Impact of Biochar on Manure Carbon Stabilization and Greenhouse Gas Emissions

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Previous studies indicate that biochar additions sometimes increase soil respiration and CO₂ emissions which could partially offset C credits associated with soil biochar applications. Little is known, however, about the impact of biochar on the mineralization of manure in soil systems and how interactions between biochar and manure impact C sequestration and greenhouse gas (GHG) emissions from soils. We studied the effect of biochar and dried-swine manure additions on changes in soil bulk density (Dₚ), total soil organic carbon (SOC), and emissions of N₂O and CO₂ during a 500-d soil column incubation study. The addition of biochar to the soil increased SOC content measured after the 500-d incubation by 17.6 to 68.8%, depending on the treatment. Biochar additions reduced N₂O emissions measured once near the end of the incubation. The N₂O emissions were weakly correlated with Dₚ, suggesting that enhanced soil aeration contributed to the reductions in N₂O emissions. Biochar additions consistently increased CO₂ emissions (measured 13 times during the incubation) relative to no-biochar controls with cumulative CO₂-C emissions equivalent to 17 to 23% of biochar C applied. However, a distinct biochar-by-manure interaction for CO₂ flux indicated that biochar either helped stabilize manure C or the presence of manure reduced the effect of biochar on the mineralization of SOC. For the studied system, we conclude that biochar additions sequestered large amounts of highly stable C, reduced N₂O emissions, increased CO₂ emissions from the soils, and reduced rates of CO₂ emissions following a manure addition.

Abbreviations: Dₚ, soil bulk density; GHG, greenhouse gas; HDS, honest significant difference; SOC, soil organic carbon; WFPS, water-filled pore space.

The Intergovernmental Panel on Climate Change (2007) reported that human activities are responsible for rapidly increasing atmospheric concentrations of CO₂ and N₂O, and that these GHGs are contributing to changes in global climate. Agriculture is one of the main contributors of GHGs to the atmosphere, but also has the potential to mitigate global climate change through the adoption of best management practices that reduce GHG emissions and increase C sequestration in soils.

Increasing the levels of SOC by conventional soil management practices such as conservation tillage, no-till, and perennial cropping systems can take many years, and there is a significant uncertainty about the C sequestration potential of these systems (Baker et. al., 2007; Denman et al., 2007). By contrast, biochar applications to agricultural soils is rapidly emerging as a new management option with the potential for long-term sequestration of large amounts of C in the soil (Glaser et al., 2002; McHenry, 2008; Steinbeiss et al., 2009; Tenenbaum, 2009). Biochar, also termed as charcoal, agrichar, and black carbon, is a co-product of pyrolysis, the thermochemical decomposition of organic material in the absence of oxygen (Scott and Piskorz, 1984). During this process, 20 to 50% of biomass C is converted to recalcitrant forms of C (Mok et al., 1992). Therefore, as noted by Laird (2008), pyrolysis coupled with C return through biochar applications addresses the dilemma of soil degradation from the systematic biomass removal for bioenergy production.

Biochar is effective for sequestering large amounts of C because of its inherent stability in soil environments. The chemistry of biochar is complex (Masiello,
forcing that is 298 times that of CO₂. Nitrous oxide is emitted under optimal laboratory conditions, which led Kuzyakov et al. (2009) to suggest a half-life of biochar under natural soil conditions of about 1400 yr.

