Biochar properties and impact on soil CO_2 and N_2O emissions

by

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TABLE OF CONTENTS

NOMENCLATURE

ASVM	Acid-Soluble Volatile Matter
BEOC	Bicarbonate-Extractable Organic Carbon
DOC	Dissolved Organic Carbon
FC	Fixed Carbon
GHG	Greenhouse Gas
IC	Inorganic Carbon
LOC	Labile Organic Carbon
OC	Organic Carbon
SOC	Soil Organic Carbon
VM	Volatile Matter
WFPS	Water-Filled Pore Space

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ABSTRACT

Biochar application to soil has been proposed as a means to sequester carbon and mitigate anthropogenic greenhouse gas (GHG) emissions. However, biochar directly influences soil GHG emissions in a complex-interactive manner that remains poorly understood, and hence further understanding of biochar-soil interactions is needed to evaluate biochar's efficacy as a tool for greenhouse gas mitigation. The goals of this dissertation are to (1) quantify the organic and inorganic alkalis of several biochars, (2) quantify the impact of biochar properties on GHG emissions from diverse soils, and (3) identify mechanisms by which biochar properties influence GHG emissions from soils. To achieve these goals, biochar alkalis and thermochemical properties were analyzed, and three laboratory incubations as well as two field studies and one greenhouse study were conducted, employing eight biochars, two field sites, and four cropping systems. It was found that biochar properties are highly diverse, and effects on GHG emissions varied with respect to biochar properties and soil properties. Both carbonates and bicarbonate-extractable organic carbon in biochar contributed directly to very short term CO_2 emissions but did not influence N_2O emissions. Effects of biochar on N_2O emissions were found to be more complex, with biochar increasing, decreasing or not affecting emissions depending on the context. Results highlight perturbation of N transformations, direct sorption of N, and enhanced water retention as potential mechanisms of biochar's influence on N_2O emissions and suggest that multiple mechanisms likely operate simultaneously.

CHAPTER 1. GENERAL INTRODUCTION

Biochar is thermochemically decomposed biological material that is intended for application to the soil. Themochemical decomposition can be achieved by pyrolysis, which occurs at high temperatures in the absence of oxygen, or by gasification, which also occurs at high temperatures but with a small, controlled, amount of oxygen present. During these processes, 20-50% of the carbon (C) in the feedstock is converted into condensed aromatic C, which is highly resistant to microbial degradation and can persist in the soil for thousands of years (Kuzyakov et al. 2009; Augustenborg et al. 2012). Furthermore, the pyrolysis process can be engineered to produce biofuels, including syngas, bio-oil, or biocrude, each of which can be combusted to generate heat and power or refined to generate drop-in liquid transportation fuels and other products. Application of the biochar co-product to soils can be used to sequester C and offset greenhouse gas (GHG) emissions that result from the biofuel production process (Lehmann et al. 2006).

According to the International Panel for Climate Change (IPCC), human activities need to become net C negative before the end of the 21st century to avoid irreversible climate change (Hare 2009). The conversion of labile biomass C into recalcitrant biochar C via pyrolysis and subsequent application to the soil could contribute significantly to achieving this imperative (Laird 2008). The long-term C sequestration potential of soil biochar applications has been substantiated by studies of *Terra Preta* soils in the Amazon rainforest. These soils contain large quantities of pyrolyzed organic matter with radiocarbon ages of several thousand years (Lehmann et al. 2006). Beyond C

sequestration, the pyrolysis and gasification of biomass can offset GHG emissions by simultaneously generating renewable energy, which could partially replace fossil fuels. Furthermore, once in soil, biochar has the potential to increase crop production and to decrease N_2O and CH_4 emissions from soils. Such outcomes will further increase soil C sequestration. This combination of C sequestration and fossil fuel offsets means that biochar generation and land application could reduce up to 12% of the current net anthropogenic GHG emissions per year (in CO_2 equivalents), compared to a maximum of 10% that could be offset by bioenergy without the co-production of biochar (Woolf et al. 2010). However, due to the complexity of soil-biochar interactions, the effect of biochar on soil GHG emissions is not well understood. The impact of biochar on GHG emissions is anticipated to depend on the properties of the biochar and the context in which it is applied to soil. A mechanistic understanding of soil-biochar interactions is therefore needed to predict both agronomic and environmental outcomes of soil biochar applications.

Biochar-soil interactions are of particular concern because inappropriate biochar-soil combination may result in high GHG emissions, thereby reducing or negating C sequestration efforts (Yu et al. 2013). Carbon dioxide (CO_2), methane (CH_4) and nitrous oxide (N_2O) emissions from soil depend on numerous soil properties including soil pH, texture, porosity, aeration, water holding capacity, oxygen availability, and availability of C substrates. Biochar can alter these soil properties with cascading effects on microbial activity, nutrient cycling, net primary production, and therefore soil GHG emissions. These changes also alter the mineralization rate of biogenic soil organic

matter (SOM) and the rate at which new humic substances are formed from plant and animal residues (Lehmann et al. 2006; Rogovska et al. 2011; Zimmerman et al. 2011). Hence, the diversity of both biochar and soil properties and processes necessitates a deep mechanistic understanding of soil-biochar interactions before appropriate biochars can be chosen for specific contexts.

Few previous studies have encompassed a wide range of soil contexts. 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 and Lehmann 2010; Novak et al. 2010; Smith et al. 2010; Cross and Sohi 2011; Luo et al. 2011; Rogovska et al. 2011; Augustenborg et al. 2012; Kammann et al. 2012; Liu et al. 2012; Yoo and Kang 2012; Zhang et al. 2012). Spokas and 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 GHG emissions 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. The goals of this dissertation are to (1) quantify the organic and inorganic alkalis of several biochars, (2) quantify the impact of biochars on GHG emissions from diverse soils, and (3) identify mechanisms by which biochar properties influence GHG emissions from soils.

CHAPTER 2. QUANTIFICATION OF BIOCHAR STRUCTURAL, CARBONATE AND OTHER ORGANIC AND INORGANIC ALKALIS

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Abstract

Various sources of biochar alkalinity have been identified in the literature – including surface functional groups, soluble organic compounds, carbonates, oxides and hyroxides – but no single study has compared their relative quantities within multiple biochars. Here we quantified four categories of alkalis – low-pK_a structural, other organic, carbonates, and other inorganic alkalis – in eight diverse biochars using a novel suite of methods. We hypothesized that quantities of alkalis in each category will be sensitive to biochar feedstock and pyrolysis conditions. While relative quantities of alkalis were shown to vary widely among biochars, carbonates and other soluble inorganic alkalis consistently constituted the majority of lignocellulosic biochar alkalinity, emphasizing the importance of these often ignored short-term alkalinity sources. Furthermore, the influence of pyrolysis temperature varied with respect to feedstock and other pyrolysis conditions, suggesting that biochar production parameters influence biochar alkalinity in a complex and interactive manner. Results also indicated that, while most biochar alkalis are likely associated with base cations, biochar alkalinity is not a simple function of elemental composition or thermal analysis metrics. More research is needed to characterize soluble biochar alkalis other than

carbonates, and to establish causal relationships among biochar production parameters and the composition of biochar alkalis.

Introduction

Biochars are solid co-products of biomass pyrolysis that are suitable for use as a soil amendment (Lehmann et al. 2006; Schimmelpfennig and Glaser 2012). Biochars are diverse materials with a wide range of chemical and physical properties (Singh et al. 2010a; Brewer et al. 2011; Kloss et al. 2012; Wang et al. 2014). Application of biochar to soil can both sequester carbon and improve soil quality, but such benefits are dependent on properties of both the soil and the biochar. Biochar application has been shown to increase soil cation exchange capacity (CEC), water-holding capacity, nutrient retention, and pH, and also to decrease soil bulk density and greenhouse gas emissions (Joseph et al. 2010; Laird et al. 2010a,b; Rogovska et al., 2011). The effects of biochar amendments on soil are complex, because some biochar-soil interactions have cascading impacts on soil agroecosystems (Joseph et al. 2010).

One of the most influential biochar properties is alkalinity, because changes in pH impact many soil processes, including nitrogen mineralization, mineral precipitation, and ion exchange (Joseph et al. 2010; McCormack et al. 2013). If sufficiently large, variability in total alkalinity and the distribution of alkalis among biochars may become confounding factors in pH-sensitive research (Lehmann et al. 2011). Many biochars have been shown to increase and buffer soil pH, but the nature of biochar alkalis, the influence of feedstock and biochar production conditions on biochar alkali properties,

and how widely biochar alkali properties may vary among biochars remain poorly understood (Yuan et al. 2011a; Yuan et al. 2011b; Xu et al. 2012). Hence, mechanistic understanding of how specific biochar alkalis interact with soil is imperative to further pH-related biochar research, especially if biochar alkalinity and alkali distributions prove to be as widely variable as other biochar properties.

