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Effectiveness of Combined Biochar and Lignite with Poultry Litter on Soil Carbon Sequestration and Soil Health

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Abstract

Healthy soils are important to ensure satisfactory crop growth and yield. Poultry litter (PL), as an organic fertilizer, has proven to supply the soil with essential macro and micronutrients, enhance soil fertility, and improve crop productivity. Integrating this treatment has the potential to improve soil physical and biological properties by increasing soil carbon, C. However, rapid decomposition and mineralization of PL, particularly in the hot and humid southeastern U.S., resulted in losing C and reduced its effect on soil health. Biochar and lignite have been proposed to stabilize and mitigate C loss through application of fresh manure. However, their combined effects with PL on C sequestration and soil health components are limited. A field experiment was conducted on Leeper silty clay loam soil from 2017 to 2020 to evaluate the combined effect on soil properties when applying biochar and lignite with PL to cotton (*Gossypium hirsutum* L.). The experimental design was a randomized complete block involving nine treatments replicated three times. Treatments included PL and inorganic nitrogen, N, fertilizer with or without biochar and lignite, and an unfertilized control. Application rates were 6.7 $Mg \cdot ha^{-1}$ for PL, 6.7 $Mg \cdot ha^{-1}$ for biochar and lignite and 134 $kg \cdot ha^{-1}$ for inorganic N fertilizer. Integration of PL and inorganic fertilizer with biochar and lignite, resulted in greater soil infiltration, aggregate stability, plant available water, reduced bulk density and penetration resistance as compared to the sole applications of PL and inorganic fertilizer.

Keywords

Soil Health, Lignite, Biochar, Poultry Litter

1. Introduction

Poultry litter (PL) has proven to supply the soil with macro and micronutrients that can reduce the demand for synthetic fertilizers and improve soil fertility and crop productivity [1] [2] [3]. In addition, PL has the potential to enhance soil organic matter content while improving soil health. However, the rapid decomposition of organic resources in the hot and humid southeastern U.S. results in C loss which contributes to environmental pollution [4] and makes PL less effective for improving soil health. Since soil C/organic matter is considered a key indicator of soil structure [5], innovative technologies and practices are needed to conserve and protect organic matter from decomposition.

Recently, the application of biochar in agriculture has become more common [6] [7]. Biochar is an organic C-rich by-product, which is characterized by stable aromatic organic matter, high surface area, and variable functional groups [8] [9]. These characteristics of biochar generate its ability to be highly resistant to microbial degradation and are not easily mineralized by soil microbes [10]. Besides being a highly stable C pool, biochar can also play a role in stabilizing C of freshly added manure via mechanisms of sorption and physical protection [11]. Due to its porosity structure, soil C can be adsorbed or absorbed on the surface or in the pores of biochar, thereby preventing C from decomposition [12]. Many soil properties including soil bulk density, water holding capacity, storage of soil organic C and N, hydraulic conductivity, and soil aggregation have been reported to improve after the application of biochar [8] [13] [14] [15] [16]. However, the combined effects of biochar with PL and inorganic fertilizer on soil C sequestration and health is still lacking.

Like biochar, lignite is a carbon-rich product, which is highly resistant to microbial degradation and remains in the soil for several years [17]. Lignite has a similar chemical composition to biochar's in terms of C, H, N and O proportions [18]. Due to its high CEC or direct binding of NH_3, Chen *et al.* [19] reported that addition of lignite to cattle feedlot reduced NH_3 volatilization; however, lignite application to agriculture fields is limited and the combined effect of lignite with organic and inorganic fertilizer on C sequestration and soil health is scare in the literature. Because of the high stability [11] and cation exchange capacity [20] of biochar and lignite, they have contributed to C sequestration and soil quality improvement [21] [22]. Yet, co-application of biochar and lignite with manure and inorganic fertilizer on soil health did not receive adequate research attention, particularly in the southeastern agroecosystem. It is possible that synergies may exist between different organic amendments, and thus, co-application may represent the best option for maximizing the delivery of a range of ecosystem services. To date, it is largely unknown if the integration of biochar and lignite with PL and an inorganic fertilizer affect soil characteristics or not. It was hypothesized that combined application of PL with biochar and lignite improves soil physical, chemical and biological properties of soils. Field experiments were carried out to test this hypothesis with the objective of determining the combined effects of PL and inorganic fertilizer with biochar and lignite on soil's chemical, physical and biological properties in southeastern U.S. agroecosystems.

2. Materials and Methods

A field experiment was conducted for four years (2017-2019) on Leeper silty clay loam (fine, smectitic, nonacid, thermic Vertic Epiaquepts) soil at the Plant Science Center of the Mississippi Agricultural and Forestry Experimental Station (MAFES), Mississippi State to evaluate the impact of co-application of poultry litter with biochar and lignite on soil physical, chemical and biological properties. The experiment site has a subtropical humid climate. The mean annual temperature is 18˚C, while January's mean is 11˚C and July's mean is 25˚C. Annual precipitation averages around 1425 mm with more than 900 mm falling between October and April. The Leeper series consists of deep, somewhat poorly drained soils that form in clayey alluvium on flood plains of the Alabama, Mississippi, and Arkansas Blackland Prairie (USDA NRCS, 2019). In spring 2017, before initiating experiment, 20 soil cores were randomly taken at 0 - 15 cm depth using a 2.5 - cm diameter soil probe, thoroughly mixed and one composite sample was taken from the experimental area. The sample was air-dried, ground to pass a 2.0-mm mesh and analyzed. Soil pH was measured on a 1:1 soil: $CaCl_2$ (0.05 M) solution using a combination electrode (Accuphast electrode, Fisher Scientific, Pittsburg, PA, USA). Total C and N were measured using a Vario Max Cube Elemental CNS Analyzer (Elementar Americas, Inc. Mt. Laurel, NJ, USA). Soil P, K, Ca, Mg, Cu, Zn, and Mn was assessed after extraction with Mehlich 3, and then the elements were quantified using Inductively Coupled Plasma–Optical Emissions Spectroscopy (ICP-OES, Varian, VistaPro; Varian Analytical Instruments, Walnut Creek, CA, USA). The modified ammonium acetate compulsory displacement method [23] was used for CEC analysis. Background soil characteristics are shown in **Table 1**. The experimental design was a randomized complete block (RCB) with three replications. The treatments consisted of a 3-by-3 full-factorial arrangement of amendments and fertilization types organized in a complete randomized design. Poultry litter was applied at the rate of 6.7 $Mg \cdot ha^{-1}$ every year based on general assumption that 55% of total N is available for plant uptake in the first year of application. Biochar and lignite were only applied at the beginning of the experiment in 2017 at the rate of 13.4 $Mg \cdot ha^{-1}$. Inorganic N fertilizer was applied at the rate of 134 $kg \cdot ha^{-1}$ every year P and K were applied based on Mississippi State Soil Testing Laboratory at the beginning of the study. The field was prepared by minimum tillage where the beds reformed without breaking down existing beds after harvesting cotton. In the spring, organic amendments (biochar, lignite, and poultry litter) were hand applied. A do-all implement (ProTank & Equipment, Bell Equipment partner, Greenwood, MS), was used to mix the materials into the soil surface without breaking down the rows while ensuring the planting bed was smooth for planting.

Table 1. Background soil characteristics at 0 - 15 cm soil depth before conducting the experiment and mean chemical properties values of poultry litter (PL), biochar (BC) and lignite (L) used in the study for four years (2017-2019) at the Plant Science Center, Mississippi State, MS.

Soil type	Soil	BC	PL	Lignite
	SCL			
pH	7.1	8.5	7.6	5.5
Moisture, %	23	6.8	26	14
Total C, $g \cdot kg^{-1}$	10.8	704	312	664
Total N, $g \cdot kg^{-1}$	0.73	2.2	33	2.6
C/N ration	14.8	320	9.5	255
P, $g \cdot kg^{-1}$	0.041	0.45	18	0.1
K, $g \cdot kg^{-1}$	0.17	1.71	28	0.2
Ca, $g \cdot kg^{-1}$	2.5	4.66	24	4.9
Mg, $g \cdot kg^{-1}$	0.11	1.06	5.4	2.0
Cu, $mg \cdot kg^{-1}$	387		344	161
Zn, $mg \cdot kg^{-1}$	780		631	138
CEC, $cmol_c \cdot kg^{-1}$	24	38[†]	---	75[†]
Surface area, $m^2 \cdot g^{-1}$	---	279[¥]	---	119

[†] Taghizadeh-Toosi *et al.* (2011). [¥] Cerato and Lutenegger, 2002.

Poultry litter was obtained from a local broiler chicken operation and biochar was produced from mixed hardwood feedstock and received from Department of chemistry at Mississippi State University. Lignite was acquired from the Mississippi Coal Company located in Ackerman, MS. At the time of application, poultry litter, biochar and lignite samples were collected and measured for initial moisture levels. The samples were then air-dried, ground and analyzed for chemical characteristics. The pH for these organic amendments was measured with deionized water (1:10 w/v). Total N and C in the poultry litter, biochar, and lignite were determined using a Vario Max Cube Elemental CNS Analyzer with dry combustion. Total P, K, Ca, Mg contents of these soil amendments was estimated by dry-ashing a 1.0-g sample according to the procedures outlined by Isaac and Kerber [24] and measured using ICP. Chemical characteristics of PL, biochar and lignite are shown in **Table 1**.

Cotton was planted using a four-row planter on 10 May 2017, 8 May 2018, and 17 May 2019 using cultivar DP 1664 and DP1614 at the seeding rate of 47,000 plants per hectare. Urea ammonium nitrate (UAN solution, 33% N) was injected to a depth of 10 cm at 134 $kg \cdot ha^{-1}$ (56 $kg \cdot ha^{-1}$ at planting and 78 $kg \cdot ha^{-1}$ at squaring). Field operation dates are shown in **Table 2**.

To monitor residual N leaching during the cotton growing season (May to October), porous ceramic suction cup lysimeters (Soil Moisture Equipment Corporation, Santa Barbara, CA) were installed in the center of each experimental plot after planting cotton. To ensure good soil-to-lysimeter contact, a thick

Table 2. Date of field operations and poultry litter (PL), biochar (BC) and lignite (L) application dates in cotton cropping system from 2017 to 2019 at the Plant Science Center, Mississippi State, Mississippi.

