Impact of six lignocellulosic biochars on C and N dynamics of two contrasting soils

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Abstract

Both soil and biochar properties are known to influence greenhouse gas emissions from biochar-amended soils, but poor understanding of underlying mechanisms challenges prediction and modeling. Here, we examine the effect of six lignocellulosic biochars produced from the pyrolysis of corn stover and wood feedstocks on CO₂ and N₂O emissions from soils collected from two bioenergy cropping systems. Effects of biochar on total accumulated CO₂-C emissions were minimal (<0.45 mg C g⁻¹ soil; <10% of biochar C), consistent with mineralization and hydrolysis of small labile organic and inorganic C fractions in the studied biochars. Comparisons of soil CO₂ emissions with emissions from microbially inoculated quartz–biochar mixtures (‘quartz controls’) provide evidence of soil and biochar-specific negative priming. Five of six biochar amendments suppressed N₂O emissions from at least one soil, and the magnitude of N₂O emissions suppression varied with respect to both biochar and soil types. Biochar amendments consistently decreased final soil NO₃⁻ concentrations, while contrasting effects on pH, NH₄⁺, and DOC highlighted the potential for formation of anaerobic microsites in biochar-amended soils and consequential shifts in the soil redox environment. Thus, results implicated both reduced substrate availability and redox shifts as potential factors contributing to N₂O emission suppression. More research is needed to confirm these mechanisms, but overall our results suggest that soil biochar amendments commonly reduce N₂O emissions and have little effect on CO₂ emissions beyond the mineralization and/or hydrolysis of labile biochar C fractions. Considering the large C credit for the biochar C, we conclude that biochar amendments can reduce greenhouse gas emissions and enhance the climate change mitigation potential of bioenergy cropping systems.

Keywords: biochar, carbon, nitrogen dynamics, nitrous oxide, priming, soil

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Introduction

The pyrolysis-biochar-bioenergy platform has been proposed as a means for producing carbon-negative bioenergy, with the potential to offset up to ~12% of anthropogenic greenhouse gas (GHG) emissions (Lehmann et al., 2006; Laird, 2008; Woolf et al., 2010). During pyrolysis, agricultural residues, manure, and green wastes are thermochemically transformed under high temperature (~300–800 °C) and oxygen-limited conditions into gaseous, liquid, and solid coproducts. Biochar, the solid coproduct of pyrolysis, is predominantly composed of condensed aromatic carbon (C), making most biochars biologically recalcitrant enough to persist in soils for hundreds to thousands of years (Keiluweit et al., 2010; Mašek et al., 2013). Application of biochar to soil additionally restores soil C and nutrients lost from bioenergy cropping systems as a result of biomass harvesting (Laird & Chang, 2013; Rogovska et al., 2016).

The stable C in biochar originates from atmospheric carbon dioxide (CO₂); hence, the application of biochars to soil constitutes a potential agricultural management option for removing C from the atmosphere, sequestering it for extended periods of time (>100 years), thereby helping to mitigate climate change (Lehmann et al., 2006; Roberts et al., 2010; Woolf et al., 2010; Kauffman et al., 2014). Biochar amendments to soil have also been shown to increase biomass production, which provides a secondary means for increasing soil C sequestration (Laird et al., 2010; Woolf et al., 2010; Biederman & Harpole, 2013). Furthermore, biochar-amended soils have been shown under some conditions to suppress soil GHG emissions, providing a third potential C sequestration pathway. The magnitude of C sequestration achievable through this last pathway remains uncertain due to high variability in the effects of biochar on GHG emissions from soils as measured in both field and laboratory studies (Woolf et al., 2010; Archontoulis et al., 2013).
Thus, a better mechanistic understanding of biochar–soil interactions is needed to develop process-based models that can account for the variable effects of climate, management, soil type, and biochar type and thereby be used to predict average impacts of biochar on GHG emissions from soils (Archontoulis et al., 2016).

The consensus from prior research is that soil biochar amendments typically—but not always—increase emissions of CO₂ from soils. Meta analyses by Liu et al. (2016) and Song et al. (2016) both report a positive average effect of biochar amendments on soil CO₂ emissions. However, the percent increase in CO₂ emitted reported by Song et al. is about five times larger than that reported by Liu et al., thereby demonstrating how variability of reported biochar impacts among studies challenges understanding and prediction of biochar impacts on GHG emissions. Furthermore, CO₂ emissions and other C cycling indicators are not consistently correlated with any single biochar property (Spokas & Reicosky, 2009; Liu et al., 2016; Song et al., 2016). As such, the literature provides little empirical evidence that can explain the generally observed increases in CO₂ emissions following biochar amendments.

Carbon priming, or the stimulation of one C pool’s mineralization through interactions with another C pool, is one potential mechanism that may explain the observed increases in CO₂ emissions following biochar amendments. Carbon priming following biochar application can occur due to stimulation of native SOC consumption by biochar C and/or stimulation of biochar C consumption by SOC. The literature, however, is inconsistent as both positive and negative C priming effects of biochar have been reported (Keith et al., 2011; Luo et al., 2011; Zimmerman et al., 2011; Woolf & Lehmann, 2012; Whitman et al., 2014; Wang et al., 2016a). In a meta-analysis, Wang et al. (2016b) report an average biochar priming effect of −3.8% (95% confidence interval: −8.1 to 0.8% of SOC), and significant effects of soil clay content in addition to biochar feedstock, pyrolysis process, and pyrolysis temperature on priming. Thus, priming is clearly influenced by both biochar and soil properties; however, current knowledge of biochar–soil interactions is insufficient to predict the magnitude and direction of priming following amendment of given biochars to select soils—largely due to a lack of studies examining multiple soils and biochars under comparable conditions (Spokas & Reicosky, 2009).