Four broad categories of biochar alkalinity have been identified in the literature: surface organic functional groups, soluble organic compounds, carbonates (salts of bicarbonate and carbonate), and other inorganic alkalis which may include oxides, hydroxides, bisulfate, or phosphates (as orthophosphate or hydrogen phosphate) (Singh et al. 2010a; Yuan et al. 2011a). The criteria for differentiating these categories vary among previous studies, especially with respect to the pK_a ranges of surface functional groups and the reaction pH or acid concentration used to quantify total alkalinity or carbonate alkalinity (Singh et al. 2010a; Yuan et al. 2011b). Despite several studies investigating alkali categories individually or in pairs (Chun et al. 2004; Yuan et al. 2011b; Wang et al. 2014; Chen et al. 2015), there remains a lack of information in the literature regarding the relative abundance all four categories in diverse biochars. Here we aim to (1) present a suite of methods to quantify biochar surface structural alkalis, carbonates, and other organic and inorganic alkalis, (2) employ these methods to quantify the alkalis of eight diverse biochars, and (3) assess the variability of total biochar alkalinity and relative abundance of biochar alkalis among the four alkali categories. We hypothesize that total, structural, carbonate, other inorganic and other organic alkalinity will all be sensitive to both feedstock type and pyrolysis conditions.

Methods

Biochar preparation

Eight biochars made from four different feedstocks at three different temperatures and encompassing a wide range of pHs were selected for this study (Table 1). Slow pyrolysis biochars made from cellulose and corn stover were pyrolyzed in a N_2-purged muffle furnace for ~1 h. A mixed wood gasification biochar (MW6g) was obtained from ICM, Inc. (http://www.icminc.com/), and a hardwood slow pyrolysis biochar (HW5s) was obtained from Royal Oak (size #10 charcoal, 0.5-2mm, http://royal-oak.com/). Two fast pyrolysis biochars, RO5f and CS5f, were obtained from the Center for Sustainable Energy Technologies at Iowa State University, and they were sieved to <0.50 mm to exclude sand particles (sand is used in the fluidizing medium in the fast pyrolysis process, and it is not completely separated from the biochar during the production process). All slow pyrolysis and gasification biochars were ground to <0.50 mm to minimize the influence of particle size on chemical analyses.

Biochar pH

Biochar pHs were measured in duplicate by mixing biochar in a 10:1 water:biochar (mL:g) slurry, allowing the slurry to equilibrate for 1 hr, and then measuring the pH of the supernatant with a pH meter (Fisher) equipped with a H^+ electrode.

Proximate analysis

Ash, fixed carbon (FC) and volatile matter (VM) of untreated and acid-washed lignocellulosic biochars were quantified in triplicate by thermogravimetric analysis using a Mettler TGA/DSC 1 (Choi et al. 2014). Biochar samples weighing 10-20 mg were heated to 105°C and held at that temperature for 40 min to remove water. Then samples were heated to 900°C in an N_2 atmosphere at a rate of 10°C min^{-1}; next the temperature was held at 900°C for 20 min; and lastly the samples were exposed to air at 900°C for 30 min. VM and FC were determined as the percentage of mass lost under N_2 purge and after exposure to air for 30 min at 900°C, respectively. Ash content was determined as the percentage of the initial mass remaining after the sample had been exposed to air at 900°C. To compare the amounts of ash and VM removed during acid washing, the ash and VM contents were normalized to the FC content. The acid-soluble ash was calculated as the difference between FC-normalized ash value of the untreated and acid-washed biochars. Acid-soluble VM was determined similarly. This calculation was based on the assumption that FC is not acid-soluble.

Total elemental analysis

C, H, and N of the seven lignocellulosic biochars were analyzed in triplicate using a Vario Microcube (Elementar) combustion analyzer. Inorganic elements were determined by x-ray fluorescence (XRF) spectroscopy using a Philips PW 2404 X-ray spectrometer (XRF) equipped with a rhodium X-ray tube operated at 3600 watts (Lawrinenko 2014). The spectrometer was flushed with helium during all

measurements. All measurements were corrected for tube drift via monitor samples (AUSMON-silicate minerals reference monitor and CA69, a carbonate rock reference monitor). Specimens were presented to the spectrometer as 2 g of loose powders in disposable sample cups sealed with polypropylene film (6-μm thick). Calibration standards were prepared by spiking a low-ash biochar derived from cellulose slow-pyrolyzed at 700 °C (prepared in the same manner as slow pyrolysis corn stover biochars) with different percentages of reagent grade potassium chloride and standard reference materials derived from coal ash, minerals and wood (NIST 1633a, NIST 2691, USGS Nod-A-1, NIST 2910, and AWP Std I; see Table S2.2). These mixtures were combined and co-ground in a SPEX Shatterbox puck mill for two minutes. Due to the similarity between the CE5s and the cellulose biochar used to make the calibration standards, CE5s was excluded from XRF analysis.

X-ray diffraction

X-ray diffraction patterns of four untreated and acid-washed biochars (CS5f, CS3s, CS6s and MW6g) were obtained with a Siemen D5000 x-ray diffractometer equipped with a graphite monochromator using Cu Kα radiation generated at 40 KV and 30 mA. Step scan mode was used with a step size of 0.05° 2θ and a dwell time of 7 seconds per step. A scintillation counter detector was used with fixed 0.5° divergence and 1.5° anti-scattering slits. Random powder mounts of biochars were analyzed at ambient temperature and humidity.

Total alkalinity and ion release

Methods for quantifying total alkalinity were previously evaluated by Fidel (2012). It was found that equilibration of 0.05 M HCl with biochar for 72 h (50:1 solution:biochar ratio) consistently yielded the highest total alkalinity upon titration of the resultant extract relative to other HCl concentrations and equilibration times tested. Use of HCl concentrations of less than 0.05 M resulted in lower measurements of total alkalinity due to incomplete reaction with biochar alkalis. Using higher concentrations of HCl, such as 0.1 M or 1 M, resulted in lower estimates of biochar alkalinity, likely due to the dissolution of acid-soluble alkalis and subsequent interference with titration. Thus, for the purposes of this study, total alkalinity is defined as the capacity of the untreated biochar to accept protons from a 0.05 M HCl solution.

Total alkalinity was quantified via reaction with HCl and subsequent back titration (Fidel 2012). Briefly, biochar was first shaken rapidly on an automatic shaker table with 0.05 M HCl solution at a 50:1 solution:biochar (v:w) ratio for 72 hours (pH ≤ 2). Two biochars, HW5s and MW6g, increased the final solution pH to > 2, and so these biochars were equilibrated at a 100:1 solution:biochar ratio. Biochar-solution slurries were filtered to <0.45 μm, and the extracts were titrated to pH 8.2 (phenolphthalein indicator) with standardized 0.05 M NaOH. The 0.05 M HCl solution was also titrated as a blank. Total alkalinity, as the mmol of H^+ reacted per gram of air-dry biochar, was calculated from the difference between the amount of acid titrated in the sample subtracted from the blank. To quantify elements released during the reaction with HCl,

acidic extracts generated during total alkalinity analysis were analyzed in triplicate for Na, K, Ca, Mg, Si, S, P, Fe, Al, and Mn using a Thermo Scientific™ iCAP™ 7400 ICP-OES Analyzer.

Low-pK_a structural and other organic alkalinity

We define "organic alkalinity" as the quantity of protons taken up by organic functional groups in biochar, including both functional groups directly bonded to biochar's condensed aromatic matrix and functional groups in acid-soluble organic compounds. We define "structural alkalinity" as the concentration of functional groups directly bonded to biochars' aromatic carbon framework structure and that are protonated after equilibration with 0.05 M HCl. In principle, the 0.05 M HCl solution used to quantify total alkalinity should react with all functional groups with pK_as between the pH of the biochar-equilibrated HCl (1.3-2.0) and the initial biochar pH prior to the addition of HCl (Table 2.1). The Boehm titration, in which surface functional groups are quantified by equilibration with solutions of known pK_a ($NaHCO_3$, Na_2CO_3 and NaOH) is capable of quantifying structural alkalis within the discrete pK_a ranges defined by the reactants (5-6.4, 6.4-10.3 and 10.3-13) (Boehm 1994; Goertzen et al. 2010). However, the lignocellulosic biochars in this study have pHs ranging from 7.1-10.3, and hence the functional group concentration within the 6.4-to-biochar pH range cannot be quantified using the Boehm titration. Therefore, we sub-divided organic alkalis into the following categories: (1) *low-pK_a structural alkalis* with pK_as ranging from 5.0 to 6.4, and (2) *other organic alkalis*, which include both high pK_a structural alkalis with pK_as

between 6.4 and the biochar pH, and soluble organic alkalis such as acetate or formate (Lin et al. 2012; Liu et al. 2015).

