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Original Research Article

Effects of Biochar and Poultry Manure on the Composition of Phosphorus Solubilizing Fungi and Soil Available Phosphorus Concentration in an Oxisol

ABSTRACT

Introduction: Anexperiment was conducted to evaluate the effect of biochar solely applied or in combination with poultry manure on the composition of soil phosphorus solubilizing fungi, available P concentration and selected properties of Oxisol in Ghana.

Methods: Cocoa husk biochar (CHB), prepared using Lucia biomass pyrolytic stove at a temperature of 400 ^{0}C was applied solely at 0, 39 and 65 t ha^{-1} or in combination with 10 t ha^{-1}, poultry manure, to the soil. Treatments were arranged in a completely randomized designwith three replications.

Results: The population of phosphorus solubilizing fungi increased in amended soils significantly (P= .05) above that of the control. The increases in fungal biomass followed; T0 < biochar < biochar + poultry manure. *Aspergillus, Fusarium* and *Penicillium* were dominant genera with few of the microbes in the genera *Colletotrichum*. The increase in phosphorus solubilizing fungi correlated positively (r = 0.96, P= .05) with increase in available phosphorus. Then again, biochar solely applied or in combination with poultry manure significantly (P = .05) increased pH, total organic carbon and cation exchange capacity. Mineral N however significantly (P = .05) increased only in combined biochar and poultry manure treatments.

Conclusion: Biochar solely applied or in combination with poultry manure enhanced phosphorus solubilizing fungal biomass and availabilityof phosphorus in the soil with combined poultry manure and biochar having the highest influence.

Keywords:

Biochar, Poultry Manure, Oxisol, Phosphorus Solubilizing Fungi, Available Phosphorus

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1. INTRODUCTION

Phosphorus is a major soil nutrient required by plants for biochemical and physiological functions (1). Biochemically, it is involved in energy storage and transfer in the form of ADP and ATP, photosynthesis, cell division, transfer of genetic characters from one generation to the other, cell enlargement and nutrient movement within the plant. Physiologically it plays vital role in early root formation, reproduction, seed formation and improving disease resistance. In Ghana, most soils in crop production areas, notably the humid agro ecological zones are highly degraded hence characterized by low acidity (pH < 5) and phosphorus deficiency. These soils are mainly Oxisols and Ultisols covering about 15% of potential agricultural lands in Ghana (2). The deficiency may be due to precipitation of phosphorus by soluble iron, aluminum and manganese ions which dominates exchange complex in acidic soils (3). Then again, high amounts of P in the form of organic and inorganic fertilizers applied to these degraded soils may be held in insoluble forms by these acidic cations rendering phosphorus unavailable to growing plants. To improve phosphorus availability for crop production on these soils, pragmatic measures are needed to reduce acidity and metal toxicity of these soils hence the application of biochar and manure in this work.

It has been suggested that biochar solely applied or in combination with manure for soil improvement could induce changes in soil; including increasing pH, altering soil microbial composition and P cycling in soil. Biochar may increase P availability in soils both directly through P addition from decomposable organic and inorganic P fractions in biochar or indirectly through impact on soil chemical (4), physical (5, 6) and biological processes. Then again biochar is known to influence soil P availability and plant uptake of P and as a result of modification of soil pH which subsequently impacted the activity of P complexing metals (Al^{3+}, Fe^{3+}) and also enhanced microbial metabolic activities (5). However earlier research findings on biochar interaction with the microbial community has not been consistent. Biochar application resulted in enhanced (7), inhibited (8) or no effects (9) on microbial biomass. The variations in the effect of biochar on soil microbial biomass is dependent on biochar physical and chemical properties and on soil properties. These may include biochars' ability to supply labile compounds; that is leachable/labile C fraction and micro and macro nutrients (10), biochars' pores and surfaces providing habitat for microbes and acting as a refuge site or microhabitat for colonizing microbes (11), alkaline nature of biochar which potentially provides a more stable chemical environment for microbes (10) and toxic organic compounds

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such as ethylene, found on biochar which may inhibit microbial biomass (12). It is therefore important to investigate the effect of biochar prepared from locally available resources on soil phosphorus solubilizing fungi.

Soil phosphorus solubilizing microbes (PSMs) have the potential to mineralize insoluble P forms (13). The mineralization process is accomplished through the production of organic acids. Acids produced directly affects the availability of P by; lowering the soil pH, competing with P for adsorption sites on the soil and forming soluble complexes with metal ions associated with insoluble P.In acid soils, PSMs release protons which helps to decrease the negative charge of adsorbing surfaces to facilitate the sorption of P ions (14). Then again, organic acids, for instance, carboxylic acids produced by PSMs mainly solubilized Al-P and Fe-P through direct dissolution of mineral phosphate as a result of anion exchange of PO_4^{3-} by acidic anion, or by chelation of both Fe and Al ions associated with phosphate sorption (15). Carboxylic anions replace phosphate on sorption complexes by ligand exchange (14) and chelate both Fe and Al ions associated with phosphate. Moreover, carboxylates form water-soluble complexes with Fe and Al, thereby decreasing the free Fe and Al ion concentration in the rhizosphere soil solution. This leads to increased solubilization of Fe^{3+} or Al^{3+} and thus release of P bound to Al/Fe oxides or bound to clays and organic matter via Fe/Al bridges (16). Little information is however available on the effect of biochar, singly applied or in combination with poultry manure on soil phosphate solubilizing fungi and the consequent effects on P availability. In view of this a greenhouse experiment was conducted to determine the effect of biochar solely applied or in combination with manure on the composition of soil phosphorus solubilizing fungi, available P concentration and selected soil properties.

2. MATERIALS AND METHODS

2.1 Soil

Highly weathered tropical soil (0-20cm depth) was sampled from the Agricultural Research Farm, Aiyinasi, in the Western Region of Ghana. It was characterized as sandy with pH averaging 4.17± 0.02, Organic Carbon of 0.35 ± 0.03%, Total N of 0.05 ±0.01%, Total P of 0.30 ± 0.02% and the soil classified as an Oxisol. It covers most agricultural lands of the Western region of Ghana. The soil was mixed thoroughly, air-dried and sieved through a 2-mm mesh prior to the experiment.

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2.2 Biochar

Biochar was produced from cocoa husk by slow-pyrolysis process using Lucia biomass pyrolytic stoves 'top-lit updraft' (Worldstove, Italy). Cocoa husk was collected from farms at Jukwa, a farming community in the Central Region of Ghana. The husks were sun dried, crushed and loaded into pyrolytic stoves and lit to start the fire. The initial yellow colour of the flames was monitored until it started to give off black smoke. Immediately black smoke was seen evolving, charring of the feedstock was assumed to be complete and the fire was put off. Pyrolysis was done at 450℃ and within 45 minutes under oxygen-limited condition. The char produced was milled and sieved through a 2 mm sieve before used. Biochar was characterised by pH (H_20) 10.3± 0.01, Total C (75.8± 4.91%), Total N (0.52± 0.05%), Total P (0.18± 0.02%), C:N (145.8), C:P (421.1).