Biochars have been shown to contain a small fraction of both labile organic carbon (LOC) and inorganic carbon (IC), two C pools that are anticipated to influence CO₂ emissions from biochar-amended soils (Jones et al., 2011; Ameloot et al., 2013; Cayuela et al., 2014). Biochar LOC is a microbial substrate that induces a short-term increase in CO₂ emissions upon addition of biochar to soil and may also indirectly influence N₂O emissions by providing C substrate for N₂O-producing microbes (Cayuela et al., 2013; Liu et al., 2014). Conceptually, biochar LOC is not a discrete, chemically extractable fraction but rather a functional fraction of biochar C that is by definition bioavailable within a relatively short period of time after application to the soil. Soluble organic C isolated from biochar using water, strong acids, and/or oxidizing agents could potentially serve as a proxy for LOC (Bruun et al., 2011; Pereira et al., 2011; Lin et al., 2012). However, research relating soluble C fractions from biochar to CO₂ or N₂O emissions of biochar-amended soils remains limited, and research evaluating bioavailability of soluble organic compounds extracted from diverse biochars has been inconclusive.

Information regarding the influence of biochar IC on soil CO₂ and N₂O emissions is similarly lacking. Hydrolysis of biochar IC was shown to account for up to half of the CO₂ emitted from a hardwood biochar in a short-term incubation study (Jones et al., 2011); however, most studies do not distinguish CO₂ emissions coming from IC and LOC (Ameloot et al., 2013). Furthermore, biochar IC may indirectly influence C or N cycling by increasing soil pH, which may in turn shift microbial community structure and function (Jones et al., 2011; Ameloot et al., 2013; Cayuela et al., 2013; Harter et al., 2013).

Long-term impacts of biochar on soil C and N cycles have been attributed to the chemical and physical properties of biochar’s recalcitrant aromatic C framework. For example, a commonly cited mechanism for reduction in CO₂ or N₂O emissions is the sorption of labile biogenic organic compounds to biochar surfaces, which reduces the availability of the C and N in these compounds to soil microbes (Jin, 2010; Rogovska et al., 2011; Ameloot et al., 2013; Angst et al., 2013; Clough et al., 2013). In addition to sorption, biochar may influence C and N cycling by altering the soil redox environment. Biochar micropores may retain oxygen during wetting cycles and/or retain water during drying cycles, creating contrasting redox environments on a μm to mm scale (Harter et al., 2013; Cayuela et al., 2014). Furthermore, the condensed aromatic C of biochar behaves as a semiconductor, potentially providing a conduit connecting sources and sinks of electrons and facilitating redox reactions (Cayuela et al., 2013). Impacts of biochar on substrate availability and soil redox environment can have a profound influence on overall C and N cycling, especially with regard to N transformations and N₂O emissions (Bateman & Baggs, 2005; Clough & Condron, 2010).

A deeper understanding of connections between biochar properties and their impacts on soil C and N cycling is imperative to modeling biochar impacts on
agroecosystems, and to the overall successful implementation of the pyrolysis-biochar-bioenergy platform. Understanding of these connections is currently limited by a lack of studies encompassing more than one biochar and soil. Few previous studies have encompassed a wide range of soil conditions. Instead, most have focused on less than five biochars in four or fewer soils, with one or no organic amendments added (Spokas et al., 2009; Van Zwieten et al., 2009; Clough et al., 2010; Kimetu & Lehmann, 2010; Novak et al., 2010; Smith et al., 2010; Cross & Sohi, 2011; Luo et al., 2011; Rogovska et al., 2011; Augustenborg et al., 2012; Kammann et al., 2012; Liu et al., 2012; Yoo & Kang, 2012; Zhang et al., 2012). Spokas & Reicosky (2009), however, studied GHG emissions from combinations of three soils and 16 biochars. Their results showed that GHG emissions varied with both soil and biochar characteristics, but were not correlated with the specific biochar and soil properties measured. Such results indicate a need for more basic research to understand the chemical, physical, and biological mechanisms influencing biochar impacts on GHG emissions. This study aimed to (i) examine the effect of six lignocellulosic biochars on soil CO2 and N2O emissions and (ii) to identify key biochar properties that influence soil C and N cycling. We hypothesize that (i) hydrolysis of IC and mineralization of LOC contribute to the often-observed increases in CO2 emissions when biochar is added to soils, (ii) acid- and/or base-soluble OC can serve as a proxy for the LOC fraction of biochar, (iii) biochar amendment to soil causes little or no positive priming of biochar C or biogenic soil organic C, and (iv) biochars will reduce N2O emissions following the addition of fertilizer due to changes in soil pH, redox environment, and/or availability of microbial substrates.

Materials and methods

Biochar and soil preparation

Six biochars made from four different feedstocks at three different temperatures, previously shown to have diverse properties, were selected for this study (Fidel et al., 2017). Slow pyrolysis biochars made from corn stover were pyrolyzed in an N2-purged muffle furnace for ~1 h at 300, 500, and 600 °C highest heating temperature (CS3s, CS5s, and CS6s, respectively). A mixed wood gasification biochar (MW6g) was obtained from ICM Inc. and hardwood slow pyrolysis biochar (HW5s) was obtained from Royal Oak® (http://royal-oak.com/; #10 sieve size). Fast pyrolysis corn stover biochar, CS5f, was obtained from the Center for Sustainable Energy Technologies (CSET) at Iowa State University. Sand is used as a fluidizing media in the CSET fast pyrolysis process, and as a result, the CSET char contained significant amounts of coarse sand (>0.5 mm). The CSET char was sieved to <0.50 mm to separate relatively pure CS5f biochar from the sand particles. All other biochars were ground to ~0.50 mm to minimize the influence of particle size. Biochar properties, as analyzed in Fidel et al., 2017, are reported in Table S1.

Two soils of contrasting textures and parent materials, Soil A and Soil B, were collected in November of 2013 from two research field sites in Iowa. Both fields were under continuous corn cropping systems. Soil A, a loess-derived soil with silt loam texture, was collected from a site on the Iowa State University Armstrong Research and Demonstration Farm and is mapped as Exira (fine-silty, mixed, superactive, mesic Typic Hapludoll). Soil B, a glacial till-derived soil with a loam texture, was collected from the Boyd Farm in Boone Co., IA, and is mapped as Clarion (fine-loamy, mixed, superactive, mesic Typic Hapludoll). Both sampling locations were on eroded hillslopes and consequently contained less clay than is typical for their mapped soil series: Soil A contained 15% sand, 80% silt, and 5% clay, whereas Soil B contained 48% sand, 42% silt, and 10% clay (particle size determined by the hydrometer method, Bauder & Gee, 1979). Soil A contained a similar amount of total C (1.9%) as Soil B (1.6%); however, only Soil A effervesced upon exposure to hydrochloric acid. The soil samples were collected from the top 5 cm following corn harvest at both sites, refrigerated for one month, and sieved (<4 mm) before use. Any visible plant residues remaining after sieving were removed by hand.

