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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2021.90.98

Research Article
Soil pH and Microbial Properties as Affected by Integrated Use of Biochar, Poultry Manure and Urea

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Abstract

Background and Objective: The use of biochar, a charred organic material, is becoming a sustainable technology that leads to the improvement of highly weathered tropical soils. A study was conducted at the Teaching and Research Farm of the University of Calabar to compare the effects of integrated use of Biochar (B), Poultry Manure (PM) and urea on the pH and microbial properties of soil-grown with *Amaranthus cruentus*. **Materials and Methods:** A total of 15 treatments consisting of a sole and combined use of biochar, poultry manure, urea and control were fitted on a randomized complete block design with 3 replications. The organic amendments were applied 2 weeks before sowing while urea was applied 2 weeks after sowing. Composite soil samples (0-15 cm) were taken before and after the experiment for pH and microbial analysis. **Results:** Soil pH was raised from a strongly acidic level to values ranging from 5.73-6.8 (moderately acid-slightly acid, respectively) pH level after the experiment with the highest obtained in Full B+½PM amended soil. The fungal population was maximum in PM (sole and combinations) (18×10^3-20×10^3 CFU g^{-1}) and minimum in sole biochar and control ($\times^{-}10\times10^3$ CFU g^{-1}). The bacterial population exhibited a similar trend with the maximum value in PM (sole and combinations) (98×10^6-148×10^6CFU g^{-1}) and minimum in control ($\times^{-}36\times10^6$CFU g^{-1}). **Conclusion:** This suggests that biochar addition alone did not cause any apparent alteration to the microbial population and diversity compared to its complementary use.

Key words: *Amaranthus*, biochar, microbial properties, poultry manure, soil pH, urea fertilizer, N fixation

Citation: Iren, O.B. and V.F. Ediene, 2021. Soil pH and microbial properties as affected by integrated use of biochar, poultry manure and urea. Pak. J. Biol. Sci., 24: 90-98.

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Biochar is a charred organic material, in the form of charcoal, produced from feedstock to be used in amending degraded soils, sequester soil carbon content and improve soil fertility[1]. It consists of a fast degradable portion and a recalcitrant portion which is very resistant to biological and chemical oxidation and can persist within the soil system for centuries to millennia to completely mineralize[2]. Its degradation can take years[3] thus resulting in long term carbon (C) sequestration[4,5] improving soil quality and health[6,7] thereby increasing crop yields[8]. Biochar is generally of alkaline pH and may alter soil pH in a favorable direction for most crops. The ash content of biochar is primarily responsible for the modification of the soil's pH. Biochar additions in soils have been reported to increase the pH of acidic soils owing to its alkaline nature[9], enhance soil fertility and mitigate climate change by reducing soil N_2O emissions[10].

Positive effects of biochar on biological communities within soils have been reported; enhanced biological N fixation (rhizobia)[11]; improved colonization of mycorrhizal fungi. Earthworms showed a preference for biochar amended soils[12]. Rondon *et al.*[11] found out that increasing biochar amendments in the soil can increase the proportion of N derived from fixation by *Phaseolus vulgaris* (common green bean) and this increased yields. Biochar amendment is reported to change soil biological community composition and its abundance[13] and enhance systemic resistance to pathogens and diseases. However, there is little or no research on the effect of biochar fortification with poultry manure and urea fertilizer on soil microbial communities.

The study was designed to assess the effect of wood biochar fortified with poultry manure and urea fertilizer on soil microbial properties and pH of soil-grown with *Amaranthus* in acidic soil in Calabar.

MATERIALS AND METHODS

Description of the study area: The experiment was conducted at the Teaching and Research Farm of the University of Calabar, Nigeria. The University of Calabar is situated between latitudes 05°32" and 4°27" N and longitudes 07°15" and 90°28"E. The site is in a degraded rainforest vegetation zone of Nigeria, having a bimodal rainfall pattern with a long rainy season from March-July and a short rainy season from September to the first two weeks of November after a short dry spell in August otherwise called 'August break'. The mean minimum temperature varies from 21-24°C and the mean maximum temperature from 27-30°C. The mean relative humidity varies between 60-90%. The soil of the experimental area is classified as Ultisol based on the USDA system of classification[14]. The soils are very strongly acidic and generally low in nutrients. This research project was conducted from May-July, 2018.

