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Abstract

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Keywords

C-mineralization, carbon sequestration, nutrient cycling, sustainability, waste management, animal agriculture, N-immobilization, N-mineralization

Disciplines

Agriculture | Agronomy and Crop Sciences | Bioresource and Agricultural Engineering | Horticulture

Comments

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Soil nutrients and carbon dynamics in the presence of biochar-swine manure mixture under controlled leaching experiment using a Midwestern USA soil

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Abstract

Biochar application to the soil can improve soil quality and nutrient leaching loss. Recent studies have reported that surficial application of biochar to stored swine manure can reduce emissions of odorous compounds and reduce the volatilization loss of ammonia. Our working hypothesis was that the biochar-treated manure application to the soil would decrease nutrient leaching from manure and increase plant-available nutrients. The study objectives were to evaluate the impact of biochar-treated swine manure on soil total C, N, and other major and minor nutrients. Three biochars (*i*) neutral pH red-oak (RO), (*ii*) highly alkaline autothermal corn (*Zea mays*) stover (HAP), and (*iii*) mild acidic Fe-treated autothermal corn stover (HAPE) were incubated with swine manure for a month. The biochar-manure mixture was applied in triplicate to soil columns with application rate determined by the P₂O₅-P content in manure or manure-biochar mixtures after the incubation. The ammonium (NH₄⁺), nitrate (NO₃⁻), and reactive P concentrations in soil column leachates were recorded for eight leaching events. Soil properties and plant-available nutrients were compared between treatments and control manure & soil. Manure-(HAP&HAPE) biochar treatments significantly increased soil organic matter (OM) and increased soil total C, N, and improved soil bulk density. Concentrations of KCl-extractable NH₄⁺ and NO₃⁻ significantly increased in HAPE column leachates during this 4-week study and in the soil after the experiment. A significant reduction in soil Mehlich3 Cu was also observed for the manure-HAPE mixture compared with the control. Overall, the manure-biochar incubation enabled biochar to sorb nutrients from manure, and the subsequent manure-biochar mixture application to soil improved soil quality and plant nutrient availability in comparison to conventional manure application to soil. This proof-of-the-concept study suggests that biochars could be used to solve *both* environmental and agronomic challenges and further improve the sustainability of animal and crop production agriculture.

1. Introduction

Swine manure is a source of valuable nutrients (Chastain et al., 1999), but mismanagement or improper application to soils makes it a potential environmental threat. The application rate of animal manure is often exceeded than the plant requirement (Juergens-Gschwind, 1989), and this excessive

manure application to the soil can result in an unintended nutrient N loss leaching to groundwater (Beckwith et al., 1998). Corn (*Zea mays*) and soybeans (*G. max*) crop rotation is a common practice in Midwest US, and the application of swine manure to fields has been a common practice to both dispose of the stored manure and to provide nutrients for crop growth. Soybean can positively respond to swine manure application as reported by previous studies (Sawyer, J.E., 2001; Killorn, R., 1999). Manure solids, undigested feed, and bedding material in the manure help build soil organic matter, which improves soil structure and helps to increase soil water holding capacity and reduce nutrient leaching loss (Magdoff and Es, 2009). However, the use of liquid swine manure to soil may not increase soil C sequestration; instead, it can increase the native soil C decomposition (Angers et al., 2009), and as a result, leaching loss of macronutrients such as N and sedimental loss of P (Reid et al., 2018) can occur. Furthermore, losses of C and N can also occur via emissions of greenhouse gases from land-applied swine manure (Maurer et al., 2017a).

Biochar, a product of biomass pyrolysis, heating under low or no O₂ conditions, has attracted much interest as a means of solving many soil problems (Laird et al., 2008). Many biochar properties can be useful to address challenges in crop and livestock agriculture (Kalus et al., 2019). Freeze dry manure fertilization to the soil in the presence of biochar has shown a significant decrease in nutrient leaching loss, greater retention of plant nutrients, and improvement in soil C and N compared with control manure-treated soil (Laird et al., 2010a and Laird et al., 2010b). The biochar amendments followed by manure application increase the cation & anion exchange capacity of biochar, enable biochar to both adsorb and release nutrients to/from the soil, hence functioning as both a reservoir and slow-release source of plant nutrients. Biochar properties can be useful to address challenges in crop and livestock agriculture, as recently reviewed elsewhere (Kalus et al., 2019).

Application of alkaline biochar to soil can increase soil pH and reduce problems related to low soil pH, which typically occur after prolonged application of ammonium forms of N fertilizer. Studies have shown that biochar improves soil water holding capacity and is able to reduce soil bulk density (BD) (Rogovska et al., 2011). Unfortunately, most biochars have few positively charged surface sites and hence limited anion exchange capacity and ability to electrostatically adsorb nutrient anions such as PO₄³⁻ and NO₃⁻ (Lawrinenko and Laird, 2015). There are consistent data in the literature supporting the electrostatic retention of positively charged ammonium (NH₄⁺) but not of negatively charged PO₄³⁻ and NO₃⁻ on biochar surfaces (Yao et al., 2012; Fidel et al., 2018). Iron (Fe) modification of biochar surfaces has been shown to be effective in enhancing PO₄³⁻ adsorption (Wilfert et al., 2015).

In addition to biochar being proposed as a soil amendment, recent studies have shown that surficial application of biochar onto the swine manure can reduce emissions of odorous gases (Meirkhanuly, 2019), acute releases of H₂S from agitated manure (Chen et al., 2020), and reduce volatilization loss of ammonia (Maurer et al., 2017b). Biochar pH is important in this regard, and surficial treatment of an alkaline/neutral pH biochar to manure storage can change the manure pH near the manure-air interface within few days of application (Meirkhanuly et al., 2020). Depending on the manure buffering capacity, the rate of ammonia release to the atmosphere may change. Biochar can also reduce emissions of NH₃ from poultry manure when it is used as a diet supplementation for broilers (Kalus et al., 2020a); and improvement in laying performance and egg quality has been reported when biochar is used as a diet supplementation for laying hens (Kalus et al., 2020b).