Biochar IC and LOC

Biochar IC was quantified using a NaOH trap method (Fidel et al., 2017). Briefly, 2 g of biochar was stirred with 100 mL of 0.05 m HCl in a sealed 0.95-L glass container that contained 15 mL of 1 m NaOH in a separate vial. After 72 h, the NaOH vials were removed, excess CaCl2 was added to the NaOH, and the solution was titrated with HCl using phenolphthalein as an indicator.

There is no standard accepted method of quantifying biochar LOC. Here, we test acid-extractable organic C (AEOC), carbonate-extractable organic carbon (CEOC), and acid-soluble volatile matter (ASVM) as proxies for biochar LOC. The AEOC and CEOC of each biochar were quantified by equilibrating biochars with 0.05 m solutions of either HCl or Na2CO3, respectively, at a ratio of 50 mL to 1 g biochar for 24 h, and measuring the DOC of the filtered (<0.45 µm) extracts (Shimadzu TOC 5050 analyzer). To remove IC and ASVM, biochars were shaken for 24 h in 0.05 m HCl using a 50 : 1 (vol:wt) solution:biochar ratio, then filtered (0.45 µm), and washed with 1 m CaCl2 twice and finally deionized water four times (15 min per wash) to remove excess salt. Total C of the untreated and acid-washed biochars was quantified using a combustion analyzer (Vario Microcube, Elementar), and fixed C (FC) was determined by proximate analysis (method adapted from standard ASTM procedure) (Choi et al., 2014). Total organic C (OC) of the untreated biochars was calculated as the difference between total C and IC. For the acid-washed biochars, total OC was assumed to equal total C. To calculate ASVM in units of OC, the OC : FC ratios of the acid-washed biochars were subtracted from the OC : FC ratios of the untreated biochar, and this difference in ratios was multiplied by the FC content of the untreated biochar. This method
for quantifying ASVM assumes that FC content does not change during acid washing treatments.

**Soil incubation**

Biochar samples (0.05 g) were mixed with 10 g (oven-dry equivalent weight) samples of field-moist soils A and B in 150-mL serum vials in quadruplicate. Controls, used to assess the influence of biochar IC, were prepared by mixing 0, 0.5 and 1.0 mg g⁻¹ of CaCO₃ with each soil; these control treatments are designated C0, C1, and C2, respectively. Controls, used to assess the mineralization of biochar LOC, were prepared by mixing biochars with a 50/50 mixture of silt and sand-sized quartz. These ‘quartz controls’ additionally received 0.5 mL of a microbial inoculant prepared by vigorously hand-shaking 14 g of field-moist soil (7 g of each soil) with 100 mL of water 25 times, then filtering (#1 Whatman ashless filter paper), and using the filtrate as the inoculant immediately. Thus, aside from any trace amounts present in the inoculating solution, the quartz controls contained negligible biogenic (nonpyrogenic) organic C or N. The CO₂ emissions from quartz controls are therefore assumed to arise primarily from hydrolysis of inorganic biochar C and mineralization of labile organic biochar C.

The soil samples and quartz controls were equilibrated at 20 °C for 50 days, during which time soil samples were maintained at field moisture content and the quartz controls were maintained at field capacity (−1/3 bar matric potential) by weekly drop-wise addition of distilled deionized water (appropriate moisture was confirmed by mass to the nearest ±0.02 g). During this equilibration period, CO₂ and N₂O emissions were quantified on days 0, 1, 6, 8, 13, 15, 20, 36, 43, 49, 64, 71, 78, 83, 97, 113, 127, and 140 during the postfertilization incubation. For each gas flux measurement, the serum vials were capped with gray butyl septa and sealed with crimp caps, and then, 11.5-mL gas samples were collected using a syringe three times during 2- to 24-h gas accumulation periods. Longer gas accumulation periods were used when flux rates were low. Gas samples were stored in helium-flushed and evacuated airtight 6-mL Exetainer vials and analyzed for CO₂ and N₂O using a gas chromatograph equipped with a methanizer flame ionization detector (SRI Instruments) and an electron capture detector. Concentrations were measured by volume and converted to mass units using the ideal gas law (Iqbal et al., 2013).

**Postincubation soil analyses**

Following the 190-day postfertilization incubation period, soil samples were oven-dried at 105 °C prior to analysis for pH, extractable nutrients, DOC, total C, and total N. Soil pH was measured in deionized water using a 1 : 1 soil : solution ratio (Thomas et al., 1996). Plant-available nutrients were determined using the Mehlich 3 method and analyzed by inductively coupled plasma optical emission spectroscopy (Thermo Scientific™ iCAP™ 7400 ICP-OES Analyzer). Soluble NH₄⁺, NO₃⁻, and DOC were extracted using 2 M KCl (5 : 1 solution : soil ratio). Subsamples of the KCl extracts were prepared with the Berthelot and Griess-Ilosvay reagents for analysis of NH₄⁺ and NO₃⁻, respectively, and analyzed colorimetrically using a microplate reader (Hood-Nowotny et al., 2010). The KCl extracts were also analyzed for DOC using the TOC analyzer to provide an index of bioavailable C (Shimadzu TOC 5050 analyzer). Total soil C and N were determined by combustion analysis (Vario Microcube, Elementar). Organic anions were extracted using deionized water from control soils and soils amended with two of the six biocars using a solution:solit ratio of 5 : 1. These aqueous extracts were analyzed for acetate, formate, lactate, glycolate, and several other low-molecular-weight organic acids using a Dionex ICS-5000 high-pressure ion chromatograph equipped with Dionex IonPac AG11-HC guard column, ATC-3 trap column, and ERS-500 suppressor.