There are many uncertainties related to estimation of mineralization rates of biochar in soils. For example, the half-life of biochar may be affected by water regimes; Nguyen and Lehmann (2009) reported that C loss under unsaturated and alternating saturated-unsaturated conditions was significantly higher than under saturated conditions, indicating that oxidation of biochar is likely a major mechanism controlling its stability. The presence of easily metabolized organic C has been shown to accelerate biochar decomposition (Hamer et al., 2004; Kuzyakov et al., 2009), suggesting that co-metabolism contributes to biochar decomposition in soils. Furthermore, the presence of biochar has been shown to accelerate the mineralization of glucose in sand–biochar–glucose systems (Hamer et al., 2004) and appears to accelerate decomposition of forest floor humus based on the mass loss of humus in litter bags amended with biochar, humus, and a 50:50 mix of humus and biochar (Wardle et al., 2008). This stimulating effect was explained by increased nutrient retention in the biochar plus humus litterbags, which led to increased microbial activity and decomposition of humus compared with humus in litter bags without biochar. In contrast, Kuzyakov et al. (2009) showed that biochar application to soils had no effect on SOC mineralization and CO₂ flux from soil and slightly reduced CO₂ flux from loess. They suggested that sorption of nutrients and easily metabolized organic C by biochar limited nutrient availability and microbial activity in the loess systems. Spokas and Reicosky (2009) reported both increases and decreases in CO₂ emissions from soils amended with 16 different types of biochar, suggesting that biochar quality has a significant influence on the interaction between biochar and soil organic matter.

In addition to the roles of biochar as a C sequestration agent and its influence on native SOC, biochar was shown to influence emissions of N₂O, a potent GHG with an estimated radiative forcing that is 298 times that of CO₂. Nitrous oxide is emitted from soils during microbially-driven processes of nitrification and denitrification (Bremner and Blackmer, 1981; Firestone and Davidson, 1989) with the possibility of both of these processes occurring simultaneously within the same soil aggregate due to micro-site variability (Kuenen and Robertson, 1994; Granli and Bockman, 1994). Oxygen supply in the soil governs the relative contribution of those processes to the total amount of N₂O emitted. In aerobic incubation studies, N₂O production has been primarily attributed to nitrification (Bremner and Blackmer, 1978, 1979), denitrification (Paul et al., 1993; Morkved et al., 2006), or equal contributions from both (Khalil et al., 2004; Carter, 2007). Any process or treatment that influences O₂ activity in the soil will have an indirect effect on N₂O flux through its impact on nitrification and/or denitrification processes. This can explain the 98% N₂O flux suppression observed in a short-term incubation study after rewetting biochar-amended soil to 78% water-filled pore space (WFPS; Yanai et al., 2007). The authors speculated that added biochar adsorbed water and improved aeration of the soil, leading to a suppression of N₂O production. Rewetting the soil to 83% WFPS, however, simulated N₂O production from biochar-amended soils. Preliminary results of a greenhouse experiment indicated that presence of biochar in soil reduced N₂O emissions by 80% (Rondon et al., 2005). While the mechanism of reduction is not completely understood, Lehmann (2007) suggested that addition of biochar will increase C to N ratio of soil, resulting in reduced net N mineralization (and nitrification) rates. On the other hand, DeLuca et al. (2006) reported that addition of biochar to grassland soils with naturally high rates of net nitrification had no effect on the nitrification potential. Moreover, addition of biochar along with NH₄⁺ to forest soils with naturally low rates of net nitrification was shown to increase the nitrification potential.

It is clear that biochar has an effect on net GHG emissions both directly through sequestration of biochar C and indirectly through altering the physical, chemical, and microbiological properties of soils. Little is known, however, about the impact of biochar on the mineralization of additional source of organic material such as manure in soil systems and how interactions between biochar and manure impact C sequestration and GHG emissions from soils.

MATERIALS AND METHODS

The soil used in this study was a Clarion (fine-loamy, mixed, superactive, Mesic Typic Hapludoll) collected from a fallow strip between tillage trials at the Iowa State University Agronomy and Agricultural Engineering Research Farm in Boone County, Iowa. Surface soil (0–15 cm, pH 6.4) was stored field moist in plastic buckets with tight closing lids to prevent moisture loss. The soil was used within 1 mo of collection.

Lump charcoal > 1 cm was obtained from a commercial producer who uses mixed hardwood (primarily oak [Quercus spp.] and hickory [Carya spp.]) and slow pyrolysis (kilns) at about 450–500°C to produce charcoal primarily for the steel industry. The lump charcoal contained 71.5% total C and 0.72% total N by mass (determined by dry combustion using a Carlo-Erba NA1500 NSC elemental analyzer), and 63.8% fixed C, 19.7% volatiles, 13.9% ash, and 2.6% moisture by proximate analysis (performed by Hazen Research, Golden, CO). Measured BET surface area of the charcoal was 19.1 m² g⁻¹ and pH in water (1:2 solid–liquid) was 7.6 (Laird et al., 2010b). The lump charcoal was ground in a hammer mill and the <0.5 mm fraction was separated by dry sieving. Hereafter we refer to this <0.5 mm charcoal as biochar.