Clearly, there is an opportunity to explore the tantalizing question if biochar can be used to address both environmental and crop production challenges in one system. We propose a novel concept of biochar utilization that can improve the sustainability of animal and crop production agriculture. Biochar can be first used to mitigate gaseous emissions (as already proven on lab and pilot-scales)

from stored manure and retain more nutrients in the manure. When the swine manure pits are agitated and cleaned out, the mixture of swine manure and biochar will be pumped out and applied to soils. Environmental and agronomic benefits are expected due to decrease nutrient leaching from manure and increase plant-available nutrients. However, there is a gap in the literature on the use of this kind of biochar-manure mixtures as a soil amendment.

The study objectives were to evaluate the impact of biochar-treated swine manure on soil total C, N, and other major and minor nutrients. Our working hypothesis was that the biochar-treated manure application to the soil would decrease nutrient leaching from manure and increase plant-available nutrients. Three biochars (*i*) neutral pH red-oak (RO), (*ii*) highly alkaline autothermal corn stover (HAP), and (*iii*) mild acidic Fe-treated autothermal corn stover (HAPE) were incubated with swine manure for a month. This was followed by a controlled column leaching experiment for soils treated with biochar-manure mixtures followed. We investigated the impact of biochar-manure treatments on soil nutrient leachate and soil physicochemical properties (pH, bulk density, total C and N), and major plant nutrients N, P, and K. In addition, this research addressed the impact of Fe-modified biochar application on manure to sorb nutrient followed by soil application as an amendment.

2. Materials and Methods

2.1 Soil, biochar, manure, and manure-biochar incubation

Hanlon (Coarse-loamy, mixed, superactive, mesic Cumulic Hapludolls) soil collected from the Iowa State University Applied Science/Moore research farm. Soybean and corn were mainly grown in rotation in these plots. Samples of soil surface (0-10 cm) were collected and stored in buckets with lids to keep the moisture at the field level. The buckets were stored at 4°C until analysis started two months after collection.

Fast pyrolysis (500 °C) neutral pH (pH ~7.5) red oak biochar (<2 cm) was obtained from a commercial producer, and a fast pyrolysis high pH (pH~ 9.2) corn stover biochar (HAP) and Fe-modified corn-stover (500 °C) biochar (HAPE) with a moderately acidic pH were obtained by autothermal pyrolysis. A detail of the pyrolysis techniques of HAP and HAPE biochar production is given elsewhere (Polin et al., 2019 and Rollag, et al., 2020). The biochar properties, moisture, volatile matters, fixed C, ash content, total C, and total N were determined by following the method described by Rover et al. (2018).

The swine manure was collected from deep pit storage at an Iowa Select Farms facility in the fall of 2019. The manure was stored in a bucket with a lid and stored (at 22-23 °C) until incubated with biochar within one month of manure collection. About 250 g biochar was surface applied on 1000 g of manure and incubated (at 22-23 °C) in an 8.5 L glass container (10 cm i.d. & 27 cm height) for one month at atmospheric condition. After the incubation period, the biochar and manure were mixed thoroughly to homogenize and stored in airtight glass at 4 °C until analysis started one month later. A control manure sample was also incubated and mixed thoroughly under the same condition for comparison.

The mixture was analyzed for moisture, total C (TC), total N (TN), mineral content, organic matter (OM), nitrate-N, ammonium-N, P₂O₅-P, and K₂O-K, and the data was provided in % dry weight basis. Moisture and dry matter in the samples were measured by heating the samples for 16 h at 105~110 °C. Organic matter was determined by heating the samples in a muffle furnace at 550 °C for 2 h. The total C and N were analyzed by combustion using Elementar Vario Max CN Method 4.01 and 3.3,

respectively. Sample nitrate and ammonium-N were measured by KCl extraction and determined on the FIA Lab flow injection autoanalyzer.

2.2 Soil column preparation and leaching experiment

The field moist soil was dried, sieved (<2 mm), and stored in a bucket with a lid for the column preparation. Soil columns (25 cm height and 4.4 cm i.d.) were built from PVC tubes with a PVC male adapter sealed at the bottom using PVC cement. At the base of each column, 4.4 i.d. 'air filter pad' was inserted, and then ~2 g of 1 mm glass beads were added on top of that coarse sieve. Each column was filled with 250 g of dried (<2 mm) soil to a length of 15 cm, maintaining an approximate bulk density (BD) of 1.2 g/cm³. Water was then filled from the bottom of each column to the top of the soil to remove excess air trapped in the soil column and drained the water under gravity (Figure 1; Supplementary Material Figure 1).

De-ionized (DI) water (50 mL) was added from the top of each column, and the leachate was collected from the bottom to collect the baseline soil data two times during one week of column equilibration time. The columns were named depending the treatments they received, M =manure control; S = soil control; MRO = manure+red oak biochar; MHAP = manure+ highly alkaline porous biochar; MHAPE = manure+highly alkaline porous engineered biochar. The amount of biochar-manure mixture or manure addition was calculated based on the P₂O₅-P content of the mixture to make sure each column gets a recommended rate of P of 135 kg/ha (120 lb/acre) of soil for a corn-soybean rotational plot (Sawyer and Mallarino, 2016). Treatments were applied after a week of column equilibration followed by a 50 mL DI water addition from the top of each column using a beaker.

The leachate was collected overnight in labeled bottles, and the next morning the leachate was transferred to <0 °C until analysis. Leachate was collected for a total of eight times (eight events) after the treatment application. Leachates were filtered through a 0.45 µm syringe filter and analyzed for nitrate-N (vanadium III, sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride), ammonium-N (salicylate and ammonia cyanurate method), and dissolved reactive phosphorus (DRP; malachite green method; D'Angelo et al., 2001) using a Synergy HTX Multi-Mode microplate reader (BioTek Instruments, Inc.) colorimetric method (De et al., 2019; Doane & Horwáth, 2003).