**Calculations and statistical analysis**

Gas flux rates were calculated from the slopes of linear regression equations fitted to the gas concentration vs. time data for each gas accumulation period; flux rates for any samples with \( r^2 < 0.5 \) were assumed to be zero (Iqbal et al., 2013). Cumulative emissions over the 50-day prefertilization and 140-day postfertilization incubations were calculated by interpolating linearly between dates when gas flux rates were measured (‘trapezoidal interpolation’). Priming was calculated as the difference between cumulative emissions (in CO₂-C per unit time) coming from a biochar-amended soil and the sum of emissions coming from the control amended with the same biochar and C0 control for the same soil (because CO₂ emissions from quartz controls were largely negligible prior to fertilization, priming was calculated for the postfertilization period only). This calculation of priming includes (i) biochar stimulation of biogenic C (SOC and corn stover C) mineralization and (ii) biogenic C (SOC and corn stover) stimulation of biochar C mineralization. This approach measures total priming and does not distinguish the source of the CO₂-C associated with priming, whether from the biochar, native SOC, or the corn stover.

Average daily fluxes were compared using the PROC MIXED procedure and an ante-dependence repeated measures model. Total cumulative emissions were compared using ANOVA, and significance of correlations was evaluated using PROC REG or PROC STEPWISE, as appropriate. Significance was evaluated at \( \alpha = 0.05 \), and all statistical analyses were conducted using SAS statistical software (v9.2).
Results

Biochar soluble OC and IC

The sum of IC, AEOC, and CEOC ranged from 3.6 to 23.5 mg g\(^{-1}\) for the studied biochars (Table 1). Biochar IC ranged from 1.17 to 8.97 mg g\(^{-1}\), and increased in the order CS3s < CS5s < CS5f < CS6s < HW5s < MW6g. Biochar AEOC ranged from 0.1 to 10 mg g\(^{-1}\) and increased in the order HW5s < CS5s < MW6g < CS6s < CS5f < CS3s. Biochar CEOC ranged from 0.5 to 12 mg g biochar\(^{-1}\) and increased in the order CS3s < CS5s < MW6g < HW5s < CS5f < CS3s. Biochar CEOC did not consistently increase with increase in AEOC or IC.

Total soil C and N

Total C (TC) and total N (TN) of the soils, measured at the end of the 190-day incubation, are reported in Table 2. For all treatments, Soil A had higher TC and TN than Soil B, while Soil B had higher C : N ratios than Soil A. The biochar-amended soils had significantly higher TC for both Soil A (0.18–0.36%) and Soil B (0.12–0.34%) compared with the C0 controls. This increase in TC corresponded closely with the amount of biochar C added (0.26–0.36%). The carbonate controls (C1 and C2) did not have significantly higher TC than the C0 controls. Biochar and carbonate amendments did not significantly change TN for either soil. The C : N ratios of biochar-amended soils were all higher than the C : N ratios of the C0 controls.

Mehlich - extractable nutrients

Plant-available nutrients as determined by Mehlich 3 extraction for Soil A, Soil B, and the quartz controls are presented in Table S3 (Mehlich, 1984). Biochar amendments consistently increased extractable base cations for both soils, likely due to base cations present in the biochars (Fidel, 2015). Indeed, the increase in extractable base cations in biochar-amended soils was proportional to – but not always equal to – the quantity of base cations extracted from the quartz controls for each biochar. Biochar-amended Soil B samples had more extractable Fe, Mn, P, and S than the C0, C1, or C2 controls, and the increase was more than could be attributed to nutrient release from the biochars based on the quantities of these nutrients extracted from quartz controls. Biochar-amended Soil A samples also had higher levels of extractable Fe and Mn but no change in extractable P and a decrease in extractable S relative to the C0, C1, or C2 controls.

Soil and quartz-control pH

The pH of both soils decreased during the incubation from an initial value of 6.3 to final values ranging from 5.1 to 6.1 for all treatments, and the pH of decreased four of the six quartz controls decreased as well (Fig. 1). As expected, carbonate amendments (C1 and C2) consistently increased soil pH by 0.2–0.4 pH units relative to controls not receiving carbonates (C0). In agreement with previous studies, biochar amendments increased the pH relative to the Soil A C0

Table 1 Acid-extractable organic C (AEOC), carbonate-extractable organic C (CEO C), inorganic C (IC), and total labile C in mg C per g of untreated biochar (± SD)

| Biochar | AEOC  | CEOC  | IC   | Total labile C |
|---------|-------|-------|------|---------------|
| CS3s    | 10.1 ± 0.3 | 12.2 ± 0.6 | 1.2 ± 0.1 | 23.5 ± 0.7  |
| CS5f    | 2.61 ± 0.05 | 4.3 ± 0.2 | 4.5 ± 0.1 | 11.4 ± 0.2  |
| CS5s    | 0.29 ± 0.03 | 0.8 ± 0.1 | 2.51 ± 0.03 | 3.6 ± 0.1  |
| CS6s    | 2.58 ± 0.05 | 0.5 ± 0.1 | 5.5 ± 0.1 | 8.6 ± 0.2  |
| HW5s    | 0.106 ± 0.009 | 4 ± 0.2 | 9 ± 0.3 | 13.1 ± 0.4 |
| MW6g    | 0.89 ± 0.04 | 1.7 ± 0.3 | 9 ± 0.2 | 11.6 ± 0.4 |

Table 2 Total C (wt%), total N (wt%), and C:N ratio of soils determined after the 190-day incubation (± SD)