Soil columns (7.7-cm i.d. by 25 cm length = 1164 cm³ volume) were constructed of PVC tubing and PVC end caps. A drain tube was attached to the bottom of each column. A small amount of fiberglass was stuffed into the drain opening at the base of the columns, and then 100 g of coarse sand (2–5 mm) was placed in the bottom of each column. The columns were randomly assigned to one of the treatments in a randomized block design with three replicates. A 3.2-yr incubation study with 14C labeled biochar showed decomposition rates of 0.5% per year under optimal laboratory conditions, which led Kuzyakov et al. (2009) to suggest a half-life of biochar under natural soil conditions of about 1400 yr.
sand filled the concave portion of the end cap which protruded below the base of the PVC column.

Batches of field moist soil (20 kg) were tumbled in a rotary cement mixer for 20 min. During the tumbling treatment, biochar was slowly added to the soil to bring the final biochar content to 0, 5, 10, and 20 g kg⁻¹ soil (oven dry weight equivalent). The tumbling treatments produced roughly spherical soil aggregates ~1 cm in diameter.

The soil columns were packed with 1 kg (oven dry weight equivalent) of soil by tamping the columns as the soil was added. All columns were intentionally packed to an initial Db of ~1.1 g cm⁻³. Bulk density was determined by measuring the depth from the top of the column to the soil surface (average of four measurements). This measure was used to estimate the volume of the head space above the soil which was deducted from the known total volume of the column discounting the volume of sand in the bottom of each column. Thus, our estimates of Db are averages for the whole column. Mean and standard deviation values of Db for particular treatments were derived from six replications.

Treatments were randomly assigned to columns and replicated six times for a total of 48 columns. The columns were incubated in a constant temperature room (25°C and 80% relative humidity) for the duration of the study. On Day 79 of the incubation, 5 g of dried and ground swine manure were added to half of the columns. The manure was incorporated into the top 3 cm of the columns by micro tillage using a laboratory spatula. Control columns not receiving manure were tilled in a similar manner. This soil disturbance had no detectable effect on long-term changes in Db. Once each week during the incubation, all columns were leached with 200 mL of 0.005 M CaCl₂. The leachate was discontinued and air from the headspace was recirculated through the infrared gas analyzer. For each column, the CO₂ concentrations were determined by measuring the depth from the top of the column to the soil surface (average of four measurements). This measure was used to estimate the volume of the head space above the soil which was deducted from the known total volume of the column discounting the volume of sand in the bottom of each column. Thus, our estimates of Db are averages for the whole column. Mean and standard deviation values of Db for particular treatments were derived from six replications.

Measurements of Carbon Dioxide Emissions

Periodically during the incubation, an end cap was placed on top of the columns and CO₂ emissions were measured using an infrared gas analyzer. Measurements were taken 24 h after leaching of the soil columns with 200 mL of 0.005 M CaCl₂. At the time of the gas measurements, soil was at field capacity. Initial attempts to measure CO₂ were not successful as the background CO₂ concentration in the constant-temperature room gradually increased with the length of time that people were in the room. The measurement protocol was changed to include an initial purge of the headspace above each column using CO₂–free air. When the CO₂ levels in the headspace dropped to 0, the purge was discontinued and air from the headspace was recirculated through the infrared gas analyzer. For each column, the CO₂ concentrations were recorded at 30, 60, 90, 120, and 150 sec after the start of headspace air recirculation. The data were fitted to a linear model and the slope was used to estimate the flux of CO₂. Changes in CO₂ concentrations during the first 30 sec were not included in the estimation of CO₂ flux to minimize any effects of CO₂ diffusion from soil. The change in volumetric concentration was converted to a mass flux by using the ideal gas law and are expressed as µg CO₂–C m⁻² h⁻¹.