Collection and preparation of research materials: Biochar made from wood feedstock was milled using a mechanical blender and sieved with a 4 mm size plastic sieve to obtain its smooth fine powder. *Amaranthus* seeds and urea were obtained from Agricultural Development Project (ADP) office in Calabar, Cross River State while poultry manure was obtained from the University of Calabar Animal Farm.

Experimental design and treatments: A total of 15 treatments were fitted into a randomized complete block design and replicated three times to give a total of 45 experimental units. The treatments consisted of sole use of biochar, urea and poultry manure and their various combinations as shown in Table 1. Sole application of urea which was regarded as a full dose for *Amaranthus* according to Iren *et al.*[15] was 60 kg N ha^{-1} while poultry manure and biochar were singly applied at 20 t ha^{-1}.

Field studies: The experimental site was manually cleared, stumped and marked out with pegs. Flatbeds measuring 1×1 m were made manually using a spade. An alley of 1.5 m was left between blocks and 0.6 m between plots. Biochar and poultry manure was applied to specified plots using broadcast with incorporation method and left for two weeks before sowing *Amaranthus* seeds to allow mineralization to take place. Urea fertilizer was applied to specified plots two weeks after planting using the band placement method. Banding of the urea fertilizer was about 3.75 cm away from the plant on one side of the seed row and about 5 cm deep[15].

Amaranthus seeds were mixed with dried river sand before sowing to ensure the seeds were not planted too close together for proper management of the seed rate desired. The mixture was 70% sand and 30% *Amaranthus* seeds[15]. These were evenly distributed directly on drills at a distance of 10 cm between each row. The seedlings were later thinned to one plant per stand a few days after emergence at a spacing of 10 cm between plants. Therefore, the planting distance was 10×10 cm giving a plant population of 100 plants per bed equivalent to 1,000,000 plants per hectare (10,000 m^2). The plots were kept weed-free throughout the crop growing period by hand pulling because of the closeness of the plants.

Table 1: Treatment combinations

Treatments	Quantity per hectare	Quantity per plot
Control (no amendment)	0	0
Biochar (B) alone	20 t ha^{-1}	2 kg
Poultry manure (PM) alone	20 t ha^{-1}	2 kg
Urea (U) alone	60 kg N ha^{-1}	13.04 g
½ Biochar+½ urea	10 t ha^{-1} B+30 kg N ha^{-1} U	1 kg B+6.52 g U
¾ Biochar+¼ urea	15 t ha^{-1} B+15 kg N ha^{-1} U	1.5 kg B+3.26 g U
¼ Biochar+¾ urea	5 t ha^{-1} B+45 kg N ha^{-1} U	0.5 kg B+9.78 g U
Full biochar+½ urea	20 t ha^{-1} B+30 kg N ha^{-1} U	2 kg B+6.52 g U
½ Biochar+full urea	10 t ha^{-1} B+60 kg N ha^{-1} U	1 kg B+13.04 g U
½ Biochar+½ poultry manure	10 t ha^{-1} B+10 t ha^{-1} P	1 kg B+1 kg P
¾ Biochar+¼ poultry manure	15 t ha^{-1} B+5 t ha^{-1} P	1.5 kg B+0.5 kg P
¼ Biochar+¾ poultry manure	5 t ha^{-1} B+15 t ha^{-1} P	0.5 kg+1.5 kg P
Full biochar+½ poultry manure	20 t ha^{-1} B+10 t ha^{-1} P	2 kg B+1 kg P
½ Biochar+full poultry	10 t ha^{-1} B+20 t ha^{-1} P	1 kg B+2 kg P
½ Biochar+½ PM+½ urea	10 t ha^{-1} B+10 t ha^{-1} P+30 kg N ha^{-1} U	1 kg B+1 kg P+6.52 g U

Soil sampling and processing: Composite soil samples (0-15 cm) were taken before the experiment while at the end of the experiment; soil samples were taken per plot, properly labeled, placed in ice chaste and transported to the laboratory for microbial analysis. Soil samples were also taken before the experiment and at the end of the experiment per plot for soil pH determination. The samples were air-dried, sieved with a 2 mm size sieve and stored for onward analysis.

