	.676**	.644**
LC2MAP										1	.751**
DMY											1

Note: **.and * Correlation is significant at the 0.01 and 0.05 levels (2- tailed), 1MAP = one month after treatment application, 2MAP = two months after treatment application, PH = vine length, LL = leaf length, RL = root length, NOOS = number of offshoots. WOR = weight of roots, NON = number of nodules, LC = leaf chlorophyll, and DMY = dry matter yield

The biplot graph indicates that principal components (PC)1 and 2 explained 71.7% of the variation among the parameters measured (Figure 1). The biplot has five sections with five out of the six treatments occupying the vertex of the polygon. The section occupied by biochar + NPK (B1fnpk) contained most of the parameters (dry matter yield, vine length, root weight, root length, and number of nodules) while the leaf length and number of offshoots were housed in the biochar alone section (B1). The position of the control (B0) opposite the best performing treatment (biochar + NPK) indicates its poor performance in the selected parameters.

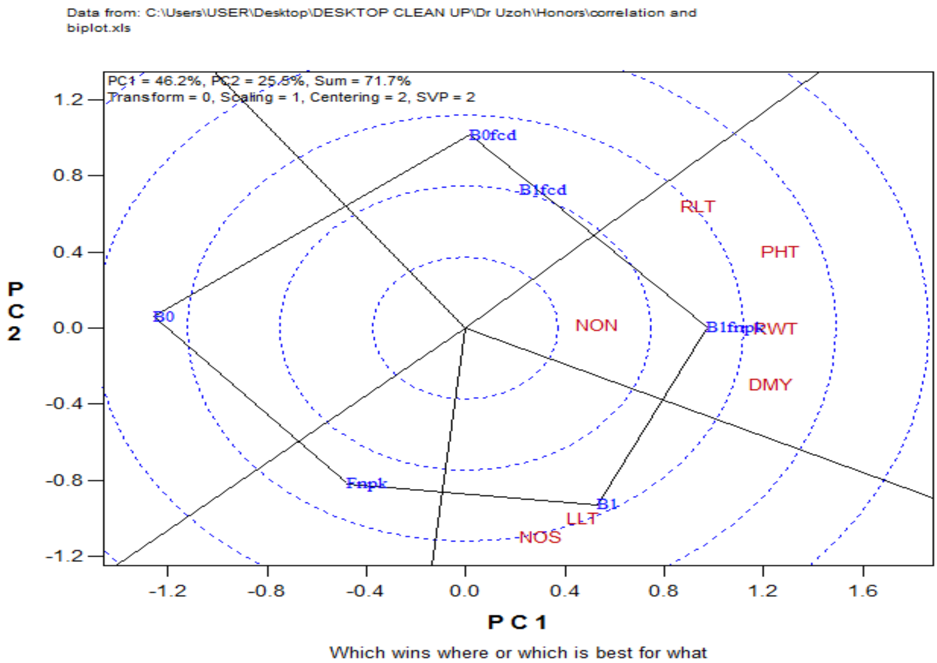

Figure 1. Graph of which-wins-where of the soil amendment treatments

Mycorrhizal colonization

The fungal mycorrhizal analysis under stereomicroscope resulted in visualization of hyphae, vesicles and arbuscules (Figures 2-5). Amendment with biochar gave the highest arbuscules, but hyphae was less visible, while in cattle dung-treated soil, there were more hyphae but fewer arbuscules. Cattle dung and biochar gave higher arbuscules than the control.

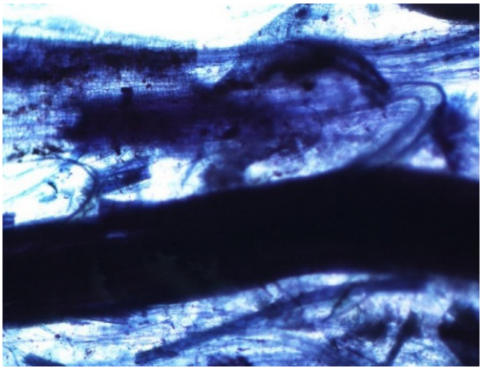

Figure 2. Cowpea roots treated with biochar alone (B1) showing darker arbuscules (38 visible arbuscules).