Planting	BC/L/PL	UAN solution		Cultivar	Harvesting
		First application	Second application		
10 May 2017	2 May 2017	13 May 2017	15 June 2017	DP 1522B2XF	24 Oct 2017
8 May 2018	2 May 2018	12 May 2018	16 June 2018	DP 1522B2XF	10 Oct 2018
17 May 2019	10 May 2019	20 May 2019	20 June 2018	DP 1646B2XF	9 Oct 2019

UAN = Urea ammonium nitrate.

slurry was prepared from the subsoil and poured into the bottom of the hole before inserting the lysimeter. The lysimeter vacuum system operated at 60 psi with a portable vacuum pump and was activated before each anticipated rain event. Leachate samples were collected 24 h after each rainfall event. Leachate volume was measured after each rain event and a sub-sample was collected for laboratory analysis. The N leached from the lysimeters was predominantly in the form of nitrate. The leachate samples were analyzed for NO_3-N using the Lachat flow injection analyzer (Lachat QuickChem FIA + 8000 Analyzer, Loveland, CO). Flow-weighted concentrations of NO_3-N in leachate were determined by dividing the total mass loss of NO_3-N (summation of concentration of NO_3-N multiplied by leachate volume collected after each rain event) by the total leachate volume collected in each month. These concentrations were recorded monthly and averaged across the growing season.

The cotton was harvested using a spindle picker. In 2019, three years after fertilization and amendment applications, post-harvest soil samples were collected and analyzed for chemical, physical, and biological properties. Four soil cores were taken between seeding rows and the edges of the bed at 0 - 15 and 15 - 30 cm depths in each plot. The cores were mixed thoroughly by corresponding depth to represent one composite sample for each depth in each plot. The soil samples were air-dried, and ground to pass through a 2 mm sieve. Total N (TN) and total C (TC) of the soil samples were determined by automated dry combustion methods using an Elementar VarioMax C/N analyzer. Soil P, K, Ca, Mg, Cu, Zn and Mn was assessed after extraction with Mehlich 3 [25] and quantified using a Inductively Coupled Plasma–Optical Emissions Spectroscopy (ICP-OES, Varian, VistaPro; Varian Analytical Instruments, Walnut Creek, CA, USA). Water stable aggregate (WSA) was measured at 0 - 15 and 15 - 30 cm depths using wet sieving [26]. Soil WSA was calculated as:

WSA = soil stable aggregates weight/(unstable aggregates weight + stable aggregates weight) * 100.

Soil bulk density was determined by using a hammer-driven core sampler with an inner ring diameter of 5.7 cm. The ring pushed into the soil and soil core was taken any excess soil was removed from the upper and lower edges of the ring. The fresh weight of each core was recorded, and the core samples were dried at 105˚C in an oven. The soil dry weight was recorded, and the bulk density was calculated by dividing the dry weight by the volume of the soil.

Soil water content was measured at 33 kPa (field capacity, FC) and at 1500 kPa (permanent wilting point, PWP). Soil samples were collected using small rings (5 cm in diameter and 1 cm depth) at the same location and time as the soil samples collected for measuring bulk density. The samples were saturated, set on ceramic plates and placed into a high-pressure chamber.

Then either 33 or 1500 kPa pressure was applied to the chamber on the top of the rings. This continued until all the water held above the matric potential of 33 or 1500 kPa had drained from the soil inside the rings. The samples were weighed and oven-dried at 105˚C for 24 hours to determine the water content at 33 and 1500 kPa water potential. Plant available water (PAW) was determined as the difference between water content at FC and PWP [27].

Soil penetration resistance was measured at four random locations between crop rows and wheel traffics in each sub-plot using a handheld cone penetrometer (Findlay Irvine Ltd., Midlothian, Scotland). The penetrometer was inserted into the soil at a rate of 2 $cm \cdot s^{-1}$ and the resistance was recorded at the 15 and 30 cm depths.

In situ infiltration was measured by a double ring infiltrometer [28] at three randomly selected locations on crop beds between seeding lines and the edge of bed in each sub-plot. Two PVC pipe rings with 12.5- and 30-cm diameter were inserted 5 cm into the soil. The short, small ring was placed inside the tall, large ring. The inner ring was filled with water until the water overflowed into the taller ring. The water level in the taller ring continued to rise to the same level as the inner ring. The time associated with the decrease in recorded water level in the inner rings was used to compute infiltration rate. n 2019, after harvesting cotton, two soil samples from 0-15 cm depth in the two middle planted rows of each plot were collected and mixed thoroughly to create one composite sample taken for soil enzyme activity analysis.

2.1. Soil Microbial Activity

Composite soil samples were processed from each plot were assayed for gravimetric moisture content, enzymatic activity, and quantitative polymerase chain reaction. Soil samples were processed for moisture content using a 10 g aliquot of each soil sample which was oven dried at 104˚C for 24 h and reweighed to assess soil moisture content. Enzymatic activity was assessed following a modified protocol from Deng *et al.* [29]. Briefly, soil cores were homogenized, and a 1.0 g aliquot (moist weight) was suspended in 120 ml sterile dH_2O. The suspension was homogenized via stomacher and transferred to a beaker with a stir bar. Soil suspensions were placed on a horizontal shaker for 30 min at 140 RPM. The soil suspensions were then subjected to the methods of Deng *et al.* [29]. Enzymatic assays for of *β*-*N*-acetyl-glucosaminidase, and *β*-glucosidase, acid phosphomonoesterase, and arylsulfatase were conducted at pH of 5.5 and 6.0, respectively. All plates were incubated at 37˚C for 1 h. Standard curves, background fluorescence (autohydrolysis) and blanks were included for all plates. All samples were performed in quadruple. Fluorescence measured outside 2 standard deviations were removed from calculations. Values were measured as methylumbelliferone (MUF) pmol·g^{-1} (dry g).

DNA was extracted from soil using a FastPrep-24 homogenizer using MP Biomedical FastDNA spin kits (MP Biomedical) following the recommended manufacturer's protocols. Prior to qPCR, DNA was serially diluted to avoid PCR inhibitory compounds. The16S rRNA (ribosomal RNA), *ureC*, and *phoA* were all measured according to Brooks *et al.* [30]. Soil fungal 18S rRNA was assessed using primers and conditions as stated in Liu *et al.* [31]. All quantitative polymerase chain reaction assays (qPCR) were performed on an Applied Biosystems StepOne Plus real-time PCR system (Applied Biosystems; Foster City, CA). The ABI PowerSybr PCR Mix (Applied Biosystems) with sybr green chemistry was used with controls including assay-positive, negative, and inhibition controls. Standard curves were generated from environmental, control stock bacterial or fungal isolates as previously described (30). All sample results were reported as absolute genomic unit per dry g soil (GU·g^{-1}). Additionally, *ureC*, *phoA*, *cbbL*R, and 18S rRNA GU·g^{-1} individual plot values were normalized by dividing by 16S rRNA values from the same plot.

2.2. Data Analysis

Statistical analysis was done using R software version 4.1.2 [32], including the lme4 [33], lmerTest [34], emmeans [35], and multcomp [36] packages. A separate general linear mixed model was fitted for each response variable. The fixed effects in the model were fertilizer treatment (with three levels: none added, fertilizer added, and poultry litter added), soil amendment treatment (with three levels: none added, biochar added, and lignite added, depth of measurement (with two levels), two-way interactions and three-way interactions. A random intercept was fit to each replicate. Response variables representing concentrations (namely total C, total N, P, K, Cu, and Zn) were log-transformed prior to analysis. Plots of model residuals were examined to ensure that the assumptions of normality and homogeneity of residuals were met. An overall F-test (ANOVA) was performed for each main effect and interaction term, using the Kenward-Roger method to estimate denominator degrees of freedom. The estimated marginal least-square means was calculated for each treatment combination averaged across soil depths, for each soil depth averaged across treatments, and for each treatment combination within each soil depth. In each case, the means were compared across treatments and/or soil depths using the Sidak adjustment for multiple comparisons to adjust the critical p-value and confidence interval. After this adjustment, pairs of means with $p < 0.05$ were considered significantly different. For the two response variables measured each year from 2017-2020 but not at multiple depths, separate general linear mixed models were fitted using the same procedure as above, but the year was a categorical fixed effect instead of soil depth, including all two-way interactions and three-way interactions between treatments and year (**Table 3**).

3. Results and Discussion

3.1. Soil Chemical Property

Soil Total C

Total C varied from 6.8 to 10.5 g·kg^{-1} with the higher end of the ranges being for biochar and PL addition. Application of PL with or without combination of biochar and lignite significantly enhanced soil total C (TC) as compared to the control (**Table 4**). The greatest soil C content was obtained with the combination of biochar with PL which was 68% greater than the control (**Table 4**). Poultry litter, biochar and lignite contained 312, 704 and 664 g TC·kg^{-1} (**Table 1**). Based on C content and the rate, application of PL, biochar and lignite, added 2.1, 9.4 and 8.9 Mg TC·ha^{-1}, respectively.

