Influence of Biochar on Nitrogen Fractions in a Coastal Plain Soil

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Interest in the use of biochar from pyrolysis of biomass to sequester C and improve soil productivity has increased; however, variability in physical and chemical characteristics raises concerns about effects on soil processes. Of particular concern is the effect of biochar on soil N dynamics. The effect of biochar on N dynamics was evaluated in a Norfolk loamy sand with and without NH_4NO_3. High-temperature (HT) (≥500°C) and low-temperature (LT) (≤400°C) biochars from peanut hull (*Arachis hypogaea* L.), pecan shell (*Carya illinoinensis* Wangenh. K. Koch), poultry litter (*Gallus gallus domesticus*), and switchgrass (*Panicum virgatum* L.) and a fast pyrolysis hardwood biochar (450–600°C) were evaluated. Changes in inorganic, mineralizable, resistant, and recalcitrant N fractions were determined after a 127-d incubation that included four leaching events. After 127 d, little evidence of increased inorganic N retention was found for any biochar treatments. The mineralizable N fraction did not increase, indicating that biochar addition did not stimulate microbial biomass. Decreases in the resistant N fraction were associated with the high pH and high ash biochars. Unidentified losses of N were observed with HT pecan shell, HT peanut hull, and HT and LT poultry litter biochars that had high pH and ash contents. Volatilization of N as NH_3 in the presence of these biochars was confirmed in a separate short-term laboratory experiment. The observed responses to different biochars illustrate the need to characterize biochar quality and match it to soil type and land use.

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In recent years, there has been considerable interest in the use of biochar from pyrolysis of renewable biomass to sequester C and improve soil productivity (Atkinson et al., 2010; Laird, 2008). Much of the stimulus for this interest has come from research on the soils of the Amazon basin, known as Terra Preta de Indio, that contain variable quantities of organic black carbon considered to be of anthrogenic origin (Atkinson et al., 2010). Although biochar and charcoal have been shown to increase soil fertility and productivity in the tropics (Chan et al., 2007; Lehmann et al., 2003; Oguntunde et al., 2004; Steiner et al., 2007; Yamato et al., 2006), the response for temperate zone soils is just beginning to be evaluated (Atkinson et al., 2010) and may not show similar responses (Gaskin et al., 2010). Biochar can improve nutrient availability (Steiner et al., 2007), cation exchange capacity (Liang et al., 2006), bulk density, and water-holding capacity (Tryon, 1948), but these effects depend on the feedstock (Gaskin et al., 2008; Novak et al., 2009b), pyrolysis conditions (Antal and Gronli, 2003; Brewer et al., 2009; Keiluweit et al., 2010; Novak et al., 2009b), and soil type (Chan et al., 2007; Van Zwieten et al., 2010). Because physical and chemical characteristics of different biochars vary widely, it is important to evaluate different biochars under specific soil and climatic regimes to increase our understanding of potential interactions before widespread use of biochars in agricultural systems.

Much of the C in biochar from pyrolysis is considered to be in a stable, aromatic form (Keiluweit et al., 2010) that is not readily available to microbes (Kuzyakov et al., 2009). However, some biochars can provide a source of metabolizable C in the short term (Deenik et al., 2010; Smith et al., 2010). This is particularly true for those created under low pyrolysis temperatures where aromaticity is less and aliphatic C content is higher than biochars produced at high temperatures (Brewer

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Abbreviations: HT, high temperature; LT, low temperature; SOC, soil organic carbon; VM, volatile matter.

et al., 2009; Bruun et al., 2008) and for those created under conditions that favor recondensation of gases produced during pyrolysis (e.g., Antal and Gronli [2003]; Deenik et al. [2010]). The metabolizable source of C may stimulate microbial activity and lead to utilization of soil mineral N (immobilization) or release of additional N (mineralization).

Variation among biochars can result in different effects on N mineralization-immobilization processes. In tropical Ferralsols, Lehmann et al. (2003) report N immobilization from the additional C supplied by charcoal. Gaskin et al. (2010) found decreased yields in corn (*Zea mays* L.) after application of 11 and 22 Mg ha^{-1} pine (*Pinus echinata* Mill.) chip biochar (made at 400°C) to a low soil organic carbon (SOC) (0.4%) Ultisol. The effect was thought to be related to N immobilization by the fresh biochar and did not persist through the second corn-growing season. Corn yields did not decline after addition of biochar made from peanut hull with an N content of 2% even though there was no evidence that the biochar N was available to corn (Gaskin et al., 2010).

Although plant-derived biochars may not be a source of mineral N, animal manure biochars can have substantial quantities of N remaining after pyrolysis. Greenhouse studies in a low-pH (4.5) and moderate SOC (1.97%) Alfisol indicate that poultry litter biochar produced at 450°C (C/N ratio, 19) and 550°C (C/N ratio, 39) with steam activation supplied N to radishes when applied at 10, 25, and 50 Mg ha^{-1} (Chan et al., 2008). Addition of N with the biochar further increased yields over the control, with a greater increase seen in the high-temperature (HT) (550°C) biochar treatment. Tagoe et al. (2008) also reported increased soybean [*Glycine max* (L.) Merr] yields with ^{15}N-labeled carbonized chicken manure (C/N ratio, 4.74) produced at 500°C and applied at 50 and 100 Mg ha^{-1} in a low pH (5.68), high SOC (10.5%) soil. Labeled N recovery by soybean was 17.6% at the 50 Mg ha^{-1} rate and only 8.9% at the 100 Mg ha^{-1} rate. The C/N ratio of this carbonized poultry manure was extremely low and may not reflect industrialized processes because it was created in a muffle furnace and may not have completely carbonized. These studies indicate some of the N in biochars derived from animal manures could be plant available when applied to the soil depending on the conditions of the pyrolysis process.