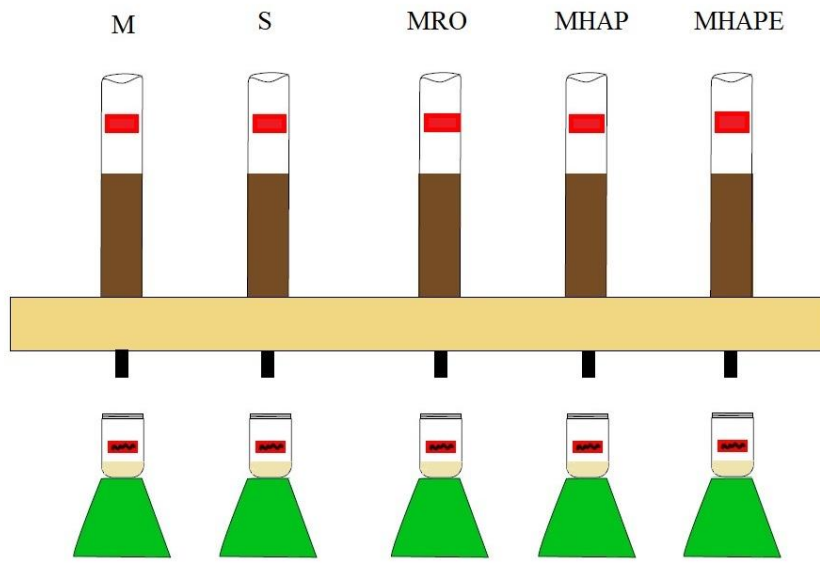


Figure 1. A schematic of the soil column leachate collection apparatus. Each column contains 250 g of soil, treated with one of five treatments. Two of these apparatuses were utilized, resulting in 15 columns; three trials for each of the five treatments). M =manure control; S = soil control; MRO = manure+red oak biochar; MHAP = manure+highly alkaline porous biochar; MHAPE = manure+highly alkaline porous engineered biochar. See Supplementary Figure 1 for a photograph of the experimental setup.

2.3 Column soil analysis

After the leachate collection was over, the columns were left for a week to drain the excess water from the clogged columns. Once the excess water drained out, the soil from each column was loosened using a long spatula and collected in Ziploc bags. The soil was then dried, sieved (<2 mm), and analyzed for pH (1:1; soil: water) following the method by McLean (1982) using the glass-electrode meter method. About 2 g soil was weighed, and soil OM was measured by loss on ignition at 360 °C by (Schulte and Hopkins, 1996) method; and the total C and N were analyzed by combustion using Elementar Vario Max CN Method 4.01 and 3.3, respectively (Nelson et al., 1996). The extractant was prepared by weighing approximately 5g soil in 200 mL Nalgene bottle, shaken with KCl at 1:5 ratio for 30 min, and then filtered through Whatman grade 1 filter paper. This extractant was used to measure KCl-extractable soil nitrate-N (vanadium III, sulfanilamide and N-(1-naphthyl)-ethylenediamine dihydrochloride) and ammonium-N (salicylate and ammonia cyanurate method) (De et al., 2019; Doane & Horwath, 2003). The Mehlich3 extractable elements were extracted by a modified method of Mehlich (1984) and analyzed by ICP-OES.

2.4 Statistical analysis

The statistical analysis was completed using R. The experiment has three biochar manure mixture, one manure control, and one soil control, with three replicates total of 15 columns. A mixed model was run to analyze the soil column leachate considering time as a factor, then Tukey's pairwise comparison was used to compare treatment effect on total nitrate-N, ammonium-N, and DRP. To

report the treatment effects on soil nitrate, ammonium, and P, a one-way ANOVA was performed. A p-value <0.05 was considered as statistically significant.

3. Results

3.1. Biochar and manure properties before mixture incubation

The soil used in the study had a pH of 7.6, containing 1.88% of total C and 0.17% of total N and a TC/TN ratio of 11.1. The swine manure used in this experiment had an alkaline pH of 9.2. Swine manure used in this study contained 37.4% TC and 18.1% of TN, and the TC/TN ratio was 2.1. As recorded, the pH of the autothermal corn stover biochar was mild acidic for HAPE (pH of 5.4) to highly alkaline (9.2) for HAP, and the pH of hardwood RO was 7.5, close to soil pH. The hardwood biochar had 78.5% TC and 0.6% TN by mass and contained 26.4% volatile matters, 15.8% ash, 54.8% fixed C by mass, as indicated by proximate analysis. Whereas the autothermal biochar HAP had 61.4% C, and 1.2% TN by mass, and contained 16.3% volatile matters, 46.8% ash, 35.0% fixed C by mass, as indicated by proximate analysis. The Fe-pretreated autothermal biochar HAPE had 36.4% TC, and 1.2% TN by mass, and contained 34.0% volatile matters, 40.0% ash, 24.0% fixed C by mass, as indicated by proximate analysis. The TC/TN ratio ranged between 30-130 among the biochars; hardwood RO had the highest carbon content (total and fixed), thus the highest TC/TN ratio. The ash content of RO biochar was the lowest among the three-biochar used in this experiment (summarized in Table 1).

Table 1: Physicochemical properties of the different biochar-manure mixtures after incubation. Except for pH, all values were reported on a % dry weight basis

Properties	Manure (control)	MHAP	MRO	MHAPE
pH	9.19	9.7 ± 2.6	9.9 ± 0.01	7.5 ± 0.01
Moisture (%)	90.8	58.1 ± 0.6	23.6 ± 3.0	49.9 ± 0.9
Mineral matter (%)	43.5	28.7 ± 0.2	45.2 ± 2.8	46.5 ± 0.4
LOI (%)	56.5	71.3 ± 0.2	54.8 ± 2.8	53.5 ± 0.4
Org-N (%)	4.4 ± 0.4	1.9 ± 0.04	0.99 ± 0.02	1.7 ± 0.01
NH ₄ -N (%)	0.69	0.04 ± 0.01	0.02 ± 0.01	1 ± 0.03
NO ₃ -N (%)	N.D.	N.D.	N.D.	N.D.
P ₂ O ₅ -P (%)	5.41 ± 0.06	0.7 ± 0.1	0.8 ± 0.2	0.8 ± 0.02
K ₂ O-K (%)	16.8 ± 2.6	4.6 ± 0.1	3.8 ± 0.3	5.1 ± 0.1
TC (%)	38.2	51.3 ± 4.5	50.2 ± 3.2	36.3 ± 1.2
TN (%)	5.4 ± 0.08	2.0 ± 0.1	1.0 ± 0.1	2.7 ± 0.04
TC/TN	7.1	26.3 ± 2.6	51.8 ± 4.4	13.3 ± 0.6

Note: N.D. = not detected; ± calculated for n = 3; MRO = manure+red oak biochar; MHAP = manure+ highly alkaline porous biochar; MHAPE = manure+highly alkaline porous engineered biochar.