After three years of PL applications, soil TC was greater by 28% as compared to unfertilized control (8.97 vs. 6.78 g·kg^{-1}, a net increase of 2.2 g·kg^{-1} (**Table 4**). Our results agree with Parker *et al.* [37] who reported application of PL to cotton at the rate of 6.7 Mg·ha^{-1} significantly increased soil TC by 26% at the 0- to 15-cm depth as compared with unfertilized control. Integration of biochar and lignite with PL significantly increased soil TC by 16% (10.4 vs. 8.98 g·kg^{-1}) and 18% (10.6 vs. 8.98 g·kg^{-1}) a net increase 1.42 and 1.62 g·kg^{-1}, respectively as compared to PL alone (**Table 4**). The combined effect of biochar and poultry litter as soil amendments on soil TC is confirmed by the findings of Frimpong *et al.* [38], who concluded that C accumulation and sequestration are stimulated by addition of biochar to the manure application. There was no difference in soil TC obtained between biochar and lignite when they combined with PL (**Table 4**). Application of PL to cotton either alone or in combination with biochar and lignite greatly increased soil TC by 20% (8.97 vs. 7.27 g·kg^{-1}) and by 43% (10.4 vs. 7.27 g·kg^{-1}) as compared to inorganic N fertilizer, respectively (**Table 4**). In addition, the combination of biochar with PL resulted in greater soil C than application of PL (**Table 4**). This increase of the total C in the combined biochar with PL was because of the C not only added by the biochar and PL but also additional C in the topsoil layers was added as microbes break down crop residues storing more C in the soil [39] which contribute to organic matter buildup. Although inorganic N is expected to benefit soil C by increasing plant residue [40], differing concentrations were not seen in soil TC obtained between inorganic N fertilizer and unfertilized control treatments (**Table 4**). In contrast to these results, Rasool *et al.* [41] reported that application of inorganic fertilizer increased crop biomass, which ultimately enhanced soil TC as compared to the control. However, Mulvaney *et al.* [42] reported that application of inorganic fertilizer N could enable microorganisms to consume soil C more readily and result in soil C reduction. The combination of biochar and lignite with inorganic fertilizer resulted in greater soil TC as compared to inorganic fertilizer alone because biochar is an important material that adds an abundance of carbon to the soil [43]. Increasing the soil's organic matter not only affects soil fertility, but it also improves soil physical conditions [44]. Total soil C significantly decreased as soil depth increased. Reduction in soil TC with increasing depth can be explained by the accumulation of organic amendments and residues in the upper soil layer. Averaged across fertilization treatments, soil TC at 0 - 15 cm depth was significantly greater by 43% than at 15 - 30 cm depth (10.6 vs 6.9 g·kg^{-1}, a net 3.5 g·kg^{-1}) (**Table 4**).

3.2. Soil Nutrient Contents

When compared to unfertilized treatment, application of PL significantly increased soil total N and Mehlich-3 extractable P, K, Cu, and Zn as compared to the unfertilized treatment (**Table 4**). Application of PL to cotton significantly increased soil TN by 33% as compared to unfertilized control (0.842 vs. 0.632 g·kg^{-1}, a net increase 0.21 g·kg^{-1}) (**Table 4**). The nutrient constituents in biochar are insufficient to provide a substantial amount of nutrients needed to support plant growth if solely applied [45]. Integration of biochar and lignite with PL on soil TN was not different from PL application alone. This is possibly due to low nutrient contents of the biochar and lignite used in this study (**Table 1**), thus no additive effects (**Table 4**). In contrast to our results, Seehausen *et al.* [46] reported that combination of biochar with manure resulted in greater soil TN as compared to manure application alone. They reported addition of biochar to the manure reduced leaching losses of N. Co-applied biochar with inorganic N fertilizer increased soil TN by 16% as compared to inorganic N fertilizer alone (1.183 vs. 1.018 g·kg^{-1}) (**Table 4**). This could be related to the reason that addition of biochar to inorganic fertilizer decreased the ammonia loss due to the temporary adsorption of NH_4^+ onto the biochar surface [47] [48]. This combination of biochar and lignite with PL significantly increased soil TN by 24% (0.783 vs. 0.632 g·kg^{-1}) and 27% (0.803 vs. 0.632 g·kg^{-1}), respectively, as compared to the unfertilized control (**Table 4**). Although PL and inorganic N fertilizer were applied to cotton at approximately equivalent N rate, soil TN with PL was 15% less than soil TN with inorganic fertilizer (0.985 vs. 0.842 g·kg^{-1}) (**Table 4**).

Application of PL to cotton for three years resulted in significant increases in soil P, K, Zn, and Cu concentrations as compared to the unfertilized control (**Table 4**) and these effects were mainly occurred in the top 15 cm of the soil. Application of PL either alone or in combination with biochar or lignite substantially increased soil P were compared to the control and inorganic N fertilizer treatments (**Table 4**). Since the N:P ratio in PL was smaller than N:P ratio of plant uptakes, the application of PL to cotton based on N needs of plants resulted in soil P buildup. There was no significant difference in soil P content between the inorganic fertilizer and the unfertilized control, which received no supplemental P (**Table 4**). Integration of inorganic N fertilizer with biochar and lignite had similar effects on soil P as compared to sol application of inorganic N fertilizer and unfertilized control treatments (**Table 4**). However, combination of PL with biochar significantly increased soil P by 9% (88.9 vs. 81.6 mg·kg^{-1}, a net increase of 7.3 mg·kg^{-1}) (**Table 4**) compared to PL alone. This indicates that biochar has a positive response to soil P and enhances available P in the soil solution. Averaged across fertilization treatments, soil P at 0 - 15 cm depth was significantly greater by 4-fold (79.4 vs. 20.2 mg·kg^{-1}) than at 15 - 30 cm depth (**Table 4**), indicating less potential of P leaching with PL application in this non-irrigated study. Application of PL significantly increased soil K, Cu and Zn concentrations compared to inorganic N fertilizer and unfertilized control (**Table 4**). Similar amounts of soil K, Cu and Zn contents were obtained between inorganic N fertilizer and the control. Integration of PL with biochar and lignite significantly reduced soil K, Cu and Zn concentrations by 19%, 41% and 18% compared to PL alone (**Table 4**). The reduction in soil K, Cu and Zn with the addition of biochar and lignite could be related to the high cation exchange capacity [11] [20], and the highly specific surface area [49] of these organic amendments in which K, Cu and Zn were temporarily adsorbed and removed from the soil solution.

Table 3. Analysis of variance (ANOVA) probability values for the effect, fertilization and amendments treatment and their interaction on soil total C (TC), Total N (TN), phosphorus (P), potassium (K), copper (Cu), zinc (Zn), bd (bulk density), PR (penetration resistance) and WSA (water stable aggregate) in post-harvest samples at Mississippi Plant Science Center, Mississippi State, MS.

model term	TC	TN	P	K	Cu	Zn	bd	PR	PAW	WSA
fertilizer/poultry litter	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
soil amendment (biochar/lignite)	<0.0001	0.23	0.69	0.4	<0.0001	0.29	<0.0001	<0.0001	<0.0001	<0.0001
depth	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.5
fertilizer × amendment	<0.0001	0.32	0.024	0.0032	0.011	0.13	<0.0001	<0.0001	<0.0001	<0.0001
fertilizer × depth	0.00034	0.018	0.26	0.42	0.00023	0.0028	0.41	<0.0001	<0.0001	<0.0001
amendment × depth	0.0047	0.58	0.02	0.11	0.014	0.12	0.025	<0.0001	<0.0001	0.0014
fertilizer × amendment × depth	0.9	0.85	0.36	0.86	0.45	0.7	0.4	<0.0001	<0.0001	0.075

Table 4. Effects of poultry litter and inorganic N fertilizer either alone or in combination with biochar and lignite applied to cotton on soil total C (TC), total N (TN), phosphorus (P), potassium (K), copper (Cu) and zinc (Zn) at Mississippi Plant Science Center, Mississippi State, MS in 2019.

		TC	TN	P	K	Cu	Zn
		g·kg^{-1}		mg·kg^{-1}	g·kg^{-1}	mg·kg^{-1}	
Control	Control	6.69 a	0.618 a	13.7 ab	0.165 a	0.755 b	1.48 bc
Control	Biochar	9.07 def	0.648 ab	12.8 a	0.163 a	0.76 b	1.62 c
Control	Lignite	9.61 efg	0.633 ab	14.5 ab	0.184 a	0.683 b	1.64 c
Fertilizer	Control	7.07 ab	0.99 cd	14.3 ab	0.158 a	0.764 b	1.14 abc
Fertilizer	Biochar	7.73 bc	1.05 d	15.4 ab	0.167 a	0.7 b	0.85 ab
Fertilizer	Lignite	8.15 cd	0.886 cd	16.4 b	0.165 a	0.477 a	0.706 a
Poultry litter	Control	8.86 de	0.829 c	64.2 c	0.424 c	5.74 c	11 d
Poultry litter	Biochar	10.1 fg	0.794 bc	66.2 c	0.347 bc	4.27 c	12.1 d
Poultry litter	Lignite	10.4 g	0.791 bc	58.2 c	0.331 b	4.47 c	10.4 d
Depth							
0 - 15 cm		10.5 b	0.925 b	48.2 b	0.279 b	1.62 b	6.32 b
15 - 30 cm		6.95 a	0.677 a	11.6 a	0.168 a	1.05 a	0.983 a

‡Means within a column followed by different letters are significantly different ($P < 0.05$).

3.3. Soil Physical Properties

3.3.1. Bulk Density

Application of PL with or without biochar and lignite significantly reduced bulk density when compared to the control (**Table 5**). Poultry litter application reduced bulk density by 3% as compared to the control and inorganic N fertilizer (1.22 vs. 1.27 g·cm^{-3}). Tejada and Gonzalez [50] reported that chicken manure application as an organic fertilizer to a clay loam soil resulted in a 20% decrease in bulk density compared to the control. Soil amended with organic manure has been reported to have lower bulk density, higher porosity, and moisture content relative to chemical fertilizer [51]. In general, decreased bulk density is associated with the low particle density of organic matter in the soil from PL application. Bronick and Lal [52] reported that organic components play a role in lowering bulk density because the soil mineral fractions are diluted with organic components improving aggregation. Addition of biochar and lignite to PL reduced bulk density compared to PL alone (**Table 4**). The reduction in bulk density with biochar application could be related to the porous nature and low bulk densities of biochar [53]. Soil bulk density was similar between inorganic N fertilizer and unfertilized control treatment (**Table 5**). The lowest bulk density of 1.193 g·cm^{-3} was obtained when both biochar and lignite were combined with PL and the greatest value was recorded in the control and inorganic fertilizer treatments. In addition, the improvement in soil physical properties due to biochar application could be related to providing better habitat for soil microorganisms [54] improved soil organic matter and aggregation [17]. The results agree with Pagliali & Antisari [55] who reported reduced bulk density is associated with the beneficial effects of total organic carbon C added to the soil from organic amendments on aggregate formation and increase in soil macro pore volume. Soil bulk density significantly increased by increasing soil depth. Averaged across fertilization treatments, soil bulk density was significantly less by 3% in the surface soil (0 - 15 cm) than in subsurface (15 - 30 cm) (1.22 vs. 1.26 g·cm^{-3}) (**Table 5**). This can be attributed to the greater soil C content in the surface soil (**Table 4**) as also reported by Celik *et al.* [56] and more compaction in the subsurface layer resulting from continuous cultivation practice with intercultural operations [57]. Reduction in soil bulk density is important for increasing crop root systems which enhances crop nutrients and water use efficiency.