Laboratory analysis: Samples of biochar and soil were subjected to chemical analysis using standard procedures as outlined by Jones[16]. Soil pH was determined in 1:2.5 soils: water ratio with a pH meter.

Soil microbial analysis

Media preparation and microbial analysis method

Soil extract agar: One thousand grams each of the experimental soil was weighed into a different conical flask containing 1 liter (1000 mL) of distilled water and stirred vigorously using a stirring rod. The supernatant was filtered and the extract was used to prepare the agar. Fifteen grams of powdered Agar were added to the soil extract boiled to dissolve and sterilized by autoclaving at a temperature of 121°C and 151 bs pressure for 15 min.

Malt extract agar: Malt extract agar was used as a medium for fungi. Then, 26 g of agar was suspended in 1000 mL of distilled and 20 mL of malt extract was added. The mixture was mixed properly and sterilized by autoclaving at a temperature of 121°C in inch for 15 min.

Cultivation and enumeration of bacteria in the experimental plots: One gram of each sample was weighed and thoroughly shaken in 10 mL of distilled water. An aliquot (1.0 mL) was transferred aseptically into the next tube and diluted serially in one-tenth stepwise to 10^{-6} dilution[17]. From the dilution of 10^{-5} of each soil sample, 0.1 mL aliquot was transferred aseptically onto freshly prepared nutrient Agar plates[18]. The inoculated plates were inverted and incubated at 37°C for 24-48 hrs after which the plates were examined for growth. The discrete colonies which developed were counted and the average counts for triplicate cultures were recorded as total viable bacterial count in the sample.

Isolation, characterization and identification of bacteria from the experimental soils: Pure culture of bacteria was obtained by aseptically streaking representative colonies of different morphological types that appeared on the culture plates on a freshly prepared nutrient agar plate which were incubated at 30°C for 24 hrs. Discrete bacteria colonies that developed were sub-cultured on nutrient agar slopes and incubated at 30°C for 24 hrs. These served as pure stock for subsequent characterization tests. Standard characterization tests were performed in duplicates: Gram staining, catalase test, coagulate test, sugar fermentation test, motility test, methyl red test, Voges Proskauer test, indole test and citrate utilization test. The pure cultures were identified on the basis of their cultural, morphological and physiological characteristics in accordance with the method by Weaver *et al.*[19].

Cultivation and enumeration of fungi: Each sample (1 g) was thoroughly shaken into 10 mL of sterile distilled water and diluted serially. From the dilution of 10^{-3} of each soil sample, 0.1 mL aliquot was transferred aseptically onto freshly prepared malt agar plate to which 0.2 mL of 0.5% of Ampicillin had been added to inhibit the growth of bacteria[18]. The

inoculated plates were inverted and incubated at 30°C (room temperature) for 5-7 days. The colonies which developed were counted and the average count for the triplicate cultures was recorded as total viable fungi in the sample.

Isolation, characterization and identification of fungi: Pure culture of fungi was obtained by subculturing discrete colonies onto freshly prepared malt agar plates and inoculated at 30°C for 5-7 days. The fungal isolates which developed were further subcultured onto agar slopes and incubated at room temperature for 5-7 days. The isolates which developed were pure cultures which were stored in the refrigerator as stock cultures for subsequent characterization test. The following standard characterization tests were performed in duplicates: Macroscopic examination of fungal growth was carried by observing the colony morphology-Diameter, colour (pigmentation), texture and surface appearance. Microscopic examination was done by needle mount method[19,20] and observing sexual and asexual reproductive structures the sporangia, conidial head, arthrospores and the vegetative mycelium. Sugar (glucose, lactose, fructose, sucrose, galactose, maltose and mannose) fermentations were carried out for species identification. The complete identification of fungal isolates was done by comparing the results of their cultural, morphological and biochemical characteristics with those of known taxa[19].