3.2. Incubation effect on biochar-manure physicochemical parameters

The addition of biochar to the manure changes the physical appearance of manure (Supplemental Figure S2). After the one-month incubation, the control manure was liquid slurry with yellow patches on the surface, possibly representing a microbial colony developed during incubation and a persistent manure odor. On the contrary, no such color or odor was observed for any of the biochar samples. During incubation, biochar absorbed the manure moisture, and after mixing to homogenize, its texture resembled loose soil. The moisture content of biochar increased several folds by soaking the manure moisture (Table 2). As a result of incubation, the pH increased for all biochar-manure mixtures. An increase in total C for the biochar-manure mixture was observed for both MHAP biochar mixtures except RO; however, the TC/TN ratio dropped for all biochar-manure mixtures

compared with the biochar. The nutrient N, P, and K contents of all biochars increased during incubation with manure (Table 1).

3.3. Nutrients in leached water

Dissolved N and P leached out from all columns irrespective of treatments were low during this eight-week study. However, the MHAPE treated columns released significantly high total NO_3^- -N ($p = 0.003$) and NH_4^+ -N ($p = 0.003$). An upward trend with time was observed for the cumulative NO_3^- -N and NH_4^+ -N concentration in MHAPE treated column leachate during the last six weeks (leaching events) (Figure 1), whereas control manure-treated columns NO_3^- -N started to increase in leachate on 7th and 8th leaching events. At the beginning of the column experiment, the MHAPE had about 1% of nitrate (Table 1); higher concentrations in comparison to other treatments.

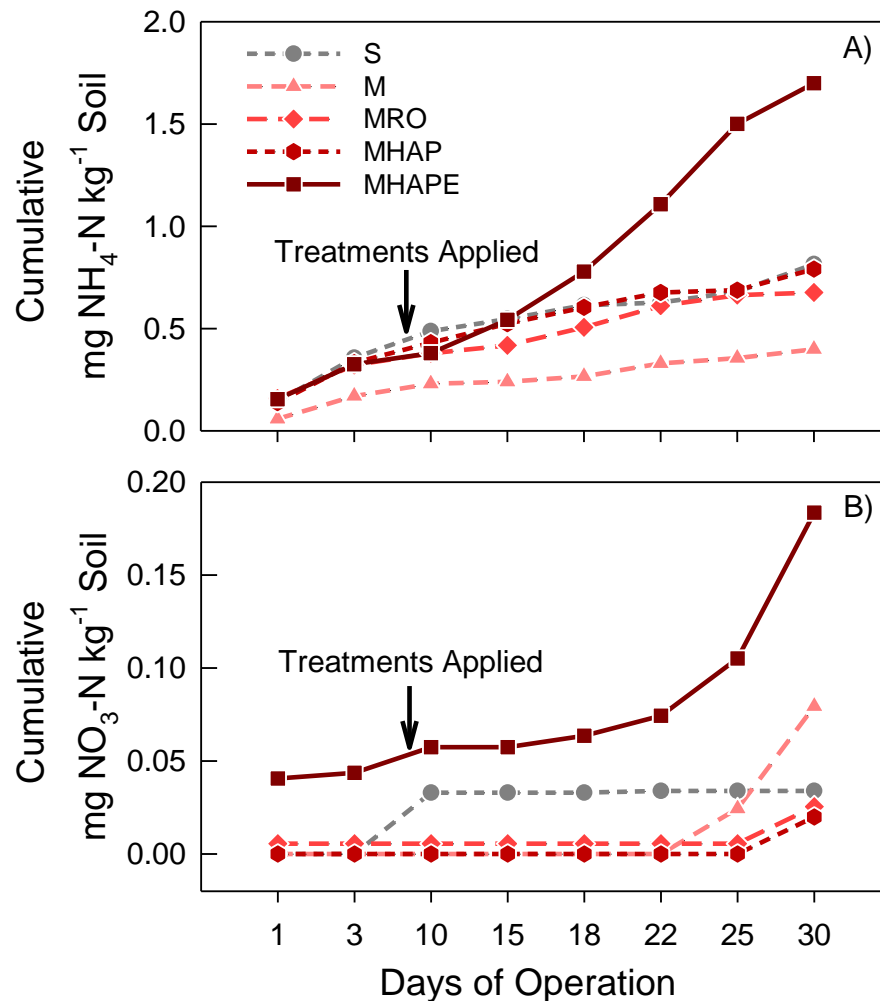


Figure 2. The effect of soil treatment on leachate cumulative concentrations of (A) ammonium and (B) nitrate. The mean was calculated for a sample of 3 replications. S = soil control; M = manure control; MRO = manure+ red oak biochar; MHAP = manure+highly alkaline porous biochar; MHAPE = manure+highly alkaline porous engineered biochar.

The relatively short (proof-of-the-concept) leaching experiment was not suitable to observe the impact of treatments on soil nutrients in the long term. An upward trend with time for the cumulative dissolved P in leachate for all treated columns was observed during the eight events of the leaching

study; soil control columns released significantly ($p < 0.05$) higher amount of total DRP. However, no impact of manure or manure-biochar mixture application to soil columns were observed during the course of this study (Figure 3).