Biochar surface functional groups were quantified in triplicate using the integrated "sparge-barium-barium" Boehm titration method developed specifically for biochars by Fidel et al. (2013). Briefly, soluble alkalis were removed from a subsample of each biochar by washing with 0.05 M HCl once, 1 M $CaCl_2$ twice, and then with deionized water four times (50:1 solution:biochar ratio). Separate samples of acid washed biochars were shaken with 0.05 M solutions of the Boehm reactants $NaHCO_3$, Na_2CO_3, and NaOH. $NaHCO_3$ extracts were acidified to pH <2 and sparged to remove carbonates, then titrated with NaOH. Na_2CO_3 and NaOH extracts were centrifuged with $BaCl_2$ to remove carbonates and DOC, and then the supernatant was acidified. Acidified extracts were titrated with NaOH (phenolphalein indicator) to determine the amount of protons donated to the Boehm reactants by the biochars' organic functional groups. The quantity of protons donated to the $NaHCO_3$, Na_2CO_3 and NaOH Boehm reactant solutions was used to calculate the concentrations of functional groups with pK_as less than 6.4, 10.3, and 13, respectively. The lower limit of the pK_a range in which functional groups were quantified was set by the initial pH of the pre-treated (acid-washed) biochars (pH ~5). Concentrations of functional groups with pK_as in the 5.0-6.4, 6.4-10.3, and 10.3-13 ranges were determined by difference.

Although functional groups in the higher pK_a ranges (6.4-10.3 and 10.3-13) were not used to directly quantify other organic alkalis, we present them here to give

perspective to the calculated other organic alkalis. Other organic alkalis were quantified as the total alkalinity minus the sum of low-pK_a structural alkalis, carbonates, and other inorganic alkalis.

Carbonate alkalinity

Biochar carbonates were quantified in triplicate using a modified NaOH trap method (Fidel, 2012). Briefly, 2 g of each biochar were stirred with 100 mL of 0.05 M HCl in a sealed Mason jar; a separate container inside of the Mason jar held 15 mL of 1 M NaOH. After 72 h, the NaOH was removed, 15 mL of 1 M $BaCl_2$ were added to the NaOH, and the solution was titrated with 1 M HCl to pH 8.2 (phenolphalein indicator) to quantify the amount of CO_2 evolved from the biochar.

Other inorganic alkalinity

"Other inorganic alkalinity" was assessed as the quantity of protons consumed by acid-soluble inorganic compounds in biochar including phosphates, sulfates, silicates, manganese hydroxides, iron hydroxides, and aluminum hydroxides. Other inorganic alkalinity was estimated as the sum of P, S, Si, Mn, Fe and Al solubilized during reaction with 0.05 M HCl, in mmol g^{-1}. The concentration of each element was adjusted for the pH of the biochar and acid reaction solution relative to the pK_as of the alkalis of interest. Thus, for biochars with a pH of ~7 (RO5f and CS3s), other inorganic alkalinity (in meq g^{-1} of biochar) was calculated from element concentrations (in brackets) obtained by analysis of acidic extracts (in meq g^{-1} of biochar):

Other inorganic alkalinity = 0.5[P] + 0.5[S] + 0.5[Mn] + 0.5[Fe] + [Al]

And for biochars with pHs between 7.5 and 8.5 (CS5f and HW5s):

Other inorganic alkalinity = [P] + 0.5[S] + [Mn] + [Fe] + [Al]

For biochars with pHs between 8.5 and 9.5 (MW6g):

Other inorganic alkalinity = [P] + 0.5[S] + 0.5[Si] + [Mn] + [Fe] + [Al]

Lastly, for biochars with pH > 9.5 (CS5s and CS6s):

Other inorganic alkalinity = [P] + 0.5[S] + [Si] + [Mn] + [Fe] + [Al]

This estimation of other inorganic alkalinity assumes that oxides and hydroxides of base cations do not contribute significantly to biochar inorganic alkalinity. Although oxides and hydroxides of base cations – such as CaO, KOH or $Mg(OH)_2$ – have been posited as a hypothetical source of biochar alkalinity, their presence in biochars has not been verified. Furthermore, all biochars used here were exposed to the atmosphere for several months, and oxides or hydroxides of base cations should in theory react with CO_2 in the atmosphere to form carbonates. Thus, we consider it unlikely that the biochars analyzed in this study contain oxides or hydroxides of base cations, and we have not included them in our analysis of inorganic alkalis.

Table 2.1. Biochar production parameters and pHs expressed as means of two replicates.

Biochar	**Feedstock**	**Pyrolysis Temperature (°C)**	**Process**	**pH**
CE5s	Cellulose	500	Slow pyrolysis	6.4
RO5f	Red Oak	500	Fast pyrolysis	7.1
CS5f	Corn stover	500	Fast pyrolysis	8.4
CS3s	Corn stover	300	Slow pyrolysis	7.3
CS5s	Corn stover	500	Slow pyrolysis	10.1
CS6s	Corn stover	600	Slow pyrolysis	10.3
HW5s	Hardwood	~500	Slow pyrolysis	7.9
MW6g	Mixed wood	~600	Gasification	8.8

Results and Discussion

Proximate analysis

Volatile matter (VM), fixed carbon (FC), and ash varied considerably among biochars, ranging from 16-36%, 40-78%, and 1-29%, respectively (Figure 2.1). Among biochars made at 500°C, wood biochars had higher FC (65-66%) than the corn stover biochars (51-58%). Ash contents were higher for corn stover biochars than for hardwood biochars, but the mixed wood biochar had an ash content comparable with that of the corn stover biochars. The cellulose biochar had the highest FC (78%), and the lowest ash content (1%), likely due to the low ash content of the feedstock. The highest temperature slow pyrolysis biochar (CS6s) had the highest FC:VM ratio (4.4:1), followed by CE5s (3.7:1), MW6g (3.3:1), CS5s (3.2:1), HW5s (2.9:1), RO5f (2.8:1), CS5f (2.5:1) and CS3s (1.1:1). Biochars made from corn stover consistently exhibited increasing FC and FC:VM ratios with increasing pyrolysis temperature and retention time. The relatively

large range of FC:VM ratios among lignocellulosic biochars made from the same feedstocks under the different pyrolysis conditions, compared with the relatively small range of FC:VM ratios among biochars made from the different feedstocks under the same pyrolysis conditions, emphasizes the importance of pyrolysis condition impacts on aromatic condensation of lignocellulosic feedstocks.

In most cases, a substantial portion – but not all – of biochar ash was acid-soluble, whereas a relatively small portion of VM was soluble. Soluble ash represented a larger proportion of total ash among wood biochars than among corn stover biochars, most likely due to lower Si concentrations in wood biochars. However, ash content and soluble ash did not vary consistently with pyrolysis temperature.

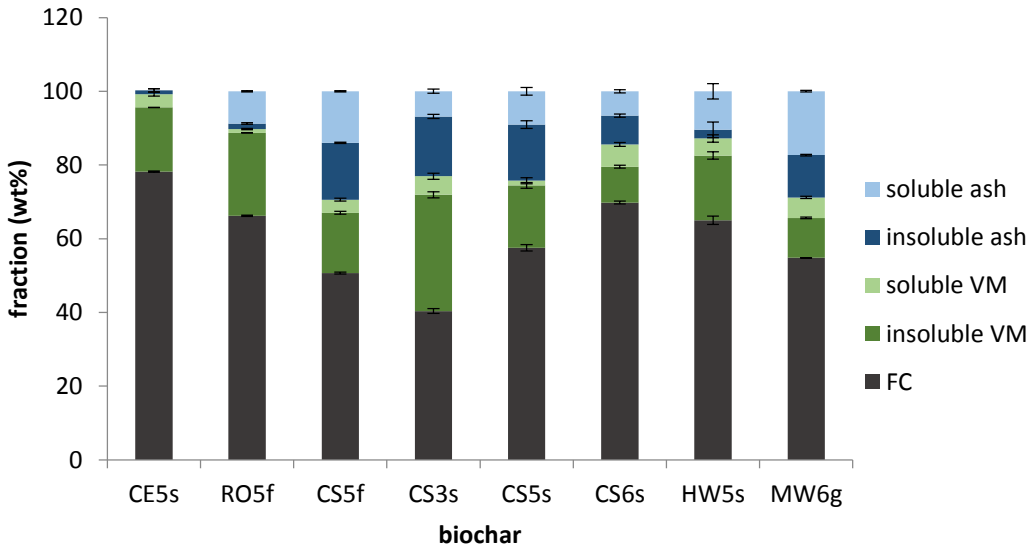

Figure 2.1. Volatile matter (VM), fixed C (FC), and ash contents of the studied biochars determined by proximate analysis. Soluble ash and soluble VM are defined as the ash and VM removed by washing with 0.05 M HCl normalized relative to FC, which is assumed to be acid insoluble. Error bars represent standard deviations of the mean (n =3).