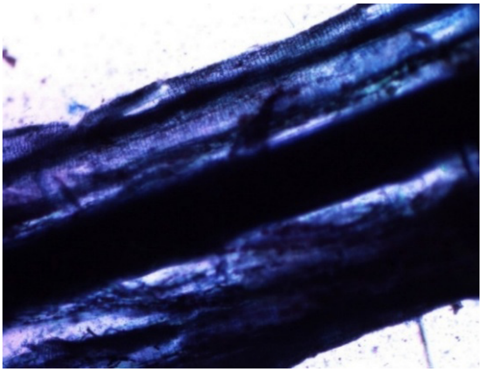

Figure 3. Cowpea roots treated with biochar + cattle dung with less arbuscules (10 visible arbuscules)

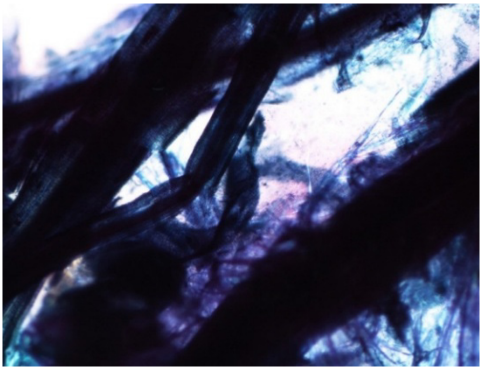

Figure 4. Cowpea roots of control had no visible arbuscules, but the hyphae observed were turbid.

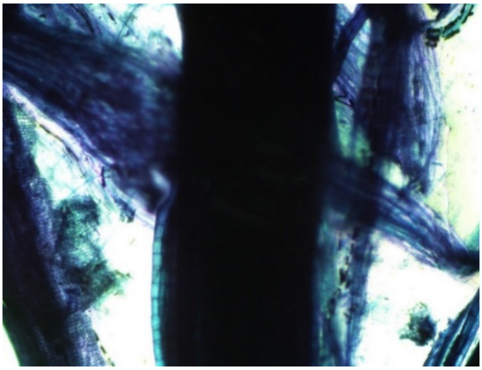

Figure 5. Cowpea roots treated with cattle dung showing both the arbuscules (16 visible arbuscules) and hyphae.

Mycorrhiza spore count in the soil

Effect of the treatments was evaluated on the mycorrhiza spore count in the soil after cultivation of cowpeas. Five mycorrhizae species were isolated from most of the treated soil, which include *Acaulospora, Glomus, Scutelospora, Gigaspora* and *Entrophospora*. Among these isolates, three namely, *Acaulospora, Glomus* and *Entrophospora* were significantly affected by the treatments. There was variability in the overall occurrence and the sequence of occurrence in both treated and untreated soils. Glomus had the highest number of spores in all the soils. It had hundreds of spores in almost all the treatment variants. *Acaulospora* also had hundreds of spores, *Entrophospora* occurred in tens while *Scutelospora* was also in tens but fewer than *Entrophospora. Gigaspora* spores were found only in control, biochar plus NPK and NPK amended plots in few numbers. Apart from *Acaulospora*, which was higher in biochar amended plot, the control plot had higher spores than the amended plots. In control plots, Glomus and *Entrophospora* had the highest soil spores (365, 100). Sole biochar amended plots and biochar plus NPK amended plots had the next highest *Entrophospora* (232) and Glomus (268) spores, respectively. The biochar plus cattle dung amended plots had the least spores of all the mycorrhiza species. Sole biochar amended plots had the highest *Acaulospora* spores (308), which was significantly ($p < 0.05$) the same as control plots (289). Considering the amended organic and inorganic soils, apart from sole biochar, NPK or biochar plus NPK had higher mycorrhiza spores than cattle dung or biochar plus cattle dung in most of the isolates. Figure 6 shows the mycorrhiza spores while Figures 7 – 11 shows the different species with their characteristic features.