Biochar may affect nitrification. Several reports indicate that nitrification is increased in the presence of charcoal from wildfires (Berglund et al., 2004; DeLuca et al., 2002; DeLuca et al., 2006). This effect may be due to sorption of phenolic compounds in forest soils that inhibit nitrifiers (DeLuca et al., 2006), an increased population of ammonium oxidizing bacteria (Ball et al., 2010), or increased soil pH (Ball et al., 2010). Although wildfire charcoal increased nitrification, fresh biochar applied to pasture soils was shown to decrease nitrification by Clough et al. (2010), who attributed this effect to inhibition of nitrifiers by the presence of α-pinene, a condensate product on the fresh biochar.

In addition to the above effects, several studies have reported reduced leaching of N in soils with biochar amendments (Ding et al., 2010; Laird et al., 2010; Major et al., 2010). These reports include increased N retention in a variety of Oxisols from the tropics (Major et al., 2009), low-SOC (23.0 g kg^{-1}) sandy silts from China (Ding et al., 2010), and high-SOC Hapudolls (Laird et al., 2010) and a variety of biochars from high-temperature (660°C) bamboo (*Bambusa* spp.) to hardwood (estimated 500°C).

The addition of fresh C sources to soils influences the lability or recalcitrance of existing soil mineral and organic N fractions (Juma et al., 1984). A desire to determine the size of the labile organic N pool for estimating the amount of N needed as fertilizer has led to the development and testing of a number of N availability indices (Keeney, 1982; Griffin, 2008; Sharifi et al., 2007; Schomberg et al., 2009; Kwon et al., 2009; Gilmour and Mauromoustakos, 2011). Nitrogen indices were created in an attempt to characterize the potential availability of organic N, which may be present as proteinaceous materials (proteins, peptides, and amino acids), amino sugars, and less available heterocyclic N compounds, including purines and pyrimidines (Stevenson, 1996; Schulten and Schnitzer, 1998; Olk, 2007). Two promising N availability indices—extraction with hot (100°C) KCl (Gianello and Bremner, 1986) and direct distillation with NaOH (Stanford, 1978)—extract overlapping but different pools of soil organic N (Sharifi et al., 2007). The hot KCl method is believed to be representative of the extractable mineral N pool and easily hydrolyzable microbial cells (Sharifi et al., 2007). Sodium hydroxide distillation is a harsher extractant and recovers a greater amount of hydrolyzable organic N, a portion of which is probably adsorbed to mineral surfaces. The NH_4–N extracted by the NaOH steam distillation method primarily consists of exchangeable NH_4–N, amino sugars, amides, and certain amino acids (Stanford, 1978; Greenfield, 2001; Sharifi et al., 2007; Kwon et al., 2009) but does not include organic N derived from bound or fixed NH_4–N or chitin. The NaOH distillable fraction has been found to be correlated with mineralizable N (Sharifi et al., 2007), Illinois Soil Test N (Roberts et al., 2009), and total organic N (Sharifi et al., 2009). Using these two indices along with extractable mineral N and total N provides a mechanism for evaluating how additions of various biochars affect short- and longer-term labile N fractions.

Because we need a better understanding of how biochar produced from different feedstocks and pyrolysis conditions influences N dynamics, we measured soil N fractions after incubation of soil with and without N fertilizer addition in the presence of various biochars. We hypothesized that (i) low-temperature biochars have a higher negative surface charge, which creates a greater capacity to retain NH_4–N, and (ii) low-temperature biochars with labile C provide a substrate for microbial biomass and increase N retention.

Materials and Methods

Biochar influences on soil N fractions were evaluated in a Norfolk loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) with and without added N as NH_4NO_3. The soil was collected from a field near Florence, South Carolina in the middle Coastal Plain physiographic region of South Carolina. The soil has a low SOC content (3.2 g kg^{-1}) and an acidic pH (4.8). The field has a long history (+20 yr) of row-crop production, including corn, cotton (*Gossypium hirsutum* L.), soybean, and wheat (*Triticum aestivum* L.). Additional information on soil chemical and physical properties, along with agricultural management history of the collection site, can be found in Novak et al. (2009b).