Elemental composition

Carbon, H, and N contents of the lignocellulosic biochars are shown in Table 2.2. Among slow pyrolysis corn stover biochars produced at 300, 500 and 600°C, %C increased with increasing pyrolysis temperatures, whereas %H decreased with increasing pyrolysis temperature. Wood biochars had higher %C and lower %H than corn stover biochars produced under the same pyrolysis conditions. The %N in the corn stover biochars were consistently higher than the %N of the wood biochars, and %N decreased with increasing pyrolysis temperature among the slow pyrolysis corn stover biochars. Acid washing tended to slightly increase %C and %H, but it did not consistently affect %N. The increase in %C and %H following acid washing are likely due to the removal of ash, which contains relatively little C and H compared with the acid-insoluble, condensed aromatic C in biochar.

The lignocellulosic biochars had widely varying amounts of inorganic elements (Table 2.3). Combined, K, Mg, Ca, and Si comprised 87-97% of all inorganic elements measured. Si was the most abundant inorganic element in the fast pyrolysis biochars and the corn stover biochars, whereas Ca was the most abundant in the slow pyrolysis hardwood and gasification mixed wood biochars. Among the corn stover biochars, K content increased with increasing pyrolysis temperature, whereas Si, Mg, and Ca decreased. Trace elements (Ni, Cu, Zn, Sr, and Ti) together comprised <1% of all inorganic elements quantified via XRF (see Table S2.1).

Table 2.2. Percent C, H, and N of the eight biochars, both untreated and acid washed, plus untreated cellulose biochar (n = 3; n.d. = not determined; ± average standard deviation)

	%C (±1%)		**%H** (±0.06%)		**%N** (±0.02%)	
biochar	untreated	acid washed	untreated	acid washed	untreated	acid washed
CE5s	96	n.d.	2.73	n.d.	0.18	n.d.
R05f	74	75	3.03	3.23	0.24	0.20
CS5f	51	53	2.40	2.60	0.59	0.71
CS3s	52	55	3.82	3.84	1.65	1.43
CS5s	60	66	2.61	2.88	1.16	1.30
CS6s	68	74	2.00	2.06	0.61	0.65
HW5s	73	76	2.72	2.81	0.44	0.48
MW6g	63	70	1.90	2.05	0.60	0.54

Table 2.3. Elements in untreated lignocellulosic biochars quantified via XRF (wt%) (single determination, RSD = relative standard deviation of the method for each element)

	Element										
biochar	**Na**	**K**	**Mg**	**Ca**	**Si**	**P**	**S**	**Cl**	**Al**	**Fe**	**Mn**
RO5f	<0.02	0.54	0.1	0.99	5.72	0.01	0.02	<0.05	<0.01	0.16	0.039
CS5f	0.025	1.38	1.32	1.59	13.57	0.19	0.05	0.59	0.087	0.11	0.012
CS3s	0.034	1.09	1.19	1.25	10.54	0.26	0.08	0.06	0.27	0.18	0.008
CS5s	0.025	1.98	1.01	1.2	7.77	0.3	0.05	0.19	0.17	0.15	0.008
CS6s	<0.02	4.74	0.76	0.66	5.54	0.24	0.06	1.4	0.022	0.03	0.011
HW5s	<0.03	0.48	0.21	8.14	1.95	0.02	0.04	<0.05	0.094	0.07	0.094
MW6g	0.167	0.86	0.74	5.23	3.57	0.06	0.15	0.22	0.36	0.44	0.036
RSD (%)	4	3	9	3	16	7	4	7	18	1	7

X-ray diffraction

The XRD patterns of untreated and acid washed CS5f, CS3s, CS6s, and MW6g are shown in Figure 2.2. Quartz peaks were observed for all untreated biochars, carbonate (as calcite, dolomite, butschliite and/or natrite) peaks were observed for CS5f, CS6s, and MW6g, and sylvite peaks were observed for CS5f and CS6s biochars. These three minerals were identified by multiple XRD peaks and are consistent with the dominant inorganic elements present in the untreated biochars (Table 2.3). The XRD patterns also indicated the presence of small quantities of other minerals tentatively identified as natrite, dolomite, and/or butschliite based on single peaks. Following acid washing, peaks attributed to sylvite, calcite, natrite, dolomite, and/or butschliite were either no longer observable or greatly diminished in intensity. Overall, mineral phases observed in the biochars were consistent with the literature but did not show consistent trends with respect to feedstock or pyrolysis temperature (Yuan et al. 2011b; Kloss et al. 2012). A more in-depth discussion of XRD is presented in the supplemental information.

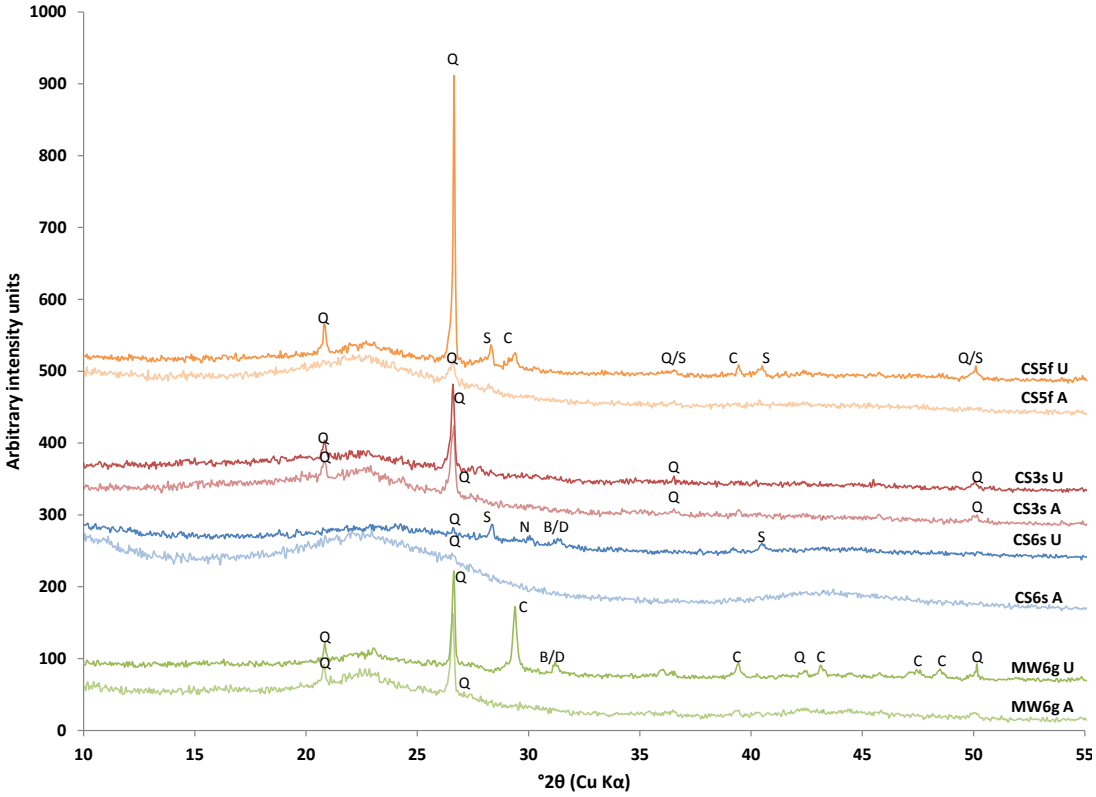

Figure 2.2. XRD patterns of untreated (U) and acid-washed (A) CS5f, CS3s, CS6s and MW6g biochars. Peaks are labeled with probable mineral phases (Q = quartz, S = sylvite, C = calcite, D = dolomite, B = buetchliite, and N = natrite).

Element release

Calcium, K, and Mg comprised the majority of ions released, with Na, Si, Al, Mn, P and S found at much lower concentrations (Table 2.4). In general, corn stover biochars released greater quantities of base cations than hardwood biochars, with the exception of MW6g. MW6g released 2.60 meq g^{-1} of soluble base cations, probably from ion-rich ash residue left in the auger bed gasifier after previous runs (Albert Bennett, *personal communications*).

The percentage of total inorganic elements solubilized on reaction with 0.05 M HCl for 72 hr varied widely with respect to element and biochar (Table 2.5). Sodium was the most acid-soluble element, whereas Si was consistently the least soluble inorganic element. The solubility of Ca and Mg did not show consistent trends with respect to biochar feedstock, but the solubility of K in was generally higher for corn stover biochars compared with wood biochars. No single biochar exhibited consistently high element solubility across all elements. The moderate (38-44%) solubility of K and Ca in CS5f, CS6s and MW6g supported the diminished intensity of sylvite and calcite peaks observed in the XRD patterns following acid washing. Conversely, the low solubility of Si supported the persistence of quartz peaks following acid washing. The solubility of P was widely variable (8-60%), suggesting that P may be present in biochar as multiple species of varying solublility, such as orthophosphate, pyrophosphates, or various organically bound P compounds. That base cations were rarely 100% soluble despite the typically high solubility of chlorides and carbonates in acid suggests that some cations may have

been occluded by low-solubility minerals or by the condensed aromatic biochar matrix. It should be noted that total Cl (as determined by XRF) increased with acid-soluble K, and Cl was always less than acid-soluble K, suggesting that some – but not all – acid-soluble K was associated with Cl, and also confirming the observation of sylvite in CS5f and CS6s (Figure S1).