3.3.2. Penetration Resistance (PR)

Poultry litter treatment significantly reduced soil PR by 24% compared to the control (1.50 vs 1.97 MPa) and by 16% compared to inorganic N fertilizer treatment (1.50 vs. 1.80 MPa) (**Table 5**). The results agree with Feng *et al.* (2019) who reported that applying 6.7 Mg pelleted poultry litter·ha^{-1} to cotton for 4 years significantly reduced penetration resistance in the top 5 cm layer, as compared with the N fertilizer and control treatments. Integration of biochar and lignite with PL was more effective in reducing soil penetration resistance than PL alone. Addition of biochar and lignite to PL significantly reduced penetration resistance by 7% (1.40 vs. 1.50 MPa) as compared to PL alone (**Table 5**). Inorganic N fertilizer also reduced penetration resistance by 9% (1.80 vs. 1.97 MPa) as compared to the control (**Table 5**), which could be related to greater addition of cotton aboveground biomass and crop residue into the soil (data not shown) with consequent increase in soil total C content than the control [58]. Addition of biochar to inorganic N fertilizer plots significantly reduced penetration resistance by 12% (1.74 vs. 1.97 MPa) and 3% (1.74 vs. 1.80 MPa), respectively as compared to the control and inorganic fertilizer alone (**Table 5**). The lowest resistance value of 1.40 MPa was obtained when biochar was added to PL plots and the greatest value was recorded with the control. The combination effect of biochar and lignite on soil penetration resistance were similar (**Table 5**); therefore, and the values were greater than the control and inorganic N fertilizer. These results agree with Zhang [59] who reported a significant decline in soil strength with application of organic amendments. Reduction in bulk density and improvement in aggregate stability in the top 0 - 15 cm depth may explain the lower soil penetration resistance where organic amendments were applied. Soil penetration resistance increased with increasing soil depth in all the treatments, most likely due to higher intrinsic bulk density of the soil at deeper layers [60]. Averaged across fertilization treatments, soil penetration resistance at 0 - 15 cm depth was significantly less by 29% than 15 - 30 cm depth (1.32 vs. 1.87 MPa) (**Table 5**).

Table 5. Effects of poultry litter and inorganic N fertilizer applied to cotton either alone or in combination with biochar and lignite applied to cotton on soil bulk density (ρd), penetration resistance (Pr), plant available water (PAW), water stable aggregate (WSA) and infiltration at 0 - 15 and 15 - 30 cm depth at Mississippi Plant Science Center, Mississippi State, MS in 2019.

Fertilizer	amendments	bd	PR	PAW	WSA
		$g \cdot cm^{-3}$	MPa	%	
Control	Control	1.27 c	1.97 e	19.1 a	52.2 a
Control	Biochar	1.19 a	1.46 b	33.4 ef	72.7 cd
Control	Lignite	1.18 a	1.41 a	34.8 f	73.8 d
Fertilizer	Control	1.27 c	1.8 d	23.5 b	53 ab
Fertilizer	Biochar	1.26 c	1.74 c	27.3 c	54.3 ab
Fertilizer	Lignite	1.27 c	1.7 c	28.3 cd	55.2 b
Poultry litter	Control	1.22 b	1.48 b	29.7 d	70.3 c
Poultry litter	Biochar	1.19 a	1.4 a	32.5 e	73.2 cd
Poultry litter	Lignite	1.2 ab	1.38 a	33 ef	74.5 d
Depth					
0 - 15 cm		1.2 a	1.32 a	27.3 a	64.2 a
15 - 30 cm		1.25 b	1.87 b	30.9 b	64.5 a

‡Means within a column followed by different letters are significantly different ($P < 0.05$).

3.3.3. Water Stable Aggregate (WSA)

Applying PL for three years significantly increased soil aggregate stability (WSA) by 35% as compared to the control (52% vs. 70 %) (**Table 4**). Soil WSA at 0 - 15 cm depth in plots treated with PL was greater than the plots treated with inorganic N fertilizer. The greater aggregate stability with PL than inorganic N fertilizer and the control could be related to increased soil total carbon with PL applications (**Table 4**) which promotes the formation and stability of soil macro-aggregates and stabilizes aggregates [61]. The beneficial role of manure on formation and stabilization of aggregates could be attributed to the marked effect on certain polysaccharides formed during decomposition of organic residues by microbial activity [62]. Palaniappan [63] has reported that the humic substances penetrate the interlamellar spaces of clay minerals, influence the interaction of clay with other soil constituents and promote formation of aggregate. There were no differences obtained in WSA between inorganic N fertilizer and the control treatment. Although application of inorganic N fertilizer increases crop residue, which could contribute to soil organic matter [5] and soil aggregation, the effect of applying inorganic N fertilizer on WSA in the present study was not better than the unfertilized treatment (**Table 5**). Integration of biochar and lignite with PL was more effective in increasing soil WSA than broiler litter alone. Addition of biochar and lignite to PL significantly increased WSA by 6% as compared to PL alone (70% vs 74%) (**Table 5**). Soil aggregate stability ranged from 52% in the control to 75% with integration of biochar and lignite with PL (**Table 5**). Biochar and lignite have similar effects on soil aggregate stability when combined with PL and inorganic fertilizer (**Table 5**). Averaged across fertilization treatments, soil WSA at 0 - 15 cm depth was significantly less by 2% than 15 - 30 cm depth (62.8% vs 63.8%) (**Table 4**)

3.3.4. Infiltration

Keeping rainwater into the soil enhances crop growth and yield in dryland fields. After four years of PL application at the rate of 6.7 $Mg \cdot ha^{-1}$ to cotton significantly increased water infiltration rate from 106 $mm \cdot h^{-1}$ in the control plots to 146 $mm \cdot h^{-1}$ in PL treated plots (38% increase) (**Figure 1**), indicating more rainwater would percolate during rainfall events. Increase water infiltration with PL application could be related to an increase in macrospores created by soil microbial activity because of poultry manure application [64]. Soil organic matters generated from animal by-products, are reported to bind soil particles together into clods and aggregates, improve structure and permeability, and subsequently influence the infiltration rate [65]. Sarkar *et al.* [66] also reported that addition of organic materials had increased moisture-retention capacity and infiltration rate of the surface soil. Inorganic N fertilizer treatment did not affect soil infiltration as compared to unfertilized control (111 vs 106 $mm \cdot h^{-1}$) and was less effective in improving infiltration rate than PL (111 vs. 146 $mm \cdot h^{-1}$). The lower infiltration values with inorganic N fertilizer and unfertilized control than with PL is most likely related to low C input (**Table 4**), high soil penetration resistance and bulk

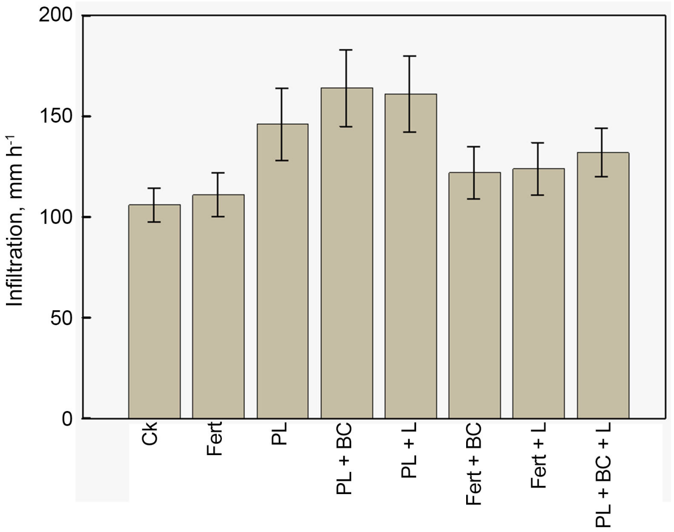

Figure 1. Effects of poultry litter (PL) and inorganic N fertilizer (Fert) either alone or in combination with biochar (BC) and lignite (L) applied to cotton on post-harvest water infiltration at Mississippi Plant Science Center, Mississippi State, MS in 2019. Error bars represent standard deviation of the means.

density (Table 5) with those treatments. Addition of biochar and lignite to PL significantly increased water infiltration by 12% (146 vs. 164 mm·h^{-1}) as compared to PL alone (Figure 1). Addition of biochar and lignite to inorganic N fertilizer plots increased water infiltration by 9% (111 vs. 122 mm·h^{-1}) as compared to inorganic fertilizer alone (Figure 1) The greatest water infiltration value of 164 mm·h^{-1} was obtained when both biochar and lignite were added to PL plots and the smallest value was recorded with the unfertilized control.

3.3.5. Plant Available Water (PAW)

There were no differences in plant available water content obtained between PL and biochar treatments (Table 5). Application of PL significantly increased soil water content more than both inorganic fertilizer and the control (Table 5). The combination of biochar and lignite with PL resulted in greater plant available water in the soil than PL and biochar application alone (Table 5). No difference in soil water content was obtained with application of inorganic N fertilizer and unfertilized control (Table 5). However, combination of biochar with inorganic N fertilizer resulted in greater soil water content than the control (Table 4). Averaged across fertilization treatments, no difference in soil water content was obtained with increasing soil depth (Table 5). The improved moisture content of the soil due to biochar was adduced to the porous nature of biochar which would have allowed it to retain water in its micro and mesopores [67]. The improved moisture content with poultry manure application can be adduced to the mulching effect and improved moisture retention which improves soil structure and macro porosity [68]. Adekiya [67] also found that poultry manure application enhanced the moisture content of the soil as compared to the control.