The biochars used in this study were created from five different feedstocks: peanut hulls (*Archis hypogaea*), pecan shells (*Carya illinoensis*), poultry litter (*Gallus gallus domesticus*), switchgrass (*Panicum virgatum* L.), and hardwood waste products. The first four feedstocks were air-dried, ground, and sieved to pass a 1- to 2-mm sieve and then exposed to slow (1–3 h) pyrolysis under a continual stream of N_2 gas. Low-temperature (LT) (≤400°C) and HT biochars (Table 1) were created to provide variations in structural and surface characteristics (Novak et al., 2009b). These biochars were produced at the University of Georgia, Athens, Georgia (peanut hull); USDA–ARS Southern Regional Research Center, New Orleans, LA (poultry litter); and North Carolina Agricultural and Technical State University, Greensboro, NC (pecan shell and switchgrass). The hardwood waste product biochar (CQuest; Dynamotive Energy Systems, Vancouver, BC) was made by a fast pyrolysis process consisting of a 1- to 2-s exposure to 450 to 600°C. All biochars were ground to pass a 0.25-mm sieve for use in the incubation experiment.

For each biochar, a single estimate of the percentage fixed C, volatile matter (VM), ash content, and elemental (C, H, O, N, and S) analysis was determined on an oven dry–weight basis by Hazen Research, Inc. (Golden, CO) following the ASTM D 3172 and 3176 standard methods (ASTM, 2006) (Table 1). The percentages of fixed C and VM are estimated by mass balance from a sequential muffle procedure where fixed C is calculated by difference (total mass – [VM + ash + moisture]). Further details are reported in Novak et al. (2009b).

For the laboratory incubation, 5 g of biochar (≈40–44 Mg ha^{-1}) was mixed with 225 g of slightly moistened soil. An additional 225 g of soil was mixed with 0 or 31 mg N as NH_4NO_3 (equivalent to 0 and 140 kg N ha^{-1}). The biochar and N treatment portions of soil were wetted with a small amount of deionized water and thoroughly mixed together. The mixed soil (450 g total) was added to the incubation container (10.3 cm inner diameter by 8.5 cm tall), tapped down to a bulk density of 1.2 g cm^3, and wetted with deionized water to 10% (w/w), which represents the upper range (5–10%) of field capacity for a typical Norfolk Ap horizon. This allowed a headspace of 2 to 3 cm above the soil for adding water. Drainage holes in the bottom of the containers were covered with nylon mesh fabric to retain soil while allowing drainage during subsequent leaching events. Control treatments with and without N were prepared similarly to the other treatments but with no added biochar. Four replications of each treatment were arranged in randomized blocks and incubated for 127 d at room temperature (18–26°C) and 35 to 83% relative humidity. Water content was readjusted to 10% (w/w) twice weekly. Soils were leached at 28, 63, 90, and 118 d using 1.2 to 1.3 pore volumes of deionized water. Leachate was collected until free drainage had ceased (usually 2 d) and analyzed for mineral N content. Nine days after the last leaching event, 120 g of soil was removed from the pots and air-dried for 3 d before shipping to the USDA Agricultural Research Service laboratory in Watkinsville, Georgia for N fractions analysis.

Procedures described in Schomberg et al. (2009) were used to measure hot- and cold-KCl–extractable NH_4–N and NO_3–N, NaOH-distillable NH_4–N, and total C and N. All analyses were performed on air-dried samples with weights adjusted to a dry-weight basis. Inorganic N was estimated as the sum of NH_4–N and NO_3–N from soil samples (3 g) extracted with 2 mol L^{-1} KCl. Mineralizable N was calculated by subtracting cold KCl NH_4–N from hot KCl NH_4–N, which was determined by weighing 3 g of soil into a 50-mL centrifuge tube, adding 20 mL of 2 mol L^{-1} KCl, and incubating the samples at 100°C for 4 h in a water bath (Gianello and Bremner, 1986). Samples were allowed to cool to room temperature and filtered. Extracts were frozen until analyzed for NH_4–N. The NO_3–N and NH_4–N in the above extracts was determined with an automated analyzer (Keeney and Nelson, 1982). Resistant N was calculated by subtracting the hot KCl NH_4–N from NaOH-distillable NH_4–N, which was determined on a 5-g sample that was added to a distillation flask with 40 mL of 12.5 mol L^{-1} NaOH (Sharifi et al., 2007). Forty milliliters of distillate were collected in a flask containing 3 mL of 4% boric acid solution. The NH_4–N content of the distillate was measured by titrating the distillate with a standard solution of 0.005 mol L^{-1} HCl in the presence of a mixed indicator (bromocresol green and methyl red). Recalcitrant N was determined by subtracting NaOH NH_4–N and cold NO_3–N from total N. Total N and C were determined using a TruSpec CN analyzer (LECO Corp., St. Joseph, MI).

