Impacts of Biochar and Other Amendments on Soil-Carbon and Nitrogen Stability: A Laboratory Column Study

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There have been a large number of recent soil incubation studies examining the potential of biochar as a soil amendment tool for enhancing soil fertility and C sequestration. However, direct comparisons of biochar's effects to that of other nontraditional soil amendments are lacking. In addition, most soil incubation studies with biochar examined gaseous emissions in closed systems that do not allow for water flow through (i.e., leaching). Thus, three amendments—humic acid (HA), water treatment residual (WTR), and biochar produced from oak at 650°C were incubated with Crosby silt-loam (fine, mixed, active, mesic Aeric Epiaqualfs) soil at a rate of 1% (w/w) in triplicated soil-columns. Effects of biochar and the other amendments on gaseous (CH_4, CO_2, and N_2O) emissions and leaching of total organic C (TOC), NO_3^-, and NO_2^- were simultaneously monitored over a 257-d incubation period. Biochar-amended soil had reduced mean cumulative leaching of TOC by 30%, nitrate by 33%, and nitrite by 34%, compared to the control (control: 93, 75, and 2 mg kg^{-1} for TOC, nitrate, and nitrite, respectively), likely due to sorption by the biochar. The WTR-amended soil exhibited similar leaching trends; however, HA-amended soil leached significantly greater ($p < 0.05$) TOC than either WTR or biochar. No amendments had any significant impact on gaseous emissions due to the high variability within each treatment. We hypothesize that the labile C and N compounds, which otherwise become emitted by microbial activity and produced significant differences in treatments, were probably leached out from the system as both leaching and gaseous sampling events took place at the same time due to the experimental settings employed. Thus, it is probable that the labile C and N compounds most likely to be oxidized and emitted in gaseous forms in any closed system incubations were either protected via mineral sorption or lost as leachate in the open column system in the present experiment. Considering both leaching and gaseous losses, C and N mean residence times (MRT) of 50 and 15 yr were calculated for the biochar-amended soil compared to 28 and 12 yr for the control, and 518 and 1.5 million years considering only gaseous losses, respectively. These findings show that estimates of soil-C and N stability that do not take into account aqueous losses of these elements (leaching) may tremendously overestimate C and N-MRT. Comparison with a field study using the same soil and amendment types suggests that analyses from laboratory incubation may serve as reliable proxies for trends in field soil responses to amendments.

Abbreviations: AWC, available water capacity; CEC, cation exchange capacity; DOC, dissolved organic C; GHG, greenhouse gas; GWP, global warming potential; HA, humic acid; MRT, mean residence times; OM, organic matter; SA, surface area; TOC, total organic C; VM, volatile matter; WFPS, water filled pore space; WTR, water treatment residual.

Materials such as biochar, HA, and WTRs are being considered as soil amendments with the potential to improve soil fertility and soil C and N stability. An understanding of the changes in dynamics of soil C and N

Supplemental Material available online
Soil Sci. Soc. Am. J.
doi:10.2136/sssaj2014.01.0025
Received 21 Jan. 2014.
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likely to occur following these additions to soil is required to identify strategies for improving soil structure, increasing productivity, and minimizing greenhouse gas (GHG) emissions. Humic acid can improve soil characteristics by buffering pH, chelating micronutrients (Kudeyarova, 2007; Mackowiak et al., 2001; Motojima et al., 2012), and enhancing cation exchange capacity (CEC) and available water capacity (AWC) of soil (Senesi and Plaza, 2007; Sharif et al., 2002; Soler-Rovira et al., 2010; Tahir et al., 2011). Coal-derived HA substances, the type used in this study, can increase water retention, AWC, and aggregate stability of degraded soils (Piccolo et al., 1996). Water treatment residuals are by-products of the water clarification process, which also have been used as soil amendment (Ippolito et al., 2011). These materials can also improve soil physical properties (Hsu and Hseu, 2011; Ippolito et al., 2011; Soler-Rovira et al., 2010; Tahir et al., 2011) and reduce heavy metals runoff (Fan et al., 2011; Mahmoud, 2011). However, data on impacts of HA and WTRs on GHG emissions and soil-C and N stability over time are scarce.

Biochar, the carbonaceous product of biomass combusted under O-limited conditions, is currently being widely studied for its effects as a soil amendment. Emissions of GHGs from biochar-amended soils have been studied extensively but mostly in closed system laboratory settings that do not allow for leaching (Bruun et al., 2012; Jones et al., 2011; Rogovska et al., 2011; Rondon et al., 2005; van Zwieten et al., 2009). Nevertheless, it has been shown by these methods that the MRT or the average time of C residing in its respective soil pool at steady state (Six and Jastrow, 2002), in biochar alone and biochar-amended soil depends on biochar pyrolysis temperature (Fowles, 2007; Lehmann, 2007; Lehmann et al., 2006) and duration of charring (Peng et al., 2011). For example, Zimmerman (2010) observed an inverse relationship between biochar production temperature (250–650°C) and both abiotic and microbial C mineralization rate with estimated MRT from this biochar-only study ranged from 375 to 21×10^4 years. In addition, Peng et al. (2011) reported that MRT of biochar-C derived from rice straw, ranging from 244 to 1700 yr, and generally increased with increase in charring temperature (250–450°C). Volatile matter (VM) content of biochar, consisting of labile aliphatic alcohol and acid functional groups (Mukherjee et al., 2011) and O/C and H/C ratios (Spokas et al., 2012; Spokas et al., 2009; Spokas and Reicosky, 2009) are significantly correlated with the CO_2 emissions from biochars (Peng et al., 2011; Zimmerman, 2010). These findings show that there is a labile component of biochar rich in organic functional groups, and this VM may also be responsible for an initial peak of CO_2 flux from biochar-amended soils (Aarna and Suuberg, 1998; Jones et al., 2011; Mukherjee and Lal, 2013; Smith et al., 2010).

While some recent studies have examined the stability or MRT of C in biochar-amended soil through measurements of gaseous emissions only (Zimmerman and Gao, 2013), the authors are aware of none that have measured both gaseous and leaching losses simultaneously. The sole study to date (Sarkhot et al., 2012), which documented both gaseous and leaching losses, (i) did not estimate MRT of C and N from elemental losses, and more importantly (ii) unlike the current study, collected both gaseous and leachate samples under two separate but similar incubation settings. Thus, one objective of this study was to obtain a better estimate of C and N stability in biochar-amended soil through simultaneous measurement of gaseous and aqueous losses from soil columns. Second, research information on MRT of soil-N under any amendment is scanty and a researchable priority. Third, there have also been very few direct comparisons of C and N stability in biochar-amended soil to that of the same soil amended with other materials, though such a comparison could be informative as to the interactive mechanisms at play. For example, like biochar, HA is a C-rich material that is refractory to some degree. In contrast, WTR has little refractory C but has a high surface area and porous structure (Mukherjee et al., 2014b). Thus, enhanced C or N stability caused by either of these two soil amendments may point to a greater importance of C chemical lability or sorptive protection, respectively. Fourth, since these same amendment type and soil combinations were previously used in field studies of C and N gaseous emission (Mukherjee et al., 2014b), they provide a test of whether soil columns may yield results that can be representative and substituted for more laborious and costly field studies. The results were also used to estimate the relative effect of each amendment on global warming potential (GWP), concerning which information is scarce for HA and WTR.