2.3 Poultry manure

Poultry droppings without litter (10 days old) was collectedfrom a battery cage, air dried at room temperature,ground and sieved through a 2-mm sieve. The manure had the following properties; pH (7.63±0.01) Total C (33.9±0.47%), Total N (3.18±0.09%), Total P (1.24±0.02%), C:N (10.9±0.26%).

2.4 Pot experiment

Completely randomized design (CRD) was used for the pot studies, with biochar and poultry manure as the experimental factors.Soils were pre-incubated at 45% water filled pore space (WFPS) for 7 days prior to the start of the experiment to revamp microbial activity (17). Three levels of CHB(0, 39 and 65 t ha^{-1}) solely or combined with poultry manure (10 t ha^{-1}) were applied to soil respectively to obtain five treatments.The treatments were; T0: control, T1: 39 t ha^{-1}, T2: 65 t ha^{-1}, T3: 39 t ha^{-1} + 10t ha^{-1} poultry manure and T4: 65 t ha^{-1} + 10t ha^{-1}poultry manure. These treatments were replicated three times.

Biochar singly or in combination with manure were thoroughly mixed with 1 kg equivalent oven-dried soil and packed into pots at a bulk density of 1.3 gcm^{-3}. Pots were incubated at 26 - 35 ^{0}C and moisture content maintained at 60 % of WFPS on weight basis for the duration of the experiment.

Six weeks after incubation (WAI),soils in each pot were mixed thoroughlyto attain a homogenous mixture and divided into parts and stored. For microbial analysis, representative soil samples from each

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amendment were stored in sterilised zip lock bags and sent to the Molecular Biology and Biotechnology Laboratory, University of Cape Coast. The samples were processed immediately for the isolation of fungi. Moisture content, mineral nitrogen (MN),pH, available phosphorus (AvP), soil organic carbon (SOC) and cation exchange capacity of the soil were also determined.

2.5 Laboratory analyses of soil after addition of amendments

Soil pH were determined for soil (soil to water ratio of 1: 2.5), biochar (biochar to water ratio of 1:5) and manure (manure to water ratio of 1:2.5) (w/v) using Suntex 701 Model pH meter. Soil moisture content was determined by oven dry method where the weight of soils before and after oven drying for 48 hours at 105 ^{0}C were calculated. Soil mineral N (total of nitrate and ammonium) was extracted with 2 M KCl at a soil to solution ratio of 1: 10 (w/v) and determined by steam distillation (17).Soil AvP was determined by Bray 1 method using Bray 1 extracting solution with soil to solution ratio of 1: 10 (w/v).Cation exchange capacity (CEC by bases) was determined by extracting basic cations($Ca^{2+,}$ $Mg^{2+,}$ K^{+} and Na^{+}) with1 N Ammonium acetate (CH_3COONH_4)solution in soil to solution ratio of 1: 10 (w/v). Exchangeable Ca^{2+} and Mg^{2+} in the extract were determined using the Atomic Absorption Spectrophotometer (Buck Scientific Model, 210 VGP) whiles K^{+} and Na^{+} concentrations were determined with a flame photometer (Jenway, PFP 7).Soil organic carbon was determined by wet oxidation by adding 0.1667 M Potassium dichromate ($K_2Cr_2O_7$) solution and 20 ml concentrated Sulphuric acid (H_2SO_4) to 0.5 g soil, after which the mixture was back titrated with 0.5 M ferrous solution.

2.6 Characterisation and identification of phosphorus solubilizing fungi

Isolation of fungi from soil was done using soil dilution plate method (18). One gram of soil was weighed from each treatment into sterile test tube containing 9 ml distilled water and serially diluted to 10^{-5}. One millilitre aliquots of the dilution were dispensed onto a 120mm Petri dishes and 20ml molten potato dextrose agar(PDA) - antibiotic mixture, was poured into the plates. The plates were swirled to mix the contents andinoculated at room temperature for 5 days for fungal growth. Colonies growing on the plates were counted after this period and fungal populations expressed as colony forming unit per gram oven dry soil (cfu/g).Pure cultures of the fungal species were prepared and preserved in 10 % glycerol at -20

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^{0}C.Two weeks after preservation, cultures were mounted inLactophenol cotton blue (LPCB) (19) and observed under the microscope. Reproductive and vegetative structures such as the spores and hyphae were examined at a magnification of x40.To determine the phosphorus solubilizing potential of isolates, National Botanical Research Institute's phosphate (NBRIP) broth (contained l^{-1}: glucose, 10 g: $Ca_3(PO_4)_2$, 5 g; $MgCl_2.6H_2O$, 5 g; $MgSO_4.7H_2O$, 0.25 g; KCl, 0.2 g, $(NH_4)_2SO_4$, 0.1 g) was used as selective media to identify phosphate solubilizing fungi. Fungal cultures were inoculated onto NBRIP broth containing TCP(5 %) v/v and the amount of phosphorus solubilized were used as determinant of phosphate solubilisation.

2.7 Statistical analyses

Data generated were analysed using Statistical Product and Service Solutions (SPSS) for Windows; version 22 (SPSS Inc., Chicago IL, USA). Analysis of variance (ANOVA) was used to determine significant differences among treatmentsusing Least Significant Difference (LSD) at 5% level of probability. Correlation analyses were used to examine relationships between soil properties.

3. RESULTS

3.1 Composition and fungal genera in soils amended with biochar and poultry manure

The population of fungi isolated from all treatments have been displayed in Figure 1.

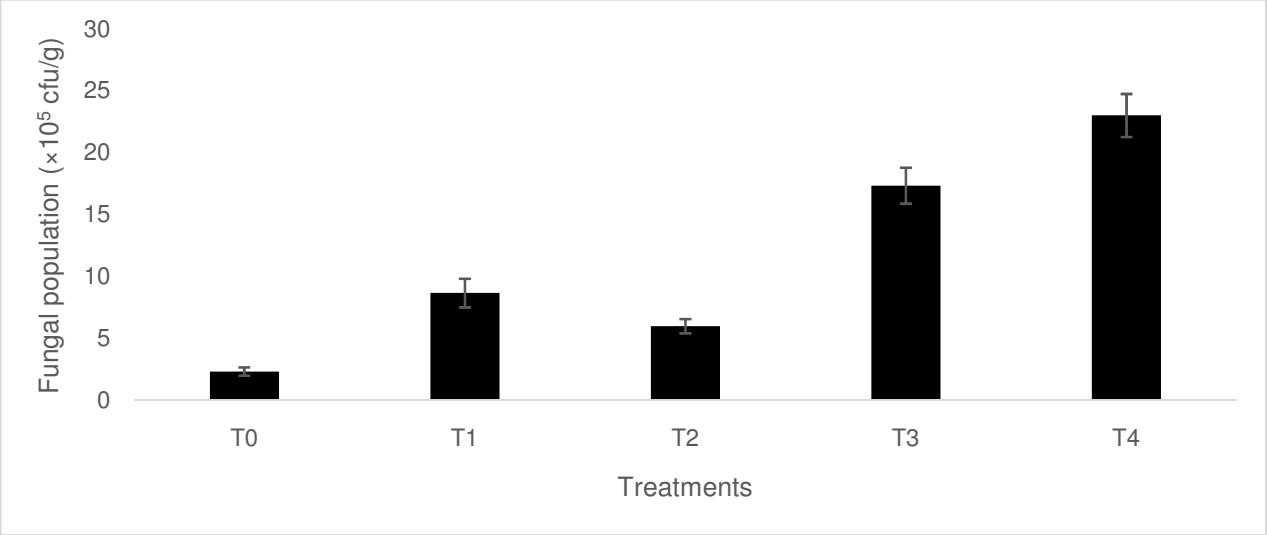

Figure 1: Composition of fungi in soils amended with biochar alone and biochar plus poultry manure (vertical bars denote standard errors of the mean (n = 3))

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The population of fungi increased significantly in amended soils (P = .05) compared with the control. The increases in total fungal biomass followed control< biochar < biochar + poultry manure.