We evaluated the biochar effects on ammonia volatilization when co-applied with NH_4NO_3 in a short-term (7 d) incubation at 25°C using the biochar treatments described above. Two

Table 1. Chemical and physical characteristics of biochar used in the incubation study.†

Biochar	Temp.‡	Surface area	pH	Ash	Volatile	Fixed C	C	H	O	N	S	Carbon fractions aliphatic	carbonyl	aromatic	carboxylic
	°C	$m^2 g^{-1}$		% dry wt.								% total C			
Peanut hull	400	0.52	7.9	8.2	38.8	53.5	74.8	4.5	9.7	2.7	0.09	35	3	57	5
	500	1.22	8.6	9.3	18.1	72.6	81.8	2.9	3.3	2.7	0.10	12	3	82	3
Pecan shell	350	1.01	5.9	2.4	61.6	36.0	64.5	5.3	27.6	0.26	0.01	49	5	42	4
	500	222	7.2	5.2	9.7	85.1	91.2	1.5	1.6	0.51	0.01	29	0	58	14
Poultry litter	350	1.10	8.7	35.9	36.7	27.8	46.1	3.7	8.6	4.9	0.78	36	3	57	4
	700	9.00	10.3	52.4	14.1	33.5	44.0	0.3	<0.01	2.8	1.0	na§	na	na	na
Switchgrass	250	0.4	5.4	2.6	74.4	20.7	55.3	6.0	35.6	0.43	0.05	63	3	29	5
	500	62.2	8.0	7.8	13.4	78.8	84.4	2.4	4.3	1.07	0.06	12	3	82	3
CQuest	500	1.28	5.7	8.6	33.7	57.7	71	3.4	16.3	0.3	0.02	14	20	52	14

† Data modified from Novak et al. (2009b).

‡ Temperatures indicate highest temperature. The CQuest material was made by fast pyrolysis (400–600°C); the others were made by a slow pyrolysis process.

§ Analysis not available due to the high ash content of the biochar material.

50-mL beakers containing 25 g of soil plus biochar (control had no biochar) were placed in a 1-L jar. Two beakers of soil were used to increase the surface area for volatilization. A third 50-mL beaker containing 30 mL of 0.05 mol L^{-1} H_2SO_4 to trap NH_3 was added to the jar. An aqueous solution of N (5 mL deionized H_2O with 0.098 g NH_4NO_3) was applied to the soil surface to wet the soil to 55% water-filled pore space. The N rate was increased from 31 mg kg^{-1} used in the long-term incubation study to 666 mg kg^{-1} N to allow greater sensitivity in measuring volatile N losses. Incubations were conducted in two blocks with two replications per block for a total of four replications. Two blanks (an empty container with the H_2SO_4 trap) were included in each block to correct for ambient NH_3. At Day 7, absorbed NH_3 was determined by titrating a 10-mL aliquot of the H_2SO_4 with standardized 0.1 mol L^{-1} NaOH to an endpoint of pH 7.

All statistical analyses were conducted using the SAS Institute Inc. Enterprise Guide v. 4.0. The Mixed procedure was used for evaluating biochar source, pyrolysis temperature, and N treatment effects on N fractions. All three effects were considered as class variables in the analysis of variance. Differences among means were calculated using the PDIFF option in the LSMEANS statement, and Tukey's adjustment for multiple means comparisons was used to determine significance at $P \leq 0.05$. A similar process was used to evaluate biochar source and pyrolysis temperature effects on NH_3 volatilization in the short-term incubation experiment. Volatilization data were log transformed before analysis to normalize the data and retransformed for presentation. Correlation analysis between treatment and response effects was also conducted within Enterprise Guide.

Results and Discussion

Biochar Characterization

Characteristics of the biochars as reported by Novak et al. (2009b) are presented in Table 1. Two key biochar characteristics were expected to influence N dynamics and fractions (pH and the C form). The pH and ash content was consistently less when the biochars were produced at lower temperatures (≤400°C). The high-ash biochars with alkaline minerals were expected to increase soil pH, particularly in our sandy, poorly buffered soil.

The second characteristic of interest was the amount and form of C in the biochar, particularly the percentage of carbon that might be in a more labile form. Two potential indicators of this are VM% and the O/C ratio. The VM% is thought to include aliphatic C as well as other C forms that decompose below 900°C. The O/C ratio is based on a classification continuum for C combustion residues proposed by Hedges et al. (2000). Highly stable C residues, such as graphite, have a very low O/C ratio (below 0.2). The dividing line between biochar or charcoal and biomass is an O/C ratio of 0.6. Spokas (2010) has proposed that the O/C ratio can be used to predict biochar half-life in soils.

For the biochars in this study, those produced at 500°C or above (HT biochars) contained a lower aliphatic fraction compared with the LT biochar of the same feedstock (Table 1). Similarly, the VM% is lower in biochars produced above 500°C. The O/C ratios are smaller for the HT biochars (ranging from 0.002 to 0.03) compared with the LT biochars (ranging from 0.10 to 0.48), indicating a more stable C at higher pyrolysis temperatures. Based on the VM and O/C indicators, the LT switchgrass and the LT pecan shell biochars should have the greatest amount of labile C. The addition of labile C would be expected to stimulate microbial activity and increase the size of the microbial biomass. This could result in immobilization of N initially but in the longer-term increase N mineralization as the microorganisms begin to die off.

Soil pH Changes in the Long-Term Incubation

Soil pH was influenced by biochar type, temperature, and addition of N, as indicated by the significant three-way interaction in Table 2. Soil pH was lower in the control soils (with and without N addition) than in soils with biochar (Table 3). The addition of NH_4NO_3 fertilizer decreased soil pH compared with the unamended soil; however, the effect varied with biochar feedstock and pyrolysis temperature. The smallest changes in soil pH were observed with switchgrass, LT pecan shell, and CQuest biochars. These biochars have relatively low ash contents (Table 1).