3.3.6. Leachate Volume and NO_3-N Contents

Soil leached volume, the amount of water collected at 45 cm depth, is highly influenced by rain events. The amount of rain during cotton growing season from May to October was in the order 2018 < 2017 < 2019. The highest quantity of leached volume was obtained from the control plots in all three growing seasons and was accompanied by having the lowest cotton aboveground biomass productions (data not shown). Averaged across treatment per year, the amount of leached water was 479, 369 and 613 ml in 2017, 2018, and 2019 respectively. Leached volume with organic amendments was smaller than inorganic fertilizer and the control. For example, PL application significantly reduced leached volume of water by 13%, 8%, and 7% as compared to inorganic N fertilizer and by 14%, 16%, and 15% compared to the control in 2017, 2018, and 2019 (**Table 6**), indicating a significant reduction in subsurface water flow with PL. Combination of biochar with PL significantly reduced leached volume by approximately 34% each year as compared to poultry litter alone (**Table 6**). This is most likely related to the hydrophobicity property of biochar [69] and the potential of biochar in enhancing soil water holding capacity and leaching reduction [70] [71]. Novake *et al.* [71] reported that the structural properties of biochar can help to retain water and nutrient concentrations. The integration effects of biochar and PL on leached volume of water reductions are most likely associated with the effect of organic matter added to the soil by these organic amendments altering the soil water holding capacity [72]. It appeared that the combination of these two amendments had an additive effect and showed the highest soil water retention as evidenced by the lowest amount of leached water obtained in 2017, 2018, and 2019 (**Table 6**). This synergism suggests that mixing biochar with PL represents a strategy to retain more water in the soil and reduce the runoff from the field.

Table 6. Effects of poultry litter and inorganic N fertilizer applied either alone or in combination with biochar and lignite on the volume of leachate and NO_3-N concentration in leached water averaged across leachate collection events in each growing season at Mississippi Plant Science Center, Mississippi State, MS.

Treatment	amendment	Mean leachate volume			Mean leached NO_3-N		
		2017	2018	2019	2017	2018	2019
		ml			$mg \cdot L^{-1}$		
Control	Control	[‡]610 a	501 a	791 a	7.5 d	8.1 d	7.6 e
Poultry litter (PL)	Control	521 b	425 c	680 c	14.4 bc	15.6 bc	17.6 bc
Fertilizer (FRT)	Control	600 a	460 b	730 b	21.9 a	22.8 a	24.6 a
Poultry litter (PL)	Biochar (BC)	335 e	275 e	420 h	8.5 d	10.7 d	12.2 de
Poultry litter (PL)	Lignite (L)	347 e	285 e	450 g	10.6 cd	12.2 cd	13.4 cd
Fertilizer (FRT)	Biochar (BC)	488 c	325 d	633 d	16.3 b	17. b	17.8 bc
Fertilizer (FRT)	Lignite (L)	457 d	315 d	590 e	14.3 bc	16.7 b	18.7 b
PL + BC + L		358 e	322 d	532 f	7.2 d	7.4 e	7.0 e
	$LSD_{(0.05)}$	28.3	18.6	24.7	4.11	4.10	4.45

[‡] Means within a column followed by different letters are significantly different ($P < 0.05$).

The combination of biochar with inorganic N fertilizer also significantly reduced the magnitude of leached volume by 19%, 29%, and 14% as compared to inorganic N fertilizer alone (**Table 6**). This was also related to the addition of organic C from carbon-rich biochar to inorganic N fertilizer plots which resulted in reducing leached volume by improving soil water holding capacity. Increasing soil water holding capacity may reduce irrigation frequency or irrigation volume, which in turn may reduce the risk of N leaching loss, from inorganic N fertilizer applications [73].

Like biochar, combination of lignite with PL and inorganic N fertilizer resulted in a reduction of leached volume as compared to PL and inorganic fertilizer applications alone in 2017, 2018, and 2019 (**Table 6**), indicating the potential of lignite in preventing soil water from loss by the addition of stable C to the soil. No differences on the quantity of leached volume were obtained between biochar and lignite when combined with PL in all four years. The N leached into the soil was predominantly in the form of nitrate and was strongly increased by fertilization and rain events.

In all years, mean NO_3–N concentrations in the leachate was significantly less in the control, than in plots that received PL and inorganic N fertilizer (**Table 6**). The highest NO_3–N concentrations in the leached water were obtained with inorganic N fertilizer in 2017, 2018, and 2019 (**Table 6**). Although PL and inorganic N fertilizer were applied to cotton at an equivalent N rate, mean NO_3-N concentrations in the leached water from PL was significantly less by 34%, 32%, and 28% than inorganic N fertilizer applications in 2017, 2018, and 2019, respectively. The greater NO_3-N concentration in the leachate from inorganic N fertilizer most likely related to the solubility of N and potential leaching loss mainly at the time of application (data not shown) compared to PL because PL derived-N is in the form of organic N and slowly mineralized to the soluble form during the cotton growing season. The combination of biochar with PL significantly reduced the mean NO_3-N content in leached water by 41%, 31% and 32% in 2017, 2018, and 2019 as compared to PL alone. However, lignite did not affect the mean NO_3-N content in leached water as compared to PL alone (**Table 6**). No difference in mean NO3-N concentrations in leached water was obtained between lignite and biochar when they combined with PL in all four-growing seasons (**Table 6**). It appears that adsorption of N by lignite and biochar did not have a negative effect on available N in the soil as evidenced by plant N uptake and no difference in plant N uptake was obtained between biochar and lignite when they were combined with PL in 2017, 2018, and 2019 (data not shown).

Like PL, the combination of biochar and lignite with inorganic N fertilizer significantly reduced the mean NO_3-N in leached water as compared to inorganic N fertilizer treatment alone (**Table 6**). The reduction of NO3-N could have resulted from ion exchange and electrostatic adsorption to the biochar's surface functional groups [74] [75], thus, rendering less available N [76] for leaching. No difference in NO_3-N in leached water was obtained between biochar and lignite when they combined with inorganic N fertilizer treatment (**Table 5**). Like biochar, addition of lignite to inorganic N fertilizer significantly reduced NO_3-N in leached water as compared to the application of inorganic N fertilizer and no differences in NO_3-N concentrations were obtained between biochar and lignite (Table 6). This indicates lignite and biochar are more effective in controlling N when combined with PL and inorganic N fertilizer than individual fertilization. Thus, biochar and lignite are agronomically and environmentally sound.

3.4. Soil Enzyme and Gene Levels

Overall, soil enzymatic levels were not significantly influenced by treatments; however, there were numerous genes that were significantly influenced by treatments (Table 7). Soil for biological samples was collected mid-season in the final year, to maximize the cumulative effects of treatments; however, it is possible that effects were only observable early in the study. Soil β-glucosidase activity varied minimally, per treatment, from a mean of 8.96×10^4 to 1.97×10^5 pmol dry·g^{-1} while soil β-*N*-acetyl-glucosamindase activity varied from a low mean of 5.35×10^4 to 1.20×10^5 pmol dry·g^{-1}. Mean soil phosphomonoesterase and arylsulfatase activity were also not significantly different due to the treatment effect with means varying between 1.56×10^5 and 2.74×10^5 pmol dry·g^{-1}. Analysis of enzyme activity based on fertilizer (poultry litter, inorganic fertilizer, control) and C amendment (lignite and biochar) demonstrated no effects or obvious trends.

Table 7. Absolute soil bacterial gene levels (GU·g^{-1}), and the analysis of variance (ANOVA) probability significance for the effect of fertilization or soil amendment treatments on normalized gene levels in experimentally treated plots at the end of the final growing season at Mississippi Plant Science Center, Mississippi State, MS.

Treatment	amendment	16S	18S	ureC	phoA	cbbLR		18S/16S	ureC/16S	phoA/16S	cbblR/16S
							Treatment Combinations	Normalized Ratio (gene/16S) Probability			
Control	Control	6.3×10^{11}	3.06×10^{11}	1.7×10^8	8.64×10^5	3.93×10^7	BC or L		*		
Poultry litter (PL)	Control	7.08×10^{11}	8.72×10^{11}	3.44×10^8	6.73×10^5	6.75×10^7	PL, PL + BC, and PL + L or Control		*		
Fertilizer (FRT)	Control	7.62×10^{11}	3.7×10^{11}	2.77×10^8	8.73×10^5	5.00×10^7	PL or all other treatments				*
Poultry litter (PL)	Biochar (BC)	6.49×10^{11}	4.28×10^{11}	2.72×10^8	9.18×10^5	4.11×10^7	PL, PL + BC, and PL + L or all other treatments	*			
Poultry litter (PL)	Lignite (L)	5.86×10^{11}	4.35×10^{11}	2.37×10^8	1.19×10^6	3.98×10^7					
Fertilizer (FRT)	Biochar (BC)	6.83×10^{11}	4.25×10^{11}	2.55×10^8	2.74×10^6	4.72×10^7					
Fertilizer (FRT)	Lignite (L)	7.53×10^{11}	3.17×10^{11}	3.13 × 108	9.4×10^5	4.33×10^7					
PL + BC + L		6.74×10^{11}	7.62×10^{11}	2.72×10^8	2.64×10^6	5.03×10^7					

[‡] Means within a column followed by different letters are significantly different ($P < 0.05$), * indicates significance between treatment combinations ($P < 0.05$).

Soil 16S rRNA absolute gene levels varied little from a high (mean = 7.62×10^{11} GU·g^{-1}) to a low 5.86×10^{11} in inorganic fertilizer and PL + biochar treatments, respectively. Fungal 18S rRNA levels also varied little from a high value (mean = 8.72×10^{11}) to a low 3.06×10^{11} GU·g^{-1} in PL and control treatments, respectively. Similarly, soil *ure*C levels were also greatest in PL applied plots (mean = 3.44×10^{8} GU·g^{-1}), which was approximately three times greater than control levels. While not significant, these levels suggest that addition of organic matter from PL or addition of genes from PL [77] tended to increase ureC levels. Soil *pho*A levels were greatest in PL + biochar + lignite and inorganic fertilizer + biochar treatments (mean~2.7×10^{6} GU·g^{-1}). The lowest *pho*A levels were found in PL plots (mean = 6.73×10^{5}). Soil *cbbL*R GU·g^{-1} levels were greatest in PL treatments (mean = 6.75×10^{7} GU·g^{-1}), while control plots were approximately 1.5 times lower.