A total of ten fungal species were isolated from control and amended soils. Eight of the isolates were identified to belong to five genera (Figures 2 to 9). These are *Aspergillus flavus, A. niger*, *Fusarium*, *Penicillium*, *Colletotrichum*and*Phytophthora spp.*

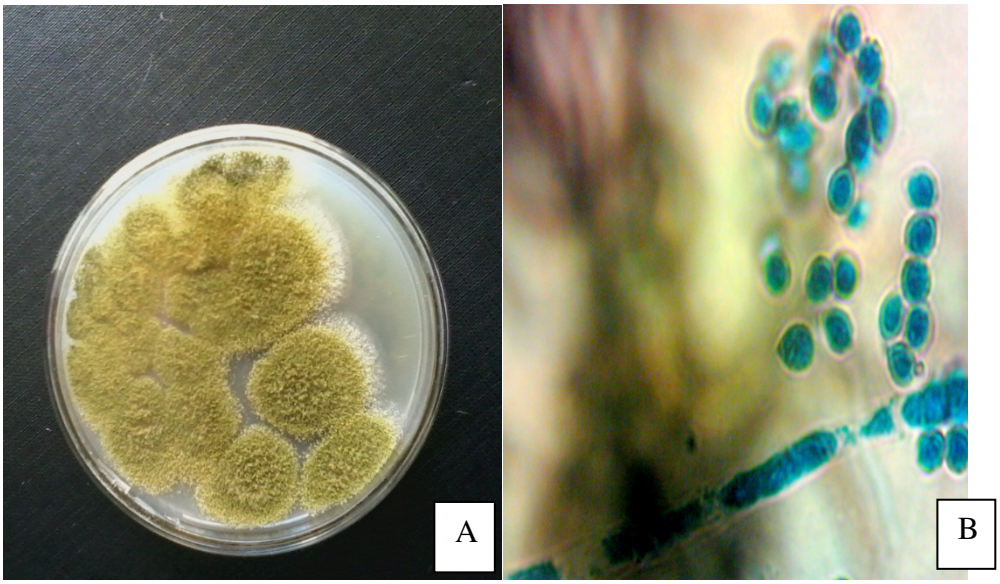

Figure 2: *Aspergillus flavus*(A) Colonies of (B) Spores (magnification ×40)

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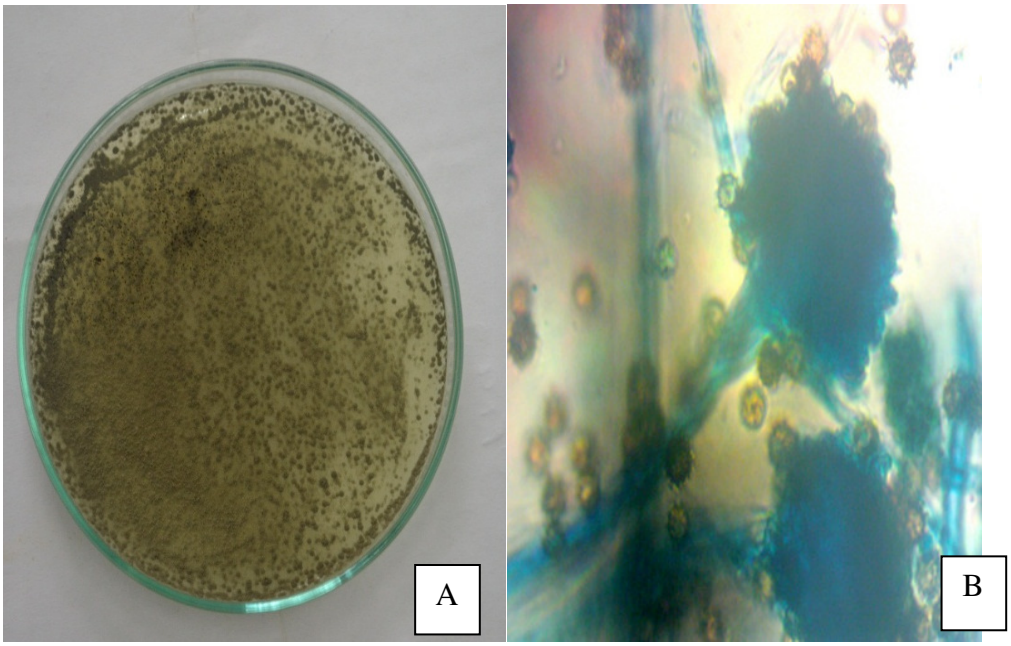

Figure 3: ***Aspergillus niger*** **(A) colonies (B) Spores (magnification ×40)**