| Treatment | Total C (%) | Total N (%) | C:N         |
|-----------|-------------|-------------|-------------|
|           | Soil A  | Soil B  | Soil A  | Soil B  | Soil A  | Soil B  |
| C0        | 2.56 ± 0.07 | 1.86 ± 0.08 | 0.290 ± 0.007 | 0.18 ± 0.01 | 8.6 ± 0.3 | 10.3 ± 0.6 |
| C1        | 2.63 ± 0.09 | 1.92 ± 0.08 | 0.305 ± 0.013 | 0.18 ± 0.01 | 8.6 ± 0.5 | 10.4 ± 0.6 |
| C2        | 2.43 ± 0.04 | 1.92 ± 0.11 | 0.278 ± 0.005 | 0.18 ± 0.01 | 8.8 ± 0.2 | 10.6 ± 1.0 |
| CS3s      | 2.80 ± 0.10 | 1.98 ± 0.19 | 0.298 ± 0.006 | 0.18 ± 0.02 | 9.4 ± 0.4 | 11.1 ± 1.4 |
| CS5f      | 2.68 ± 0.05 | 2.20 ± 0.09 | 0.279 ± 0.005 | 0.17 ± 0.02 | 9.6 ± 0.3 | 12.6 ± 1.4 |
| CS5s      | 2.70 ± 0.12 | 2.06 ± 0.14 | 0.285 ± 0.013 | 0.18 ± 0.01 | 9.5 ± 0.6 | 11.4 ± 1.2 |
| CS6s      | 2.75 ± 0.06 | 2.13 ± 0.15 | 0.284 ± 0.008 | 0.18 ± 0.01 | 9.7 ± 0.3 | 12.0 ± 1.1 |
| HW5s      | 2.86 ± 0.11 | 2.14 ± 0.09 | 0.290 ± 0.008 | 0.18 ± 0.01 | 9.9 ± 0.5 | 12.1 ± 0.9 |
| MW6g      | 2.78 ± 0.04 | 2.12 ± 0.15 | 0.285 ± 0.004 | 0.17 ± 0.02 | 9.8 ± 0.2 | 12.7 ± 1.5 |
control by 0.3–0.5 pH units (Yuan & Xu, 2011; Rogovska et al., 2014). By contrast, the biochar amendments decreased Soil B’s pH by 0.3–0.5 pH units relative to that of the Soil B C0 control. The quartz controls had final pHs ranging from 5.4 to 7.2, which increased with increase in biochar IC and monovalent:divalent cation ratio (CS3s < CS5f < CS5s < HW5s < MW6g < CS6s). The pH of the CS6s quartz control increased relative to the starting pH, most likely due to the high pH (10.3) and high K⁺ concentration of the CS6s biochar (Fidel, 2015). Final pHs of biochar-amended soil A and B samples also increased with biochar IC and monovalent:divalent cation ratio, thereby demonstrating the impact of biochar alkalis on soil pH.

Soil DOC

Biochar amendments had opposite impacts on 2M KCl-extractable DOC for soils A and B (Fig. 2). Extractable DOC for the quartz controls was below the detection limit. Soil A samples amended with all six biochars had lower DOC levels than the C0, C1, and C2 controls (35–58 mg kg⁻¹ for biochar treatments vs. 122–169 mg kg⁻¹ for the controls). By contrast, Soil B samples amended with all six biochars had higher DOC levels than the C0, C1, and C2 controls (158–213 mg kg⁻¹ for biochar treatments vs. 81–90 mg kg⁻¹ for the controls). Carbonate amendments decreased DOC concentrations for Soil A but not for Soil B, and biochar amendments decreased DOC for Soil A samples by twice as much as carbonate amendments (Figs 2 and S1). Thus, the results indicate a biochar-by-soil interaction that cannot be attributed to carbonates.

To help elucidate the mechanism by which the biochar amendments differentially influenced pH and DOC for soils A and B, water-extractable organic anions were quantified for a subset of treatments (C0, CS5s, and HW5s) for each soil (Fig. S2). Organic anion concentrations increased with increase in DOC levels, and the ratio of organic anion C to DOC was consistently higher among biochar-amended soils than the C0 control soils. Compared with their respective C0 controls, biochar-amended Soil A samples had slightly lower concentrations of the dominant organic anions (lactate, acetate, glycolate, and formate), whereas biochar-amended Soil B samples had substantially higher concentrations of these organic anions.

Soil and quartz-control NH₄⁺ and NO₃⁻

Both soil and biochar affected 2 M KCl-extractable NH₄⁺-N and NO₃⁻-N; a biochar-by-soil interaction effect on was also apparent (Table 3). Differences in extractable NH₄⁺ and NO₃⁻ among biochar treatments for each soil were minimal. Extracts from Soil A C0 controls had larger quantities of both NH₄⁺ and NO₃⁻ and higher NH₄⁺:NO₃⁻ ratios compared with extracts from the Soil B C0 controls. Carbonate amendments did not cause significant changes in extractable NH₄⁺ or NO₃⁻ concentrations for either soil. Total reactive nitrogen, Nr (Nr = NH₄⁺-N + NO₃⁻-N), extracted from the soil samples exceeded added fertilizer Nr, likely due to mineralization of soil organic N and/or corn stover N. For Soil A, the six biochar treatments induced a ~50% decrease in NH₄⁺ and a ~10–20% decrease in NO₃⁻ relative to the C0 controls; consequently, total Nr was lower and NH₄⁺:NO₃⁻ ratios were slightly lower in biochar-amended soils compared with C0 controls. By contrast, biochar treatments induced a ~200% increase in extractable NH₄⁺ and a ~25–50% decrease in extractable NO₃⁻ relative to the C0 controls for Soil B. Furthermore, Soil B samples amended with corn stover biochars (CS3s, CS5f, CS5s, and CS6s) had 8–37% lower NO₃⁻ concentrations than those amended with wood biochars.
Soil B samples decreased rapidly during the first 22 days of the prefertilization incubation, and after 44 days, emission rates were similarly low for all treatments and for both soils, at approximately 5–15 µg CO₂-C g⁻¹ day⁻¹.

**Equilibration period CO₂ emissions**

Cumulative CO₂ emissions for soils A and B during the 50-day prefertilization equilibration period are shown in Fig. 3a. Cumulative CO₂ emissions from the quartz controls were negligible (<3 µg CO₂-C g⁻¹) during the prefertilization period (*data not shown*). Initially Soil B had higher CO₂ emissions than Soil A, and emissions on day 0 from all biochar and carbonate-amended Soil B samples were higher than emissions from the Soil B C0 controls (Fig. S3). However, emission rates from Soil B samples decreased rapidly after the first 22 days of the prefertilization incubation, and after 44 days, emission rates were similarly low for all treatments and for both soils, at approximately 5–15 µg CO₂-C g⁻¹ day⁻¹.