Table 5. Effect of soil amendment on soil mycorrhiza spore count

Soil Treatment	*Acaulospora*	*Glomus*	*Scutelospora*	*Gigaspora*	*Entrophospora*
Control (0 tha^{-1})	289^{a}	365^{a}	11^{a}	6^{a}	100^{a}
Biochar (5 tha^{-1})	308^{a}	232^{ab}	11^{a}	0^{a}	83^{ab}
Cattle dung (5 tha^{-1})	243^{a}	177^{b}	17^{a}	0^{a}	62^{abc}
NPK (60 kg ha^{-1})	243^{a}	198^{ab}	23^{a}	13^{a}	29^{bc}
Biochar +cattle dung	82^{b}	90^{b}	0^{a}	0^{a}	20^{c}
Biochar +NPK	209^{a}	268^{ab}	19^{a}	14^{a}	58^{abc}

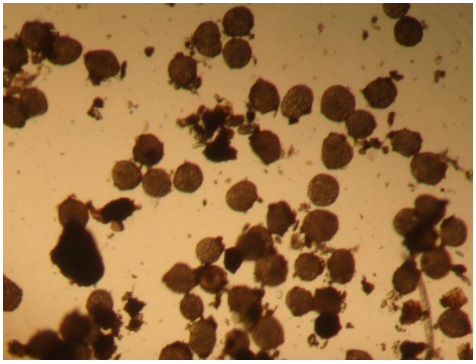

Figure 6. Mycorrhiza spores

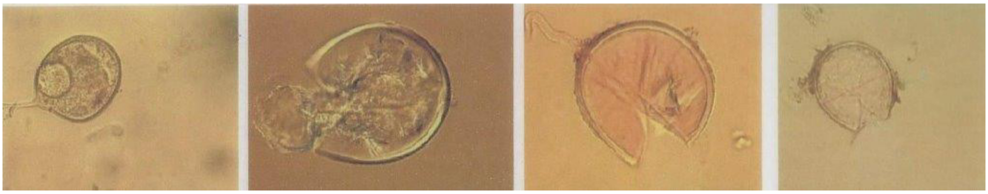

Figure 7. *Glomus intradices*

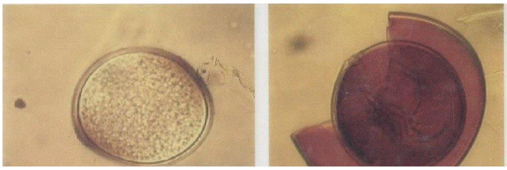

Figure 8. *Scutelospora calospora*

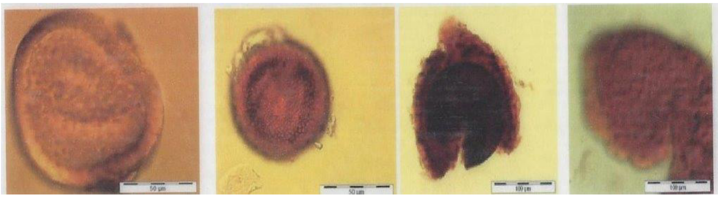

Figure 9. *Entrophospora colombiana*

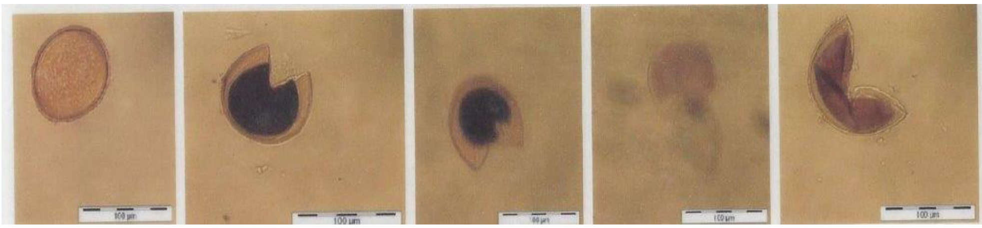

Figure 10. *Acaulospora mellea*

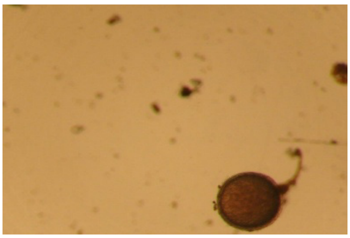

Figure 11. *Gigaspora* species

Discussion

Effect of soil amendments with biochar on root nodules and root properties

Roots are a significant part of a plant because it is an interface between nutrient and water absorption from the soil and any amendment added to it and the crop growth and development. Properties of the root are therefore very vital because these affect how the root accomplishes its task of nutrient and water absorption into the plant. Biochar soil amendment, according to Xiang *et al.* (2017) may change root development and properties, which inevitably affect crop performance. In this research, the sole application of biochar or in combination with NPK or cattle dung soil amendments increased root length and weight over the control. Xiang *et al.* (2017) found that responses of root traits associated with 13 variables under biochar application had increased root biomass (+32%), root volume (+29%) and surface area (+39%). They also found that biochar increased the number of root nodules, just as was confirmed with findings of this research. In both years, soil amendment increased the number of root nodules. The reason for observed increase in root properties was likely as a result of improvement in soil properties by those soil amendments. Biochar increases soil pH, nutrient status and cation exchange capacity (CEC) (Vanek and Lehmann, 2015), which invariably should improve root properties in biochar-applied plots (Macdonald *et al.*, 2014; Olmo *et al.*, 2016). Integration of NPK or cattle dung into biochar amendment enhanced root length and weight. Alburquerque *et al.* (2015) observed that biochar applied with NPK was found to interactively influence root growth.