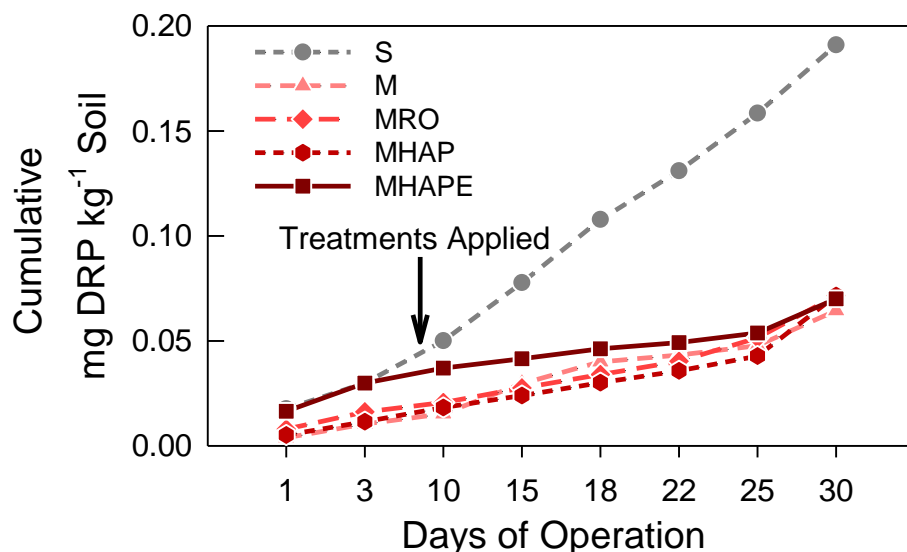


Figure 3. The effect of soil treatment on cumulative leachate concentrations of orthophosphate. The mean was calculated for a sample of 3 replications. S = soil control; M = manure control; MRO = manure+ red oak biochar; MHAP = manure+highly alkaline porous biochar; MHAPE = manure+highly alkaline porous engineered biochar.

3.4. Treatment effect on column soil properties

The columns were freely drained for the first four leachings, but the rate of water leaching slowed upon treatment application; specifically, the manure-treated columns. After eight events of leachate collection, the experiment was stopped due to longer leaching time and ponding on column surfaces. Columns with soil and biochar-manure treatments were relatively better drained in comparison to manure columns. The initial BD was 1.2 for all columns. After the leaching experiment, the soil BD ranged from 1.17 to 1.6 g/cm³, and for manure treated columns ended with higher BD in comparison to biochar+manure receiving columns. The application of manure and manure-biochar mixtures to the soil columns resulted in an increasing trend to the soil OM content, soil TC, and soil TN relative to control soil columns (Table 2).

Table 2: Column soil physicochemical properties after leaching events were completed, including the percentage of organic matter (OM), pH, percentage of carbon (TC), the make-up of organic nitrogen (TN), and the total carbon to nitrogen (C/N) ratio. (Mean \pm standard deviation for $n=3$ replicates; values in parentheses represent p -values; **Bold** signifies statistical significance).

Soil Treatment	pH	OM (%)	TC (%)	TN (%)	C/N ratio
M	7.43 \pm 0.05	3.11 \pm 0.07	2.18 \pm 0.35	0.18 \pm 0.01	11.06 \pm 1.07
S	7.53 \pm 0.05	3.08 \pm 0.14	1.90 \pm 0.11	0.18 \pm 0.009	11.84 \pm 0.12
MRO	7.60 \pm 0.08 ($p=0.045$)	3.25 \pm 0.01	2.29 \pm 0.15	0.19 \pm 0.01	10.57 \pm 0.44

MHAP	7.57 ± 0.09	3.41 ± 0.02 (p= 0.001)	2.40 ± 0.19	0.19 ± 0.008	11.87 ± 0.53
MHAPE	7.30 ± 0.08	3.37 ± 0.01 (p= 0.004)	2.41 ± 0.11	0.21 ± 0.009	12.63 ± 0.63

Note: S = soil, M = manure MRO = manure+red oak biochar; MHAP = manure+ highly alkaline porous biochar; MHAPE = manure+highly alkaline porous engineered biochar.

The TC/TN ratios were between 10.6 and 12.6. Manure-HAP and MHAPE biochar treated columns had significantly ($p < 0.05$) higher OM than manure or soil control columns. There was no significant change in TC and TN observed among manure or manure-biochar treatments. A slight change was observed on soil pH; manure-RO was significantly ($p = 0.04$) higher among all columns. The pH was mostly got buffered for the manure and manure-biochar treated columns and ranged between 7.3 and 7.6, i.e., close to soil pH. Before application to soil, the pH of all manure and manure-biochar mixtures, except HAPE, were highly alkaline (Table 1).

Before application to soil columns, manure had a %TN value of 5.4, highest among any manure-biochar samples (Table 1). After the leaching events, an increase in the TN was observed for all manure-biochar treated soil columns compared to the manure or soil control columns. Manure-HAPE had 2.7% of TN before addition to soil columns, the highest %TN among the manure-biochar mixtures used in this study significantly increased the soil TN. In addition, MHAPE biochar treatment significantly increased soil NO_3^- -N ($p = 0.009$) and NH_4^+ -N ($p = 0.001$) concentration after the leaching experiment (Figure 4).