The HT pecan shell, LT and HT peanut hull, and LT and HT poultry litter biochars increased soil pH above 7.0. Although the LT peanut hull biochar has similar ash content to the CQuest biochar, there was a stronger pH response with peanut hull biochar addition. The different responses are likely due to the content of various minerals in the ash, particularly CaO. Gaskin et al. (2010) reported high Ca concentrations in

Table 2. Analysis of variance results for evaluating the effect of adding various biochars with and without nitrogen fertilizer on measured parameters in a 127-d incubation with four leaching events.

Effect	Soil			Leached	N fraction			
	pH	C	N	N	Inorganic	Mineralizable	Resistant	Recalcitrant
	Pr > F							
Biochar†	<0.0001	<0.0001	<0.0001	<0.0001	0.0948	0.9630	0.2335	<0.0001
Temp.‡	<0.0001	<0.0001	0.0120	<0.0001	0.7132	0.2975	<0.0001	0.0236
N§	<0.0001	0.5783	0.1828	<0.0001	0.3908	0.2055	0.9400	0.1867
Biochar x temp.	<0.0001	<0.0001	<0.0001	<0.0001	0.0856	0.5445	0.0385	<0 .0001
Biochar x N	<0.0001	0.5841	0.0337	<0.0001	0.1301	0.7313	0.4600	0.0275
Temp. x N	0.0025	0.0663	0.5448	<0.0001	0.7217	0.1426	0.7483	0.5921
Biochar x temp. x N	0.0008	0.9286	0.2024	0.0012	0.1110	0.0324	0.1280	0.1819

† Biochar refers to feedstock.

‡ Temp. refers to pyrolysis temperature.

§ N refers to treatments with and without NH_4NO_3 fertilizer.

peanut hull biochar. Novak et al. (2009a) observed in a previous experiment that the addition of pecan shell biochar containing significant quantities of Ca, K, Mn, and P to Norfolk soil increased soil pH and decreased exchangeable acidity, S, and Zn after 67 d and two leaching events. In our study, HT pecan shell biochar had a greater effect on soil pH than LT pecan shell biochar.

The greatest increase in soil pH was with the poultry litter biochars, which contain large amounts of ash (Table 1). The alkalinity of the poultry litter biochar was great enough to buffer the system against pH changes after N fertilizer addition. This was unlike some of the other feedstocks where addition of N resulted in lower soil pH values. Novak et al. (2009b) found in their long-term incubation experiment that soil chemical characteristics were influenced by the type of biochar added, with the greatest changes associated with the addition of poultry litter biochars.

Soil Carbon Effects in the Long-Term Incubation

As expected, the addition of the biochars significantly influenced C concentration of the soil (Table 2). This effect was dependent on pyrolysis temperature, as indicated by the significant biochar-by-pyrolysis temperature interaction (Table 2). Increases in soil C were greater for most biochars when made at higher pyrolysis temperatures, except for the HT and LT poultry litter biochars, where the soil C contents were similar and somewhat less than most of the other biochars (Table 3). The smaller increase in soil C with the poultry litter biochars was a result of their large ash content, and our additions were made based on the weight of the biochars rather than on equal additions of C. On average, soil C concentrations increased 5.5 times with the addition of the biochars compared with the unamended soil. The smallest increase was 3.7 times for the poultry litter biochar; the greatest increase was 7.1 times for the peanut hull biochar.

Soil Nitrogen Effects in the Long-Term Incubation

Soil Total Nitrogen

Soil total N concentrations were influenced by addition of the biochars, with the effect being influenced by pyrolysis temperature or N treatment (Table 2). The biochar feedstock-by-pyrolysis temperature interaction was largely due to a difference in response observed between poultry litter biochars and the other biochars. For most of the biochars, soil total N was not different between the HT and LT biochars (Table 3). However, for the poultry litter biochars, the LT biochar increased soil total N more than the HT biochar. Although the analysis of variance

Table 3. Effect of adding various biochars made at two pyrolysis temperatures with and without addition of nitrogen fertilizer (NH_4NO_3) on soil pH, total carbon, total nitrogen, and total leached nitrogen in a 127-d incubation with four leaching events.