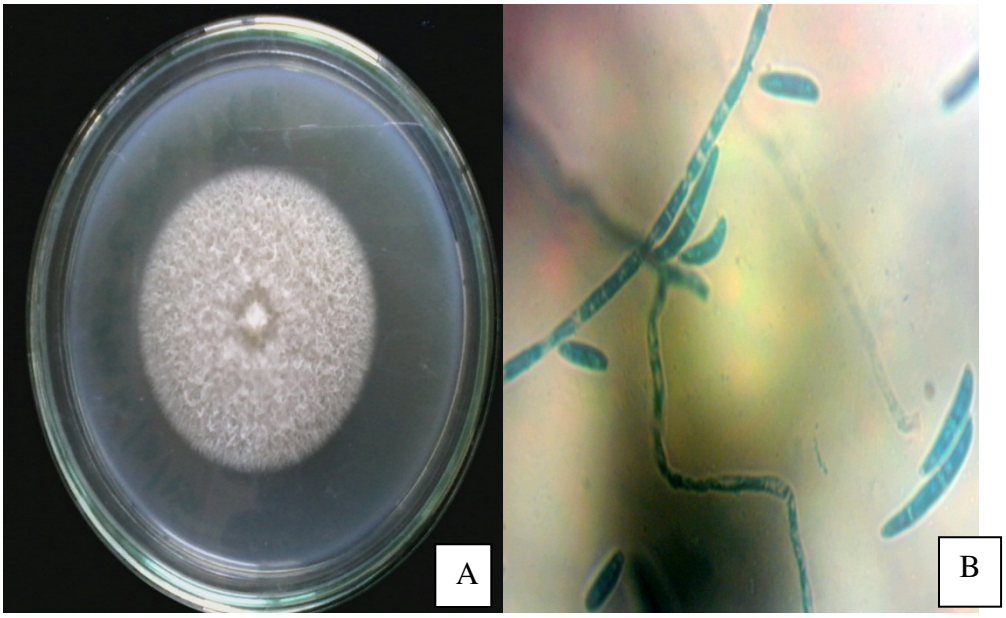

Figure 4: ***Fusarium sp 1*** **(A) colony (B) Spores (magnification ×40)**

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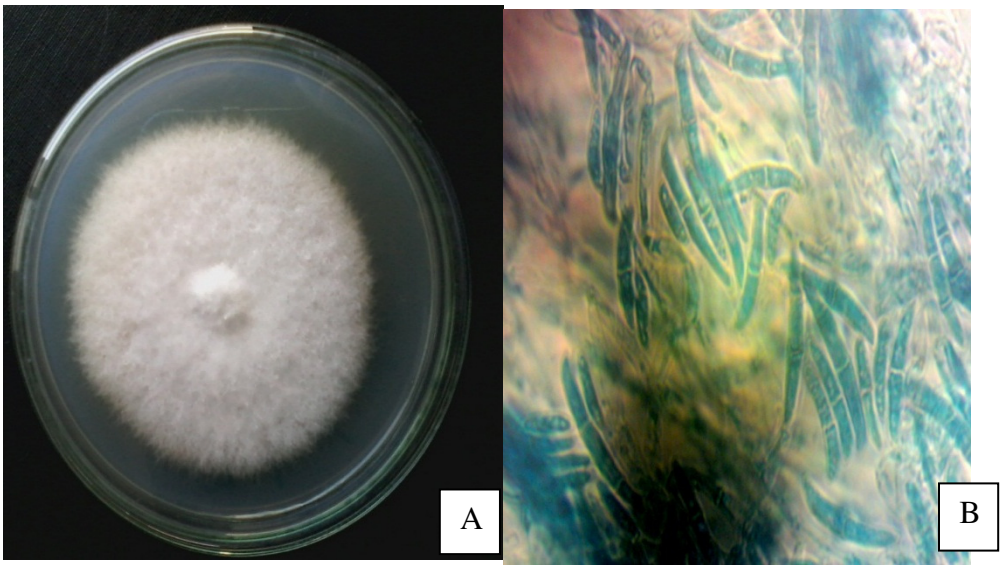

Figure 5: ***Fusarium sp 2*** **(A) colony (B) Spores (magnification ×40)**

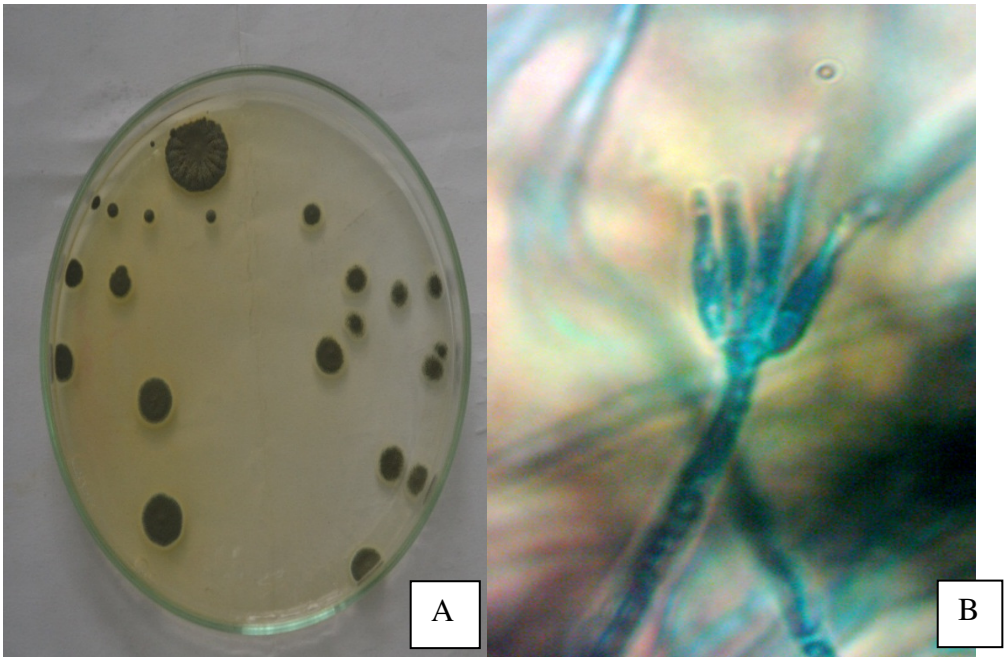

Figure 6: ***Penicilliumsp 1*** **(A) Colonies (B) Spores (magnification ×40)**

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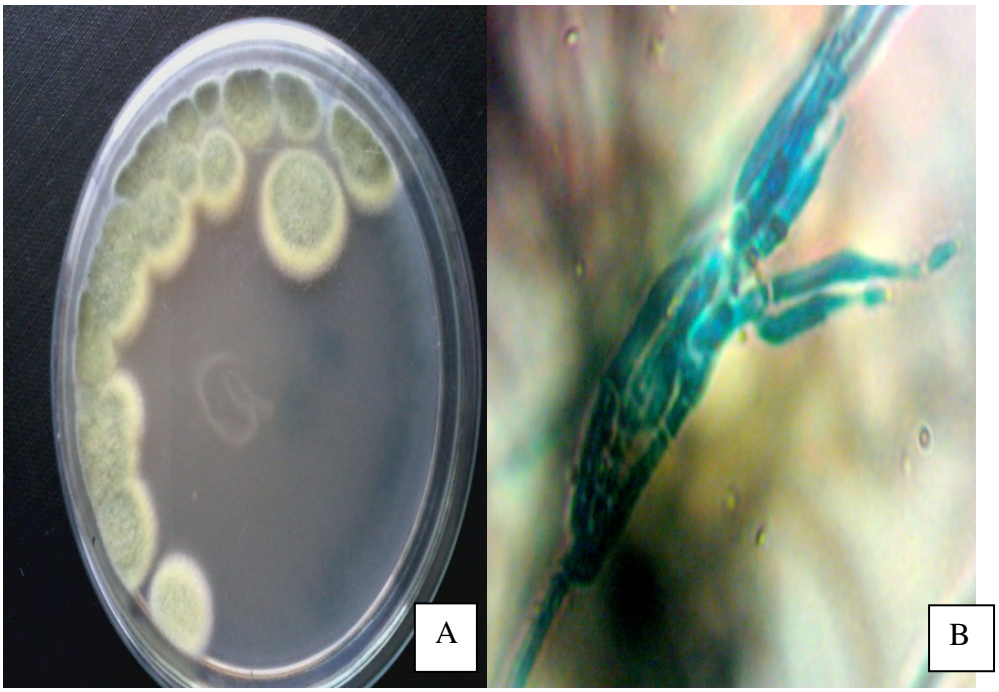