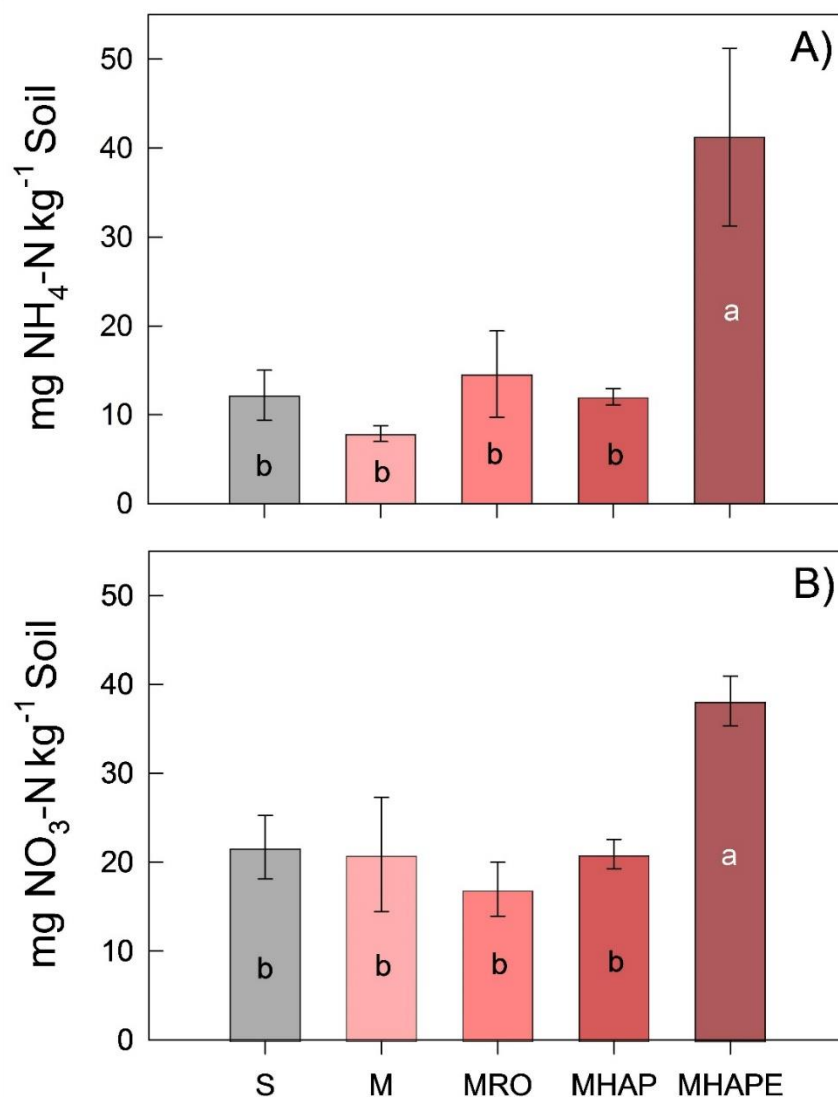


Figure 4. The effect of soil treatment on soil concentration of (A) ammonium and (B) nitrate. Each bar represents an average of the three replicates for that group. Error bars show the standard deviation of $n=3$. S = soil control; M = manure control; MRO = manure+red oak biochar; MHAP = manure+highly alkaline porous biochar; MHAPE = manure+highly alkaline porous engineered biochar. Letters mark a significant difference between treatments at $p<0.05$.

The concentration of P_2O_5 -P was higher in manure compared to any manure-biochar mixtures applied, and that was reflected in the soil Mehlich3 extractable P after column leaching. Manure addition to soil columns had the highest soil Mehlich3 extractable P (Figure 5) and significantly higher ($p<0.001$) in comparison to other treatments. Among the manure-biochar mixture treated soils, manure-RO ($p<0.05$) and manure-HAP ($p<0.05$) significantly increase Mehlich3 soil P and K than control soil. The treatment, manure-HAPE did show a small numerical increase in Mehlich3 P (not significant; $p>0.05$). However, Mehlich3 soil K concentration was highest and significantly higher ($p<0.001$) than other manure biochar treatments (Figure 6) though the manure had a higher concentration of K_2O -K compared with the manure-biochar mixtures.

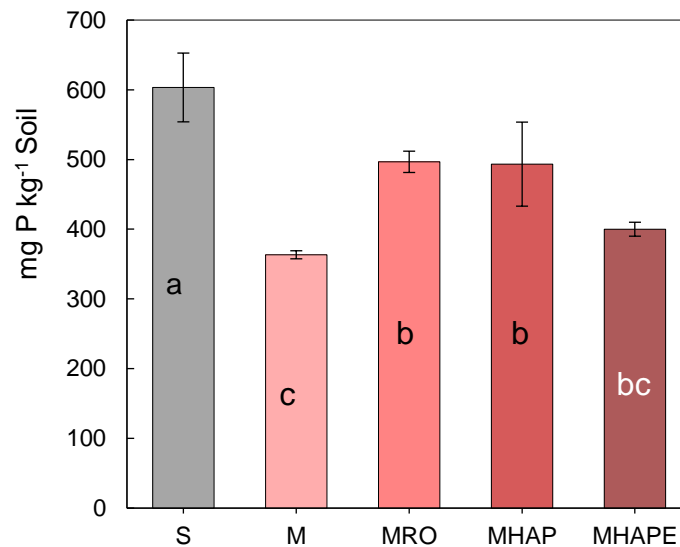


Figure 5. Comparison between treatments of the Mehlich3 extractable phosphorus. Each bar represents an average of the three replicates for that group. Error bars show the standard deviation of $n=3$. S = soil control; M = manure control; MRO = manure+red oak biochar; MHAP = manure+highly alkaline porous biochar; MHAPE = manure+highly alkaline porous engineered biochar. Letters mark a significant difference between treatments at $p<0.05$.

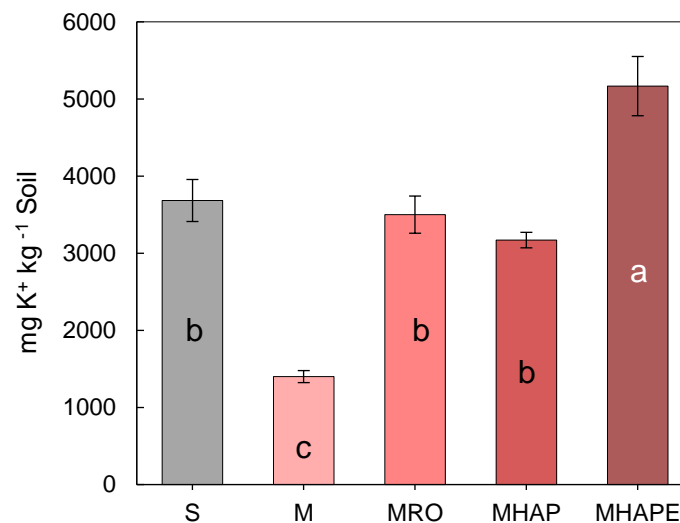


Figure 6. Comparison between treatments of the Mehlich3 extractable potassium. Each bar represents an average of the three replicates for that group. Error bars show the standard deviation of $n=3$. S = soil control; M = manure control; MRO = manure and red oak biochar; MHAP = manure and highly alkaline porous biochar; MHAPE = manure and highly alkaline porous engineered biochar. Letters mark a significant difference between treatments at $p<0.05$.