Biochar	Pyrolysis temp.	N	Soil pH	Soil C		Soil N		Leached N
	°C	mg		g kg⁻¹				mg pot⁻¹
Control		0	5.6 l†	3.1	e	0.28	cd	8.1 ghi
		31	5.2 m	2.8		0.22		36.2 a
Peanut hull	400	0	7.6 d	18.1	b	0.69	b	8.7 gh
		31	7.1 f	18.8		0.78		30.6 bcd
	500	0	7.8 c	19.5	ab	0.68	b	8.5 gh
		31	7.4 e	19.5		0.71		27.7 cde
Pecan shell	350	0	6.3 h	15.5	c	0.42	c	5.7 ghi
		31	6.0 j	15.4		0.31		33.4 ab
	500	0	7.8 c	21.5	a	0.42	c	9.8 g
		31	7.7 c	20.3		0.39		26.2 de
Poultry litter	350	0	8.4 b	10.7	d	1.02	a	16.0 f
		31	8.3 b	11.1		1.01		31.9 abc
	700	0	9.0 a	11.6	d	0.77	b	3.4 ij
		31	9.0 a	10.3		0.62		15.0 f
Switchgrass	250	0	6.2 i	12.5	d	0.32	cd	0.8 j
		31	5.8 k	12.9		0.33		32.0 abc
	500	0	7.0 f	19.6	ab	0.43	c	5.3 ghij
		31	6.6 g	19.3		0.45		25.2 e
CQuest	500‡	0	6.6 g	17.2	bc	0.38	cd	4.1 hij
		31	6.2 i	17.2		0.37		28.5 bcde
Effect			**Tukey's 95% Confidence Interval**					
Biochar			0.05	1.2		0.07		1.8
Temp.			0.03	0.6		0.04		1.0
N			0.03	0.6		0.04		1.0
Biochar x temp.			0.08	2.0		0.13		3.1
Biochar x N			0.08	2.0		0.13		3.1
Temp. x N			0.05	1.2		0.07		1.8
Biochar x temp. x N			0.13	3.2		0.20		5.0

† Letters within columns indicate differences between means based on Tukey's multiple means comparison procedure. Letters for pH and leached N indicate differences based on the three-way interaction; those for C and N indicate differences for the biochar by temperature interaction.

‡ The CQuest biochar was made by fast pyrolysis where temperatures varied between 450 and 600°C

indicated a significant biochar-by-N interaction, no true differences were found in the means comparisons using Tukey's adjusted P values (Table 3). Somewhat surprising was the lower soil total N where poultry litter biochar was added with N fertilizer (0.90 vs. 0.82 g kg^{-1} for the 0 and 31 mg N treatments, respectively), although this effect was not significant.

Nitrogen Retention in the Long-Term Incubation

The cumulative N leaching results indicated that most of the added N was removed from the system after 127 d and four leaching events (Table 3). The significant three-way interaction indicated that biochar feedstock, pyrolysis temperature, and N fertilizer addition combined to influence N loss from the system (Table 2). The amount of N leached from the control soil without added N was 8.1 mg. The LT peanut hull, HT peanut hull, LT pecan shell, HT pecan shell, HT switchgrass, and CQuest biochar treatments without added N leached similar amounts of N. Although the LT poultry litter biochar with no added N released inorganic N over the course of the incubation (Table 3), LT switchgrass biochar appeared to retain N. This is plausible considering that microbial oxidation of the LT switchgrass biochar required N immobilization because it was composed of more readily decomposable compounds (carbohydrates, hemi-cellulose, etc.) (Novak et al, 2009b).

With added N, the control soil had the greatest amount of leached N, but similar amounts were leached from soils with the LT pecan shell, LT poultry litter, and LT switchgrass biochars. For most biochars, the amount of leached N was similar between LT and HT biochars, but this was not the case for the poultry litter biochar, where much less N leached with the HT than the LT biochar. In the N-added treatments, we also noticed that higher pyrolysis temperature biochars tended to have slightly smaller amounts of N leached (Table 3).

Nitrogen Fractions in the Long-Term Incubation

The cumulative N leaching results indicated reduced N loss with some biochars; consequently, we expected to find increases in some of the labile N fractions. Analysis of variance results for the different labile N fractions are presented in Table 2. There were no significant treatment effects on the inorganic N fraction remaining in the soil (Table 4). The inorganic N content of the control soil (8.4 mg kg^{-1}) was not different from the other treatments. A lack of response to fertilizer addition reflects the very sandy texture and low nutrient holding capacity of this soil. We expected to find some residual added N at the end of the incubation, particularly with addition of biochars, but did not find it in the inorganic N fraction.

For the mineralizable soil N fraction, the ANOVA indicated a significant three-way interaction; however, none of the treatment means was found to be different when compared using Tukey's adjusted *P* values (Table 4).

The amount of resistant soil N remaining was influenced by biochar feedstock and pyrolysis temperature (Table 2). The significant interaction between these factors is the result of a smaller resistant N fraction in the HT poultry litter biochar treatment (Table 4). The HT poultry litter, HT pecan shell, and HT peanut hull biochar treatments had similar lower amounts of resistant N. These three treatments also significantly increased soil pH (Table 3), which can accelerate loss of native organic C and its associated resistant N fraction. Curtin et al. (1998) demonstrated that increases in soil pH due to the application of liming materials significantly increased mineralization of N in soil organic matter. Lower amounts of resistant N would be expected to cause an increase in leaching loses, but we observed an opposite response (Table 3).