Table 2.4. Concentrations of inorganic elements released from the untreated biochars after 72 hr reaction with 0.05 M HCl (n = 3; RSD = relative standard deviation).

	Concentration (g kg^{-1})									
biochar	**Na**	**K**	**Mg**	**Ca**	**P**	**S**	**Si**	**Mn**	**Fe**	**Al**
CE5s	*	*	*	*	*	*	*	*	*	*
RO5f	0.32	4.6	0.38	12.0	0.22	0.05	0.59	0.05	0.09	*
CS5f	0.47	18.4	4.72	15.3	2.8	0.29	0.86	0.09	0.38	0.17
CS3s	0.58	13.0	4.38	9.63	3.57	0.34	1.37	0.07	0.68	0.31
CS5s	0.82	24.9	4.85	11.7	4.29	0.25	1.69	0.08	0.76	0.92
CS6s	1.03	53.0	3.19	6.2	2.67	0.11	1.79	0.10	0.00	0.18
HW5s	0.90	2.0	0.60	23.4	0.29	0.12	0.74	0.29	0.1	0.2
MW6g	3.73	5.0	3.05	41.3	0.17	0.79	5.83	0.20	0.00	0.67
RSD (%)	22	4	17	17	20	33	19	13	38	33

*below detection limit

Table 2.5. Percentage of total elements soluble in 0.05 M HCl (n = 3).

biochar	**Na**	**K**	**Mg**	**Ca**	**P**	**S**	**Si**	**Mn**	**Fe**	**Al**
	----------% ----------									
RO5f	*	28.5	12.5	41.5	61.2	11.0	0.3	4.1	1.9	*
CS5f	61.8	44.5	11.9	32.0	48.0	17.9	0.2	25.4	11.5	6.7
CS3s	56.1	39.7	12.3	25.7	45.2	14.6	0.4	29.5	12.7	3.9
CS5s	100	41.9	16.0	32.4	47.0	16.9	0.7	34.7	16.8	18.1
CS6s	*	37.5	13.9	31.2	37.9	5.9	1.1	30.1	*	27.8
HW5s	*	13.9	9.1	9.6	40.5	10.7	1.3	10.4	4.6	7.1
MW6g	74.2	19.6	13.6	26.3	8.8	17.4	5.4	18.4	*	6.3

*below detection limit

Total alkalinity

Total alkalinities varied widely among biochars (0.23-2.26 meq g^{-1}), with MW6g and CE5s having the highest and lowest total alkalinities, respectively (Figure 2.3). Slow pyrolysis corn stover biochars exhibited increasing total alkalinity with increasing pyrolysis temperature. Compared with CS5s, CS5f had a higher total alkalinity, suggesting that heating rate may affect total alkalinity in addition to pyrolysis temperature. Total alkalinity varied more widely among wood biochars than among corn stover biochars: MW6g had a total alkalinity over six times higher than RO5f, whereas the total alkalinity of CS6s was less than three times higher than that of CS3s. The large range of total alkalinities among wood biochars despite similarities in feedstocks and pyrolysis temperatures may be attributed to other pyrolysis parameters or feedstock handling procedures, including heating rate, the partial pressure of oxygen within the reactor, and feedstock particle size, which have been shown to influence various biochar properties (Sun et al. 2012). Thus, the biochar feedstock, and pyrolysis temperature, and heating rate would appear to have unique and interactive impacts on total alkalinity.

Total alkalinity was strongly correlated with the equivalents of acid-soluble base cations released among the seven lignocellulosic biochars (r^2 = 0.95) (Figure 2.4). The cellulose biochar, CE5s, was an outlier of the regression line due to its non-detectable levels of acid-soluble base cations, but its total alkalinity was still low (0.23 meq g^{-1}). Furthermore, biochar pH increased with increasing biochar alkalinity and increasing

proportions of K and Na relative to Ca and Mg (Table 2.1, Table 2.5, and Figure 2.3). For example, CS6s had the highest ratio of monovalent-to-divalent cation equivalents (2.5:1) and the second highest alkalinity, and the highest pH; MW6g on the other hand, had the highest alkalinity but a lower monovalent-to-divalent cation ratio (0.1:1), and consequently its pH was lower than CS6s. Thus, among the lignocellulosic biochars examined here, biochar alkalis were predominantly associated with base cations, and higher proportions of acid-soluble monovalent cations among total base cations corresponded with higher biochar pHs.

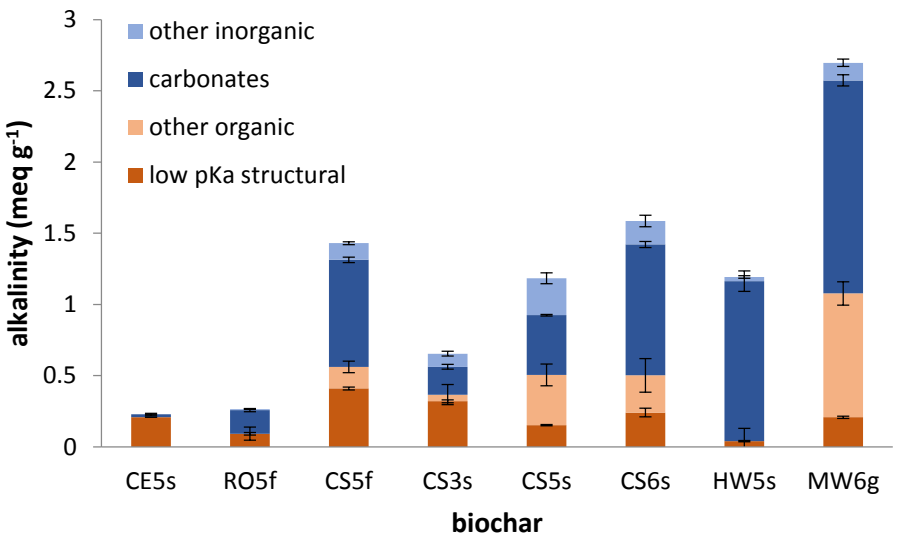

Figure 2.3. Biochar alkalinity attributed to low pK_a structural alkalis ($5 \leq pK_a \leq 6.4$), other organic alkalis, carbonates, and other inorganic alkalis, in meq per gram of biochar. Error bars represent the standard deviation of the mean (n = 3).

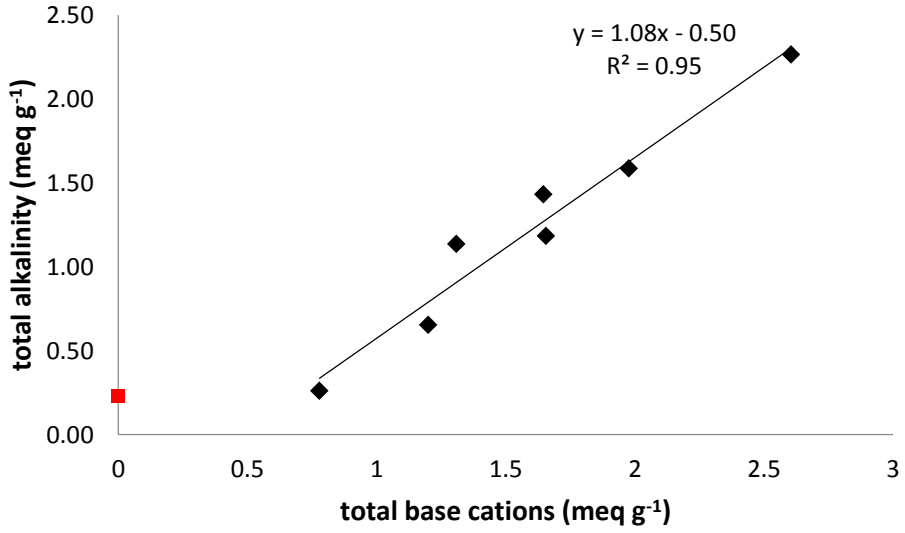

Figure 2.4. Total soluble base cations (Na + K + Ca + Mg) and total alkalinity (meq g^{-1}) of all eight untreated biochar. Each data point represents the mean of three measurements, and regression was calculated for lignocellulosic biochars only (red square = CE5s; black diamonds = lignocellulosic biochars).

Organic alkalinity

Structural functional group concentrations in three discrete pK_a ranges, as determined by the modified Boehm titration, are shown in Figure 2.5. The total functional group concentration and the distribution of functional group concentrations among the three pK_a ranges varied widely among biochars. CS5f had the highest total functional group concentration (2.46 meq g^{-1}) and HW5s had the lowest (0.16 meq g^{-1}). Functional group concentrations within the 5.0-6.4, 6.4-10.3 and 10.3-13 pK_a ranges varied from 0.04-0.207 meq g^{-1}, 0-0.39 meq g^{-1}, and 0-1.68 meq g^{-1}, respectively. Effects of feedstock and temperature on functional group concentrations were inconsistent across biochars. However, all lignocellulosic biochars' functional group distributions were skewed towards the higher pK_a ranges, whereas the cellulose biochar did not contain significant quantities of functional groups with $pK_a > 6.4$, suggesting that lignin and/or ash may be involved in the formation of these functional groups.