Cumulative release of CO₂–C evolved during a period of 268 d (Days 54–322) was calculated as described by Li et al. (2009). Cumulative release of CO₂–C for the remainder of the 500-d (before Day 54 and after Day 322) incubation period were predicted based on slopes of linear regressions fitted to the first or last three CO₂–C flux measurements. Analysis of variance was performed on the cumulative CO₂–C emission data to test for the main effects of the treatments and their interactions. The least square means of cumulative CO₂ emissions were calculated for each treatment and differences were examined using Tukey’s HSD (honest significance difference). All statistical analyses were performed using SAS software (SAS Institute, Cary, NC). Graphical representations of the data were made in SigmaPlot (Systat Software, Inc., Chicago, IL).

Measurements of Nitrous Oxide Emissions

Nitrous oxide emissions were measured only once on Day 414 of the incubation. Measurements were taken 24 h after leaching of the soil columns with 200 mL of 0.005 M CaCl₂. At the time of the gas measurements soil was at field capacity. Gas samples were collected by fitting an end cup on the top of each column. The end cups had an opening fitted with butyl rubber septa, through which a syringe needle was inserted and 10-mL gas samples were withdrawn. Headspace N₂O concentrations were determined at 15-min intervals for each column, and after 1 h, columns were reopened. The headspace volume ranged from 0.46 to 0.60 L depending on Db. Air samples were immediately transferred to previously evacuated air tight glass vials (6 mL) fitted with butyl rubber septa. Samples were analyzed for N₂O with an SRI gas chromatograph. Nitrous oxide was measured using a 63Ni electrode capture detector, with a stainless steel column (HaySepD, 0.3175 cm diam. by 74.54 cm long). The N₂O flux was determined from concentration plotted against time. The changes in volumetric concentration were converted to a mass flux using the ideal gas law and are expressed as µg N₂O-N m⁻² h⁻¹ (Holland et al., 1999).

Diagonostics performed on the N₂O data revealed heterogeneity of variance, therefore the Box-Cox procedure (Box and Cox, 1964) was used to find a suitable transformation. Analysis of variance was performed on the log-transformed N₂O data to test for the main effects of the treatments and their interactions. The least square means of log transformed N₂O flux were calculated for each treatment and differences were examined using Tukey’s HSD. A model using weighted least squares was fitted to describe the relationship between Db and N₂O flux.

Soil Analysis

On termination of the incubation experiment (Day 500), the intact soil cores were removed from the columns using Ar gas pressure supplied through drain openings at the bottom of the columns to help push the soil core out of the columns. The soil cores were sectioned into 0 to 3 cm (depth of manure incorporation), 3 to 6 cm, and 6-cm bottom depth segments. Samples were air-dried, ground, and analyzed for concentrations of C by dry combustion using a Carlo Erba NA1500 NSC elemental analyzer (Haake Buchler Instruments, Paterson, NJ). Carbon concentrations were determined twice for each sample and the average for the two determinations was used. As the pH of the original soil was 6.4 and pH values for all soil samples excavated from the
columns was <7.5 (Laird et al., 2010b), the total C measurements are assumed to represent only organic C. The total mass of C in each column was determined as the sum of products for SOC concentration and soil mass for each depth segment. The mass of each depth segment was estimated using the total mass of soil added to each column, the volume of the increment, and average column D_b determined on Day 481 of the incubation. Diagnostics performed on the SOC data revealed heterogeneity of variance, therefore the Box-Cox procedure (Box and Cox, 1964) was used to find a suitable transformation. Analysis of variance was performed on the log-transformed SOC data to test for the main effects of the treatments and their interactions. The least square means of the increments, and average column D_b determined on Day 481 of the incubation, the Db for columns receiving biochar averaged 7% lower than the D_b of the control columns. Similar results were observed by Oguntunde et al. (2008) who reported 9% lower D_b for charcoal kiln-site soils compared with adjacent field soils. The effect of biochar on D_b can be partially explained by the low particle density (high internal microporosity) of biochar. This explanation, however, does not account for the nonlinear response of D_b to the amount of biochar added (Fig. 1; Laird et al., 2010b). Another possible explanation for the reduced D_b is that the biochar amendments increased aggregate stability. Piccolo et al. (1997) reported that soils amended with coal-derived humic acid had greater total porosity and macroporosity, and lower D_b relative to controls; and Glaser et al. (2002) hypothesized that coal-derived humic acids were bonded to minerals forming organo-mineral complexes with exposed hydrophobic polyaromatic backbones that reduced penetration of water into the pores leading to increased aggregate stability.