Figure 7: ***Penicilliumsp 2*** **(A) Colonies (B) Spores (magnification ×40)**

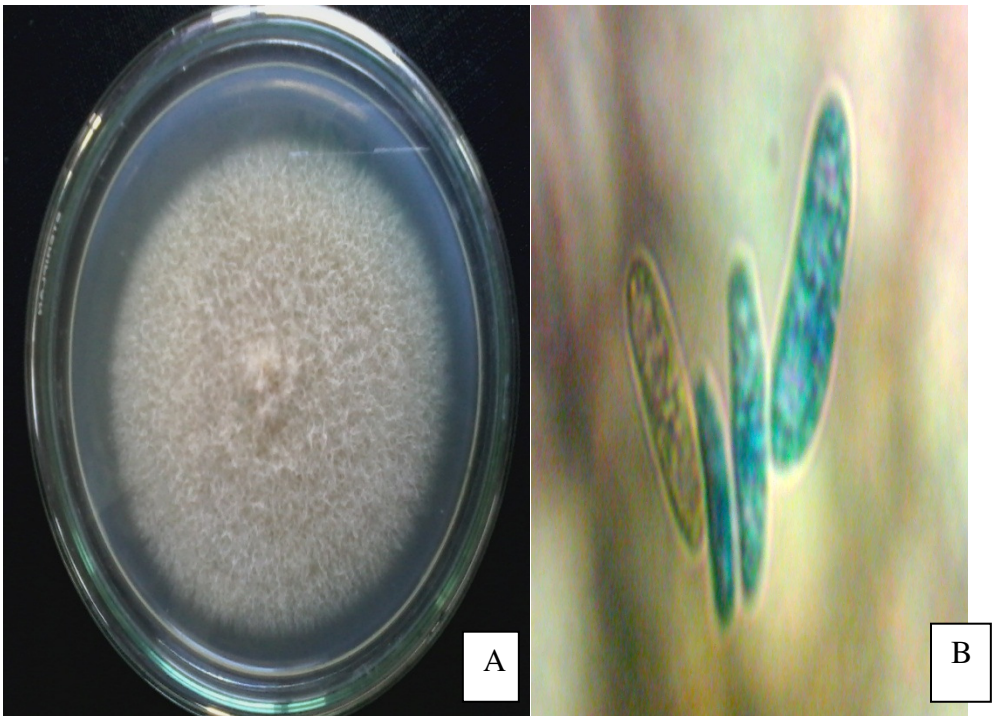

Figure 8: ***Colletotrichumsp*** **(A) Colony (B) Spore (magnification ×40)**

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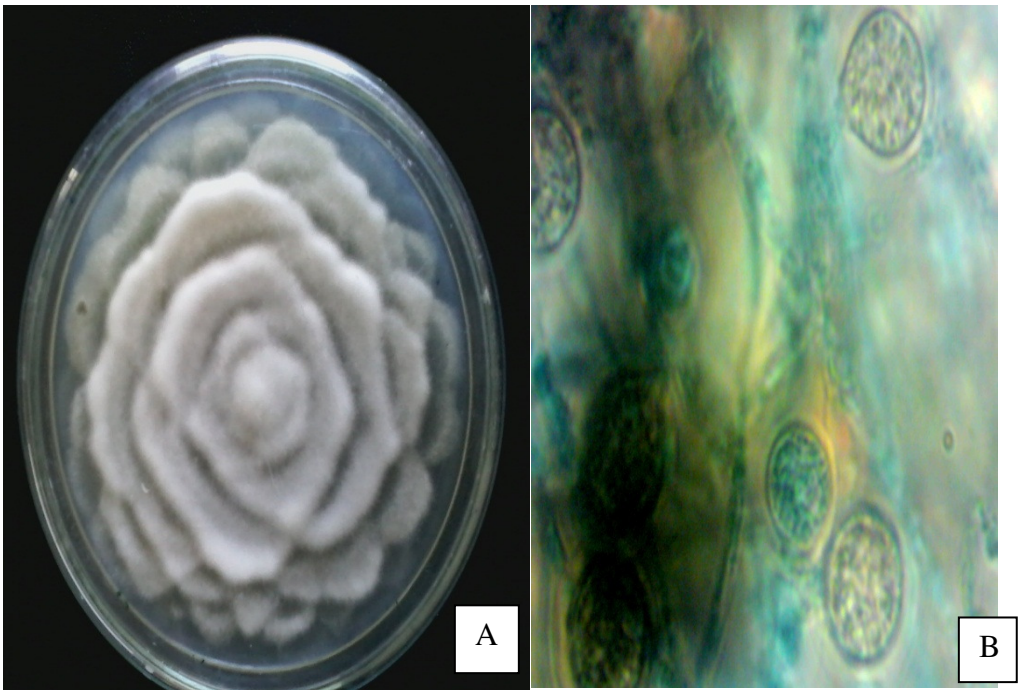

Figure 9: *Phytophthorasp* (A) colony (B) Spores (magnification ×40)

3.2 Identification of phosphorus solubilizing fungi

Estimated available P in NBRIP broth as a result of inoculating it with isolates of fungi were as shown in Figure 10. It was observed that all the isolates,apart from *phytophthorasp,*showed relatively high solubilization of phosphorus. *Aspergillus niger* had the highest solubility ability recording P concentration of 84.67±4.63 µg ml^{-1} in NBRIP broth. This was followed by *A. flavus* and *Penicillium (SP1) and (SP2)*, recording 71.08±4.0, 53.87±3.49 and 48.93±5.43 µg ml^{-1} respectively. *Fusarium (sp2, sp1)* and *Colletotrichum* solubilized 38.54±2.42, 22.68±3.13 and 18.13±2.06 µg ml^{-1} of P respectively.

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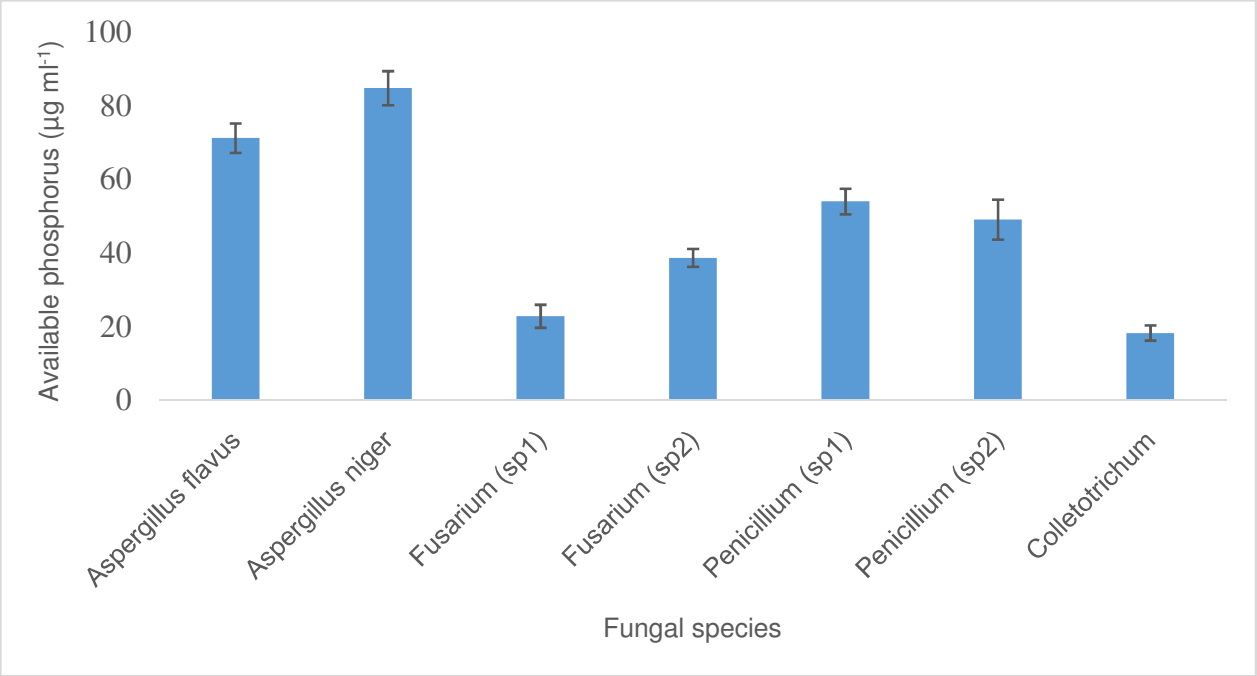