The response of the more recalcitrant N fraction was influenced by interactions between biochar feedstock and pyrolysis temperature and between biochar feedstock and N treatment (Table 2). The results for the recalcitrant N fraction primarily reflect differences in the initial N content of the biochars (Tables 2 and 4). The LT and HT poultry litter and the LT and HT peanut hull biochars had more recalcitrant N compared with the other treatments (Table 4). The LT poultry litter biochar had more recalcitrant N than all other treatments. Similarly for the biochar-by-N treatment interaction, soil with the poultry litter biochar and without added N had the greatest amount of recalcitrant N (867 mg kg^{-1}), followed by poultry litter with N, peanut hull with N, and peanut hull without N (783, 716, and 656 mg kg^{-1}, respectively). This group was significantly different from the remaining biochar–N level combinations, which were not different from each other. The poultry litter and peanut hull biochars had the greatest N contents of any of the treatments (Table 1). Although these two biochars contain greater amounts of N than the other biochars, most of the N remaining after pyrolysis would be expected to be largely incorporated into the aromatic C structures and not available to microorganisms (Hilscher et al., 2009; Knicker, 2007). The poultry litter and peanut hull biochars had an O/C ratio of <0.2, which indicates a stable form of C in the Hedges et al. (2000) black C continuum model and is consistent with the large amount of N in the recalcitrant fraction.

Overall, the results from the evaluation of the various labile N fractions indicate little effect due to the addition of the different biochars. This was somewhat surprising because we thought LT biochars might have some available C to influence N cycling. We were puzzled by the leaching data because of the contrasting results for the poultry litter biochar treatments. We suspected that where the biochars increased soil pH above 7, N may have been lost due to NH_3 volatilization. We analyzed the inorganic N content of the incubation study archived soil samples (nonreplicated) collected on day 0 to determine if there were indications of N loss before leaching. For the 31 mg N treatment, losses ranged from 22 to 93% of the NH_4–N fraction (data not shown) with addition of the biochars. The greatest losses were with LT and HT peanut hull, LT and HT poultry litter, and HT pecan shell biochars, which all had pH values greater than 7.0 at the end of the incubation (Table 2). Volatilization of NH_3 from ammonium-based fertilizers is known to increase significantly as soil pH increases above 7 (Fenn and Kissel, 1975). The alkaline properties of these biochars most likely lead to N losses due to NH_3 volatilization. Although several researchers have observed increased soil pH with biochar additions, no previous reports of N losses due to volatilization with additions of biochar have been reported (Chan and Xu, 2009).

Confirmation of Ammonia Volatilization in Short-Term Incubation

We conducted a short-term (7-d) experiment to support our hypothesis that initial losses of N were due to NH_3 volatilization induced by the addition of high-ash alkaline biochar. A significant biochar feedstock-by-pyrolysis temperature interaction was present in the results ($p \leq 0.0001$). Ammonia losses were small for most of the biochars and the control (Table 5). Losses of NH_3 were most apparent where the pH of the soil was near or above 7.0. Losses were particularly great for the HT poultry litter biochar, where 53% of the applied NH_4–N was volatilized in our closed incubation system.

As would be expected, NH_3 loss was related to the soil pH ($r = 0.695$; $p = 0.05$). We also found a high degree of correlation with the biochar properties such as percent ash ($r = 0.890$; $p = 0.003$), the sum of base cations ($r = 0.888$; $p = 0.003$), and the sum of total minerals ($r = 0.887$; $p = 0.003$). The pH of the soil/biochar mixture was also highly correlated with the percent ash ($r = 0.772$; $p = 0.024$), the sum of base cations ($r = 0.780$; $p = 0.022$), and the sum of total minerals ($r = 0.762$; $p = 0.028$) in the biochar.

These results indicate the potential for losses of NH_4–N when applying biochars with high ash and alkaline properties. Most biochars are reported to increase soil pH (Clough and Condron, 2010). The range of volatilization reflects differences in the ash content of the biochar, a characteristic that is dependent on the feedstock and the pyrolysis temperature (Gaskin et al., 2008; Novak et al., 2009b). Brewer et al. (2009) report that ash content of switchgrass from fast pyrolysis is dominated by SiO_2, whereas ash contents of hardwood biochars are dominated by CaO. Gaskin et al. (2008) reported greater Ca, K, and Mg concentrations in peanut hull and poultry litter biochars than in pine chip biochars from slow pyrolysis. Greater ash content is also associated with higher pyrolysis temperatures (Keiluweit et al., 2010; Novak et al., 2009b).

Although the results may be different under field situations, applying N to a low-buffer-capacity soil where an alkaline biochar has been recently applied could result in N fertilizer losses, which can reduce yields and cause economic loss to producers. Further research under field conditions is needed to determine how great this impact may be for different soils and biochar application methods.

Conclusions

Biochar has been reported to affect N cycling in several ways. The first is a reduction in leaching losses. We expected to find

Table 4. Effect of adding various biochars made at two pyrolysis temperatures with and without the addition of nitrogen fertilizer (NH_4NO_3) on mineral nitrogen and soil nitrogen fractions after a 127-d incubation and four leaching events.