MATERIALS AND METHODS

Materials

Soil chosen for the laboratory incubation experiment was collected from 0- to 10-cm depth intervals at the Waterman Farm of the Ohio State University, Columbus, OH. It was classified as a Crosby silt loam. Soil was air-dried, ground, and passed through a 2-mm sieve. Commercial coal-derived HA was obtained from Sigma Aldrich, MO and used as is. The Al-WTR was collected from a water treatment plant in Columbus, OH and used after air-drying. Biochar was produced from laurel oak (*Quercus laurifolia* Michx.; collected in Gainesville, FL) wood, cut to 5 by 5 by 30 cm and combusted for 3 h at 650°C in a closed kiln sealed loosely to allow smoke to exit. Detailed information on chemical and physical characteristics of the oak-650 biochar has been presented elsewhere (Kasozi et al., 2010; Mukherjee et al., 2011; Zimmerman, 2010). The coarse size fraction (0.25–2 mm) of the biochar, separated by sieve, was used in the experiment, as that fraction (i) was used in the field component of this research (Mukherjee et al., 2014b), (ii) has been used by a number of recent studies (Case et al., 2012; Githinji, 2013; Jones et al., 2011; Troy et al., 2013; Zhang et al., 2012), and (iii) has the potential to be chosen by growers for field application based on previous experiments (Mukherjee and Zimmerman, 2013; Mukherjee et al., 2011). General characteristics of the soil and three amendments are presented in Supplemental Table S1.

Experimental

All experimental treatments, including a soil control with no amendment, were triplicated in columns made from transparent rigid PVC tubes (U.S. Plastic Corp), each 30.5 by 7.6 cm (length by internal diameter) in size (Fig. 1). Thoroughly mixed combinations of 10 g of each amendment and one kilogram of soil were placed in each column and incubated at room temperature (~25°C). This rate of application corresponds to a 15 Mg ha^{-1} amendment level, assuming 1.5 Mg m^{-3} bulk density (ρ_b) of the 0- to 10-cm layer. Each column was sealed on both ends with duct tape and fitted end-caps. The sediment column was 39.5 cm in height (including the end-caps length) and had an overlying headspace of 17 cm. The upper end-cap was fitted with two rubber septa to draw gaseous samples and introduce water and the lower end-cap was fitted with a valve attached to a tube for control of leachate collection (Fig. 1). The bottom part of the core contained landscape fabric and Whatman filter papers to prevent loss of solids.

At the start of the experiment, soils were wetted from the top with 400 cm^3 of distilled water to achieve water filled pore space (WFPS) of 69% (assuming a soil bulk density of 1.5 Mg m^{-3} and a particle density of 2.65 Mg m^{-3}). Thereafter, columns were leached with 200 cm^3 of distilled water (equivalent to 35% WFPS), initially once in a week for the first 2 mo, then once in a month for the next 2 mo, and 2 mo later for the remaining samplings. The leachates were stored in a refrigerator before chemical analyses performed within 2 wk of sampling. Gaseous samples were withdrawn from the headspace of the core by inserting a 20-cm^3 syringe through the rubber septa before each leaching of the cores. Gas samples were stored in 20 cm^3 air tight previously evacuated glass vials and concentrations of CO_2, CH_4, and N_2O in the glass vials were determined using a gas chromatograph (GC-2014, Shimadzu Corp., Japan). The flux of GHG emissions was calculated following Shrestha et al. (2009):

$$F = \left(\delta G * 10^{-6} / \delta t\right)\left(V_C / A\right)\left(M / V\right)\ \mathrm{d}^{-1} \qquad [1]$$

where, F = gas flux (g m^{-2} d^{-1}), δG = change in gas concentration in ppm over time δt in a day, V_C = volume of chamber (m^3), A = soil surface area within the chamber (m^2), V = ideal gas volume in headspace (0.0245 m^3), and M = molar weight (g) of the gas. Soil ρ_b in the columns was determined periodically by measuring the change in height of the soil column (average of three measurements) following Rogovska et al. (2011).

Chemical Analyses

The VM content of each material was determined as weight lost after combustion in a ceramic crucible loosely covered with a ceramic cap at 850 to 900°C for 6 min (ASTM, 1990; Mukherjee et al., 2011). Total C and N concentrations in the soils were determined on 250 μm sieved and ground samples using an elemental analyzer (Vario Max, Elemntar Americas, Inc., Germany) by dry combustion (900°C) method (Nelson and Sommers, 1996). Dissolved TOC and NO_3^-–NO_2^- in the leachates were analyzed using a TOC analyzer (TOC-VCPH/CPN, Version 2, Shimadzu Corp., Japan) and an Astoria 2 micro-flow analyzer (Astoria-Pacific, Clackamas, OR), respectively (Keeney and Nelson, 1987; Nelson and Sommers, 1996). The surface area (SA) was measured on a Quantachrome Autosorb-1 instrument using N_2 and CO_2 sorptometry (N_2–SA and CO_2–SA, respectively) (Mukherjee et al., 2011).

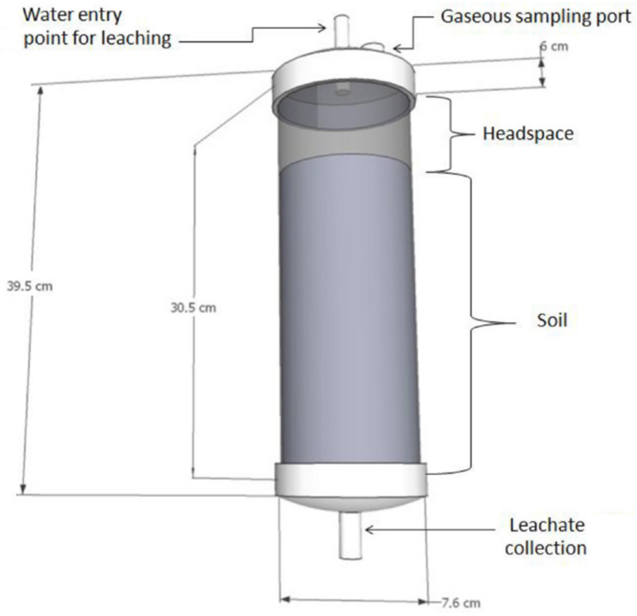

Fig. 1. Design of column used in the laboratory incubation experiment.

Data Modeling

Soil-C and N stability were estimated by calculating their MRT using the C and N losses from soil via leaching and gaseous emissions. A first order degradation rate was assumed and solved graphically such that

$$\delta A / \delta t = -k * A \qquad [2]$$

where, $\delta A/\delta t$ = rate of change of concentration of element A over time t, k = pseudo-first order decomposition rate constant, and A = initial concentration. The MRT of an element was then calculated as the inverse of k (MRT = k^{-1}). A first order model, compared to that of a second order was chosen, as the data of the current study were better fitted with this model as reported in previous studies (Peng et al., 2011; Six and Jastrow, 2002; Steinbeiss et al., 2009).