Addition of manure did not have a significant effect on D_b in this soil-column study (Fig. 1). In field experiments, application of manure has previously been shown to decrease D_b (Tiarks et al., 1974; Unger and Stewart, 1974). This decrease was attributed to the dilution effect, resulting from mixing of lower density organic material with the higher density mineral fraction of the soil, as well as increased macroporosity due to improved aggregate stability.

Soil Organic Carbon Content

The C and N content of the biochar used in this study was 71.54 and 0.72%, respectively (Laird et al., 2010b). It is not surprising; therefore, that application of biochar significantly increased the SOC content measured at the end of the study. Depending on the rate, biochar application significantly increased SOC from an average of 20.5 g kg⁻¹ in the control columns to 24.1, 28.3, and 34.6 g kg⁻¹ C in the columns receiving 5, 10, and 20 g of biochar, respectively (Table 1, Fig. 2), which correspond to 17, 38, and 69% increases in SOC, respectively, relative to the 0 g kg⁻¹ biochar controls (Table 1).

Application of 5 g manure (41.27% C and 3.51% N) on Day 79 of the incubation supplied an additional 2.06 g of C and 0.18 g of N to the manure-amended columns. Soil analysis performed at the end of the incubation period indicated that application of manure increased SOC content in the top 3 cm of soil, the depth of manure incorporation, but this increase was significant only for columns amended with 10 g kg⁻¹ biochar (Fig. 2).

Nitrous Oxide Emission

Soil columns amended with the highest rate of biochar emitted significantly less N₂O than control columns for one observation made 414 d after the biochar addition (Table 2, Fig. 3). Our observations are consistent with the results presented by

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RESULTS AND DISCUSSION

Soil Bulk Density

The soil in the columns was packed to an original D_b of ~1.1 g cm⁻³ for all treatments. Over time, soil in all of the columns compacted due to the effects of gravity and the weekly leaching events; however, columns receiving biochar treatments compacted at a slower rate than the control columns (Fig. 1). By the end of incubation, the D_b for columns receiving biochar averaged 7% lower than the D_b of the control columns. Similar results were observed by Oguntunde et al. (2008) who reported 9% lower D_b for charcoal kiln-site soils compared with adjacent field soils. The effect of biochar on D_b can be partially explained by the low particle density (high internal microporosity) of biochar. This explanation, however, does not account for the nonlinear response of D_b to the amount of biochar added (Fig. 1; Laird et al., 2010b). Another possible explanation for the reduced D_b is that the biochar amendments increased aggregate stability. Piccolo et al. (1997) reported that soils amended with coal-derived humic acid had greater total porosity and macroporosity, and lower D_b relative to controls; and Glaser et al. (2002) hypothesized that coal-derived humic acids were bonded to minerals forming organo-mineral complexes with exposed hydrophobic polyaromatic backbones that reduced penetration of water into the pores leading to increased aggregate stability.

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Soil columns amended with the highest rate of biochar emitted significantly less N₂O than control columns for one observation made 414 d after the biochar addition (Table 2, Fig. 3). Our observations are consistent with the results presented by
several authors (Yanai et al., 2007; Singh et al., 2010; Cayuela et al., 2010) but not Clough et al. (2010), who reported increases in N$_2$O emissions associated with urine patches on biochar amended soils. Soil columns amended with 0, 5, 10, and 20 g kg$^{-1}$ biochar emitted on average 10.1, 2.0, 1.8, and 1.2 µg N$_2$O-N m$^{-2}$ h$^{-1}$, respectively. Biochar columns that received manure exhibited similar reductions in N$_2$O emissions; however, the main effect of manure was not significant (Table 2). Even though manure supplied additional N, fluxes of N$_2$O between manure-and non-amended columns were not statistically different 335 d after the manure application, suggesting that excess N supplied with the manure had been lost to leaching (Laird et al., 2010a) or denitrification by the time N$_2$O fluxes were measured.