The use of the Bohem titration results to estimate the contribution of structural alkalis to total alkalinity is problematic because Bohem titrations only quantify functional group concentration for discrete pK_a ranges whereas total alkalinity includes all sources of alkalinity which are reactive at pHs up to that of biochar when equilibrated in distilled water. Hence to distinguish structural alkalis which contribute to total alkalinity ($pK_a <$ biochar pH) with those that do not ($pK_a >$ biochar pH), we divide organic alkalis into three categories: (1) structural alkalis with $5.0 \leq pK_a \leq 6.4$ ("*low-pK_a structural*

alkalis"), (2) structural alkalis with 6.4 ≤ pKa ≤ biochar pH, and (3) soluble organic alkalis with pK_a ≤ biochar pH ("*other organic alkalis*").

The majority of biochars had larger quantities of low-pK_a structural alkalis than other organic alkalis. The corn stover biochars tended to have larger quantities of low-pK_a structural alkalis compared with wood biochars. CS5f had the highest structural alkalinity (0.41 meq g^{-1}), followed by CS3s (0.32 meq g^{-1}) and CS6s (0.24 meq g^{-1}). Among the wood biochars, MW6g had the highest structural alkalinity (0.21 meq g^{-1}), and HW5s had the lowest (0.05 meq g^{-1}). The mixed wood gasification biochar (MW6g) had the largest quantity of other organic alkalis, followed by the corn stover biochars, whereas RO5f, HW5s and CE5s had negligible (≤0.001 meq g^{-1}) quantities of other organic alkalis. Among the corn stover biochars, other organic alkalinity increased with increasing concentration of functional groups in the 6.4-10.3 pK_a range, suggesting that these functional groups contributed to other organic alkalinity. Indeed, CS6s, which had a pH similar to the pK_a of the Na_2CO_3 Boehm reactant, also had an other organic alkali content (0.26 meq g^{-1}) similar to the structural functional group concentration in the 6.4-10.3 pK_a range (0.29 meq g^{-1}). The mixed wood gasification biochar was the only biochar for which other organic alkalinity (0.87 meq g^{-1}) greatly exceeded the functional group concentration in the 6.4-10.3 pK_a range (0.14 meq g^{-1}). Thus a large portion of MW6g's other organic alkalinity can probably be attributed to soluble organic alkalis such as acetate and formate, which have been previously identified in aqueous biochar extracts (Lin et al. 2012; Liu et al. 2015).

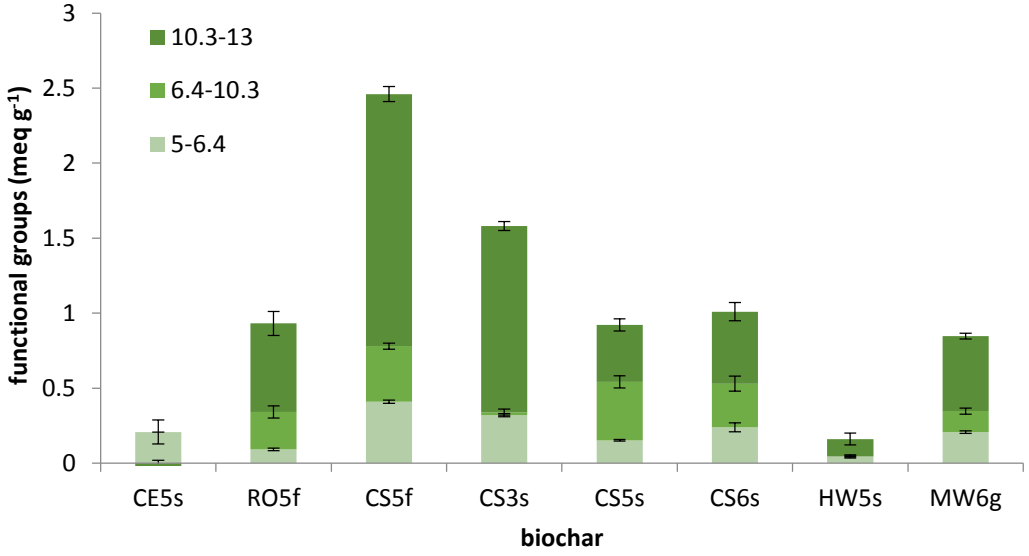

Figure 2.5. Surface organic functional group concentrations in discrete pK_a ranges (5-6.4, 6.4-10.3, and 10.3-13), as quantified via the Boehm titration. Error bars represent the standard deviation of the mean (n = 3).

Carbonates and other inorganic alkalis

The quantities of carbonate and other inorganic alkalis varied greatly among the eight biochars (Figure 2.3). MW6g had the highest carbonate alkalinity (1.50 meq g^{-1}), followed by HW5s (1.41 meq g^{-1}) whereas RO5f had the lowest carbonate alkalinity of all the lignocellulosic biochars (0.165 meq g^{-1}). Among the slow pyrolysis corn stover biochars, carbonate alkalinity increased with increasing pyrolysis temperature (CS3s<CS5s<CS6s). Compared with slow pyrolysis biochars made from similar feedstocks at the same temperature, CS5f and RO5f had higher and lower carbonate contents, respectively. Such inconsistencies in the effect of pyrolysis conditions across feedstocks are in agreement with previous evidence suggesting that pyrolysis conditions and feedstock may interact in a complex, difficult-to-predict manner (Sun et al. 2012).

Concentrations of other inorganic alkalis – here defined as non-carbonate soluble inorganic alkalis – ranged from 0-0.26 meq g^{-1}. CS5s had the highest other inorganic alkalinity, whereas CE5s, RO5f and HW5s all had negligible (≤0.03 meq g^{-1}) other inorganic alkalinities. The slow pyrolysis corn stover biochars did not exhibit a consistent trend in other inorganic alkali content with respect to pyrolysis temperature, as CS5s had the highest quantity of other inorganic alkalis (0.26 meq g^{-1}), and CS3s had the lowest (0.09 meq g^{-1}). Due to feedstock batch differences CS6s had a lower ash content than CS5s (Figure 2.1), which may explain why other inorganic alkalinity was lower for CS5s than CS6s.

Contributions of alkalis to total alkalinity

The contributions of alkalis to total alkalinity varied widely with respect to feedstock, pyrolysis temperature and heating rate. The cellulose biochar, CE5s, contained negligible ash (Figure 2.1) and hence was dominated by low-pK_a structural alkalinity (91%), whereas for the other biochars low-pK_a structural alkalinity ranged from 3 to 49%. Other organic alkalis contributed <20% of the total alkalinity to all biochars except CS5s and MW6g, for which other organic alkalis contributed 30-32% of the total alkalinity. The wood biochars consistently had a higher proportion of carbonate alkalinity (64-97%) than the corn stover biochars (30-58%). The corn stover biochars consistently had a higher proportion of other (non-carbonate) inorganic alkalinity (8-22%) than the hardwood biochars (0-5%). When combined, all inorganic alkalis contributed 44-99% of total alkalinity of the lignocellulosic biochars, but only 9% of total alkalinity of the cellulose biochar. Among all lignocellulosic biochars with pyrolysis temperatures ≥500°C, inorganic alkalis comprised >50% of total alkalinity. Thus, among the seven lignocellulosic biochars studied here, inorganic alkalinity comprised the majority or near-majority of all biochar alkalis.

Conclusions

Both total alkalinity and the distribution of alkalis among low-pK_a structural, other organic, carbonate, and other inorganic alkalis were found to vary widely with feedstock and pyrolysis conditions among the eight studied biochars. Corn stover biochars consistently had higher concentrations of other low-pK_a structural alkalis and

other inorganic alkalis than wood biochars produced under similar conditions. However, other differences in the impact of pyrolysis conditions on biochars produced from corn stover and wood on alkali quantity and composition suggest that biochar alkalinity may arise from feedstock and pyrolysis conditions in a complex and interactive manner that is difficult to predict solely from pyrolysis parameters. Furthermore, a lack of consistent correlations between VM, FC or ash content and biochar alkalinity or biochar alkali composition suggests that thermogravimetric metrics may not be good indicators of biochar alkalinity. Biochar alkalinity and alkali distributions should therefore be directly quantified until the relationship between biochar alkalinity and other biochar properties is better understood. Ultimately, more research will need to be conducted on a much broader suite of biochars to ascertain how production parameters impact biochar alkalinity and alkali composition and to fully characterize the chemical composition of the other organic and inorganic alkalis.