Normalized *ure*C levels (*ure*C/16S rRNA) were significantly different in biochar or lignite applied plots ($p = 0.0260$). While values were very similar, the addition of biochar or lignite was statistically associated with a rise in absolute *ure*C levels ($p = 0.0169$) as well. It is possible that the recruitment of urease genes, with addition of C via biochar or lignite, may be precipitated by utilization of available N compounds [78]. The influence of PL on normalized *ure*C levels was significant ($p = 0.0492$) when all PL treatments grouped and compared with control treatments; however, inorganic fertilizer treatments were not significantly different. Similarly, though not significant, there was a trend for lower *cbbL*R gene levels in plots treated with either biochar or lignite. Normalized soil *cbbL*R was significantly greater in PL treated soils compared to the other treatments, including those in combination with PL + lignite or biochar treatments. Soil fungal levels (18S rRNA) were significantly elevated ($p = 0.0317$) in PL treated plots relative to all other treatments including inorganic fertilizer only plots. When grouped together, treatments with PL were significantly greater in 18S/16S rRNA ratios, which indicates that fungi may have been selected for in PL treatments ($p < 0.01$). The ratio 18S/16S rRNA was significantly influenced by PL alone, when compared to all other treatments except for PL combined with biochar and lignite. Similarly, when all treatments were considered, inorganic fertilizer or inorganic fertilizer with lignite was lowest in 18S/16S rRNA ratio, indicating that there was a bias towards bacteria in those plots. This suggests that bacteria were enriched with readily available nutrients, rather than more recalcitrant organic matter which saprophytic fungi are more adapted towards. Absolute soil *pho*A levels, and normalized *pho*A/16S rRNA, while not statistically significant, trended towards increased levels in PL/biochar/lignite combined treatments. These results indicate that the addition of PL tended to enhance bacterial and fungal populations; albeit, total bacterial populations were not significantly increased, this indicates selection for bacterial populations carrying these functional genes rather than broad spectrum increases. Poultry litter tended to select for functional genes such as *ure*C, *pho*A, and *cbbL*R (normalized values) which is possibly due to the addition of labile organic matter or gene addition via poultry litter.

Pearson correlation of soil *β-N*-acetyl-glucosamindase and phosphomonoesterase suggested strong inverse correlation with NO_3 levels in biochar or lignite applied plots ($p < 0.05$). This suggests that activity of soil *β-N*-acetyl-glucosamindase was inhibited by NO_3 levels, and the added surface area from biochar or lignite could have reduced NO_3 vertical leaching. This keeps the source of N close to the surface and available [79], thus reducing the need for *β-N*-acetyl-glucosamindase activity. This may also suggest that phophomonoesterase activity was also affected in this same manner, suggesting that hydrolytic enzymes are regulated by nutrient availability and need [80]. Pearson correlation also suggested a medium correlation associated with K soil levels and *β-N*-acetyl-glucosaminidase activity when all plots were grouped together which may be related to addition of biochar or increased soil fertility [81]. Pearson correlation suggested strong positive correlation between total carbon and fungal 18S rRNA levels in biochar or lignite applied plots ($p < 0.05$). This trend is not surprising given the role that fungal biomass plays in C cycling and stabilization. Pearson correlation suggested a strong correlation between 16S rRNA soil levels and bulk density, while Cu and Zn were negatively correlated with 16S rRNA levels ($p < 0.05$). It is interesting to note that plots which received PL were greater in Mehlich III Cu and Zn levels when compared to those receiving inorganic fertilizer (mean Cu 5.87 vs. 0.74 ppm and Zn 13.51 vs. 2.02 ppm). Magalhães *et al.* [82] demonstrated that Cu levels from 0 to >150 ppm caused distinct bacterial community clustering around 0 - 4, 8, and 60 - >150 ppm, respectively; however, there was a decrease in 16S rRNA levels from 4 - 8 ppm, while 60 PPM saw a 3-fold increase, and a decrease at >150 ppm. Additionally, others have noted a 2-fold drop at 50 ppm Cu [83]. Pearson correlation of *pho*A suggested that BD was strongly, inversely correlated with *pho*A gene levels ($p = 0.02$) in PL applied fields, but not in inorganic fertilizer and control plots. The strong inverse correlation with bulk density, suggests that *pho*A presence and potential activity increases in a less dense soil, because of more movement of nutrients and water through the profile. Infiltration and available water content were also strongly positively correlated with *pho*A levels ($p < 0.05$). This also suggests that *pho*A levels are tied to water movement and availability.

4. Conclusion

Results of this experiment revealed that combination of poultry litter with biochar and lignite improved soil physical, chemical, and biological properties more than poultry litter alone and inorganic fertilizer alone. Inorganic N fertilizer did not affect soil C but increased soil N. The combination of biochar with inorganic fertilizer resulted in greater soil N than inorganic fertilizer alone. This is due to the integration of biochar with inorganic N which reduces N leaching loss and keeps N in place. Combined application of biochar and lignite with poultry litter and inorganic fertilizer showed a synergistic effect and offers a novel approach to sustain good soil health, improve soil fertility, promote crop productivity, and could be used as a sustainable agronomical strategy in southeast region. More field work is needed to evaluate the combined effects of biochar and lignite with organic and inorganic fertilizers in the presence or absence of cover crops using different soil types on N_2O and CO_2 emissions.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Binder, D.L., *et al.* (2002) Biosolids as Nitrogen Source for Irrigated Maize and Rainfed Sorghum. *Soil Science Society of America Journal*, **66**, 531-543. https://doi.org/10.2136/sssaj2002.0531

[2] Adeli, A., Tewolde, H., Sistani, K. and Rowe, D. (2010) Comparison of Broiler Litter and Commercial Fertilizer at Equivalent N Rates on Soil Properties. *Communications in Soil Science and Plant Analysis*, **41**, 2432-2447. https://doi.org/10.1080/00103624.2010.511374

[3] Tewolde, H., Buehring, N., Adeli, A., Sistani, K.R., Rowe, D.E. and Pratt, R.G. (2009) Cotton Response to Chicken Litter in Rotation with Corn in Clayey Soil. *Agronomy Journal*, **101**, 626-634. https://doi.org/10.2134/agronj2008.0154x

[4] Sharpe, R.R., Schomberg, H.H., Harper, L.A., Endale, D.M., Jenkins, M.B. and Franzluebbers, A.J. (2004) Ammonia Volatilization from Surface-Applied Poultry Litter under Conservation Tillage Management Practices. *Journal of Environmental Quality*, **33**, 1183-1188. https://doi.org/10.2134/jeq2004.1183

[5] Fernandez, A.L., Shaeffer, C.C., Wyse, D.L., Staley, C., Gould, T.J. and Sadowsky, M. (2016) Associations between Soil Bacterial Community Structure and Nutrient Cycling Functions in Long-Term Organic Farm Soils Following Cover Crop and Organic Fertilizer Amendment. *Science of the Total Environment*, **566**, 949-959. https://doi.org/10.1016/j.scitotenv.2016.05.073

[6] Lu, N., Liu, X.R., Du, Z.L., Wang, Y.D. and, Zhang, Q.Z. (2014) Effect of Biochar on Soil Respiration in the Maize Growing Season after 5 Years of Consecutive Application. *Soil Research*, **52**, 505-512. https://doi.org/10.1071/SR13239

[7] Pei, J., Zhuang, S., Cui, J., Li, J., Li, B., Wu, J. and, Fang, C. (2017) Biochar Decreased the Temperature Sensitivity of Soil Carbon Decomposition in a Paddy Field. *Agriculture*, *Ecosystems & Environment*, **249**, 156-164. https://doi.org/10.1016/j.agee.2017.08.029

[8] DeLuca T.H., Gundale M.J., MacKenzie M.D. and Jones D.L. (2015) Biochar Effects on Soil Nutrient Transformations. In: Lehmann, J. and Joseph, S., Eds., *Biochar Environmental Management*: *Science*, *Technology Implementation*, 2nd Edition, Routledge, London, 421-454.

[9] He, Z., Uchimiya, S.M. and Guo, M. (2016) Production and Characterization of Biochar from Agricultural By-Products: Overview and Use of Cotton Biomass Residues. *Agricultural and Environmental Applications of Biochar*: *Advances and Barriers*, **63**, 63-86. https://doi.org/10.2136/sssaspecpub63.2014.0037.5

[10] Rogovska, N., Laird, D., Cruse, R., Fleming, P., Parkin, T. and Me, D. (2011) Impact of Biochar on Manure Carbon Stabilization and Greenhouse Gas Emissions. *Soil*

Science Society of America Journal, **75**, 871-879. https://doi.org/10.2136/sssaj2010.0270

[11] Liang, B., Lehmann, L., Solomon, D., Kinyangi, K., Grossman, J., O'Neill, B., Skjemstad, J.O., Thies, J., Luiza, F.J., Petersen, O.J. and Neves, E.G. (2006) Black Carbon Increases Cation Exchange Capacity in Soils. *Soil Science Society America Journal*, **70**, 1719-1730. https://doi.org/10.2136/sssaj2005.0383

[12] Shen, Y., Zhu, L., Cheng, H., Yue, S. and Li, S. (2017) Effects of Biochar Application on CO_2 Emissions from a Cultivated Soil under Semiarid Climate Conditions in Northwest China. *Sustainability*, **9**, 1482. https://doi.org/10.3390/su9081482

[13] Joseph, U.E., Toluwase, A.O., Kehinde, E.O., *et al.* (2020) Effect of Biochar on Soil Structure and Storage of Soil Organic Carbon and Nitrogen in the Aggregate Fractions of an Albic Soil Arch. *Agronomy Soil Science*, **66**, 1-12. https://doi.org/10.1080/03650340.2019.1587412

[14] Rehman, A., Nawaz, S., Alghamdi, H.A., *et al.* (2020) Effects of Manure-Based Biochar on Uptake of Nutrients and Water Holding Capacity of Different Types of Soils. *Case Studies in Chemical and Environmental Engineering*, **2**, Article ID: 100036. https://doi.org/10.1016/j.cscee.2020.100036

[15] Toková, L., Igaz, D., Horák, J. and Aydin, E. (2020) Effect of Biochar Application and Re-Application on Soil Bulk Density, Porosity, Saturated Hydraulic Conductivity, Water Content and Soil Water Availability in a Silty Loam Haplic Luvisol. *Agronomy*, **10**, 1005. https://doi.org/10.3390/agronomy10071005

[16] Zhang, Q., Song, Y., Wu, Z., *et al.* (2020) Effects of Six-Year Biochar Amendment on Soil Aggregation, Crop Growth, and Nitrogen and Phosphorus Use Efficiencies in a Rice-Wheat Rotation. *Journal of Cleaner Production*, **242**, Article ID: 118435. https://doi.org/10.1016/j.jclepro.2019.118435