**Postfertilization soil CO₂ emissions**

Immediately following the addition of corn stover and NPK fertilizer, CO₂ emissions from both soils and all treatments were >50 µg CO₂-C g⁻¹ day⁻¹ (day 0) but decreased rapidly at first and then more gradually through the duration of the 140-day postfertilization incubation (Fig. S4). By the end of the 140-day postfertilization incubation period, emission rates for all samples were <8 µg CO₂-C g⁻¹ day⁻¹. The soil*treatment*day interaction was significant on all days except for days 1 and 20 (*P < 0.05*). Significant differences between biochar-amended samples and C0, C1, and C2 controls occurred on most days during the incubation, but biochar-amended soils did not have consistently higher or lower emissions relative to the controls.

Total CO₂ evolved was similar for soils A and B during the 140-day postfertilization period (Fig. 3b). No significant effect of biochar or carbonate amendment was
observed Soil A ($P > 0.05$). However, emissions from Soil B samples amended with the CS3s, CS5f, CS5s, and HW5s biochars were significantly lower than emissions from the C1 and C2 controls ($P < 0.05$), and amendment of Soil B with the CS3s biochar reduced CO2 emissions relative to Soil B C0 control ($P < 0.05$).

Postfertilization CO2 emissions from the quartz controls

Quartz controls emitted less than one-tenth as much CO2 as the soil samples during the postfertilization incubation (Fig. S5). Initially emissions ranged from 2.5 to 5.5 µg CO2-C g$^{-1}$ day$^{-1}$, but decreased rapidly to <1.5 µg CO2-C g$^{-1}$ day$^{-1}$ by day 13. After 80 days, all quartz controls except for the CS6s quartz control emitted <0.25 µg CO2-C g$^{-1}$ day$^{-1}$. The CS6s quartz control emitted approximately 0.8 µg CO2-C g$^{-1}$ day$^{-1}$ for the remainder of the incubation. Total CO2 emissions from quartz controls increased in the order CS3s<CS5s<CS5f<HW5s<MW6g-CS6s and were positively correlated with biochar IC content and pH (Fig. 4a, b). When the CS6s outlier was excluded, the correlation with IC was very strong ($r^2 = 0.91$).

$N_2O$ emissions

During the 50-day prefertilization period, $N_2O$ emissions from both soils and quartz controls were generally below the detection limit (<0.0003 µg N2O-N g$^{-1}$ day$^{-1}$), and averages within treatments approached zero (data not shown). Following fertilization, $N_2O$ emissions from quartz controls remained below the detection limit. By contrast, $N_2O$ emissions for most soil samples were above the detection limit during days 0–8, and dropped below the detection limit after day 8 (Fig. S7; emissions after day 8 not shown). As evaluated using repeated measures analysis, the main effects of treatment, day and soil on daily $N_2O$ emissions were significant, as were the treatment*day and soil*treatment*day interaction effects ($P < 0.05$; days 0–8 only).

Cumulative $N_2O$ emissions from soils A and B during the first 8 days of the postfertilization period are shown in Fig. 5. Total $N_2O$ emissions over the initial eight-day period were significantly higher for Soil A controls than for Soil B controls. The $N_2O$ emissions from biochar-amended Soil A samples were significantly less than $N_2O$ emissions from the Soil A controls; by contrast, only Soil B samples amended with the CS3s and MW6g biochars emitted significantly less $N_2O$ relative to the Soil B C0 controls and Soil B C1-controls, respectively.

Discussion

The potential synergy of simultaneous C sequestration, soil quality enhancement, and renewable energy
production has driven rapid growth of research on the pyrolysis-biochar-bioenergy platform (Laird, 2008; Archontoulis et al., 2016). Recent studies have shown that the platform’s C sequestration potential can be augmented by the reduction in soil GHG emissions following biochar amendment; however, this is not a universal phenomenon, and biochar has been shown to increase CO2 and N2O emissions under specific conditions. Mechanisms underlying these context-specific responses remain poorly understood, in part due to a lack of studies examining emissions impacts of multiple biochars concurrently with biochar and soil property metrics relevant to C and N cycling. To foster accurate predictive modeling of biochar impacts, we have therefore quantified CO2 and N2O emissions from two soils amended with six biochars, and analyzed these soils and biochars for pH as well as extractable nutrients and C substrates.

**Influence of biochar on the soil environment and substrate availability**

Biochars caused contrasting shifts in select soil chemical properties when amended to Soil A compared with Soil B (Fig. 2, Tables 3 and S3). In previous studies, such shifts in soil DOC, NH4+, and NO3− following biochar amendment have been attributed to changes in soil pH, the soil redox environment, substrate consumption or transformation rates, and/or substrate sorption rates (Joseph et al., 2010; Laird et al., 2010; Sánchez-García et al., 2014). Here, we find evidence for one or more of these factors influencing KCl-extractable substrates in each soil.

When amended to Soil A, biochars consistently decreased KCl-extractable DOC, NH4+, and NO3−. The observed decrease in DOC could be partially explained by the biochars’ liming effect, as DOC tended to decrease with increase in soil pH among biochar-amended Soil A samples; however, this relationship only explained 32% of the variation in DOC (P < 0.05) (Fig. S8). Final DOC did not increase with decrease in total CO2; the decrease in DOC therefore could not be explained by increased DOC mineralization (Figs 2 and 3). The decrease in NO3− in response to biochar amendment, in conjunction with a lack of response to carbonate amendment, suggests that increased nitrification in response to liming was not responsible for the observed decrease in NH4+ (Parton et al., 1996; Zheng et al., 2012). Likewise, shifts in the soil redox environment to a more reducing or oxidizing regime would be expected to alter relative amounts of NH4+ and NO3− rather than causing a decrease in both. Therefore, we reject the hypotheses that biochar reduced extractable substrate concentrations in Soil A primarily through changes in pH, redox environment, substrate consumption rates, or substrate transformation rates, and conclude that biochars reduced soil DOC, NH4+, and NO3− through sorption mechanism(s).

When amended to Soil B, biochars consistently increased KCl-extractable DOC and NH4+, but decreased NO3−. Similar to Soil A, final DOC, NH4+, and NO3− concentrations of biochar-amended Soil B samples were not consistently correlated with measured soil pH, or CO2 emissions, suggesting that soil pH and decreased DOC consumption were not primary factors responsible for changes in substrate concentrations. Because substrate concentrations did not decrease universally, changes in substrate availability cannot be entirely explained by substrate sorption to biochar. By process of elimination, a shift in the redox environment is therefore the most likely explanation for the observed pH and substrate shifts in Soil B.