APPENDIX A. SUPPLEMENTAL INFORMATION FOR CHAPTER 2

Table S2.1. Concentrations of trace elements in lignocellulosic biochars (wt%; single determination; method s.e. = 0.001)

biochar	Ni	Cu	Zn	Sr	Ti
RO5f	<.003	<.003	<.001	0.0052	<.008
CS5f	0.005	0.004	0.002	0.0031	0.009
CS3s	<.003	<.003	0.003	0.0032	0.033
CS5s	<.003	<.003	0.002	0.0029	0.020
CS6s	<.003	0.009	0.002	0.0030	<.008
HW5s	<.003	<.003	<.001	0.0211	0.017
MW6g	<.003	0.031	0.011	0.0173	0.057

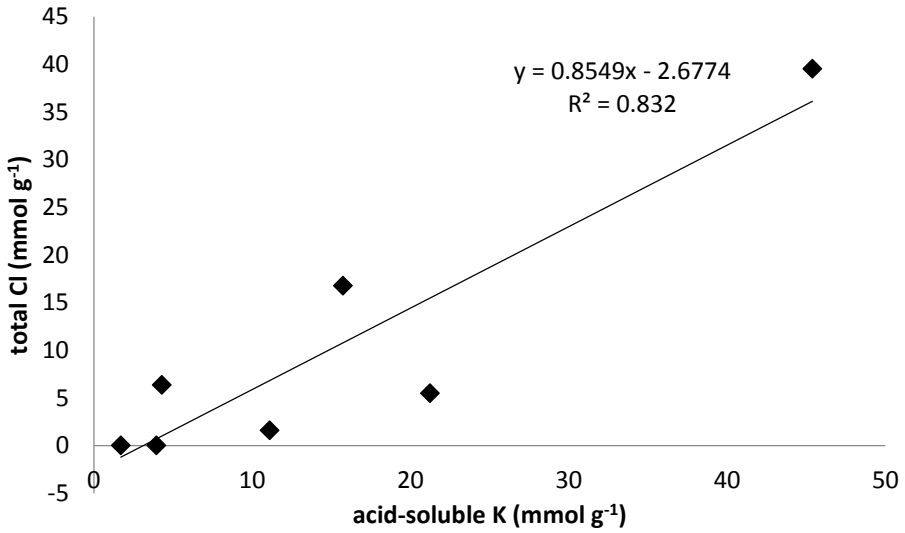

Figure S2.1. Total Cl content and acid-soluble K, in mmol per g of lignocellulosic biochar

Table S2.2. Descriptions of XRF standards, and analytes each standard was used for

Standard name	Description	Analytes
NIST 1633a	Coal fly ash (<90 µm)	Ca, Pb, Ni, K, Si, Sr, Zn
NIST 2691	Coal fly ash (<150 µm)	Al, Ca, Fe, Mn, P, K, Si, Na, Sr, S, Ti
NIST 2910	Calcium hydroxyapatite	Ca, P
NOD-A-1	Manganese nodule	Al, Ba, Ca, Fe, K, Mg, Mn, Mo, Na, P, Pb, Si, Sr Ti
AWP Std 1	Treated wood	Cr, Cu, As
Potassium chloride	Reagent grade compound	K, Cl

In-Depth Discussion of XRD Patterns

Quartz peaks (3.35 Å) were observed for all untreated biochars (Figure 2.2). Quartz peak intensities were inconsistent, suggesting that quartz may have been introduced as a contaminant, possibly from entrained soil or the sand used in the fast pyrolyzer. Sylvite peaks (3.15 Å) were observed for untreated CS5f and CS6s, but not CS3s or MW6g. Calcite peaks (3.04 Å) were observed for CS5f and MW6g. For CS6s and MW6g, a peak at 2.86 Å suggesting the presence of either dolomite or butschliite was observed. For CS6s only, a peak at 2.97 Å additionally suggested the presence of natrite. However, the presence of dolomite, butschliite, or natrite could not be confirmed by additional peaks. Thus the corn stover biochars displayed a wide variety of mineral compositions despite having the same feedstock, while the mixed wood biochar had a similar mineral composition to CS5f and CS6s despite feedstock differences.

Following acid washing, peaks attributed to sylvite, calcite, dolomite and butschliite were universally diminished in intensity, with most acid-washed biochars

exhibiting no discernable peaks for these minerals. Quartz peaks, on the other hand, did persist after acid washing, reflecting the low solubility of this mineral. In the acid washed CS5f biochar, a small peak at 3.15 Å suggests that some sylvite may have persisted after acid washing, however, this peak was only barely evident above the background noise. The presence of sylvite in acid washed biochars is best explained by occlusion of sylvite within the aromatic C matrix (Lawrinenko 2014; Lawrinenko and Laird 2015).

Comparisons with Proximate Analysis Metrics

Few consistent relationships between proximate analysis metrics and total alkalinity were observed. Total alkalinity was not correlated significantly with FC, VM, or ash content (p <0.05, r^2 <0.5). Soluble ash was not correlated with carbonate alkalinity (r^2 = 0.05), but was correlated with other inorganic alkalinity (r^2 = 0.52). Other organic alkalinity was not correlated with VM (r^2 = 0.01). Low-pK_a structural alkalinity (5.0 ≤ pK_a ≤ 6.4) was not correlated with VM (r^2 = 0.004) or FC (r^2 = 0.10) when all biochars were included. However, when lignocellulosic biochars were grouped by their FC:VM ratios, the five biochars with FC:VM > 2.6 exhibited a very strong positive correlation between the sum of FC + Ash and low-pK_a structural alkalinity (r^2 = 0.99), and biochars with FC:VM <2.6 also exhibited increasing functional group concentrations with increasing FC + ash contents (Figure S2.2). These correlations imply that soluble inorganic alkalis are associated with the soluble ash fraction of biochar, and low-pK_a structural alkalinity may be associated with the condensed aromatic fraction of biochar. However, the

relationship between low-pK_a structural alkalinity and FC + ash appears to depend on FC in a way that is not clear from the available data. Thus no consistent relationships between proximate analysis metrics were observed, but grouping the biochars by FC revealed a bimodal relationship. Hence although biochar alkali properties could not be completely systematically reduced to simplistic thermogravimetric properties within the context of this paper, we highlight the relationship between structural alkalis and FC as a potential avenue for future research.

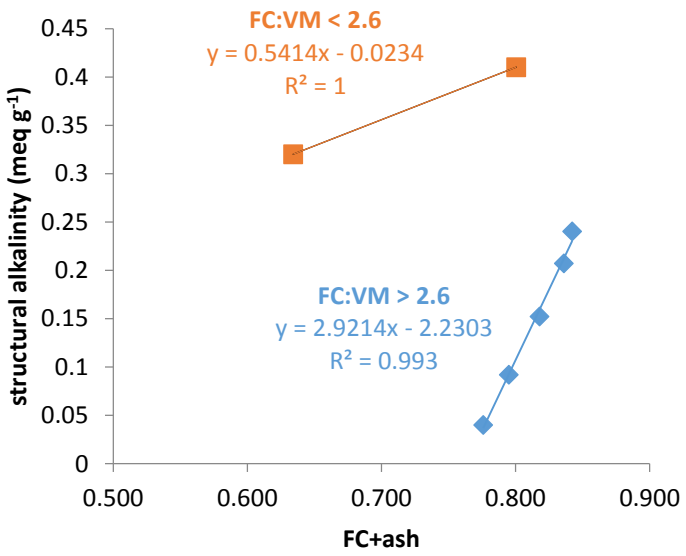

Figure S2.2. Correlation between low pK_a structural alkalinity of lignocellulosic biochars and the sum of FC + Ash (biochars with: FC:VM < 2.6 = orange squares, FC:VM > 2.7 = blue diamonds). Each data point represents mean of three measurements.

CHAPTER 3. EFFECT OF SIX LIGNOCELLULOSIC BIOCHARS ON CO_2 AND N_2O EMISSIONS FROM TWO SOILS

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A paper to be submitted to *Soil Biology and Biochemistry*

Abstract

Biochar application to soil has been proposed as a means for carbon sequestration, but biochar's efficacy as a carbon sequestration tool will depend on how biochar influences soil carbon dioxide ($_{CO2}$) and nitrous oxide (N_2O) emissions. Carbon dioxide and N_2O emissions from biochar-amended soils have been shown to vary with respect to both soil and biochar properties, but the underlying mechanisms of biochar-soil interaction influencing greenhouse gas emissions remain poorly understood. Here we examine the effect of six lignocellulosic biochars on CO_2 and N_2O emissions from two contrasting soils. None of the biochars consistently affected cumulative CO_2 emissions after 140 days following fertilization from both soils, but four biochars did reduce CO_2 emissions from one of two soils and cause negative priming of C. Five biochars significantly reduced N_2O emissions from one soil, and two biochars consistently reduced N_2O emissions from both soils. Furthermore, the magnitude of N_2O suppression varied with respect to both biochar and soil. Reduced NO_3^- concentrations in biochar-amended soils relative to controls corresponded with reduced or equivalent N_2O emissions, suggesting that biochar may influence N_2O emissions by reducing NO_3^- availability.