[17] Lehmann, J., Abiven, S., Kleber, M., Pan, G., Singh, B.P., Sohi, S. and Zimmerman, A. (2015) Persistence of Biochar in Soil. In: Lehmann, J. and Joseph, S., Eds., *Biochar Environmental Management*: *Science*, *Technology Implementation*, 2nd Edition, Routledge, London, 233-280. https://doi.org/10.4324/9780203762264-17

[18] Paramashivam, D., Clough, T.J., Carlton, A., Gough, K., Dickinson, N., Horswell, J., Sherlock, R.R., Clucas, L. and Robinson, B.H. (2016) The Effect of Lignite on Nitrogen Mobility in a Low-Fertility Soil Amended with Biosolids and Urea. *Science Total of Environment*, **543**, 601-608. https://doi.org/10.1016/j.scitotenv.2015.11.075

[19] Chen, D., Sun, J., Bai, M., Dassanayake, K.B., Denmead, O.T. and Hill, J. (2015) A New Cost-Effective Method to Mitigate Ammonia Loss from Intensive Cattle Feedlots: Application of Lignite. *Scientific Reports*, **5**, Article No. 16689. https://doi.org/10.1038/srep16689

[20] Laird, D., Fleming, P., Wang, B.Q., Horton, R. and Karlen, D. (2010) Biochar Impact on Nutrient Leaching from a Midwestern Agricultural Soil. *Geoderma*, **158**, 436-442. https://doi.org/10.1016/j.geoderma.2010.05.012

[21] Sun, J., Bai, M., Shen, J., Griffith, D.W.T., Denmead, O.T., Hill, J., Lam, A.R. and Mosier, D. (2016) Chen, Effects of Lignite Application on Ammonia and Nitrous Oxide Emissions from Cattle Pens. *Science of the Total Environment*, **565**, 148-154. https://doi.org/10.1016/j.scitotenv.2016.04.156

[22] Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A. and Joseph, S. (2007) Agronomic Values of Green Waste Biochar as a Soil Amendment. *Australian Journal of Soil Research*, **45**, 629-634. https://doi.org/10.1071/SR07109

[23] Rhoades, J.D. (1982) Cation Exchange Capacity. In: Page, A.L., Ed., *Methods of Soil Analysis*. *Part* 2: *Chemical and Microbiological Properties*, 2nd Edition, Agronomy

Monograph 9, American Society of Agronomy Madison, 149-157. https://doi.org/10.2134/agronmonogr9.2.2ed.c8

[24] Isaac, R.A. and Kerber, J.D. (1977) Atomic Absorption and Flame Photometry: Techniques and Uses in Soil, Plant, and Water Analysis. In: Walsh, L.M., Ed., *Instrumental Methods for Analysis Soils and Plant Tissue*, SSSA, Madison, 17-37. https://doi.org/10.2136/1971.instrumentalmethods.c2

[25] Mehlich, A. (1984) Mehlich-3 Soil Test Extractant: A Modification of Mehlich 2 Extractant. *Communications in Soil Science and Plant Analysis*, **15**, 1409-1416. https://doi.org/10.1080/00103628409367568

[26] Nimmo, J.R. and Perkins, K.S. (2002) Aggregate Stability and Size Distribution. In: Dane, J.H. and Topp, G.C., Eds., *Methods of Soil Analysis*. *Part* 4. *Physical Methods*, Soil Science Society of America, Madison, 317-328. https://doi.org/10.2136/sssabookser5.4.c14

[27] Brady, N. and Weil, R. (2017) The Nature and Properties of Soils. 15th Edition, Pearson, New York.

[28] Reynolds, W., Elrick, D. and Youngs, E. (2002) Single-Ring and Double- or Concentric-Ring Infiltrometers. In: *Methods of Soil Analysis*: *Part* 4 *Physical Methods*, American Society of Agronomy, Madison, 821-826.

[29] Deng, S., Popova, I.E., Dick, L. and Dick, R. (2013) Bench Scale and Microplate Format Assay of Soil Enzyme Activities Using Spectroscopic and Fluorometric Approaches. *Applied Soil Ecology*, **64**, 84-90. https://doi.org/10.1016/j.apsoil.2012.11.002

[30] Brooks, J.P., Adeli, A., Smith, R.K., McGrew, R., Lang, D.J. and Read, J.J. (2019) Bacterial Community Structure Recovery in Reclaimed Coal Mined Soil under Two Vegetative Regimes. *Journal of Environmental Quality*, **48**, 1029-1037. https://doi.org/10.2134/jeq2018.09.0349

[31] Liu, J., Sui, Y., Yu, Z., Shi, Y., Chu, H., Jin, J., Liu, X. and Wang, G. (2015) Soil Carbon Content Drives the Biogeographical Distribution of Fungal Communities in the Black Soil Zone of Northeast China. *Soil Biology and Biochemistry*, **83**, 29-39. https://doi.org/10.1016/j.soilbio.2015.01.009

[32] R Core Team (2021) R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna. https://www.R-project.org

[33] Bates, D., Maechler, M., Bolker, B. and Walker, S. (2015) Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, **67**, 1-48. https://doi.org/10.18637/jss.v067.i01

[34] Kuznetsova, A., Brockhoff, P.B. and Christensen, R.H.B. (2017) lmerTest Package: Tests in Linear Mixed Effects Models. *Journal of Statistical Software*, **82**, 1-26. https://doi.org/10.18637/jss.v082.i13

[35] Lenth, R., Singmann, H., Love, J., Buerkner, P. and Herve, M. (2018) Package "Emmeans". R Package Version 4.0-3. http://cran.r-project.org/package=emmeans

[36] Hothorn, T., Bretz, F., *et al.* (2008) Simultaneous Inference in General Parametric Models. *Biometrical Journal*, **50**, 346-363. https://doi.org/10.1002/bimj.200810425

[37] Parker, M.A., Nyakatawa, E.Z., Reddy, K.C. and Reeves, D.W. (2002) Soil Carbon and Nitrogen as Influenced by Tillage and Poultry Litter in North Alabama. *Proceeding Southern Conservation Tillage Conference for Sustainable Agriculture*, Auburn, 24-26 June 2002, 283-287.

[38] Frimpong, K.A., Amoakwah, E., Osei, B.A. and Arthur, E. (2016) Changes in Soil Chemical Properties and Lettuce Yield Response Following Incorporation of Bio-

char and Cow Dung to Highly Weathered Acidic Soils. *Journal of Organic Agriculture and Environment*, **4**, 28-39.

[39] Stetson, S.J., Osborne, S.L., Schumacher, T.E., Eynard, A., Chilom, G., Rice, K., Nichols, A. and Pikul, J.L. (2012) Corn Residue Removal Impact on Topsoil Organic Carbon in a Corn-Soybean Rotation. *Soil Science Society of America Journal*, **76**, 1399-1406. https://doi.org/10.2136/sssaj2011.0420

[40] Dick, R.P. (1992) A Review, Long-Term Effects of Agricultural Systems on Soil Biochemical and Microbial Parameters. *Agricultural Ecosystems Environment*, **40**, 25-36. https://doi.org/10.1016/0167-8809(92)90081-L

[41] Rasool, R., Kukal, S.S. and Hira, G.S. (2007) Soil Organic Carbon and Physical Properties as Affected by Long-Term Application of FYM and Inorganic Fertilizers in Maize-Wheat System. *Soil and Tillage Research*, **101**, 31-36. https://doi.org/10.1016/j.still.2008.05.015

[42] Mulvaney, R.L., Khan, S.A. and Ellsworth, T.R. (2009) Synthetic Nitrogen Fertilizers Deplete Soil Nitrogen: A Global Dilemma for Sustainable Cereal Production. *Journal of Environmental Quality*, **38**, 2295-2314. https://doi.org/10.2134/jeq2008.0527

[43] Glaser, B., Haumaier, L., Guggenberger, G. and Zech, W. (2001) The Terra Preta Phenomenon: A Model for Sustainable Agriculture in the Humid Tropics. *Naturwissenschaften*, **88**, 37-41. https://doi.org/10.1007/s001140000193

[44] Edwards, L., Mudie, P.J. and de Vernal, A. (1991) Sea Surface Temperature Reconstruction Based on Dinoflagellates from the North Atlantic. Pangaea.

[45] Siedt, M., Schäffer, A., Smith, K.E., Nabel, M., Roz-Nickoll, M. and van Dongen, J.T. (2020) Comparing Straw, Compost, and Biochar Regarding Their Suitability as Agricultural Soil Amendments to Affect Soil Structure, Nutrient Leaching, Microbial Communities, and the Fate of Pesticides. *Science of Total Environment*, **751**, Article ID: 141607. https://doi.org/10.1016/j.scitotenv.2020.141607

[46] Seehausen, M.L., Gale, N.V., Dranga, S., *et al.* (2017) Is There a Positive Synergistic Effect of Biochar and Compost Soil Amendments on Plant Growth and Physiological Performance? *Agronomy*, **7**, 13. https://doi.org/10.3390/agronomy7010013

[47] Widowati, U.W., Soehono, L.A. and Guritno, B. (2011) Effect of Biochar on the Release and Loss of Nitrogen from Urea Fertilization. *Journal Agriculture Food Technology*, **1**, 127-132.