Biochar-manure mixture addition to soil columns did not impact the soil Mehlich3 extractable Ca and Fe concentrations (Table 3). However, MHAPE treatment had a significantly lower concentration of Mehlich3 extractable Mg ($p = 0.03$) and Cu ($p = 0.005$). All manure-biochar treatments had a significantly ($p<0.05$) low Mehlich3 extractable Zn compared to the control manure samples, and

only MRO treatment had significantly low Mn ($p = 0.02$) compared to the control manure but not to the control soil column.

Table 3: Column soil physicochemical properties after leaching events were completed, including the elements, Ca, Mg, Fe, Cu, Mn, and Zn in mg/kg. (Mean \pm standard deviation for $n=3$ replicates; values in parentheses represent p -values; **Bold** signifies statistical significance).

Treatment	Ca (g/kg)	Mg (g/kg)	Fe (g/kg)	Cu (g/kg)	Mn (g/kg)	Zn (g/kg)
M	19.75 \pm 0.14	5.77 \pm 0.10	4.77 \pm 0.09	0.03 \pm 0.002	0.60 \pm 0.05	0.06 \pm 0.004
S	20.26 \pm 1.19	5.84 \pm 0.45	4.01 \pm 1.2	0.03 \pm 0.003	0.97 \pm 0.12 ($p=0.007$)	0.04 \pm 0.007 ($p=0.003$)
MRO	19.68 \pm 0.46	5.58 \pm 0.19	4.07 \pm 0.72	0.02 \pm 0.007	0.99 \pm 0.18 ($p=0.02$)	0.05 \pm 0.006 ($p=0.047$)
MHAP	18.82 \pm 0.27	5.38 \pm 0.10	4.22 \pm 0.17	0.03 \pm 0.002	0.8 \pm 0.03	0.05 \pm 0.006 ($p=0.01$)
MHAP-E	18.92 \pm 1.07	5.13 \pm 0.22 ($p=0.03$)	4.78 \pm 0.02	0.01 \pm 0.0013 ($p=0.0005$)	0.60 \pm 0.07	0.04 \pm 0.003 ($p=0.003$)

Note: S = soil, M = manure MRO = manure+red oak biochar; MHAP = manure+ highly alkaline porous biochar; MHAPE = manure+highly alkaline porous engineered biochar.

4. Discussion

Substrate quality is an important deciding factor of its decomposition rate; a low TC/TN ratio means high quality and a readily available substrate to decomposing microbial group (Condron et al., 2010). The high decomposition of soil OM can result in low C-sequestration and high loss of C to the atmosphere. Manure with TC/TN ratio of ~ 7 after incubation and even lower before incubation (~ 2) supports the notion of N mineralization loss to the atmosphere, more likely as NH_3 than C loss as CH_4 or CO_2 . Biochar (with a very high C/N ratio) application to manure and incubation may have resulted in an N immobilization and improved the biochar TC/TN ratio. The application of manure-biochar MRO of TC/TN ratio 51.8 ended up to 10.6 to the soil, and similarly, other manure-biochar mixtures resulted in a TC/TN ratio near the soil TC/TN ratio (~ 12) upon application to the soil as an amendment. Also, an increase in total C and N speculates that the mixture has the capacity to improve C sequestration compared to manure only treatment and a better microbial habitat than biochar with a high TC/TN ratio. Hardwood (RO) biochar is capable of altering soil physicochemical properties in a way that improves nutrient leaching loss from manure. Biochar application significantly increases the soil specific surface area, is also capable of holding the plant available moisture considerably, and improved other plant nutrients availability effectively than soil (Laird et al., 2010b). Our data also supports the finding of Laird et al., 2010a resulting in a low BD and relatively high OM content in soil with biochar treatments to the manure-biochar soil system.

An increase in N-mineralization with biochar application is consistent with previous studies that interpret biochar addition as linked with increases in soil microbial respiration (Laird et al., 2010a; Rogovska et al., 2011), which results in soil N mineralization. Application of Fe-modified manure-biochar (MHAPE) treatment to soil resulted in a higher rate of N-mineralization than any other manure-biochar mixture or manure controls. In comparison to other treatments, the MHAPE column released more nitrate and ammonium to the leachate. In plant-soil systems, these inorganic N forms are favorably taken up by plants directly (Tisdale et al., 1985). However, high mobility of nitrate, the N loss in this anion form from the soil in leachate, is well known (Syswerda et al., 2012) and ends up in groundwater contamination. The leaching experiment recorded a 0.4% of nitrate-N of the total KCl-extractable soil nitrate that ended in the MHAPE water leachate. In comparison, about 3.5% of the KCl-extractable soil nitrate ended up in manure columns water leachate. This observation suggests that Fe modification to biochar increased the N-mineralization, and simultaneously, it

positively impacted on nitrate sorption onto biochar surface with less leaching loss compared to manure control.

All the manure and manure-biochar treated columns received 135 kg/ha (120 lb/acre) P at the beginning, and only the soil-treated column released significantly high P in leachate during the leaching experiment. At the end of the leaching experiment, the manure treated columns had a significantly ($p < 0.05$) higher amount of Mehlich3 P than manure-biochar treated columns. This observation suggests that manure-biochar mixtures had stored the P in the soil in other forms contributing to soil total P and not to the plant-available form to be extracted by Mehlich3. The excess P in manure-biochar could be associated with the mineral phases of biochar or associated with OM, which was not evaluated or reported in this study. The lowest P content of Mehlich3 P in Fe-modified biochar among all manure-biochar treatments suggests a P sorption on oxy-hydroxide phases of Fe-biochar (Bakshi et al., 2019) were not extracted with Mehlich3.

In addition to other major and minor plant nutrients, swine manure is a good source of K (Chastain et al., 1999). A significantly high K ($p < 0.05$) concentration was found in our manure and manure-biochar treated columns compared with control soil columns. Soil K in other manure-biochar columns, MHAP and MRO, did not statistically differ from manure-treated soil column. However, MHAPE had a significantly ($p < 0.05$) high amount of soil K in comparison to all treatments. This result suggests that Fe-modified biochar is a good source of soil K, and incubation of it with manure had further increased the K content.