The relative effect of each treatment on GWP was calculated by summing the weighted daily gaseous fluxes by numerical integration of the area underlying the gas flux curve using the trapezoid rule (Dendooven et al., 2012). The GWP of CH_4 and N_2O were assumed to be 23 and 296 times that of CO_2 over the time horizon of 100 yr (USEPA, 2007).

Statistical Analyses

All data are presented as means ± standard deviations of three measurements. Differences between treatments were analyzed using Tukey's test in PROC GLM in SAS version 9.2

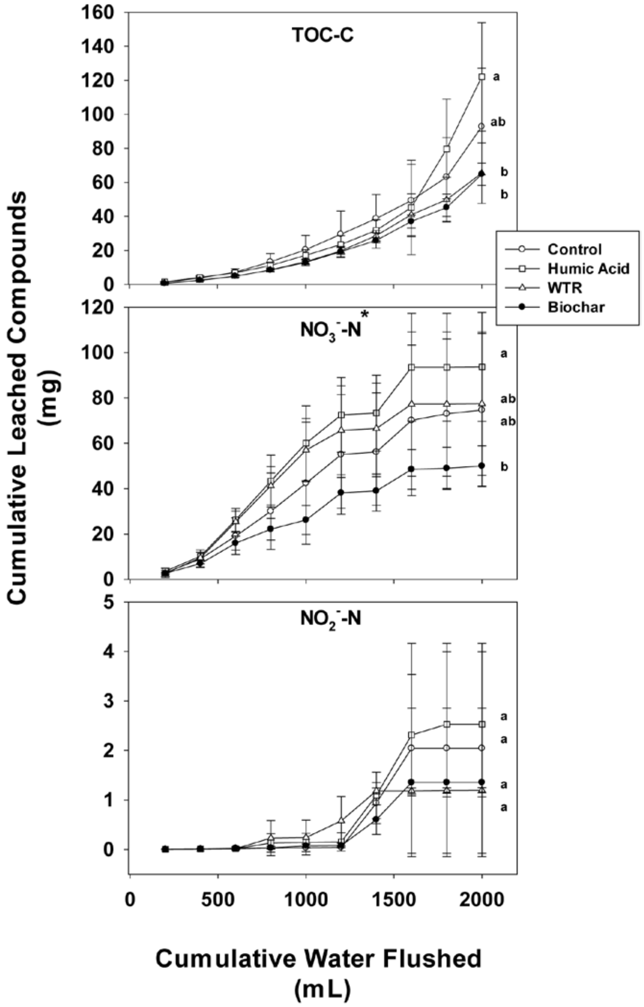

Fig. 2. Cumulative values of parameters labeled with different letters at right are significantly different at $p < 0.05$, *: significant only at $p < 0.1$ for NO_3^-–N (Note: different scales in *y*-axes). TOC, total organic C; WTR, water treatment residual.

(SAS, 2012). Treatment differences were considered significant when $p < 0.05$. All the graphical figures were created using Sigmaplot 12.0 (Sigmaplot, 2012).

RESULTS AND DISCUSSIONS

Impacts of Amendments on Elemental Leaching

In general, leaching losses were lower in soil with biochar in comparison with other amendments or the control soil. Temporal leaching losses in the control ranged from 1.2 to 29.7, 1.1 to 14.0, and 0.0 to 1.1 $\mu g\ g^{-1}$ TOC, NO_3^-, and NO_2^-–N, respectively, compared with 0.7 to 19.3, 0.5 to 12.0, 0.0 to 0.8 $\mu g\ g^{-1}$, respectively, in biochar-amended soil (Supplemental Fig. S1). Additionally, while NO_3^- and NO_2^-–N releases were variable over time (Supplemental Fig. S1) and reached a plateau after $1.5 \times 10^{-3}\ m^3$ flush volumes (Fig. 2), TOC releases from amended soils were progressively continuous (Supplemental Fig. S1, Fig. 2). While soil treated with HA lost greater cumulative NO_3^- and NO_2^-–N via leaching compared to the control, soil amended with biochar and WTR had lower losses of cumulative TOC, NO_3^-, and NO_2^- by 30, 33, and 34%, and 29, −4, and 41% (negative sign indicates increase), respectively, compared to the control (Fig. 2).

Despite having added the greatest amount of total C (Supplemental Table S1), the least amount of dissolved organic C (DOC) was released by biochar-amended soil during the incubation period among all the treatments (Fig. 2). Previous studies have found differing results in regards to the effects of biochar on soil C leaching. For example, charcoal incorporation resulted in increased microbial biomass, which in turn enhanced DOC in the leachates of biochar-amended soil under a field study (Bell and Worrall, 2011). Another field study conducted in a savanna Oxisol indicated that DOC was 267% greater in the leachates of biochar-amended soils relative to a control. This increase was proportional to the increase in water flux in biochar-amended soils likely due to its lower ρ_b, increased saturated hydraulic conductivity, and water infiltration (Major et al., 2010). However, other processes may influence leaching from biochar-amended soils. For example, soils with biochar pyrolyzed from grass at lower temperature (400°C) released greater C, N, and P than those made from wood (oak) at a higher temperature (650°C) suggesting that these leached elements can also be derived from the biochar itself (Mukherjee and Zimmerman, 2013). Sorption of the aromatic phenols and carboxylic acids abundant in biochar leachate can be readily sorbed by soil minerals (Kaiser and Guggenberger, 2000) and catechol, a model soil organic matter (OM) compound that can be sorbed readily to biochar (Kasozi et al., 2010). Laboratory column experiments with grass biochar amended-soils revealed that 20 to 40% of biochar-C leachate was sorbed by a sandy Entisol, whereas none was sorbed by a clayey Utilsol (Mukherjee and Zimmerman, 2013) suggesting influence of soil types on sorption. In another study, soil DOC was found to be sorbed to biochar-mineral complexes formed in soil aggregates (Lin et al., 2012). However, the extent to which these sorbed components may be later desorbed or are microbially available is an area for future research.

Thus, it is likely that biochar sorption is responsible for lower leaching of DOC and N compounds in the biochar-amended soil of this study relative to the control (Fig. 2). This is despite its having the highest total C and VM content (Supplemental Table S1) and the lowest soil-ρ_b or highest aeration (Fig. 3). Although biochar had the highest surface area (Supplemental Table S1), WTR also likely sorbed soil OM by specific bonding as WTR possesses abundant Fe and Al-hydroxides (Agyin-Birikorang and O'Connor, 2007; Ahmad et al., 2012; Ippolito et al., 2011; Miller et al., 2011). On the other hand, the increase in dissolved C in leachates of the HA-treated soil compared to the control may have been due to enhanced microbial colonization and biomass as well as leaching of the HA itself (Fig. 2).