Water-extractable organic acid anions were analyzed to confirm the hypothesized shift to a more reducing environment in Soil B with biochar amendments (Fig. S2). Results showed that the increase in DOC was largely driven by an increase in lactic, acetic, glycolic, and formic acids — known byproducts from fermentation of corn stover (Datta, 1981; Thanakoses et al., 2003; Xu et al., 2010). Hence, biochars apparently promoted O2-limited conditions favorable to fermenting microbes in Soil B but not in Soil A. Because all samples were incubated at −1/3 bar-equivalent moisture with regular exposure to ambient air, it is unlikely that the whole biochar-amended Soil B samples entered an entirely anaerobic state. The presence of some extractable NO3− (Table 3) is further evidence that the biochar-amended Soil B soils were not entirely anaerobic. Therefore, fermentation of corn stover likely occurred in O2-depleted microsites within or near biochar particles. The formation of anaerobic microsites would also explain the observed increase in NH4+, decrease in pH, increase in Mehlich-extractable Fe and Mn, and decrease in extractable S observed for Soil B amended with biochar relative to C0 (control). The observed slight reduction in NO3− could be explained by reduced NH4+ oxidation and/or enhanced denitrification within anaerobic microsites in Soil B. Alternatively, NO3− may have been reduced through sorption to biochar in Soil B samples (as occurred in Soil A). Thus, for Soil B, we attribute the increase in NH4+ and DOC with biochar amendment to the formation of anaerobic microsites, and the decrease in NO3− to reduced O2 availability and/or sorption of NO3− to biochar.

**Influence of biochar LOC and IC**

The CO2 emission results support the hypothesis that biochar IC contributes to CO2 emissions in biochar-amended soils, but do not provide evidence for
influence of AEOC or CEOC. Effects of biochar on total CO2-C emissions accumulated over the 190-day incubation were minimal (<0.4 mg C g−1 soil higher than C0 controls) relative to the total biochar C added (2.6–3.6 mg C g−1 soil), consistent with a small labile C fraction representing 1–10% of biochar C. Based on CO2 emissions from the quartz controls and carbonate controls, biochar IC likely comprised a significant but variable fraction (10–100%) of total labile biochar C. Biochar IC explained 91% of the variation in the average total CO2-C evolved from quartz–biochar mixtures (140 days postfertilization; P < 0.05; Fig. 4a), and 82% of variation in average total CO2-C evolved from biochar-amended Soil B samples (190 days; P < 0.05) (Fig. S9b). Total CO2-C emitted from Soil A (which contained residual carbonate particles from agricultural lime applied in 2011) was not correlated with biochar IC (P > 0.05), suggesting biochar IC effects likely depend on native soil IC (Fig. S9a). Total CO2-C emitted from quartz–biochar mixtures increased nonlinearly with increase in final pH, but this did not occur for the biochar-amended soils (Fig. 4b). Although biologically mediated pH effects and abiotic carbonate effects – which may both positively impact CO2 emissions – cannot be fully distinguished here, the short-term nature of the Soil B and quartz-control CO2 responses to both carbonate controls and biochar amendments suggests that abiotic carbonate hydrolysis contributed significantly to CO2 evolved. Among biochar-amended soils, CO2-C emitted was not significantly correlated with AEOC or CEOC (or AEOC + CEOC, or AEOC + CEOC + IC); we therefore conclude that these soluble biochar OC fractions were not good indicators of labile biochar OC. However, biochar IC could not explain all of the variability in CO2 emissions, suggesting that other forms of labile biochar OC did contribute to CO2 emissions. Unlike AEOC and CEOC, biochar ASVM-C explained 82% of variation in total CO2-C emitted from biochar-amended Soil A samples, suggesting that ASVM-C is effective for estimating LOC in biochars in some contexts (Fig. S9c). Thus, observed CO2 emissions are overall consistent with a small labile C fraction representing <10% of biochar C – and predominately comprised of IC.

Soil C priming

Primed effects ranged from −0.34 to 0.05 mg C g−1, with most biochar–soil combinations resulting in negative or negligible priming of native SOC and/or corn stover C (Fig. 6). Thus, the hypothesis that biochar amendment would cause nonsignificant or minimal positive priming was supported. Positive priming effects were small in magnitude relative to C sources (≤0.1 mg C g−1 soil), equal to ≤4% of biochar C, ≤7% of added corn stover C, and ≤2% of soil C. In the case of Soil A, some biochars enhanced consumption of SOC and/or corn stover C (positive priming), while others inhibited consumption (negative priming); in the case of Soil B, all biochar amendments inhibited consumption of SOC and/or corn stover C (negative priming). Individual biochars induced contrasting priming effects in each soil, thereby confirming that priming is not solely a function of biochar properties. Average C primed was not consistently correlated with any measured biochar properties. Observed negative priming in Soil B is consistent with the hypothesis that biochars facilitated the formation of anaerobic microsites in this soil, which would have inhibited the mineralization of OC.

We acknowledge that differences in pH between quartz controls and biochar-amended soil samples may have also influenced calculated priming effects; however, given the small magnitude and short-term nature of CO2 response to carbonate amendments, these pH differences were likely not a major factor affecting respiration rates. We furthermore acknowledge that differences in abiotic conditions between substrate controls and treatments – here quartz controls and biochar-amended soils – present an ever-present challenge to data interpretation in priming studies. Namely, minerals present in soils such as phyllosilicates and sesquioxides may preserve biochar in biochar-amended soils to a greater degree than the quartz matrix, thus potentially producing an underestimate of positive priming or overestimate of negative priming. However, the evidence for negative or negligible priming observed in this study is consistent with other studies and with the biochar-facilitated formation of anaerobic microsites in Soil B; we therefore infer minimal influence of pH and mineralogical differences between soils and quartz controls on priming.