Effect of soil amendments on growth parameters and dry matter yield

Amendments used in this research improved cowpea growth parameters and, most significantly, the dry matter yield. All the amendments had higher dry matter yield than the control. Applying biochar to soil can increase crop yields (Quilliam *et al.*, 2013) and biochar applications may be helpful in developing more sustainable food production systems (Liu *et al.*, 2017). In this study, biochar, cattle dung, NPK or their combination positively affected cowpea growth parameters and dry matter yields. This agrees with the result of Suthar (2012).

The combined insights from the correlation analysis and the GGE biplot reveal key relationships between dry matter yield and other agronomic parameters, as well as the differential performance of treatments in promoting specific traits. The correlation analysis underscores the importance of traits like root length and leaf chlorophyll content in driving DMY. The strong positive correlation between DMY and RL highlights the critical role of robust root development in cowpea biomass production. Longer roots likely improve water and nutrient uptake, particularly under stress conditions, which translates to greater dry matter accumulation (Santos *et al*., 2020). Similarly, the significant positive correlations between DMY and LC, particularly at two months after treatment application, suggest that maintaining higher chlorophyll content during later growth stages is crucial for enhanced photosynthetic efficiency and biomass production. Wanjiku *et al* (2023) observed a significant increase in cowpea leaf chlorophyll content as the plant advances in age.

Leaf length at different growth stages also contributes positively to DMY, with LL1MAP and LL2MAP correlating significantly with RL and LC2MAP. This indicates that leaf expansion, coupled with enhanced chlorophyll content, optimizes photosynthetic capacity and resource capture, thereby supporting higher biomass yield. These findings align with Atugwu *et al*. (2023), who observed a significant correlation between cowpea leaf length and dry matter yield. Interestingly, vine length at an earlier stage shows a negative correlation with DMY, suggesting that excessive early vegetative growth in height may divert resources from other processes critical for dry matter accumulation.