Figure 10: Amount of phosphorus solubilized by different fungal species in NBRIP broth (µg ml^{-1})

Table 1: Phosphorus solubilizing fungi identified in amended and unamended samples

Fungal isolates	T0	T1	T2	T3	T4
Aspergillus flavus	+	+	+	+	+
Aspergillus niger	+	+	+	+	+
Fusarium (sp1)	+	+	+	+	+
Fusarium (sp2)	+	+	+	+	+
Penicillium (sp1)	-	-	-	+	+
Penicillium (sp2)	+	+	+	+	+
Colletotrichum	-	-	-	+	+

(+) present, - (Absent)

Table 1 presents the occurrence of phosphorus solubilizing fungi in control and amended soils. It would be observed that most frequently isolated fungal species were *Aspergillus niger, Aspergillus flavus, Fusarium and Penicillium spp.*

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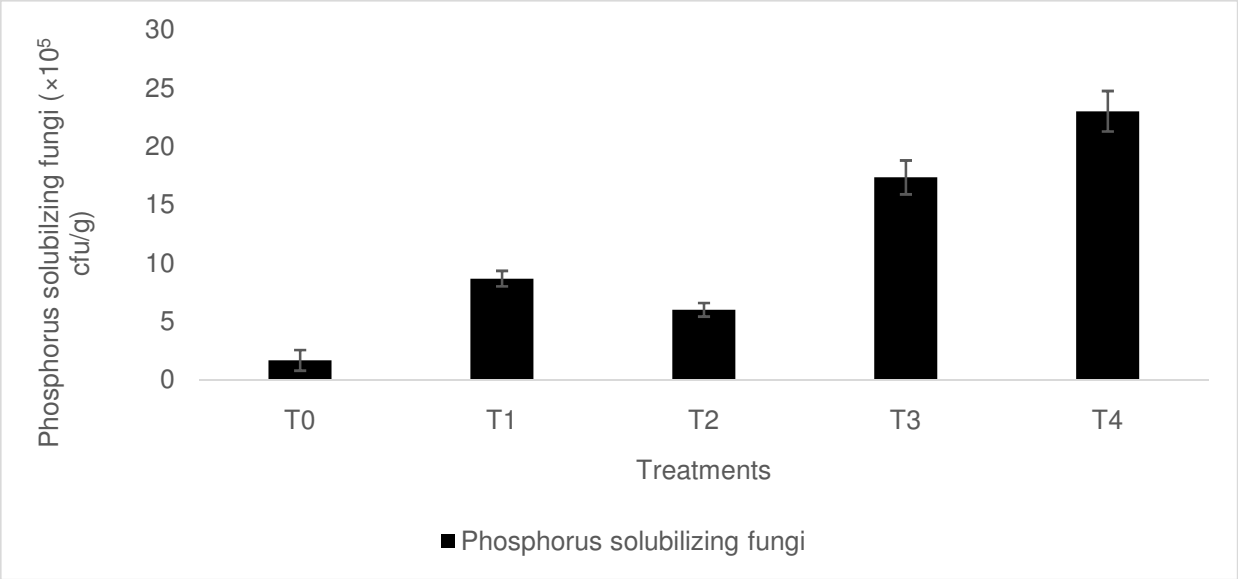

Figure 11: Population of phosphorus solubilizing fungi in control and amended soils (vertical bars denote standard errors of the mean (n = 3))

The population of phosphorus solubilizing fungi increased in soils following the incorporation of biochar only or in combination with poultry manure. At 39 t ha^{-1} (T1) of CHB application, the population of phosphorus solubilizing fungi increased significantly (P = .05). However, when biochar rate was increased to 65 t ha^{-1} (T2) fungal biomass reduced compared with what was observed at 39 t ha^{-1} (Figure 11).

3.3 Species composition of phosphorus solubilizing fungi

Figure 12 shows the species composition of phosphorus solubilizing fungi in soil treatments.

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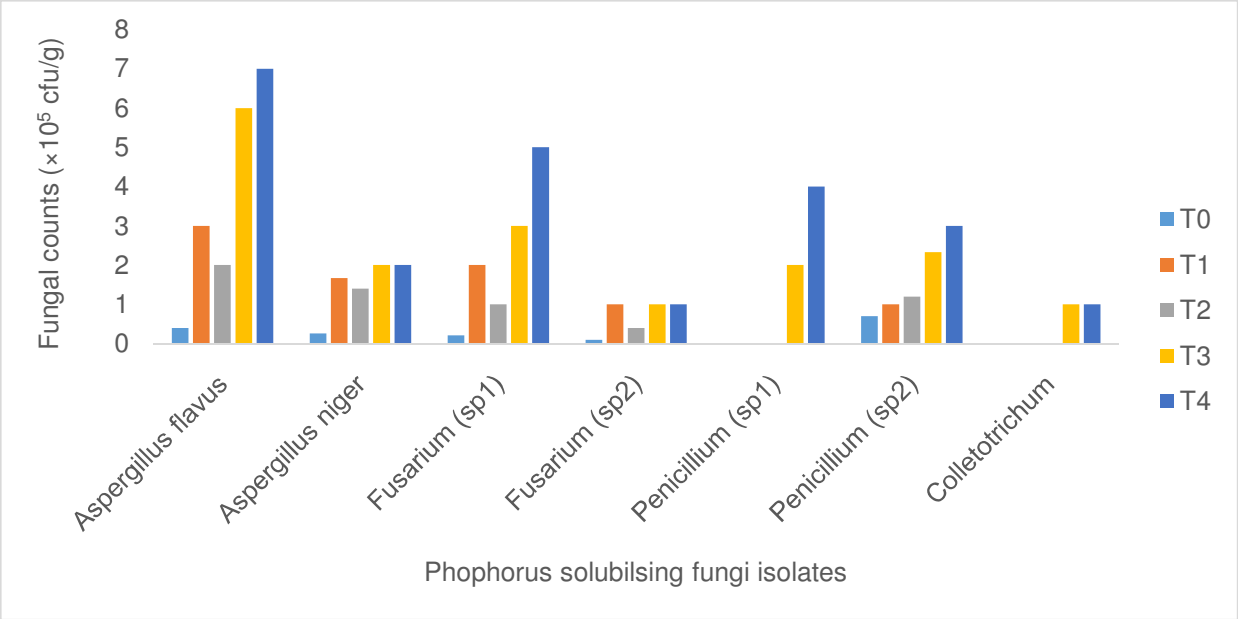