![Fig. 6 Soil and corn stover C primed by biochars, after fertilization. Primed C was calculated as the difference between the sum of the quartz–biochar mixture and C0 emissions, minus the emissions of the respective biochar-amended soils (in CO2-C). Error bars indicate ± SE.](image-url)
IC and OC on CO2 emitted. The results support the hypotheses that (i) biochar IC contributes to short-term CO2 emissions, (ii) biochars do not significantly accelerate mineralization of biogenic SOC when biochar IC and LOC are accounted for, and (iii) biochars reduce N2O emissions by altering the soil redox environment and/or reducing available OC and inorganic N substrates. We did not observe any evidence for the influence of biochar carbonates on N2O emissions. No single acid- or base-soluble biochar OC fraction was able to consistently serve as a proxy for mineralizable biochar OC; however, results showed a clear, soil-specific influence of biochar IC and OC on CO2 emitted. Consistent reductions in extractable NO3− with biochar amendment support previous studies positing NO3− sorption to biochar as a mechanism for reducing N2O emissions, while contrasting responses of other soil properties to biochar amendment highlight the ability of biochars to influence microsite redox status in a context-specific manner. Thus, a convergence of evidence suggests that sorption of substrates to biochar and changes in the soil redox environment are key mechanisms driving biochars’ effects on CO2 and N2O emissions – and that the dominant mechanisms likely vary by soil (Cayuela et al., 2014; Sánchez-García et al., 2014). Further research is necessary to understand and model the mechanisms governing interactions between biochar properties and soil properties that result in contrasting soil responses to biochar amendments (Jeffery et al., 2015). Specifically, developing accurate models for C and N cycling in bioenergy cropping systems incorporating biochar necessitates research examining (i) biochar sorptive and redox properties and their interactions with soil properties, and (ii) how biochar–soil–management interactions occur in the field, such as in the presence of plants (Whitman et al., 2014; Weng et al., 2015). Such models would help decision-makers choose optimum biochars and management techniques for specific regions, soils, and bioenergy cropping systems, and thereby realize the full climate change mitigation potential of the pyrolysis-biochar-bioenergy platform.

Evidence for N2O emission suppression and potential mechanisms

In agreement with Cayuela et al. (2014) and Cayuela et al. (2015), biochar amendments suppressed N2O emissions by 32% on average, and variations in the magnitude of suppression revealed interactive effects of biochar and soil (Fig. 5). It has been previously posited that biochars could suppress N2O emissions by (i) increasing or buffering soil pH, thereby favoring N2O reduction to N2, (ii) introducing toxic compounds that inhibit activity of NH4+-oxidizing or NO3−-reducing microbes, (iii) sorbing soluble C and/or N substrates, thereby reducing their bioavailability, (iv) increasing soil microporosity, thereby influencing soil water dynamics and making water and/or oxygen more available to microbes involved in the N cycle, and (v) acting as a sink, source, or conduit for electrons, thereby catalyzing biochemical and abiotic redox reactions (Cayuela et al., 2013, 2014; Wells & Baggs, 2014; Case et al., 2015).

In the present study, consistently lower final soil NO3− concentrations in biochar-amended soils relative to controls (with and without carbonate) support the hypothesis that biochars reduce N2O emissions by reducing NO3− availability (Cayuela et al., 2014; Kammann et al., 2015). However, the final NO3− concentrations may not reflect concentrations at the time detectable N2O emissions were measured (days 0–8). Alternatively, the concurrent reduction in final DOC from biochar-amended Soil A samples relative to controls suggests that biochars may reduce the availability of C substrates needed for microbial N2O production (Rogovska et al., 2011). In the case of Soil B, biochars likely facilitated the formation of anaerobic microsites with low pH and abundant bioavailable C (see section Influence of biochar on the soil environment and substrate availability), resulting in reduced oxidation of NH4+ to NO3−, inhibited reduction of NO3− to N2O, and/or enhanced reduction of N2O to N2 (Baggs, 2011; Harter et al., 2013). Indeed, it has been posited that biochars can influence the soil redox potential by (i) acting as semiconductors, (ii) introducing redox-active species such as iron oxides and quinoid moieties, and (iii) creating unique micro- and nanopore environments combining semiconductive boundaries, redox-active species, and laminar flow (Joseph et al., 2013, 2015; Klüpfel et al., 2014; Weng et al., 2015). Thus, the N2O emission results indicate that biochar and soil properties interact to affect soil N2O emissions in a complex manner, potentially through multiple context-specific mechanisms tied to C or N substrate availability and/or the soil redox environment (Sánchez-García et al., 2014).

Implications

The results support the hypotheses that (i) biochar IC contributes to short-term CO2 emissions, (ii) biochars do not significantly accelerate mineralization of biogenic SOC when biochar IC and LOC are accounted for, and (iii) biochars reduce N2O emissions by altering the soil redox environment and/or reducing available OC and inorganic N substrates. We did not observe any evidence for the influence of biochar carbonates on N2O emissions. No single acid- or base-soluble biochar OC fraction was able to consistently serve as a proxy for mineralizable biochar OC; however, results showed a clear, soil-specific influence of biochar IC and OC on CO2 emitted. Consistent reductions in extractable NO3− with biochar amendment support previous studies positing NO3− sorption to biochar as a mechanism for reducing N2O emissions, while contrasting responses of other soil properties to biochar amendment highlight the ability of biochars to influence microsite redox status in a context-specific manner. Thus, a convergence of evidence suggests that sorption of substrates to biochar and changes in the soil redox environment are key mechanisms driving biochars’ effects on CO2 and N2O emissions – and that the dominant mechanisms likely vary by soil (Cayuela et al., 2014; Sánchez-García et al., 2014). Further research is necessary to understand and model the mechanisms governing interactions between biochar properties and soil properties that result in contrasting soil responses to biochar amendments (Jeffery et al., 2015). Specifically, developing accurate models for C and N cycling in bioenergy cropping systems incorporating biochar necessitates research examining (i) biochar sorptive and redox properties and their interactions with soil properties, and (ii) how biochar–soil–management interactions occur in the field, such as in the presence of plants (Whitman et al., 2014; Weng et al., 2015). Such models would help decision-makers choose optimum biochars and management techniques for specific regions, soils, and bioenergy cropping systems, and thereby realize the full climate change mitigation potential of the pyrolysis-biochar-bioenergy platform.
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