RESULTS AND DISCUSSION

The soil used was loamy sand and strongly acidic (pH 5.1). It was low in organic carbon (1.15%), total N (0.08%) and exchangeable K (0.11 cmol kg^{-1}) but high in available P (31.02 mg kg^{-1}) and had exchangeable acidity of 2.00 cmol kg^{-1}. The exchangeable bases (Ca, 2.4 cmol kg^{-1}; Mg, 1.2 cmol kg^{-1}; K, 0.11 cmol kg^{-1}, Na, 0.07 cmol kg^{-1}) were low. Generally, the low soil organic carbon, N and K contents indicates low fertility status thus necessitating the need for additional nutrient supply.

Table 2 shows the nutrient concentration of biochar (1.3% N, 0.05% P, 1.72% Ca, 1.92% K and 1.05% Mg). It had an alkaline pH of 7.8 and an organic carbon content of 35.9%. This confirms the report by Chintala *et al.*[9] who stated that biochar contains an ash component that is usually alkaline and therefore could potentially increase soil pH if added to acidic soil. The organic carbon content of the biochar used falls within that reported by Chan and Xu[21] who stated that organic carbon in biochar can vary from 0-91%. The high C:N ratio shows the potential of the biochar to decompose slowly.

The effects sole and complementary use of biochar, poultry manure and urea fertilizer on soil pH are shown on Table 3. Soil pH was raised from a strongly acidic level of 5.1 and 5.4 obtained before experiment and control, respectively to values ranging from 5.733 (moderately acid)-6.8 (slightly acid) pH level after the experiment. The highest pH value was obtained in the soil amended with Full B+½PM (6.8), followed by ½B+Full PM (6.7) and ¼B+¾PM (6.6). This means that biochar when combined with poultry manure (organic manure) has a more positive effect on the pH level of the soil than when combined with urea (inorganic fertilizer). This confirms the assertion made by Chintala *et al.*[9] who reported that biochar contains an ash component that is usually alkaline and could potentially increase soil pH if added to acidic soils. Therefore, combining biochar with poultry manure is of the added advantage as poultry manure contains basic nutrients such as calcium and magnesium which displace hydrogen and aluminum ions that cause acidity. This agrees with the result obtained by Iren *et al.*[15] who then attributed the ability of organic manures to increase soil pH to the presence of these basic cations.

The soil pH values obtained in the different treatments differed from each other with a Standard Deviation (SD) of ±0.394 and a coefficient of variability (CV) of 6.279% showing the level of variability among treatments.

Mean population count: The microbial properties of the soils are discussed to Table 4-5 and Fig. 1a-b. The probable bacterial isolates in the rhizosphere soils of the control and sole biochar plots include *Arthrobacter* spp., *Agromyces* sp., *Bacillus* spp., *Klebsiella* spp., *Micrococcus* spp., *Pseudomonas* spp. and *Nocardia* spp. while the fungal isolates were *Aspergillus niger*, *Nigrosopra* spp., *Penicillium* spp., *Pullularia* spp. and *Rhizopus* spp., while the bacterial isolates for sole biochar plots were *Arthrobacter* spp., *Agromyces* sp., *Bacillus* spp., *Klebsiella* spp.,

Table 2: Chemical composition of the amendments used

	Chemical composition (%)						
Amendment	N	P	K	Ca	Mg	O.C	pH (H_2O)
Biochar	1.3	0.05	1.92	1.72	1.05	35.90	7.8
Poultry manure	2.6	0.21	2.70	1.44	0.67	36.90	7.6
Urea	46	-	-	-	-	-	-

Table 3: Effect of sole and complementary use of biochar, poultry manure and urea on soil pH