The reduction in N$_2$O flux for biochar-amended columns was weakly correlated with the reduction in D$_b$ ($R^2 = 0.34$, $P < 0.01$). As discussed above, reduced D$_b$ due to biochar application implies increased porosity and probably increased oxygen diffusion within the soil. This observation is in agreement with results of other studies that documented substantial differences in N$_2$O flux between compacted and uncompacted agricultural soils (Bhandral et al., 2007). The observed differences can be attributed to reduction in total pore volume due to compaction, and hence increased WFPS, decreased oxygen diffusion through soil and increased denitrification rates (Ruser et al., 2006; Ball et al., 2008). Assuming that most of the N$_2$O evolved during denitrification, we hypothesize that the decrease in D$_b$ due to biochar addition led to fewer anaerobic sites and, therefore, reduced rates of denitrification.

It is noteworthy that there were no significant differences in N$_2$O flux between columns amended with 5, 10, or 20 g of biochar kg$^{-1}$ of soil and no significant differences in D$_b$ were observed for those treatments at the time the N$_2$O measurements were performed. Spokas et al. (2010) recently attributed reductions in N$_2$O emissions to the production of ethylene by biochar and its inhibiting effect on nitrification. In this study, we did not measure ethylene.

**Carbon Dioxide Emission**

Among columns not receiving manure, biochar application significantly increased CO$_2$ emissions for all measurement dates during the incubation (Fig. 4). The stepwise increase in CO$_2$ emissions with increasing biochar additions may be partially explained by decreases in D$_b$. A decrease in D$_b$ will in general enhance gas exchange and increase the partial pressure of O$_2$ in the soil pores, which should increase the activity of aerobic microorganisms. Greater CO$_2$ emissions from biochar-amended soil may also be attributed to inner porosity of biochar. As noted by Pietikäinen et al. (2000), the high internal porosity and high capacity of biochar to adsorb organic compounds, may enhance the habitat for microorganisms, and accelerate the decomposition of adsorbed organic compounds. In a study comparing the capacity of several adsorbents with similar internal porosity to bind organic compounds from leaf litter extracts, it was found that activated C had the greatest capacity to adsorb the dissolved organic compounds followed by charcoal and pumice (Pietikäinen et al., 2000). Basal respiration for the charcoal, however, was three to four times greater than for pumice and activated C (Pietikäinen et al., 2000), suggesting that biochar pores and surfaces are an excellent habitat for soil microorganisms (Warnock et al., 2007).

The observed stimulatory effect of biochar application on soil respiration is in agreement with the findings of Wardle et al. (2008), who observed that mass loss of humus in litterbags containing both charcoal and humus in Swedish forest soils was much higher than that of charcoal-only treatments.

<table>
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<th>Source</th>
<th>df</th>
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<th>N$_2$O MS</th>
<th>N$_2$O F value</th>
<th>CO$_2$ SS</th>
<th>CO$_2$ MS</th>
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<td>2.69</td>
<td>2.69</td>
<td>75.12**</td>
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<tr>
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<td>0.81</td>
<td>0.27</td>
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** Significant at the 0.01 level.
† NS, not significant.
greater than predicted if the two components were considered separately. Considered in isolation, the accelerated rate of respiration implies an accelerated rate of soil organic matter mineralization, which as noted by Wardle et al. (2008), would partially offset the ability of biochar to sequester C in soils. However, an increased rate of microbial activity also implies enhanced nutrient cycling, which coupled with decreased nutrient leaching (Laird et al., 2010) and hence enhanced C fixation through photosynthesis. The balance between increased C input through photosynthesis and enhanced SOC mineralization will influence the net impact of biochar on soil C sequestration.