Figure 12: Species composition of phosphorus solubilizing fungi in biochar amended soils only and biochar plus manure amended soils (× 10^5cfu/g)

The soils were dominated by three main genera and these were *Aspergillus, Fusarium* and *Penicillium* with few of the microbes in the genera *Colletotrichum.* At application rate of 29 tha^{-1} (T1) and 65 tha^{-1} (T2) *Aspergillus flavus* increased by 6.50 folds and 4.00 folds respectively. Similarly, *Aspergillus niger*, increased by 5.42 and 4.38 folds at biochar application rates of 29 tha^{-1} (T1) and 65 tha^{-1} (T2). Similarly, the population of *Fusarium sp1* increased by 8.52 folds and 3.76 folds at biochar application rates of 29 tha^{-1} (T1) and 65 tha^{-1} (T2) respectively. The population of *Fusarium sp2* increased by 9.00 folds when biochar was applied at 39 tha^{-1} (T1) and at 65 tha^{-1} an increase by 3.00 folds was observed. Then again, *Penicillium sp2* increased by 0.43 and 0.71 respectively, when biochar was applied at rates of 39 tha^{-1} (T1) and at 65 tha^{-1}.The population of each fungal species increased in combined biochar and poultry manure than observed in sole biochar application. At application of 10 tha^{-1} poultry manure respectively with 39 tha^{-1} and 65 tha^{-1} biochar, *Aspergillus flavus* increased by 14 folds and 16.5 folds. In the same combination involving manure and biochar, *Aspergillus niger* increased by 6.69 and 6.70 folds; Fusarium *sp1* increased by 13.29 and 22.81, Fusarium sp2 increased by 9 folds respectively, *Penicillium sp2* increased by 2.33 and 3.29 folds. Then again, two new fungal species were identified in the combined

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treatments. These were *Colletotrichum* and *Penicillium sp1*. The population of *Penicillium sp1* increased by 1 fold whiles no increase was recorded for *Colletotrichum sp*.

3.4 Effect of biochar and/or poultry manure on selected soil properties

Following 6-weeks incubation of treatments, sole biochar and in combination with poultry manure influenced pH, Available P, Total Organic Carbon, Mineral N and Cation Exchange Capacity (Table 2).

Table 2: Chemical properties of control and amended soils at day 42 following incorporation of biochar and/or poultry manure

Treatments	pH	Av. P (mg kg^{-1})	TOC (%)	MN (mg kg^{-1})	CEC ($cmol_c kg^{-1}$)
T0	4.15 ± 0.07a	1.04 ± 0.03a	0.21 ± 0.10a	6.40 ± 0.11a	2.05 ± 0.07 a
T1	5.63 ± 0.01b	4.70 ± 0.05b	0.55 ± 0.07b	7.03 ± 0.13a	3.86 ± 0.14b
T2	5.91 ± .01d	4.94 ± 0.11b	0.81 ± 0.06c	6.55 ± 0.08a	4.03 ± 0.16b
T3	5.76 ± 0.02c	17.06 ± 0.51c	1.20 ± 0.10d	146.63 ± 3.16b	5.24 ± 0.16c
T4	6.10 ± 0.03e	18.61 ± 0.50d	1.34 ± 0.11e	186.50 ± 3.64c	5.54 ± 0.07d

Values are expressed as mean ± SD. Different letters following the data in the same column denote significance (*P* = .05). AvP – available phosphorus; TOC – total organic carbon; MN –mineral nitrogen; CEC – cation exchange capacity.

Table 3: Simple linear correlation between soil fungal biomass, phosphorus solubilizing fungal biomass and soil properties in soils amended with biochar only or in combination with poultry manure

Soil Microbial properties		pH	AvP	CEC	TOC	MN
Total Fungi	r value	0.670**	0.961**	0.899**	0.869**	0.949**
Phosphorus solubilizing	r value	0.687**	0.959**	0.906**	0.870**	0.941**

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microbes

** Correlation is significant at the 0.01 level

Biochar solely applied or in combination with poultry manure increased pH significantly (P = .05) above the control (Table 2). The results also show that sole application of biochar or biochar combined with poultry manure increased AvP, CEC and TOC of the amended soils and were significantly higher (P = .05) than the control. There were no significant increases in mineral N (sum of Nitrate and Ammonium N) concentration in sole biochar amended soils however significant increases were observed when biochar was applied in combination with manure (Table 2).

4. DISCUSSION

4.1 Biochar and manure effect on phosphorus solubilizing fungal biomass

Increase in fungal biomass and population of phosphorus solubilisers could be associated with the effect of biochar and or manure which induced changes in soil properties consequently influencing the microbial community. Soil pH, Total organic carbon, available P, CEC and mineral N increased significantly in amended soils which correlated positively with fungal composition (Table 3). The addition of biochar improved soil properties and might have created a favorable environment for microbial proliferation (10). Furthermore, the sorption of readily decomposable organic compounds, dissolved organic carbon and chemisorption of ammonium (NH_4^+) at biochar surfaces due to the presence of functional groups, could indicate its suitability as a favourable microbial habitat (11). The increase in fungal biomass observed in our study is in contrast with suggestions that biochar could affect microbial biomass negatively, due to the presence of volatile organic compounds (VOCs) in the labile fractions (10) and high C/N ratio of some biochar (20). Although C/N ratio of biochar used in this study was high, it is possible that the rates used in this study were appropriate to improve fungal proliferation and sustain their activity and did not suppress its proliferation. However, the decreased fungal population at 65 tha^{-1}could be attributed to high C/N ratio of the biochar used for this study (20).High C/N ratio of the biochar used might have caused rapid mineralization of labile carbon leading to reduced soil nitrogen. Nitrogen limitation in soil might have

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resulted in the turnover of microbial biomass. When manure was included in the combination with biochar higher microbial biomass was observed and this could be explained by the supply of C, N, and P from mineralization of poultry manure. Poultry manure used in this study was rich in C, N and P and these elements are rich source of microbial substrate. Its addition to the soil may be a plausible reason for the increase in fungal population. Previous findings have shown that manure supplied macro, micro nutrients and enhanced soil carbon pool (21). This created a good environment for microbial growth, survival and proliferation (22, 23).

4.2 Effect of biochar and/or poultry manure on available P and other selected soil properties

Biochar solely applied or in combination with poultry manure significantly (P = .05) increased soil pH, AvP, TOC and CEC. Increase in pH, AvP, TOC and CEC in treatments followed the order control > biochar > biochar + poultry manure.