Biochar addition also consistently resulted in reduced leaching of NO_3^- and NO_2^- over time compared to the other two amendments (Fig. 2). Other studies have also reported reduced leachate of N from biochar-amended soils (Dempster et al., 2012b; Laird et al., 2010; Lehmann et al., 2003; Major et al., 2012; Mukherjee and Zimmerman, 2013; Novak et al., 2009). Release of inorganic N has also been found to depend on the pyrolysis temperature of biochar (Mukherjee and Zimmerman, 2013) and high VM content of biochar (Deenik et al., 2010). Both low temperature (300°C) hardwood derived and dairy effluent-mixed "enriched" biochar reduced NH_4 and NO_3–N from coarse loamy soil, which was attributed to sorption and immobilization of inorganic-N by the specific biochar. Thus, reduced leaching of N from biochar-amended soil may be attributed to: (i) reduced rate of nitrification as biochar inhibits nitrification processes in soil (Dempster et al., 2012a; Dempster et al., 2012b), (ii) retention of ammonium via biochar sorption because of the high CEC of biochar (Chan et al., 2008; Clough et al., 2013; Liang et al., 2006; Novak et al., 2009), (iii) enhanced microbial uptake of inorganic N and cycling into the organic pool with subsequent sorption of organic N on the soil–biochar surface (Guerena et al., 2013; Sarkhot et al., 2012), and (iv) N immobilization due to high concentration of soluble phenolic compounds present in the VM of biochar (Deenik et al., 2010; Mukherjee et al., 2011), which may serve a high C/N source of C for soil microbes (Deenik et al., 2010). While these processes might have occurred in the soil columns of the present study, direct adsorption of NO_3^- onto the biochar surface (Clough et al., 2013) could also be a prominent pathway, as the oak-650 biochar used here has previously been found to develop anion exchange capacity over time (Mukherjee et al., 2014a). While WTR had no significant effect on N leaching, HA increased NO_3^-–N leaching from the amended soil, perhaps due to higher denitrifier activity stimulated by addition of labile C (Clough et al., 2013).

Impacts of Amendments on Greenhouse Gas Emissions

No amendments had any significant impact on gaseous emissions due to the high variability within each treatment. We hypothesize that the labile C and N compounds, which were otherwise utilized and emitted by microbial activity and produced significant differences in treatments were probably leached out from the system, as both leaching and gaseous sampling events took place at the same time as per the experimental setting. As differences in gaseous emissions were not statistically significant under various amendments, the discussion or their implications on this aspect is limited, especially considering that the standard errors are quite high (Fig. 4, Supplemental Fig. S2).

However, there are few previous data on gaseous emissions from soils amended with HA and WTR, and the mechanisms by which biochar amendment affect gaseous emissions of soils are poorly understood (Mukherjee and Lal, 2013; Mukherjee and Lal, 2014)—which warrant discussion, especially in the cases when they may link to leaching. The high pH of both biochar and WTR used in the present study (Supplemental Table S1) may have suppressed CO_2 efflux through the formation of secondary carbonate precipitation (Lehmann et al., 2011). More likely, however, the same sorption that led to decreased C leaching in the biochar-amended soil also resulted in the reduced C mineralization through sorptive protection. Higher temperature biochars, such as the oak-650 biochar used here, have been shown to be more likely to cause negative priming, that is, the reduction in soil C mineralization by this mechanism (Zimmerman et al., 2011). On the other hand, this process did not, apparently, occur in soils treated with HA treatment, as it decreased aeration (Fig. 3) and CO_2 emission compared to control at the same time (Fig. 4). Abiotic release of C through leaching (Fig. 2) may have depleted the labile C source for the microbial community to exploit for HA treated soil (Fig. 4, Supplemental Table S2). The much smaller reduction in CO_2 emission observed for HA and WTR (Fig. 4) may be attributed to similar sorptive-protective mechanisms, only operative to a much lesser extent.

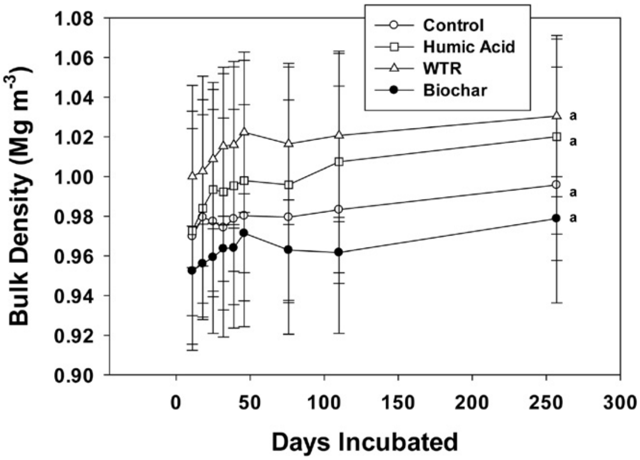

Fig. 3. Change in bulk density during incubation period; different letters indicate those treatments that are significantly different at $p < 0.05$. WTR, water treatment residual.

Implications of Gaseous Emissions and Leaching from Amended Soils

Losses via both leaching and gaseous emissions were added to estimate C and N stability (MRT) in amended soils (Table 1, Columns 1 and 3). For comparison, MRT were also calculated using losses through gaseous emissions only (Table 1, Columns 2 and 4). Biochar amendment significantly ($p < 0.05$) increased MRT of C by 79% compared to the control, when both leaching and gaseous C-loss pathways were included in the MRT calculation (Table 1). However, other amendments did not have any significant effects on calculated MRTs. Because losses of C due to leaching were about twice those of gaseous losses of C, inclusion of leaching in MRT of C calculations had a strong effect, resulting in MRT of C that were, on average, about 12 times less than those calculated from gaseous emissions alone. Similarly, MRT of N calculated from gas and leaching data were about five orders less than those calculated from gaseous emissions only (Table 1). These calculations assume that all gaseous and aqueous C and N losses were accounted for by the measured species. This may not be the case if significant denitrification took place, producing an efflux of N_2 from the columns, which was not measured in the present study. Likewise,