Application of a readily available source of C to soils typically causes a rapid increase in microbial metabolic activity and a sharp increase in soil respiration. However, this increase in respiration is relatively short-lived as CO$_2$ emissions soon peak and then rapidly diminish due to exhaustion of the readily-available substrate (De Nobili et al., 2001; Dumale et al., 2009). An increase in respiration was observed in the present study as manure application on Day 79 of the incubation significantly increased CO$_2$ emissions measured on Days 83, 90, 97, 104, and 111 for all manure-amended columns (Fig. 4). On Days 157, 187, and 322 (78–243 d after addition of manure), there was no significant difference in CO$_2$ emissions between no-biochar control columns that received manure (B0M) and those that did not receive manure (B0), indicating that microbial activity had declined to original levels. On Days 187 and 322 however, CO$_2$ emissions from biochar-amended columns that received manure were significantly lower than CO$_2$ emissions from biochar-amended columns that did not receive manure. This suggests that, after having exhausted the readily available C, the microbial population was not able to mineralize the remaining biogenic soil organic matter as efficiently.

Cumulative release of CO$_2$–C for the 268 d during which CO$_2$ emissions were measured (Days 54–322 of the incubation) showed a linear increase in C losses with increasing biochar applications for both manured and nonmanured columns (Fig. 5). There was a significant interaction between biochar and manure applications on cumulative CO$_2$–C emissions (Table 2). Application of manure to columns that received no biochar resulted in a 36% increase in cumulative CO$_2$–C emissions; while application of manure to biochar-amended columns resulted in 24, 11, and 8% increases in cumulative CO$_2$–C emissions for B5M, B10M and B20M treatments, respectively for the period of monitoring (Days 54–322 of the incubation). Significant differences in
cumulative CO$_2$–C emissions between manure and no-manure biochar treatments were observed only for B0 and B5 treatments (Fig. 5). While cumulative CO$_2$ emissions from manure- and biochar-amended columns was greater than from columns that received biochar only, by the end of the study, CO$_2$ losses from columns receiving both manure and biochar were increasing at a slower rate than from columns that received biochar only (Fig. 6A). Although manure was incorporated only to a depth of 3 cm in the soil columns, dissolved organic compounds leached from the manure likely came in contact with biochar that was distributed throughout the column.

Subtracting cumulative CO$_2$–C emissions for the no-manure columns (B0, B5, B10, and B20) from the corresponding cumulative CO$_2$–C emissions for the manure-amended columns (B0M, B5M, B10M, and B20M, respectively) would, in the absence of a manure-by-biochar interaction, yield a mass balance estimate of the amount of manure C mineralized during the incubation period (Fig. 6B). Differences in cumulative CO$_2$–C emissions for the no-biochar controls (B0M-B0) indicate the rapid mineralization of manure C (Days 83–124), after which there was very little difference in the rate of CO$_2$ flux between the B0M and B0 columns. For the no-biochar control columns, the cumulative difference in CO$_2$–C emissions on Day 322 was 40% (13% std) of the amount of manure C added to the columns. Among columns receiving biochar amendments, peak differences in cumulative CO$_2$–C emissions occurred on Days 124 or 153, after which there was a decline in differences in cumulative CO$_2$–C emissions. By the Day 322, flux differences for B10M and B20M were significantly lower then for B0M and B5M. The results of the study demonstrate a distinct manure-by-biochar interaction which substantially reduced the rate of CO$_2$–C emission for columns receiving both biochar and manure relative to the biochar only controls after Day 124 (Fig. 6B). Based on these observations, it appears that either biochar stabilized manure C by influencing biochemical recalcitrance or physical protection of manure C (Krull et al., 2003), or the manure additions reduced the ability of biochar to enhance mineralization of soil organic matter.