Treatments	pH (H_2O)
Control	5.4
U-alone	5.5
B-alone	6.0
PM-alone	6.1
½ B+½ U	5.8
¾ B+¼ U	6.4
¼ B+¾ U	6.2
Full B+½ U	6.5
½ B+Full U	5.9
½ B+½ PM	6.5
¾ B+¼ PM	6.5
¼ B+¾ PM	6.6
Full B+½ PM	6.8
½ B+Full PM	6.7
½ B+½ PM+½ U	5.7
Mean	6.3
SD	0.394
CV	6.279

SD: Standard deviation, CV: Coefficient of variability

Micrococcus spp. and *Pseudomonas* spp., while the fungi isolates were *Aspergillus* spp., *Cladosporium* spp., *Nigrosopra* spp., *Pullularia* spp., *Penicillium* spp. and *Rhizopus* spp. Interestingly, the sole poultry liter and its combinations had more diverse species with the bacterial recording: *Agromyces* sp., *Streptococcus* spp., *Salmonella* spp., *Enterococci* spp., *Escherichia coli*, *Bacillus cereus*, *Staphylococcus* spp., *Campylobacter* spp., *Pseudomonas* spp. and *Micrococcus* spp. while fungi species included: *Trichoderma* spp., *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus fumigatus*, *Aspergillus terreus*, *Alternaria alternate*, *Nectria* spp., *Actinomycete* spp. *Bacillus* spp. was the most common occurring bacteria across the various treatments while *Aspergillus* spp. was the most common fungi specie. The ½ Biochar+½ Poultry +½ Urea combinations yielded much more communities of bacteria and fungi (Table 4-5). The bacterial isolates for the sole urea plots include *Arthrobacter* spp., *Agromyces* sp., *Bacillus* spp., *Klebsiella* spp., *Micrococcus* spp. and *Pseudomonas* spp.

The bacterial and fungal counts across the different treated soils varied from the control and sole biochar treatments (Fig. 1a-b). The sole biochar treatment yielded a slight bacterial ($\times^{-}37\times10^{6}$) population increase from the control ($\times^{-}36\times10^{6}$) but showed the least fungal ($\times^{-}10\times10^{3}$) counts and diversity amongst the treatments. There was a less drastic change in the bacterial population and diversity in response to sole biochar application than when compared with the sole urea ($\times^{-}80\times10^{6}$) and poultry manure ($\times^{-}148\times10^{6}$). The combinations of urea and biochar gave higher microbial counts and diversity than biochar alone (Table 4-5). The combinations of ½ biochar+½ urea, ¾ biochar+¼ urea, ¼ biochar+¾ urea, full biochar+½ urea and