Biochar	Pyrolysis temp.	N	N fraction			
			Inorganic	Mineralizable	Resistant	Recalcitrant
	°C	mg	mg kg⁻¹			
Control		0	8.6 a†	14.3 a	14.2 a	250 d
		31	8.2 a	9.9 a	16.7	191
Peanut hull	400	0	10.1 a	14.0 a	14.7 a	658 b
		31	14.0 a	10.6 a	18.6	743
	500	0	12.5 a	10.5 a	13.8 ab	654 b
		31	8.6 a	13.6 a	9.7	688
Pecan shell	350	0	12.1 a	9.8 a	17.4 a	391 cd
		31	11.3 a	12.1 a	17.2	276
	500	0	10.0 a	19.2 a	7.4 ab	392 c
		31	8.6 a	10.0 a	15.3	365
Poultry litter	350	0	9.6 a	12.3 a	18.8 a	986 a
		31	11.6 a	11.9 a	18.3	974
	700	0	10.7 a	14.5 a	5.1 b	747 b
		31	18.6 a	10.1 a	6.3	592
Switchgrass	250	0	8.3 a	12.0 a	18.5 a	291 cd
		31	8.8 a	11.5 a	16.5	297
	500	0	9.8 a	14.3 a	14.1 a	396 c
		31	9.9 a	10.6 a	13.3	425
CQuest	500‡	0	9.6 a	11.2 a	15.9 a	346 cd
		31	10.8 a	15.5 a	11.6	336
Effect			**Tukey's 95% confidence interval**			
Biochar			3.9	3.8	4.3	75
Temp.			2.1	2.0	2.3	40
N			2.1	2.0	2.3	40
Biochar × temp.			6.5	6.4	7.2	126
Biochar × N			6.5	6.4	7.2	126
Temp. × N			3.9	3.8	4.3	75
Biochar × temp. × N			10.5	10.3	11.6	203

† Letters within columns indicate differences between means based on Tukey's multiple means comparison procedure. Letters for inorganic and mineralizable N indicate differences based on the three-way interaction; those for resistant and recalcitrant N indicate differences for the biochar by temperature interaction.

‡ The CQuest biochar was made by fast pyrolysis where temperatures varied between 450 and 600°C

increased NH_4–N retention, particularly with the LT biochars, which should have had a greater negative surface charge due to higher O content (Cheng et al., 2008). Although we observed some reductions in cumulative N leaching, the reductions for the HT peanut hull, HT pecan shell, and HT poultry litter were most likely related to less N present in the system due to initial volatilization losses. The LT peanut hull, HT switchgrass, and CQuest biochar treatments also had reduced cumulative leaching compared with the control; however, we did not observe a related increase in the inorganic N fractions of these treatments that would be expected if the biochars increased retention. Consequently, overall we did not find evidence that the biochar treatments reduced N leaching through increased ion retention.

We expected the mineralizable N fraction would increase with the addition of biochars with higher VM%, aliphatic C contents, or high O/C ratios because these biochars might increase C availability to the microbial biomass. The biochars apparently had little impact on the soil microbial biomass because we did not see large increases in mineralizable N with the addition of biochar. Therefore, most of the C in these biochars was not available to microorganisms, except for potentially the LT switchgrass biochar. We did observe some changes in the resistant N fraction. Whether this fraction is oxidized in the presence of high-pH, high-ash biochars is an area needing further investigation. The recalcitrant fraction was related to the N content of the feedstocks, indicating that biochars contain structural N, which is largely unavailable to microbial transformation into available N forms. These results indicate that easily measurable N availability indices can be useful in evaluating the effects of biochars on soil N fractions. Additional work is needed to determine if the approach can be used with other soils and in field studies where environmental and climatic conditions may affect chemical and biological processes.

We also explored the use of VM% and the O/C ratio as a means to interpret short-term stability of various biochars and whether these indicators can be used to predict short-term responses in microbial biomass that might immobilize N. We did not see a consistent pattern with these indicators and mineralizable N.

Table 5. Soil pH and ammonia losses from a 7-d incubation of Norfolk soils with various biochar amendments.

Biochar	Temp.	Soil pH	NH_3 loss	Fraction N loss
	°C		mg N kg^{-1}	% added N
Control†		5.1	4.6 cd†	0.7
Peanut hull	400	5.8	4.8 cd	0.7
Peanut hull	500	6.6	8.9 bcd	1.3
Pecan shell	350	4.6	3.3 cd	0.5
Pecan shell	500	7.0	16.5 bc	2.5
Poultry litter	350	7.2	33.8 b	5.1
Poultry litter	700	8.0	179.3 a	26.9
Switchgrass	250	5.2	6.0 cd	0.9
Switchgrass	500	6.4	6.3 cd	0.9
CQuest	500‡	5.2	2.7 d	0.4

† Letters indicate differences between means based on Tukey's multiple means comparison procedure for the biochar by temperature interaction.

‡The CQuest biochar was made by fast pyrolysis where temperatures varied between 450 and 600°C

Our short-term experiment to directly measure NH_3 volatilization demonstrated that much of the N not found in the leachate or in the N fractions of the longer-term incubation was probably lost as NH_3. To our knowledge, this is the first report of NH_3 volatilization resulting from the addition of high-pH, high–alkaline ash biochars. Other soil organic matter and biological processes may also be affected by biochars due to changes in pH or alkaline minerals. Novak et al. (2009a) noted very high P and Na concentrations after application of these biochars to this soil. Considering the range of possible responses to different biochars within a soil type, it would be prudent for biochars to be considered similarly to other agricultural by-products and be proven to be agronomically beneficial or at least neutral and environmentally sound before widespread use. Development of biochar by-product use standards and guidelines would allow matching of biochars to particular soils and land use situations and help to avoid potential problems in the future.

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