In the present study, it is not clear how much of the CO$_2$–C came from the biochar and/or manure, and how much CO$_2$–C came from enhanced mineralization of SOC. If the biochar contained pools of both biologically available and unavailable C (Lehmann et al., 2009), then the apparent enhanced CO$_2$ emissions would be expected to continuously and exponentially diminish as the labile organic compounds oxidize, leaving behind the more refractory organic material (Zimmerman, 2010). We saw, however, a relatively constant effect of biochar additions on CO$_2$ emissions over a period of 268 d among no-manure columns and the stepwise effect of biochar on CO$_2$ emissions reemerged after the manure effect had worn off for the manure-treated columns. This implies, but does not prove, that much of the increased emissions of CO$_2$ resulting from the biochar additions were due to enhanced SOC mineralization. As noted previously, the stimulatory effect of biochar applications on soil respiration (Wardle et al., 2008) may partially offset the ability of biochar to sequester C in soils. Indeed, in our study the estimated increase in CO$_2$–C lost from no-manure columns over a 500-d incubation period due to either enhanced mineralization of SOC or mineralization of the biochar was 0.82, 1.61, and 2.48 g of additional CO$_2$–C lost for B5, B10, and B20, respectively, which is equivalent to 17–23% of the biochar C applied.

It is, however, important to estimate the net effect of biochar application on C sequestration including CO$_2$ emitted during biochar production. A very limited number of life cycle assessment studies have been conducted to assess CO$_2$ emitted during production and processing of hardwood biomass. Therefore, for the purpose of this discussion, we assume biochar was produced from corn stover with the energy required for field production, transportation, and processing of 599 kg of biochar C being 4792 MJ (Gaunt and Lehmann, 2008). For all calculations, we assume that the combustion of 1 L diesel fuel results in 51.5 MJ energy and 1.13 kg of CO$_2$–C eq. lost to the atmosphere (Gaunt and Lehmann, 2008; West and Marland, 2002). Therefore, production of 1 kg of biochar C would emit 0.176 kg CO$_2$–C eq. Subtracting CO$_2$–C eq. loss associated with biochar production and increased soil respiration from total amount of biochar C applied, the net C sequestered in no-manure columns after the 500-d incubation was 1.9, 3.8, and 8.3 g of C per column for B5, B10, and B20 treatments, respectively (Table 3). The net

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**Fig. 6.** (A) Cumulative release of CO$_2$–C from manured and nonmanured columns during a period of 268 d. (B) Differences in cumulative CO$_2$–C emissions between manured and nonmanured soil columns during a period of 268 d. Biochar treatments B0, B5, B10, and B20 include amendment of 0, 5, 10, and 20 g biochar kg$^{-1}$, respectively. Manure (M) was applied to half of the columns on Day 79 of the incubation.
C sequestered in the manure-amended columns was 3.5, 6.0, and 10.5 g of C per column for B5M, B10M, and B20M treatments, respectively. Thus in this study, 53 to 58% and 62 to 64% of C was potentially sequestered in no-manure and manure-amended columns, respectively (Table 3). The net effect of the biochar amendments was sequestration of large amounts of C and any enhanced mineralization of SOC was largely offset by the manure-by-biochar interaction among the manure-amended columns. This analysis does not consider additional GHG credits for biochar applications that may accrue from reduced N2O emissions or enhanced net primary production.

CONCLUSIONS

By the end of this soil column study, application of biochar reduced the extent of soil compaction as was evident by significant differences in Dp, whereas application of manure did not have a significant effect on Dp. Biochar additions consistently increased CO2 emissions (measured 13 times) and reduced N2O emissions (measured once) relative to controls receiving no biochar additions. The reduced N2O emissions were correlated with reductions in Dp, suggesting that enhanced aeration of the soil may have contributed to the reductions in N2O emissions. Cumulative release of CO2 increased with increasing biochar applications. Increased CO2 emissions with biochar additions either due to mineralization of biochar itself or enhanced mineralization of biogenic soil organic matter were equivalent to 17 to 23% of biochar C applied and partially offset the C sequestered by addition of the biochar. Application of manure further increased cumulative CO2 emissions; however, the rate of increase varied with biochar treatment and a distinct manure-by-biochar interaction was observed. Although the cause of the interaction is not clear, these observations indicate that either biochar enhanced stabilization of manure C or that manure inhibited biochar enhanced mineralization of soil organic matter. Considering the large amount of C sequestered by the biochar additions, the relatively small increases in CO2-C emissions, and the reduction in N2O emissions observed in this study; the biochar amendments substantially reduced net GHG emissions. Further evaluations under field conditions are needed.

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