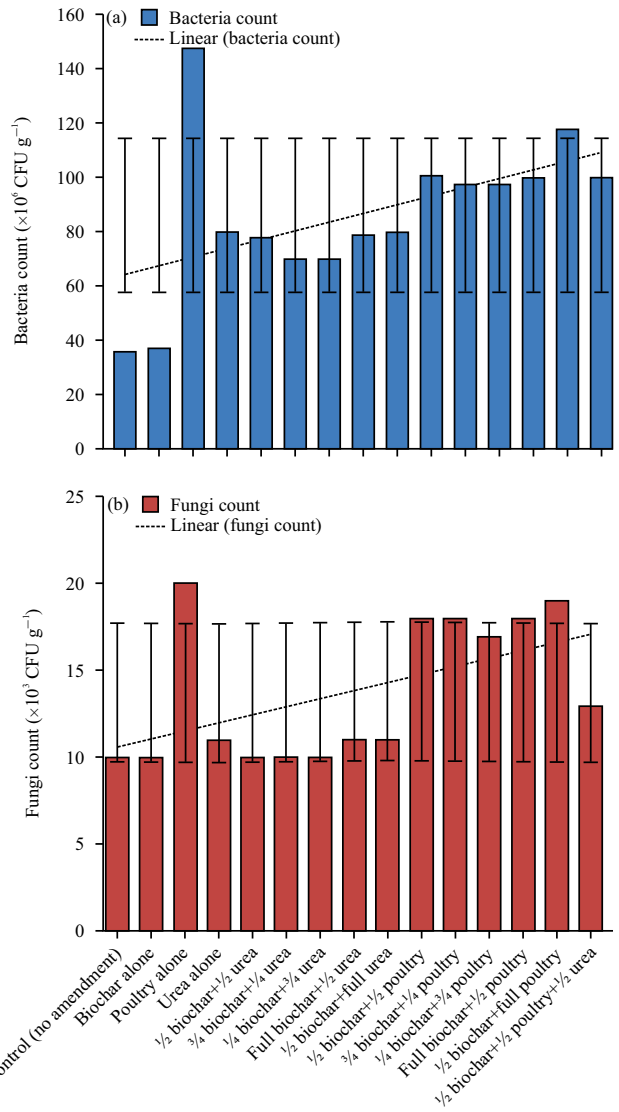

Fig. 1(a-b): Comparison of rhizosphere, (a) Bacterial and (b) Fungal communities in biochar fortified with poultry manure and urea treated soils

½ biochar+full urea, gave mean counts of 78×10^{6}, 70×10^{6}, 70×10^{6}, 79×10^{6} and 80×10^{6}, respectively. Amongst the various treatment combinations, biochar with poultry manure yielded the highest microbial (bacteria and fungi) mean counts and diversity (Table 4) with ¾ biochar+¼ poultry manure and ¼ biochar+¾ poultry manure having a mean of 98×10^{6}. This was closely followed by the ½ biochar+½ poultry manure+½ urea treatments with a mean of 100×10^{6} and the combinations of ½ biochar+full poultry manure with a mean count of 118×10^{6}. The non-alteration of the bacterial composition may be attributed to the prevailing environmental factor (high rainfall) in the study area which can influence microbial activities. It could also be a result of

Table 4: Probable bacterial Isolates and their percentage occurrence in biochar fortified with poultry manure and urea treated soils

Treatments	Probable isolates	Occurrence (%)	Treatments	Probable isolates	Occurrence (%)	Treatments	Probable isolates	Occurrence (%)
Control (no amendment)	*Arthrobacter* spp. *Agromyces* sp. *Bacillus* spp. *Klebsiella* spp. *Micrococcus* spp. *Pseudomonas* spp. *Nocardia* spp.	2.8	¾ Biochar+¼ urea	*Arthrobacter* spp. *Agromyces* sp. *Bacillus* spp. *Klebsiella* spp. *Micrococcus* spp. *Pseudomonas* spp.	5.4	¾ Biochar+¼ Poultry	*Enterococci* spp. *Escherichia coli* *Bacillus cereus* *Staphylococcus* spp. *Campylobacter* spp. *Pseudomonas* spp. *Micrococcus* spp. *Streptococcus* spp. *Salmonella* spp.	7.6
Biochar alone	*Arthrobacter* spp. *Agromyces* sp. *Bacillus* spp. *Klebsiella* spp. *Micrococcus* spp. *Pseudomonas* spp. *Nocardia* spp.	2.9	¼ Biochar+¾ urea	*Arthrobacter* spp. *Agromyces* sp. *Bacillus* spp. *Klebsiella* spp. *Micrococcus* spp. *Pseudomonas* spp. *Nocardia* spp.	5.4	¼ Biochar+¾ Poultry	*Agromyces* sp. *Enterococci* spp. *Escherichia coli* *Bacillus cereus* *Staphylococcus* spp. *Campylobacter* spp. *Pseudomonas* spp. *Micrococcus* spp. *Streptococcus* spp. *Salmonella* spp.	7.6
Poultry alone	*Streptococcus* spp. *Salmonella* spp. *Enterococci* spp. *Escherichia coli* *Bacillus cereus* *Staphylococcus* spp. *Campylobacter* spp. *Pseudomonas* spp. *Micrococcus* spp.	11.4	Full biochar +½ urea	*Arthrobacter* spp. *Agromyces* sp. *Bacillus* spp. *Klebsiella* spp. *Micrococcus* spp. *Pseudomonas* spp.	6.1	Full biochar +½ poultry	*Agromyces* sp. *Enterococci* spp. *Escherichia coli* *Bacillus cereus* *Staphylococcus* spp. *Campylobacter* *Pseudomonas* spp. *Micrococcus* spp. *Nocardia* spp. *Streptococcus* spp. *Salmonella* spp.	7.7
Urea alone	*Arthrobacter* spp. *Agromyces* sp. *Bacillus* spp. *Klebsiella* spp. *Micrococcus* spp. *Pseudomonas* spp.	6.2	½ biochar+Full urea	*Arthrobacter* spp. *Agromyces* sp. *Bacillus* spp. *Klebsiella* spp. *Micrococcus* spp. *Pseudomonas* spp.	6.2	½ Biochar+Full poultry	*Enterococci* spp. *Escherichia coli* *Bacillus cereus* *Staphylococcus* spp. *Campylobacter* spp. *Pseudomonas* spp. *Micrococcus* spp. *Nocardia* spp. *Streptococcus* spp. *Salmonella* spp.	9.2
½ biochar+ ½ urea	*Arthrobacter* spp. *Agromyces* spp. *Bacillus* spp. *Klebsiella* spp. *Micrococcus* spp. *Pseudomonas*	6.0	½ Biochar+½ poultry	*Agromyces* sp. *Streptococcus* spp. *Salmonella* spp. *Enterococci* spp. *Escherichia coli* *Bacillus cereus* *Staphylococcus* spp. *Campylobacter* spp. *Pseudomonas* spp. *Micrococcus* spp.	7.8	½ biochar+½ poultry+½ urea	*Agromyces* sp. *Arthrobacter* spp. *Enterococci* spp. *Escherichia coli* *Bacillus cereus* *Campylobacter* spp. *Klebsiella* spp. *Pseudomonas* spp. *Micrococcus* spp. *Staphylococcus* spp. *Streptococcus* spp. *Salmonella* spp.	7.7