Biochar applications in crop agriculture are often limited by the heavy metals content associated with some types of feedstock and sources (Pulka et al., 2020). Biochar is capable of sorbing heavy metals and making them less available to soil exchange sites for the plant to uptake (Zhang et al., 2013). Copper toxicity is not an issue in the Midwest USA, but a high concentration of Cu could be detrimental for soil microbial communities and plants. The MHAPE treatment showed a significant reduction in soil Mehlich3 Cu. This observation is consistent with other previous studies reported that the presence of biochar could immobilize soil Cu (Bakshi et al., 2014) and make it less available in the soil for plants to uptake.

This relatively short control leaching experiment may not be representative of soil at the field scale, and a more extended soil experiment is essential to carry out to verify the long-term impact of the manure-biochar mixture on soil nutrient availability to plants. Also, the slow leachate flow rate of the manure treated control columns made the comparisons challenging for this short-term leaching experiment. Properties of the manure-biochar mixture can vary depending on the manure type, biochar type, biochar production, and manure-biochar incubation time, which were not evaluated in this experiment. Microbial biomass was not determined in the study could be one of the limitations of this work; moreover, swine manure from only one representative source was used.

5. Conclusions

The results of this short (8 weeks) soil leaching experiment suggest that biochar-manure mixture application to agricultural soils improved soil OM, TC, TN, and BD compared to the conventional liquid swine manure treatments to the soil. At the end of the leaching experiment, an increase in the major plant-available nutrients N, P, and K concentrations in soil (depending on the biochar type) was observed for the manure-biochar treated column soil. MHAPE treatment to soil significantly increased plant-available N and K, and soil OM content compared with the control soil or manure treated soil. Although the total $\text{NO}_3\text{-N}$ and $\text{NH}_4\text{-N}$ concentrations in leachates of MHAPE columns

were significantly higher among all treatments, these values were significantly lower than soil NO₃-N and NH₄-N concentrations found at the end of the experiment. A long-term field experiment is warranted to report a long-term environmental implication of the biochar-manure mixture on plant-soil biota.

Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contributions

Conceptualization, C.B., J.K.; methodology, C.B., M.D., M.L. and A.S.; software, C.B., D.B.; validation, C.B., J.K.; formal analysis, C.B., D.B. and M.D.; investigation, C.B. and D.B.; resources, C.B., J.K., M.D. and B.C.; data curation, C.B., and J.K.; writing—original draft preparation, C.B. and D.B.; writing—review and editing, C.B., D.B., J.K., A.S. and M.L.; visualization, C.B., D.B., and M.D.; supervision, C.B. and J.K.; project administration, C.B. and J.K.; funding acquisition, J.K., C.B., A.S. and M.L. All authors have read and agreed to the published version of the manuscript.

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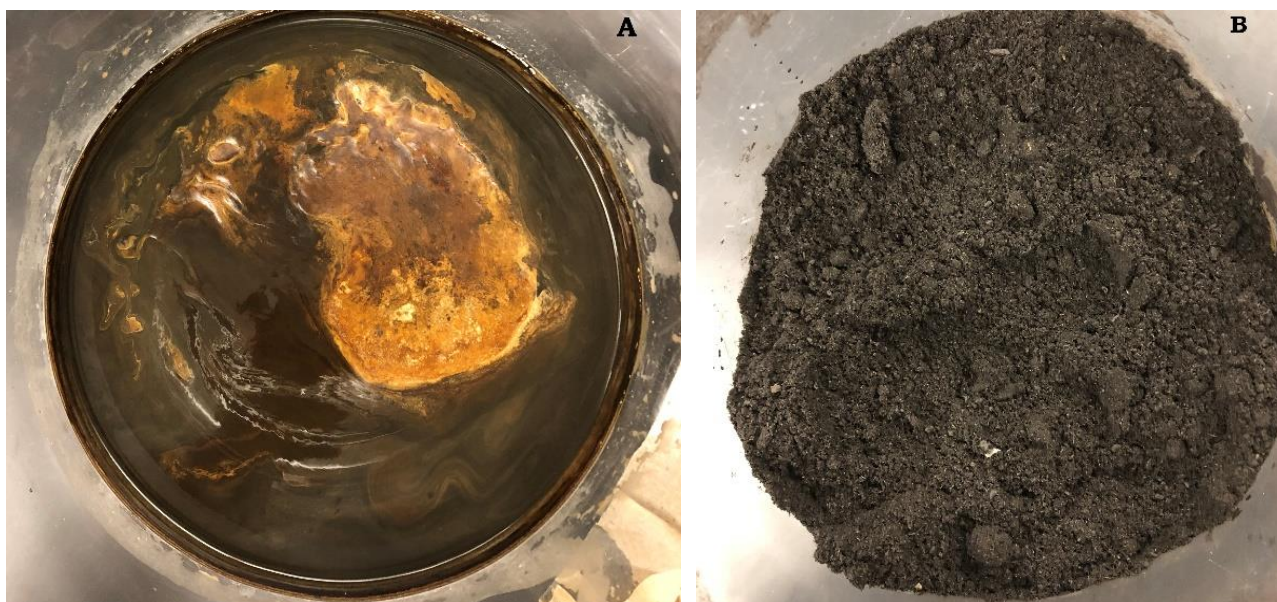
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Supplementary Material



Supplementary Figure 1. A photo of the 15 soil columns on the wooden rack for the leachate collection. Each column contains 250 g of soil, treated with one of five treatments. Two of these apparatuses were utilized, resulting in 15 columns; three trials for each of the five treatments). M =manure control; S = soil control; MRO = manure and red oak biochar; MHAP = manure and highly alkaline porous biochar; MHAPE = manure and highly alkaline porous engineered biochar.



Supplementary Figure 2. This photo of the liquid swine manure (A) and swine manure+ biochar mixture (B) showing the physical appearance of the two after one month of incubation under the lab environment. The biochar (250 g) and manure (1000 g) was mixed 1:4 (w/w) ratio. During incubation, biochar absorbed the manure moisture, and when mixed, its appearance was like loose soil. The manure was liquid slurry with yellow patches on the surface, possibly represents microbial colony developed during incubation.