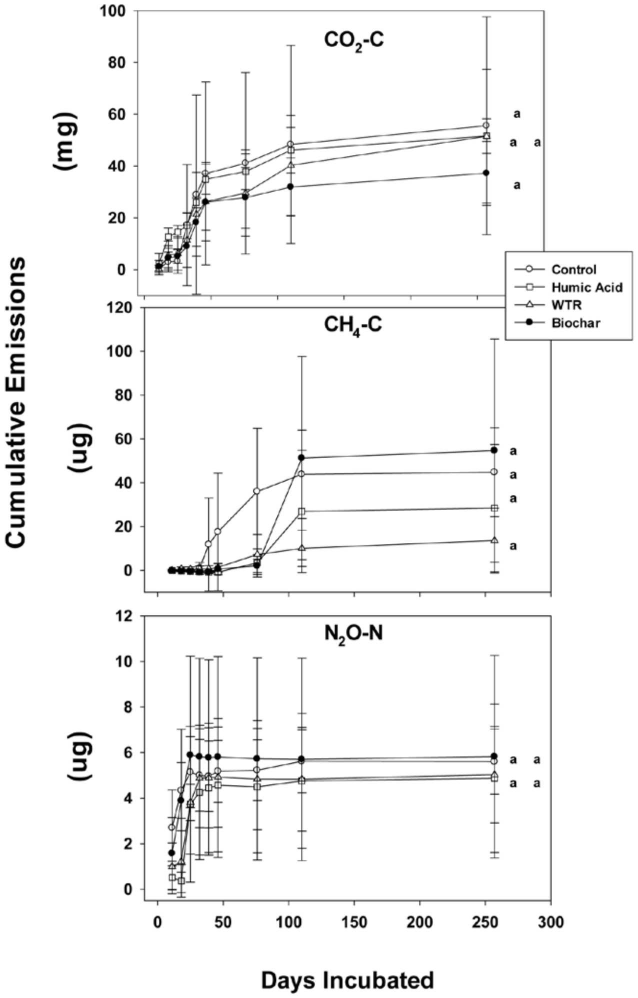

Fig. 4. Cumulative gaseous emissions of C and N from control and amended soil columns during 257-d incubation period (Note: different scales and units in *y*-axes); different letters indicate those treatments that are significantly different at $p < 0.05$. WTR, water treatment residual.

losses of aqueous inorganic C or organic N may have occurred via leaching, which were not measured in the current study.

While a number of studies either estimated half-life or MRT of pure biochar-C (Zimmerman, 2010; Zimmerman et al., 2011) or labeled biochar-C in soils (Keith et al., 2011; Knoblauch et al., 2011; Kuzyakov et al., 2009; Peng et al., 2011; Santos et al., 2012; Singh et al., 2012) by measuring gaseous emissions, data are scarce on MRT of C and N of total biochar-amended soil. Nevertheless, Santos et al. (2012) estimated 15 to 51 yr of soil-C MRT incubating (180 d) Andesitic and Granitic soils with ponderosa pine (*Pinus ponderosa* P. Lawson & C. Lawson) biochar produced at 450°C. Indicating high variability of MRT of soil-C due to differences in soil types, a high range (327–2026 yr) of MRT of soil-C was estimated from seven types of surface silt-loam soils collected from the Midwestern United States (Paul et al., 2001). The soils were similar to the control soil used in the present study. In the current experiment, the ranges of MRT of the control silty loam and amended soil-C were 19 to 50 yr considering combined gaseous and leaching losses and 362 to 518 yr considering only gaseous losses (Table 1).

Table 1. Mean residence time (MRT) of C and N calculated using first order kinetics modeling of 8.5 mo combining GHG (CO$_2$, CH$_4$, and N$_2$O) emissions and aqueous total organic C (TOC) and NO$_3^-$ and NO$_2^-$ leaching data (model-R^2 were all significant at $p < 0.05$ and ranged from 0.60 to 0.99).

Treatments	MRT of C		MRT of N	
	L + G	G	L + G	G
			(Years)	
Control soil	28bc ± 15	368a ± 212	12a ± 9	(10.8a ± 6.5) × 10^5
Soil + HA†	19c ± 4	385a ± 69	8a ± 2	(7.8a ± 6.9) × 10^5
Soil + WTR	44ab ± 14	362a ± 255	18a ± 14	(8.6a ± 5.1) × 10^5
Soil + biochar	50a ± 5	518a ± 155	15a ± 5	(15.5a ± 15.3) × 10^5

† Abbreviations: HA, humic acid; WTR, water treatment residuals; L + G, estimation based on elemental losses through combination of leaching and gaseous pathways; G, estimation based on elemental losses through gaseous pathway only.

The authors are unaware of any data on MRT of soil-N under biochar or any other amendments. One critical reason for scarcity of MRT of soil-N is that a significant amount of soil-N can be lost by leaching and must be taken into account while calculating MRT of N to avoid overestimation. The MRTs calculated from the data of the present study indicate that inclusion of leaching losses actually decrease soil C and N-MRT by up to 1 and 5 orders of magnitude, respectively (Table 1), suggesting that biochar-amended soil C and N may not be as stable as has been proposed in earlier studies considering only gaseous losses (Zimmerman and Gao, 2013). In other words, these results identify importance of inclusion of both pathways (leaching and emission) for MRT-estimation.

Comparative effects of each amendment on GWP were evaluated using the measured emissions of CO$_2$, CH$_4$, and N$_2$O. The true effect on GWP, however, includes the amount of GHG emitted during the production, transport, and application of each amendment. For example, there was an emission of 43.3% of the parent biomass C (Zimmerman, 2010) during the production of oak-650 biochar. Estimation of the full effect of each amendment on GWP was beyond the scope of this paper. In addition, while leached C and N may eventually be converted to greenhouse gases, the extent to which this occurs in each soil cannot be known with confidence, so this data was not used in GWP effect evaluation and, thus, the calculated GWP is termed "partial-GWP" as only measured gaseous emissions during the incubation period were used for calculation. For each soil treatment and the control, the largest contributor to partial-GWP was CO$_2$ (Supplemental Fig. S3). The HA, WTR, and biochar treatments each decreased partial-GWP by 2, 9, and 28%, respectively, relative to the control.

Comparison between Laboratory and Field-derived Soil Parameters

Trends in ρ_b, gaseous emissions, MRT of C, and partial-GWP established in this laboratory incubation can be compared to those measured in a field experiment performed with the same soil and amendment combinations. The main difference in the conditions of these two studies are that the field study had amendment application rates of 0.5% and was leached via rain at a rate of 6.7 cm per month in average during a period of 4 mo under soybean [*Glycine max* (L.) Merr.], whereas the laboratory study was performed under laboratory settings with 200 cm^3 leaching over 257 d. Detailed methods and results of the field study were presented elsewhere (Mukherjee et al., 2014b).

Table 2. Qualitative comparison between trends in amendment effects observed under laboratory vs. field conditions.

	ρ_b		CO_2†		CH_4		N_2O		MRT-C‡		GWP	
Treatments	**L**	**F§**	**L**	**F**	**L**	**F**	**L**	**F**	**L¶**	**F**	**L**	**F**
Soil + HA	I	D	D*	I	D	I	D	D	D	D	D	I
Soil + WTR	I	D	D*	D	D	D	D	D	I	I	D	D
Soil + biochar	D	D**	D*	D	I	D	I	D**	I*	I	D	D

*Significantly different from control at $p < 0.05$ (laboratory).

** Significantly different from control at $p < 0.1$ (field).

† Cumulative gaseous emissions (CO_2, CH_4, N_2O).

‡ Abbreviations: HA, humic acid; WTR, water treatment residuals; ρ_b, bulk density; MRT, mean residence time; GWP, global warming potential; L, laboratory; F, field; I, increase compared to control; D, decrease compared to control.

§ Field experiments, described in detail elsewhere (see Mukherjee et al., 2014b), were carried out using the same soil and amendments types as the laboratory study but with application rate of 0.5 weight % as opposed to 1% for laboratory.

¶ Combining losses of C by both leaching and gaseous pathways.