Table 5: Probable fungal Isolates and their percentage occurrence in biochar combined with poultry manure and urea treated soils

Treatments	Probable isolates	Occurrence (%)	Treatments	Probable isolates	Occurrence (%)	Treatments	Probable isolates	Occurrence (%)
Control (no amendment)	*Aspergillus niger* *Nigrosopra* spp. *Penicillium* spp. *Pullularia* spp. *Rhizopus* spp.	4.9	¾ Biochar+¼ urea	*Aspergillus* spp. *Cladosporium* spp. *Nigrosopra* spp. *Pullularia* spp. *Penicillium* spp. *Rhizopus* spp.	4.9	¾ Biochar+¼ Poultry	*Trichoderma* spp. *Aspergillus flavus* *Aspergillus niger* *Aspergillus fumigatus* *Aspergillus terreus* *Alternaria alternate* *Nectria* spp. *Actinomycete* spp.	8.7
Biochar alone	*Aspergillus niger* *Nigrosopra* spp. *Penicillium* spp. *Pullularia* spp. *Rhizopus* spp.	4.9	¼ Biochar+¾ urea	*Aspergillus* spp. *Cladosporium* spp. *Nigrosopra* spp. *Pullularia* spp. *Penicillium* spp. *Rhizopus* spp.	4.9	¼ Biochar+¾ Poultry	*Aspergillus flavus* *Aspergillus niger* *Aspergillus fumigatus* *Aspergillus terreus* *Alternaria alternate* *Nectria* spp. *Actinomycete* spp. *Nigrosopra* spp. *Pullularia* spp. *Trichoderma* spp. *Trichoderma* spp.	8.3
Poultry alone	*Trichoderma* spp. *Aspergillus flavus* *Aspergillus niger* *Aspergillus fumigatus* *Aspergillus terreus* *Alternaria alternate* *Nectria* spp. *Hoematococca* *Actinomycete* spp.	9.7	Full biochar+½ urea	*Aspergillus* spp. *Cladosporium* spp. *Nigrosopra* spp. *Pullularia* spp. *Penicillium* spp. *Rhizopus* spp.	5.3	Full biochar+½ poultry	*Trichoderma* spp. *Aspergillus flavus* *Aspergillus niger* *Aspergillus fumigatus* *Aspergillus terreus* *Alternaria alternate* *Nectria* spp. *Actinomycete* spp.	8.7
Urea alone	*Aspergillus* spp. *Cladosporium* spp. *Nigrosopra* spp. *Pullularia* spp. *Penicillium* spp. *Rhizopus* spp.	5.3	½ Biochar+Full urea	*Aspergillus* spp. *Cladosporium* spp. *Nigrosopra* spp. *Pullularia* spp. *Penicillium* spp. *Rhizopus* spp.	5.3	½ Biochar+Full poultry	*Trichoderma* spp. *Aspergillus flavus* *Aspergillus niger* *Aspergillus fumigatus* *Aspergillus terreus* *Alternaria alternate* *Nectria* spp. *Actinomycete* spp.	9.2
½ Biochar+½ urea	*Aspergillus* spp. *Cladosporium* spp. *Penicillium* spp. *Rhizopus* spp.	4.9	½ Biochar+½ poultry	*Trichoderma* spp. *Aspergillus flavus* *Aspergillus niger* *Aspergillus fumigatus* *Aspergillus terreus* *Alternaria alternate* *Nectria* spp. *Actinomycete* spp.	8.7	½ Biochar+½ poultry+½ urea	*Trichoderma* spp. *Aspergillus flavus* *Aspergillus niger* *Aspergillus fumigatus* *Aspergillus terreus* *Alternaria alternate* *Nectria* spp. *Actinomycete* spp.	6.3