The GGE biplot analysis further complements these findings, explaining 71.7% of the variation among parameters and showcasing the distinct performance of treatments. The "which-won-where" result identifies biochar + NPK as the most effective treatment, with DMY, RL, LC, root weight, and number of nodules all grouped within its polygon section. This indicates that biochar + NPK amendment promotes traits that directly contribute to enhanced biomass production, likely due to the synergistic effects of biochar and NPK in improving soil fertility, nutrient availability, and root-soil interactions. In contrast, the control was ranked low in the biplot analysis which indicates that cowpea responds to soil amendments even though it can fix nitrogen through its symbiotic relationship (Atugwu *et al*., 2023; Nkaa *et al*., 2014). The findings suggest that treatments that enhance both root system development and chlorophyll content are most effective in boosting biomass production. Yield is a polygenic trait that can be enhanced by identifying and modifying the morphological qualities that influence it (Chukwudi, 2021). Root traits, including RL and NON, appear to play pivotal roles in supporting nutrient and water uptake, which are critical for dry matter accumulation. Similarly, the strong relationship between LC and DMY highlights the importance of maintaining photosynthetic efficiency during later growth stages for maximizing yield.

Mycorrhizal colonization

The mycorrhizal colonization results showed the presence of hyphae, arbuscules and nodules in the roots. Biochar treated soil had the highest arbuscules showing high mycorrhizal colonization. Solaiman *et al*. (2010) also showed that addition of biochar increases mycorrhizal colonization. In addition, cattle dung treated soil also had arbuscules showing mycorrhizal colonization. Although cattle dung treated soil had less arbuscules than biochar-treated soil. This also is in consonance with Muthukumar and Udaiyan (2002), who noted that the effect of organic manure on mycorrhizal colonization depends on the organic manure used. The combined application, which shows evidence of mycorrhizal colonization. Gryndler *et al*. (2001) observed that organic manure and mineral fertilization increases mycorrhizal colonization by increasing hyphal length. The hyphae help in the absorption of nutrients from the soil and transport them to the rest of the plant and arbuscules penetrate the cortical cells.

Mycorrhiza spore count in the soil

Spores of *Glomus* species was found in both amended and non-amended plots and in greatest number. Several studies of agricultural soil have shown dominance of Glomus species (Gunwal *et al*., 2014; Mathimaran *et al*., 2005) in such soils. The reason for the above observation was as result of the higher colonization efficiency of Glomus species than other mycorrhiza species. The five isolates reported from this study have also been indicated to be found in agricultural soils (Jansa *et al*., 2005). Except for amended soil, organic manure-amended plots showed lower spores of mycorrhiza than inorganic fertilizer amended plots. It's assumed that in organic fertilizer-amended plots phosphorus availability is higher than in inorganic fertilizer-amended plots (Xin *et al*., 2022). And as such leads to lower mycorrhiza colonization (Garcia and Zimmermann, 2014). Johnson (2010) observed that nutrient-stressed plants release more soluble carbohydrates in root exudates, which support more mycorrhiza than plants with sufficient nutrients. It has been noted that in high availability of phosphorus, that mycorrhiza colonization or sporulation is low. Other findings of mycorrhizal response in organic amended soils have shown diverse opinions. Aleklett and Wallander (2012) reported an increase in AMF growth from adding N-rich organic residue. Organic residue with low N didn't affect AMF colonization while Hammer *et al*. (2011) and Gryndler *et al*. (2009) found an increase in plant response to mycorrhiza and spore count.

Conclusion

Biochar soil amendments significantly improved mycorrhiza root colonization and spore count, root nodulation, root properties and cowpea dry matter yield when compared either to control or, in some cases, the other treatments that were added to the soil. The study was able to explain the beneficial effect on yield attributed to biochar soil amendment. Correlation analyses show that the factors with a high correlation to dry matter yield mainly were improved by biochar soil amendment.

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