While the measured rates of gas emissions and soil properties were different, in all but a few cases, the soil amendments had the same effect on the parameters measured in laboratory and field incubations relative to the control soil (Table 2) under several biogeochemical processes (Supplemental Table S2) as described before. For example, CO_2 emissions were decreased by biochar and WTR amendment and increased by HA amendment in both the laboratory incubation and field settings (Table 2). The ρ_b measurement was different in the laboratory setting compared to the field method, which may have been reflected in the trends. However, as a proxy of aeration, only biochar treated soils decreased ρ_b in the laboratory setting, and although all the amendments decreased ρ_b of soil in the field compared to control, the response was only significant with biochar (Table 2). Natural processes such as climatic variability, freeze/thaw cycles, and run-off events are absent in any idealized laboratory setting (Spokas, 2010), and artificial periodic leaching as the case of the present study may only simulate natural rainfall to some extent; optimized microbial activity (Knicker, 2011; Spokas, 2010; Zimmerman, 2010) and absence of crop growth may be the reasons for the apparent differences in some of the trends of field vs. laboratory observations (Table 2). Nevertheless, these observations indicate that laboratory incubations may serve as good proxies for field-scale experiments and may be performed in advance of large-scale field application to predict the effects of particular amendments on specific soils.

SUMMARY

This study reports both gaseous and leached C and N components of columns with different amendments under long-term laboratory settings. The data support the following conclusions:

1. Gaseous emissions from soils were not significantly affected by any amendments because the labile C and N compounds most likely to be oxidized and emitted in gaseous forms in any closed system incubations were either protected via mineral sorption or lost as leachate in the open column system employed in this study.

2. Calculation of MRT of C and N in amended soils based entirely on gaseous losses may be overestimates because losses due to leaching can be significant.

3. Compared to that of the control, only oak biochar significantly enhanced soil-C stability by 79%, considering both leaching and gaseous losses of C.

4. From the results of this laboratory column study and similar field work performed before, it can be stated that analyses from laboratory incubation may serve as reliable proxies for trends in field soil responses to amendments. Irrespective of the differences in two experimental conditions, comparison of lab vs. field experiments demonstrates that trends in field-soil characteristics under amendments could be predicted or established by reliable laboratory settings. More research is needed, especially under field scale, measuring both gaseous and dissolved N losses is a priority to better understand the effects of these amendments on altering N cycling.

ACKNOWLEDGMENTS

The authors thank Mr. Basant Rimal for his generous help in laboratory analyses and Mr. Justin Morse for his help with the construction of the soil columns.

REFERENCES

Aarna, I., and E.M. Suuberg. 1998. Changes in reactive surface area and porosity during char oxidation. Symp. (Int.) Combust. 27:2933–2939.

Agyin-Birikorang, S., and G.A. O'Connor. 2007. Lability of drinking water treatment residuals (WTR) immobilized phosphorus: Aging and pH effects. J. Environ. Qual. 36:1076–1085. doi:10.2134/jeq2006.0535

Ahmad, Z., M.A.M. Abd-Elbasit, M. Inoue, H. Yasuda, T. Honna, and S. Yamamoto. 2012. Use of two industrial wastes as soil amendments: Effect on dissolved reactive phosphorus in runoff. Soil Sed. Contam. 21:207–226. doi:10.1080/15320383.2012.649376

ASTM. 1990. D1762–84: Standard method for chemical analysis of wood charcoal. ASTM International, Philadelphia, PA.

Bell, M.J., and F. Worrall. 2011. Charcoal addition to soils in NE England: A carbon sink with environmental co-benefits? Sci. Total Environ. 409:1704–1714. doi:10.1016/j.scitotenv.2011.01.031

Bruun, E.W., P. Ambus, H. Egsgaard, and H. Hauggaard-Nielsen. 2012. Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. Soil Biol. Biochem. 46:73–79. doi:10.1016/j.soilbio.2011.11.019

Case, S.D.C., N.P. McNamara, D.S. Reay, and J. Whitaker. 2012. The effect of biochar addition on N2O and CO2 emissions from a sandy loam soil—The role of soil aeration. Soil Biol. Biochem. 51:125–134. doi:10.1016/j.soilbio.2012.03.017

Chan, K.Y., L. Van Zwieten, I. Meszaros, A. Downie, and S. Joseph. 2008. Using poultry litter biochars as soil amendments. Aust. J. Soil Res. 46:437–444. doi:10.1071/SR08036

Clough, T., L. Condron, C. Kammann, and C. Müller. 2013. A review of biochar and soil nitrogen dynamics. Agronomy 3:275–293. doi:10.3390/agronomy3020275

Deenik, J.L., T. McClellan, G. Uehara, M.J. Antal, and S. Campbell. 2010. Charcoal volatile matter content influences plant growth and soil nitrogen transformations. Soil Sci. Soc. Am. J. 74:1259–1270. doi:10.2136/sssaj2009.0115

Dempster, D.N., D.B. Gleeson, Z.M. Solaiman, D.L. Jones, and D.V. Murphy. 2012a. Decreased soil microbial biomass and nitrogen mineralisation with Eucalyptus biochar addition to a coarse textured soil. Plant Soil 354:311–324. doi:10.1007/s11104-011-1067-5

Dempster, D.N., D.L. Jones, and D.V. Murphy. 2012b. Organic nitrogen mineralisation in two contrasting agro-ecosystems is unchanged by biochar addition. Soil Biol. Biochem. 48:47–50. doi:10.1016/j.soilbio.2012.01.013

Dendooven, L., L. Patino-Zuniga, N. Verhulst, M. Luna-Guido, R. Marsch, and B. Govaerts. 2012. Global warming potential of agricultural systems with contrasting tillage and residue management in the central highlands of Mexico. Agric. Ecosyst. Environ. 152:50–58. doi:10.1016/j.agee.2012.02.010

Fan, J.H., Z.L. He, L.Q. Ma, Y.G. Yang, X.E. Yang, and P.J. Stoffella. 2011. Immobilization of copper in contaminated sandy soils using calcium water treatment residue. J. Hazard. Mater. 189:710–718. doi:10.1016/j.jhazmat.2011.02.081

Fowles, M. 2007. Black carbon sequestration as an alternative to bioenergy. Biom. Bioenerg. 31:426–432. doi:10.1016/j.biombioe.2007.01.012

Githinji, L. 2013. Effect of biochar application rate on soil physical and hydraulic properties of a sandy loam. Arch. Agron. Soil Sci. 60:457–470. doi:10.1080/03650340.2013.821698

Guerena, D., J. Lehmann, K. Hanley, A. Enders, C. Hyland, and S. Riha. 2013. Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system. Plant Soil 365:239–254. doi:10.1007/s11104-012-1383-4

Hsu, W.M., and Z.Y. Hseu. 2011. Rehabilitation of a Sandy soil with aluminum-water treatment residual. Soil Sci. 176:691–698 10.1097/SS.0b013e318235dd99.