the application rate as biochar applied in high quantities is reported to cause a decrease in microbial activity as seen in incubation studies done by Baldock and Smernik[22]. The low activity of microbes in the sole biochar plots could also arise owing to the feedstock and the recalcitrant nature of the biochar. Although it is chronicled in the literature that biochar can be utilized by microorganisms as a carbon source, evidence still exist that biochar shows great resistance to decomposition and utilization by many microorganisms. According to Smith *et al.*[3] the degradation can take millennia. In other words, only a small fraction of biochar is easily available to microbes. Its recalcitrant nature proves to be the most limiting factor regarding the influence it has on chemical and biological processes within the soil system[23]. This is the main reason why biochar serves mainly as a tool to sequester carbon within the soil system[4-6,24].

The different bacteria and fungi species observed for the different treatment combinations are dependent on the degradability of the substance, the stage of decomposition of the treatment materials and the availability of substrate for utilization by the microorganisms. The less drastic change observed in microbial population and diversity in the rhizosphere of sole biochar applied treatment and the appreciable counts and diversity in the biochar/urea and biochar/poultry manure rhizospheres is an indication that the activities of these organisms are boosted by the urea and poultry materials. The percentage occurrence of bacteria (11.40%) and fungi (9.7%) was highest in poultry manure alone treated soils (Table 4-5).

CONCLUSION

The positive influence of biochar applied either alone or in combination with urea or poultry manure show the potentials of biochar in ameliorating acidic soils. However, the combination of biochar with poultry manure is of the added advantage as poultry manure contains basic nutrients such as calcium and magnesium which displaces hydrogen and aluminum ions that cause acidity thus creating a conducive environment for microbes to thrive. Microbial population and diversity were highest in the combined treatments of either biochar with urea or poultry manure compared to biochar alone. The results, therefore, suggest that biochar addition alone did not cause any apparent alteration to the microbial population and diversity compared to its complementary use.

SIGNIFICANCE STATEMENT

This study discovers the usefulness of using biochar either solely or in combination with either organic or inorganic source of nutrient in ameliorating acidic soils. This study will help the researcher to uncover the critical role of complementary use of biochar with other source of nutrients in creating a conducive environment for microbes to thrive that many researchers were not able to explore. Thus for increase in microbial population and diversity in soils to be attained, sole use of biochar should be minimized and other possible combinations may be arrived at.

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