The increase in pH in sole biochar amended soils could be attributed to the acid neutralising effect of the biochar and products of manure decomposition. Biochar has a strong adsorptive affinity for cations (Al^{3+}) hence attracting these cations unto its surfaces, consequently reducing exchangeable acidity (Al^{3+}, H^{+}) of the soil. Then again, biochar used in this study contain appreciable concentrations of basic cations especially Ca, Mg and K. The application of biochar to soil caused a rise in exchangeable CEC (Table 2) which correlated positively with Soil pH (r = 0.89, P = .05) and this contributed to the rise in the pH of the soil due to the release of basic cations into solution. Basic cations such as Ca, K, Mg, and silicon (Si) in biochar material can form alkaline oxides or carbonates during the pyrolysis of feedstock (27). When biochar is applied to soil, alkaline oxides or carbonates of Ca, K and Mg can react with the H^{+} and Al^{3+}, raise the soil pH, and decrease exchangeable acidity. This confirms earlier submission that biochar acts as liming material in soils due to its high carbonate concentration (25). The combined application of poultry manure and biochar augmented the pH as a result of mineralization of both amendments which released basic cations like Ca, Mg and K consequently displacing and replacing H^{+} and Al^{3+} ions at the soil exchange sites. As organic manures mineralize, importantly, calcium ions are released into soil solution and subsequently the released calcium ions get hydrolysed. The Calcium hydroxide formed reacts with soluble aluminum ions (Al^{3+}) in the soil solution to yield insoluble $Al(OH)_3$. Similarly, it could be

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possible that the rise in pH was due to ion exchange reactions which occurred when terminal OH- of Al or Fe hydroxyl oxides are replaced by organic anions. These organic anions are products (malate, citrate and tartrate) of decomposition of manure (25).

The results show that sole application of biochar or in combination with poultry manure increased AvP in experimental soils. The upsurge of P solubilizing fungal biomass coupled with other related factors like increase in pH and mineralization of organic P (i.e inositol phosphate, nucleic acid and phospholipid) from biochar and manure caused an elevation of AvP. Application of biochar influenced the upsurge in the microbial numbers due to increases in pH.Acidic condition affects microbial proliferation, in exception of few acidophiles, reducing metabolic activities (26). This limits the mineralization of phosphorus. The increase in AvP correlated positively with population of phosphorus solubilizing fungi (r = 0.96, P = .05) which demonstrates that phosphorus solubilisers played major role in increasing AvP through their metabolic activities acting on precipitated Fe-P or Al-P. In addition, biochar releases basic cations into soil, which subsequently aids in the precipitation of Al and Fe as $Fe(OH)_3$ and $Al(OH)_3$ (26). Thus, increasing availability of P in soil whiles decreasing solubility of Al and Fe (16). A significant positive relationship was found between pH and AvP concentrations (r = 0.66; P= .05), an indication that pH increases enhanced the availability of phosphorus in amended soils. Moreover, biochar ash contains some amount of decomposable phosphorus fractions (organic, inorganic orthophosphate and pyrophosphate) resulting from the conversion of organic P during charring (27). The combination of manure to biochar showed a positive synergy with the two amendments adding nutrient directly to soil and also enhancing the composition of phosphorus solubilisers.

Cation exchange capacity increased (P = .05) in amended soils than that of the control. Generally, the increase in CEC of amended soils can be attributed to biochar surface oxidation and creation of carboxylic and phenolic surface functional groups upon biochar application to soil (28). The ionization of these functional groups creates higher surface negative charges which helps in retaining more cations. Then again, it could be related to the increase in pH, affecting the levels of basic cations positively (Ca^{2+}, Mg^{2+} and K^{+}) in amended soils. A positive correlation between CEC and pH (r = 0.89; P = .05) was observed.

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Total organic C of the amended soils were significantly higher than (*P = .05*) the control. The increase of TOC in biochar amended soil may be as a result of the addition of labile carbon or the mineralization of recalcitrant C (usually about 5 % is decomposed) (29) in biochar and/or positive priming effect (30). Although most research findings have reported the recalcitrance of biochar C, it has contrary been reported that biochar may affect microbial proliferation which in turn may contribute to degradation of biochar and subsequently affect carbon release (11). Then again, the addition of biochar might cause positive priming stimulating the mineralization of native soil organic carbon (31). Significant increases (*P* = .05) in the combined treatment could be related to labile C in amendments and co metabolism due to the interaction of biochar, manure and soil. Biochar has been reported in relatedstudy to be positively primed by the addition of a labile C source such as the manure in our study (30) and this is a plausible reason for the increase of organic carbon in soils amended with biochar and manure in our study. The decomposition of biochar might have resulted from the action of metabolites of microorganism whose population was enhanced by the inclusion of manure in this study. Manure acts as carbon and energy source when it decomposes, further enhancing microbial activity. In addition, whiles biochar decomposition could be taken place, poultry manure as well was getting decomposed.

No significant increases (*P*= 0.05) in mineral N concentration in sole biochar amended soils were observed but when biochar was applied together with poultry manure, significant increases were observed. The insignificant increase in mineral N concentrations could be attributed to the immobilization of NH_4^+-N onto biochar surfaces or loss of mineral N as ammonia gas (10, 32). More so, the insignificant increases in the concentration of mineral N could be due to immobilization of N by microbial biomass. Addition of biochar increased microbial population as a result of modifying effect of biochar creating a conducive environment which stimulated the proliferation of the nitrifier community. Coupled with higher C/N ratio of biochar materials applied, increasing ammonifier and nitrifier population might have caused immobilization of mineral N in sole biochar-amended soils (12). Combined biochar and poultry manure elevated net N mineralization in nutrient deficient soil which is expected. The increase in mineral N could be as a result of the synergistic effect of the two amendments with poultry manure having a high concentration of N. Upon decomposition of the amendments especially manure, mineral N is released into solution due to higher net ammonification and nitrification. Enhanced net N mineralization was

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possible because less of NH_4^+-N released from mineralized manure became bound to the biochar due to the saturation of NH_4^+-N binding sites on biochar material by excess NH_4^+-N ammonified from manure, leaving adequate amount to be nitrified. Then again, net N mineralization could be associated with the increase of manure supplied cations (Mg, Ca, K and Na) in the soil solution (33). These ions compete with NH_4^+-N or replace adsorbed NH_4^+-N at binding sites on the biochar. The excess, non-sequestered and desorbed NH_4^+-N is nitrified leading to higher NO_3^-- N in soil samples that received combined biochar and manure amendments. The increased net N mineralization may also be due to regulation of microbial immobilization as a result of manure addition. It was expected that once biochar was included in the fraction, immobilization might occur due to the high C/N ratio of the biochar materials but the addition of poultry manure maximized net N mineralization to buffer the C/N ratio effect (34).

5. CONCLUSION

The study demonstrated that the application of biochar solely or in combination with poultry manure increased the fungal biomass and phosphorus solubilizing fungal biomass. The increases in the biomass of phosphorus solubilizing fungi correlated positively with available phosphorus concentration. Soil pH, soil organic carbon and cation exchange capacity also increased following the incorporation of biochar singly or in combination with poultry manure. The results also revealed that sole biochar application did not have any significant effect on mineral N but upon inclusion of poultry manure, mineral N increased in amended soil. The trend of improvement in soil properties followed control < biochar < biochar + poultry manure. It is conclusive that appropriate combination of biochar and poultry manure can significantly revamp microbial biomass and improve the fertility of highly weathered tropical soil.

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