Ippolito, J.A., K.A. Barbarick, and H.A. Elliott. 2011. Drinking water treatment residuals: A review of recent uses. J. Environ. Qual. 40:1–12. doi:10.2134/jeq2010.0242

Jones, D.L., D.V. Murphy, M. Khalid, W. Ahmad, G. Edwards-Jones, and T.H. DeLuca. 2011. Short-term biochar-induced increase in soil CO(2) release is both biotically and abiotically mediated. Soil Biol. Biochem. 43:1723–1731. doi:10.1016/j.soilbio.2011.04.018

Kaiser, K., and G. Guggenberger. 2000. The role of DOM sorption to mineral surfaces in the preservation of organic matter in soils. Org. Geochem. 31:711–725. doi:10.1016/S0146-6380(00)00046-2.

Kasozi, G.N., A.R. Zimmerman, P. Nkedi-Kizza, and B. Gao. 2010. Catechol and humic acid sorption onto a range of laboratory-produced black carbons (biochars). Environ. Sci. Technol. 44:6189–6195. doi:10.1021/es1014423

Keeney, D.R., and D.E. Nelson. 1987. Extraction of exchangeable ammonium, nitrate and nitrite. In: A.L. Page, editor, Methods of soil analysis: Part 2, chemical and microbiological properties. SSSA, Madison, WI. p. 648–649.

Keith, A., B. Singh, and B.P. Singh. 2011. Interactive priming of biochar and labile organic matter mineralization in a smectite-rich soil. Environ. Sci. Technol. 45:9611–9618. doi:10.1021/es202186j

Knicker, H. 2011. Pyrogenic organic matter in soil: Its origin and occurrence, its chemistry and survival in soil environments. Quaternary Int. 243:251–263. doi:10.1016/j.quaint.2011.02.037.

Knoblauch, C., A.-A. Maarifat, E.-M. Pfeiffer, and S.M. Haefele. 2011. Degradability of black carbon and its impact on trace gas fluxes and carbon turnover in paddy soils. Soil Biol. Biochem. 43:1768–1778. doi:10.1016/j.soilbio.2010.07.012.

Kudeyarova, A.Y. 2007. Application of basic chemical concepts to understanding the formation and transformation mechanisms of humic substances: A revue of publications and own experimental data. Euras. Soil Sci. 40:934–948. doi:10.1134/s1064229307090037.

Kuzyakov, Y., I. Subbotina, H.Q. Chen, I. Bogomolova, and X.L. Xu. 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by C-14 labeling. Soil Biol. Biochem. 41:210–219. doi:10.1016/j.soilbio.2008.10.016

Laird, D., P. Fleming, B.Q. Wang, R. Horton, and D. Karlen. 2010. Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 158:436–442. doi:10.1016/j.geoderma.2010.05.012

Lehmann, J. 2007. Bio-energy in the black. Front. Ecol. Environ 5:381–387. doi:10.1890/1540-9295(2007)5[381:BITB]2.0.CO;2

Lehmann, J., J.P. da Silva, C. Steiner, T. Nehls, W. Zech, and B. Glaser. 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: Fertilizer, manure and charcoal amendments. Plant Soil 249:343–357. doi:10.1023/A:1022833116184

Lehmann, J., J. Gaunt, and M. Rondon. 2006. Bio-char sequestration in terrestrial ecosystems- a review. Mitig. Adapt. Strategies Glob. Change 11:395–419.

Lehmann, J., M.C. Rillig, J. Thies, C.A. Masiello, W.C. Hockaday, and D. Crowley. 2011. Biochar effects on soil biota—A review. Soil Biol. Biochem. 43:1812–1836. doi:10.1016/j.soilbio.2011.04.022

Liang, B., J. Lehmann, D. Solomon, J. Kinyangi, J. Grossman, B. O'Neill, J.O. Skjemstad, J. Thies, F.J. Luizao, J. Petersen, and E.G. Neves. 2006. Black carbon increases cation exchange capacity in soils. Soil Sci. Soc. Am. J. 70:1719–1730. doi:10.2136/sssaj2005.0383

Lin, Y., P. Munroe, S. Joseph, and R. Henderson. 2012. Migration of dissolved organic carbon in biochars and biochar-mineral complexes. Pesquisa Agropecu. Bras. 47:677–686. doi:10.1590/S0100-204X2012000500007

Mackowiak, C.L., P.R. Grossl, and B.G. Bugbee. 2001. Beneficial effects of humic acid on micronutrient availability to wheat. Soil Sci. Soc. Am. J. 65:1744–1750. doi:10.2136/sssaj2001.1744

Mahmoud, E.K. 2011. Heavy metal reductions in soils amended with compost and water treatment residuals. Compost Sci. Util. 19:69–73. doi:10.1080/1065657X.2011.10736978

Major, J., J. Lehmann, M. Rondon, and C. Goodale. 2010. Fate of soil-applied black carbon: Downward migration, leaching and soil respiration. Glob. Change Biol. 16:1366–1379. doi:10.1111/j.1365-2486.2009.02044.x

Major, J., M. Rondon, D. Molina, S.J. Riha, and J. Lehmann. 2012. Nutrient leaching in a Colombian savanna Oxisol amended with biochar. J. Environ. Qual. 41:1076–1086. doi:10.2134/jeq2011.0128

Miller, M.L., J.H. Bhadha, G.A. O'Connor, J.W. Jawitz, and J. Mitchell. 2011. Aluminum water treatment residuals as permeable reactive barrier sorbents to reduce phosphorus losses. Chemosphere 83:978–983. doi:10.1016/j.chemosphere.2011.02.014

Motojima, H., P. Yamada, M. Irie, M. Ozaki, H. Shigemori, and H. Isoda. 2012. Amelioration effect of humic acid extracted from solubilized excess sludge on saline-alkali soil. J. Mater. Cycles Waste Manage. 14:169–180. doi:10.1007/s10163-012-0056-y

Mukherjee, A., R. Hamdan, W.T. Cooper, and A.R. Zimmerman. 2014a. Physicochemical changes in biochars and biochar-amended soils after 15-month of field-aging. Solid Earth Discuss. 6:731–760. doi:10.5194/sed-6-731-2014

Mukherjee, A., and R. Lal. 2013. Biochar impacts on soil physical properties and greenhouse gas emissions. Agronomy 3:313–339. doi:10.3390/agronomy3020313

Mukherjee, A., and R. Lal. 2014. The biochar dilemma. Soil Res. 52:217–230.

Mukherjee, A., R. Lal, and A.R. Zimmerman. 2014b. Effects of biochar and other amendments on the physical properties and greenhouse gas emissions of an artificially degraded soil. Sci. Total Environ. 487C:26–36.

Mukherjee, A., and A.R. Zimmerman. 2013. Organic carbon and nutrient release from a range of laboratory-produced biochars and biochar–soil mixtures. Geoderma 193–194:122–130. doi:10.1016/j.geoderma.2012.10.002

Mukherjee, A., A.R. Zimmerman, and W.G. Harris. 2011. Surface chemistry variations among a series of laboratory-produced biochars. Geoderma 163:247–255. doi:10.1016/j.geoderma.2011.04.021

Nelson, D.W., and L.E. Sommers. 1996. Total carbon, organic carbon, and organic matter. In: D.L. Sparks et al., editors, Methods of soil analysis, Part 3; Chemical methods. SSSA Book Ser. 5. SSSA, Madison, WI. p. 961–1010.