[48] Awasthi, M.K., Wang, Q., Ren, X., Zhao, J., Huang, H., Awasthi, S.K., Lahori, A.H., Li, R., Zhou, L. and Zhang, Z. (2016) Role of Biochar Amendment in Mitigation of Nitrogen Loss and Greenhouse Gas Emission during Sewage Sludge Composting. *Bioresources Technology*, **219**, 270-280. https://doi.org/10.1016/j.biortech.2016.07.128

[49] Van Zwieten, L., Kimber, S., Morris, S., Chan, K.Y., Downie, A., Rust, J., Joseph, S. and Cowie, A. (2010) Effects of Biochar from Slow Pyrolysis of Papermill Waste on Agronomic Performance and Soil Fertility. *Plant and Soil*, **327**, 235-246. https://doi.org/10.1007/s11104-009-0050-x

[50] Taghizadeh-Toosi, A., Clough, T.J., Sherlock, R.R. and Condron, L.M. (2012) A Wood Based Low-Temperature Biochar Captures NH3-N Generated from Ruminant Urine-N, Retaining Its Bioavailability. *Plant and Soil*, **353**, 73-84. https://doi.org/10.1007/s11104-011-1010-9

[51] Edmeades, D.C. (2003) The Long-Term Effects of Manures and Fertilizers on Soil Productivity and Quality: A Review. *Nutrient Cycling in Agroecosystems*, **66**, 165-180. https://doi.org/10.1023/A:1023999816690

[52] Bronick, C.J. and Lal, R. (2005) Comment on "Soil Structure and Management: A Review". *Geoderma*, **134**, 231-232. https://doi.org/10.1016/j.geoderma.2005.10.003

[53] Ulyett, J., Sakrabani, R., Kibblewhite, M. and Hann, M. (2014) Impact of Biochar Addition on Water Retention, Nitrification, and Carbon Dioxide Evolution from Two Sandy Loam Soils. *European Journal of Soil Science*, **65**, 96-104. https://doi.org/10.1111/ejss.12081

[54] Pietikäinen, J., Kiikkilä, O. and Fritze, H. (2000) Charcoal as a Habitat for Microbes and Its Effect on the Microbial Community of the Underlying Humus. *Oikos*, **89**, 231-242. https://doi.org/10.1034/j.1600-0706.2000.890203.x

[55] Pagliali, M. and Antisari, L.V. (1993) Influence of Waste Organic Matter on Soil Micro- and Macrostructure. *Bioresources Technology*, **43**, 205-213. https://doi.org/10.1016/0960-8524(93)90032-7

[56] Celik, I., Ortas, I. and Kilic, S. (2004) Effects of Compost, Mycorrhiza, Manure and Fertilizer on Some Physical Properties of a Chromoxerert Soil. *Soil and Tillage Research*, **78**, 59-67. https://doi.org/10.1016/j.still.2004.02.012

[57] Ghuman, B.S. and Sur, H.S. (2001) Tillage and Residue Management Effects on Soil Properties and Yields of Rainfed Maize and Wheat in a Subhumid Subtropical Climate. *Soil and Tillage Research*, **58**, 1-10. https://doi.org/10.1016/S0167-1987(00)00147-1

[58] Bharadwaj, V. and Omanwar (1992) Impact of Long-Term Fertilizing Treatments on Bulk Density, Water Contents, and Microbial Population of Soil. *Journal of the Indian Society of Soil Science*, **40**, 553-555.

[59] Zhang, H. (1994) Organic Matter Incorporation Affects Mechanical Properties of the Soil Aggregates. *Soil and Tillage Research*, **31**, 263-275. https://doi.org/10.1016/0167-1987(94)90085-X

[60] Unger, P.W. and Jones, O.R. (1998) Long-Term Tillage and Cropping System Affect Bulk Density and Penetration Resistance of Soil Cropped to Dry-Land Wheat and Grain Sorghum. *Soil and Tillage Research*, **45**, 39-57. https://doi.org/10.1016/S0167-1987(97)00068-8

[61] Angers, D.A., Pesant, A. and Vigneux, J. (1992) Early Cropping Induced Changes in Soil Aggregation, Organic Matter, and Microbial Biomass. *Soil Science Society of America Journal*, **56**, 115-119. https://doi.org/10.2136/sssaj1992.03615995005600010018x

[62] Martin, J.P. (1945) Microorganisms and Soil Aggregates, I: Origin and Nature of Some of the Aggregating Substances. *Soil Science*, **59**, 163-174. https://doi.org/10.1097/00010694-194502000-00006

[63] Palaniappan, R. (1975) Studies on Soil Organic Matter. PhD Thesis, Tamil Nadu Agricultural University, Coimbatore.

[64] Mubarak, A.R., Omaima, E.R., Amal, A.A. and Nemat, E.H. (2009) Short-Term Studies on Use of Organic Amendments for Amelioration of a Sandy Soil. *African Journal of Agricultural Research*, **4**, 621-627.

[65] Boyle, M., Frankenberger, W.T. and Stolzy, L.H. (1989) The Influence of Organic Matter on Soil Aggregation and Water Infiltration. *Journal of Production Agriculture*, **2**, 290-299. https://doi.org/10.2134/jpa1989.0290

[66] Sarkar, S., Singh, S.R. and Singh, R.P. (2003) The Effect of Organic and Inorganic Fertilizers on Soil Physical Condition and Productivity of a Rice-Lentil Cropping Sequence in India. *Journal of Agricultural Science*, **140**, 419-425. https://doi.org/10.1017/S0021859603003186

[67] Adekiya, A.O., Agbede, T.M., Aboyeji, C.M., Dunsin, O. and Simeon, V.T. (2018) Biochar and Poultry Manure Effects on Soil Properties and Radish (*Raphanus sativus* L.) Yield. *Biological Agriculture and Horticulture*, **35**, 33-45. https://doi.org/10.1080/01448765.2018.1500306

[68] Aluko, O.B., Oyedele, D.J., Aluko, O.B. and Oyedele, D.J. (2005) Influence of Organic Incorporation on Changes in Selected Soil Physical Properties during Drying of a Nigerian Alfisols. *Journal Applied Science*, **5**, 357-362. https://doi.org/10.3923/jas.2005.357.362

[69] Borchard, N., Wolf, A., Laabs, V., Aeckersberg, R., Scherer, H.W., Moeller, A. and Amelung, W. (2012) Physical Activation of Biochar and Its Meaning for Soil Fertility and Nutrient Leaching a Greenhouse Experiment. *Soil Use Management*, **28**, 177-184. https://doi.org/10.1111/j.1475-2743.2012.00407.x

[70] Basso, A.S., Miguez, F.E., Laird, D.A., Horton, R. and Westgate, M. (2013) Assessing Potential If Biochar for Increasing Water-Holding Capacity of Sandy Soils. *GCB Bioenergy*, **5**, 132-143. https://doi.org/10.1111/gcbb.12026

[71] Novak, J.M., Busscher, W.J., Watts, D.W., Amonette, J.E., Ippolito, J.A., Lima, I.M., Gaskin, J., Das, K.C., Steiner, C., Ahmedna, M., Rehrah, D. and Schomberg, H. (2012) Biochars Impact on Soil-Moisture Storage in an Ultisol and Two Aridisols. *Soil Science*, **177**, 310-320. https://doi.org/10.1097/SS.0b013e31824e5593

[72] Villamil, M.B., Bollero, G. and Darmody, R.G. (2006) No-Till Corn/Soybean Systems Including Winter Cover Crops. *Soil Science Society of America Journal*, **70**, 1936-1944. https://doi.org/10.2136/sssaj2005.0350

[73] Zheng, H., Wang, Z., Deng, Z., Herbert, H. and Xing, B. (2013) Impacts of Adding Biochar on Nitrogen Retention and Bioavailability in Agricultural Soil. *Geoderma*, **206**, 32-39. https://doi.org/10.1016/j.geoderma.2013.04.018

[74] Fidel, R.B., Laird, D.A. and Spokas, K.A. (2018) Sorption of Ammonium and Nitrate to Biochars Is Electrostatic and pH-Dependent. *Scientific Reports*, **8**, Article No. 17627. https://doi.org/10.1038/s41598-018-35534-w

[75] Yin, Q., Zhang, B., Wang, R. and Zhao, Z. (2017) Biochar as an Adsorbent for Inorganic Nitrogen and Phosphorus Removal from Water: A Review. *Environmental Science and Pollution Research International*, **24**, 26297-26309. https://doi.org/10.1007/s11356-017-0338-y

[76] Knowles, O.A., Robinson, B.H., Contangelo, A. and Clucas, L. (2011) Biochar for the Mitigation of Nitrate Leaching from Soil Amended with Biosolids. *Science of the Total Environment*, **409**, 3206-3210. https://doi.org/10.1016/j.scitotenv.2011.05.011

[77] Rothrock Jr., M.J., Cook, K.L., Lovanh, N., Warren, J.G. and Sistani, K. (2006) Development of a Quantitative Real-Time Polymerase Chain Reaction Assay to Target a Novel Group of Ammonia-Producing Bacteria Found in Poultry Litter. *Poultry Science*, **87**, 1058-1067. https://doi.org/10.3382/ps.2007-00350

[78] Geisseler, D., Joergensen, R.G. and Ludwig, B. (2012) Temporal Effect of Straw Addition on Amino Acid Utilization by Soil Microorganisms. *European Journal of Soil Biology*, **53**, 107-113. https://doi.org/10.1016/j.ejsobi.2012.09.006

[79] Haider, G., Steffens, D., Moser, G., Müller, C. and Kammann, C.I. (2017) Biochar Reduced Nitrate Leaching and Improved Soil Moisture Content without Yield Improvements in a Four-Year Field Study. *Agriculture*, *Ecosystems and Environment*, **237**, 80-94. https://doi.org/10.1016/j.agee.2016.12.019

[80] Sinsabaugh, R.L., Hill, B.H. and Follstad Shah, J.J. (2009) Ecoenzymatic Stoichiometry of Microbial Organic Nutrient Acquisition in Soil and Sediment. *Nature*,

462, 795-798. https://doi.org/10.1038/nature08632

[81] Turner, B.L. and Joseph Wright, S. (2014) The Response of Microbial Biomass and Hydrolytic Enzymes to a Decade of Nitrogen, Phosphorus, and Potassium Addition in a Lowland Tropical Rain Forest. *Biogeochemistry*, **117**, 115-130. https://doi.org/10.1007/s10533-013-9848-y

[82] Magalhães, C.M., Machado, A., Matos, P. and Bordalo, A.A. (2011) Impact of Copper on the Diversity, Abundance and Transcription of Nitrite and Nitrous Oxide Reductase Genes in an Urban European Estuary. *FEMS Microbiology Ecology*, **77**, 274-284. https://doi.org/10.1111/j.1574-6941.2011.01107.x

[83] Li, J., Zheng, Y.M., Liu, Y.R., Ma, Y.B., Hu, H.W. and He, J.Z. (2014) Initial Copper Stress Strengthens the Resistance of Soil Microorganisms to a Subsequent Copper Stress. *Microbial Ecology*, **67**, 931-941. https://doi.org/10.1007/s00248-014-0391-8