Novak, J.M., W.J. Busscher, D.L. Laird, M. Ahmedna, D.W. Watts, and M.A.S. Niandou. 2009. Impact of biochar amendment on fertility of a southeastern Coastal Plain soil. Soil Sci. 174:105–112.

Paul, E.A., H.P. Collins, and S.W. Leavitt. 2001. Dynamics of resistant soil carbon of Midwestern agricultural soils measured by naturally occurring 14C abundance. Geoderma 104:239–256. doi:10.1016/S0016–7061(01)00083–0.

Peng, X., L.L. Ye, C.H. Wang, H. Zhou, and B. Sun. 2011. Temperature- and duration-dependent rice straw-derived biochar: Characteristics and its effects on soil properties of an Ultisol in southern China. Soil Tillage Res.

112:159–166. doi:10.1016/j.still.2011.01.002

Piccolo, A., G. Pietramellara, and J.S.C. Mbagwu. 1996. Effects of coal derived humic substances on water retention and structural stability of Mediterranean soils. Soil Use Manage. 12:209–213. doi:10.1111/j.1475-2743.1996.tb00545.x

Rogovska, N., D. Laird, R. Cruse, P. Fleming, T. Parkin, and D. Meek. 2011. Impact of biochar on manure carbon stabilization and greenhouse gas emissions. Soil Sci. Soc. Am. J. 75:871–879. doi:10.2136/sssaj2010.0270

Rondon, M., J. Ramirez, and J. Lehmann. 2005. Charcoal additions reduce net emissions of greenhouse gases to the atmosphere. Paper presented at: 3rd USDA Symposium on Greenhouse Gases and Carbon Sequestration, Baltimore, MD. 21–24 Mar.

Santos, F., M.S. Torn, and J.A. Bird. 2012. Biological degradation of pyrogenic organic matter in temperate forest soils. Soil Biol. Biochem. 51:115–124. doi:10.1016/j.soilbio.2012.04.005

Sarkhot, D.V., A.A. Berhe, and T.A. Ghezzehei. 2012. Impact of biochar enriched with dairy manure effluent on carbon and nitrogen dynamics. J. Environ. Qual. 41:1107–1114. doi:10.2134/jeq2011.0123

SAS. 2012. SAS version 9.2. SAS Institute Inc., Cary, NC.

Senesi, N., and C. Plaza. 2007. Role of humification processes in recycling organic wastes of various nature and sources as soil amendments. Clean-Soil Air Water 35:26–41. doi:10.1002/clen.200600018

Sharif, M., R.A. Khattak, and M.S. Sarir. 2002. Effect of different levels of lignitic coal derived humic acid on growth of maize plants. Commun. Soil Sci. Plant Anal. 33:3567–3580. doi:10.1081/CSS-120015906

Shrestha, R.K., R. Lal, and C. Penrose. 2009. Greenhouse gas emissions and global warming potential of reclaimed forest and grassland soils. J. Environ. Qual. 38:426–436. doi:10.2134/jeq2008.0283

Sigmaplot. 2012. Sigmaplot version 12. Scientific Software Solutions Internationals, San Jose, CA.

Singh, B.P., A.L. Cowie, and R.J. Smernik. 2012. Biochar carbon stability in a clayey soil as a function of feedstock and pyrolysis temperature. Environ. Sci. Technol. 46:11,770–11,778. doi:10.1021/es302545b

Six, J., and J.D. Jastrow. 2002. Organic matter turnover. In: R. Lal, editor, Encyclopedia of soil science. Marcel Dekker, New York. p. 936–942.

Smith, J.L., H.P. Collins, and V.L. Bailey. 2010. The effect of young biochar on soil respiration. Soil Biol. Biochem. 42:2345–2347. doi:10.1016/j.soilbio.2010.09.013

Soler-Rovira, P., E. Madejon, P. Madejon, and C. Plaza. 2010. In situ remediation of metal-contaminated soils with organic amendments: Role of humic acids in copper bioavailability. Chemosphere 79:844–849. doi:10.1016/j.chemosphere.2010.02.054

Spokas, K.A. 2010. Review of the stability of biochar in soils: Predictability of O:C molar ratios. Carbon Manage. 1:289–303. doi:10.4155/cmt.10.32

Spokas, K.A., K.B. Cantrell, J.M. Novak, D.W. Archer, J.A. Ippolito, H.P. Collins, A.A. Boateng, I.M. Lima, M.C. Lamb, A.J. McAloon, R.D. Lentz, and K.A. Nichols. 2012. Biochar: A synthesis of its agronomic impact beyond carbon sequestration. J. Environ. Qual. 41:973–989. doi:10.2134/jeq2011.0069

Spokas, K.A., W.C. Koskinen, J.M. Baker, and D.C. Reicosky. 2009. Impacts of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. Chemosphere 77:574–581. doi:10.1016/j.chemosphere.2009.06.053

Spokas, K.A., and D.C. Reicosky. 2009. Impacts of sixteen different biochars on soil greenhouse gas production. Ann. Environ. Sci. 3:179–193.

Steinbeiss, S., G. Gleixner, and M. Antonietti. 2009. Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biol. Biochem. 41:1301–1310. doi:10.1016/j.soilbio.2009.03.016

Tahir, M.M., M. Khurshid, M.Z. Khan, M.K. Abbasi, and M.H. Kazmi. 2011. Lignite-derived humic acid effect on growth of wheat plants in different soils. Pedosphere 21:124–131. doi:10.1016/S1002-0160(10)60087-2

Troy, S.M., P.G. Lawlor, C.J. O' Flynn, and M.G. Healy. 2013. Impact of biochar addition to soil on greenhouse gas emissions following pig manure application. Soil Biol. Biochem. 60:173–181. doi:10.1016/j.soilbio.2013.01.019.

USEPA. 2007. Inventory of USA greenhouse gas emissions and sinks: 1990–2005. USEPA, Washington, DC.

van Zwieten, L., B. Singh, S. Joseph, S. Kimber, A. Cowie, and K. Yin Chan. 2009. Biochar and emissions of non-CO2 greenhouse gases from soil. In: J. Lehmann and S. Joseph, editors, Biochar for environmental management. Routledge, New York.

Zhang, A.F., Y.M. Liu, G.X. Pan, Q. Hussain, L.Q. Li, J.W. Zheng, and X.H. Zhang. 2012. Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain. Plant Soil 351:263–275. doi:10.1007/s11104-011-0957-x

Zimmerman, A.R. 2010. Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). Environ. Sci. Technol. 44:1295–1301. doi:10.1021/es903140c

Zimmerman, A.R., and B. Gao. 2013. The stability of biochar in the environment, biochar and soil biota. CRC Press, Boca Raton, FL.

Zimmerman, A.R., B. Gao, and M.Y. Ahn. 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. Soil Biol. Biochem. 43:1169–1179. doi:10.1016/j.soilbio.2011.02.005