Introduction

Biochars are the solid co-product of pyrolysis intended for soil application (Lehmann et al. 2006). During the pyrolysis process, organic feedstocks such as agricultural residues, manure, and green wastes are themochemically decomposed under high temperature (~300-800°C) and oxygen-limited conditions, and about 20-50% of the original feedstock is thereby converted into biochar (Keiluweit et al. 2010; Mašek et al. 2013). The key defining characteristic of biochars is their condensed aromatic carbon (C) framework, which, due to its recalcitrant nature, allows biochars to persist in soil for hundreds to thousands of years (Keiluweit et al. 2010). Therefore, the application of biochar to soil constitutes a potentially viable means for long term C sequestration and net greenhouse gas (GHG) emissions reductions (Roberts et al. 2010; Woolf et al. 2010; Kauffman et al. 2014).

Although biochar application to soil has become a well-established means for C sequestration in recent decades, the exact amount of GHG emissions biochar can offset remains difficult to predict. The effect of biochar on soil GHG emissions remains an especially uncertain component of life cycle analysis models, primarily because GHG emissions of biochar-amended soil are a product of highly complex biochar-soil interactions (Joseph et al. 2010; Cayuela et al. 2013b). The impact of biochars on soil GHG emissions has been shown to be highly context-specific, with different biochar-soil combinations yielding distinct results (Spokas and Reicosky 2009; Zheng et al. 2012; Cayuela et al. 2013b). Soil carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O)

emissions depend on myriad soil properties that biochar can influence, including pH, porosity, texture, water holding capacity, cation exchange capacity, and availability of C substrates (Cayuela et al. 2013a; Cayuela et al. 2013b). Biochar may also introduce compounds that inhibit microbial nitrogen (N) transformations, such as polycyclic aromatic hydrocarbons or ethylene (Spokas et al. 2010; Watzinger et al. 2014). Hence, several mechanisms of influence are possible, depending on the biochar and soil in question. However, in part because few studies have encompassed more than one or two biochar-soil combinations, and because biochars and soils vary between studies, the mechanisms by which biochar influences soil GHG emissions remain poorly understood. In the few studies that do include several biochar-soil combinations, most biochars have been shown to decrease emissions, but some biochar-soil combinations result in an increase in CO_2, CH_4 or N_2O emissions (Spokas and Reicosky 2009; Cayuela et al. 2013b; Lin et al. 2014). Even when three soils and sixteen biochars were used, biochar or soil properties that most influence GHG emissions of biochar-amended soils could not be attributed to biochar feedstock type, pyrolysis temperature, elemental composition or surface area (Spokas and Reicosky 2009). Such results suggest that traditionally examined biochar properties – such as elemental composition – may not be immediately relevant to soil-biochar interactions that give rise to soil GHG emissions. Thus further mechanistic research comparing more soil-relevant biochar properties with GHG emissions of biochar-amended soils is needed.

Two soil-relevant biochar properties of interest that may influence GHG emissions are labile organic carbon (LOC) and inorganic carbon (IC) (Jones et al. 2011;

Cayuela et al. 2013b). Biochars have been shown to contain a small but labile fraction of organic C that induces a short-term increase in CO_2 emissions upon addition to soil, and may also indirectly influence N_2O emissions. Conceptually, LOC is not a discrete chemical fraction but rather a *functional* fraction that can be defined as bioavailable biochar carbon that contributes to CO_2 emissions. Many studies have isolated soluble organic C fractions from biochar that could potentially contribute to LOC, with different studies employing water, strong acids, and oxidizing agents (Bruun et al. 2011; Calvelo Pereira et al. 2011; Lin et al. 2012). The size of this soluble (and *potentially* labile) organic C fraction has been shown to vary among biochars, but there is little research relating soluble fractions to CO_2 emissions of biochar-amended soil. Thus, the relationship between isolated soluble biochar fractions, the "true" biochar LOC fraction, and soil GHG emissions remains poorly understood. There is a similar dearth of information regarding the influence of the biochar IC fraction on soil GHG emissions. Most studies do not distinguish the IC fraction from the LOC fraction, and those that do only compare one or two biochar-soil combinations. Biochar IC has been shown to account for up to half of biochar C hydrolyzed in the short term (Jones et al. 2011). This fraction can directly influence CO_2 emissions by releasing CO_2 upon acidification of CO_3^{2-} (through hydrolysis), or indirectly by increasing soil pH, which can have cascading impacts on soil microbial processes (Jones et al. 2011; Cayuela et al. 2013a; Harter et al. 2014).

Here we aim to quantify the soluble OC and IC fractions of six biochars, and compare CO_2 and N_2O emissions following amendment to two soils to determine how biochar LOC and IC influence emissions. We hypothesize that (1) the biochars will

increase CO_2 emissions in the short term, due to hydrolysis of IC and mineralization of soluble OC, (2) biochar will not cause positive priming of soil OC when both biochar IC and soluble OC are accounted for, and (3) biochars will reduce N_2O emissions following the addition of fertilizer due to changes in pH or C availability caused by biochar IC or soluble OC, and/or due to perturbations of the N cycle, such as reduced NH_4^+ or NO_3^- availability.

Methods

Biochar and soil preparation

Six biochars made from four different feedstocks at three different temperatures were selected for this study (see Ch.2 Table 2.1). Slow pyrolysis biochars made from corn stover were pyrolyzed in a N_2-purged muffle furnace for ~1 h. The mixed wood gasification biochar (MW6g) was obtained from ICM Inc. and the hardwood slow pyrolysis biochar (HW5s) was obtained from Royal Oak (#10 sieve size). The fast pyrolysis corn stover biochar, CS5f, was obtained from the Center for Sustainable Energy Technologies at Iowa State University, and was sieved to <0.50 mm to exclude sand particles used as fluidization media in the fast pyrolysis process. All other biochars were ground to <0.50 mm to minimize the influence of particle size.

Two soils of contrasting textures, Soil A and Soil B, were collected in November of 2013 from two study sites in Iowa under continuous corn cropping systems. Soil A was collected from a site on the Iowa State University Armstrong Research and Demonstration Farm, and is mapped as an Exira soil with a silt loam texture (Fine-silty,

mixed, superactive, mesic Typic Hapludoll). Soil B was collected from the Boyd Farm in Boone Co., IA, and is mapped as a Clarion soil with a loam texture (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 types: soil A contained 15% sand, 80% silt and 5% clay, whereas soil B contained 48% sand, 42% silt and 10% clay (hydrometer method, Gee and Bauder 1979). From both sites, soil was collected from the top 5 cm following corn harvest, then kept refrigerated for one month and sieved to <4 mm before use. Any visible plant residues remaining after sieving were removed by hand.

Biochar LOC and IC

Because there is no one accepted method of quantifying biochar LOC, here two methods are used to provide indices of biochar LOC by quantifying different soluble biochar OC fractions. First acid-soluble volatile matter (ASVM) was quantified by comparing the fixed carbon (FC)-normalized volatile matter (VM) contents of acid washed and untreated biochars. Biochars were acid washed by shaking with 0.05 M HCl, followed by 1 M $CaCl_2$ and water at a 50:1 (vol:wt) solution:biochar ratio (see Ch. 2). The VM:FC ratios of the acid washed biochar was subtracted from the VM:FC ratio of the untreated biochar, and this difference in ratios was multiplied by the FC of the untreated biochar to calculate the ASVM. Due to the higher solution pH during the $CaCl_2$ and water washes, ASVM represents VM soluble in both highly acidic (pH 1-2) and mildly acidic (pH 4-5) solutions. Second, the bicarbonate-extractable organic carbon (BEOC) of

the six biochars was quantified by equilibrating acid-washed biochars with 0.05 M sodium bicarbonate (pH 8.0-8.5) for 24 h, and measuring the DOC content of the extracts (Shimadzu TOC 5050 analyzer). We here consider the ASVM and BEOC fractions to together constitute the "soluble OC" fraction of biochar.

Biochar IC was quantified using a NaOH trap method (see Ch. 2). Briefly, 2 g biochar were stirred with 100 mL of 0.05 M HCl in a sealed 0.95 L glass container also containing 15 mL of 1 M NaOH. After 72 hours, excess $BaCl_2$ was added to the NaOH and the solution was titrated with HCl using phenolphthalein as an indicator.

Soil incubation

Biochar samples (0.05 g) were mixed with 10 g samples of soils A and B in 150 mL serum vials in quadruplicate. To control for the influence of biochar IC, 0, 0.5 and 1.0 mg g^{-1} of $CaCO_3$ – treatments C0, C1 and C2, respectively – were also added to each soil and biochars were additionally added to a 50/50 mixture of silt and sand-sized quartz to account for mineralizeable or hydrolysable biochar C (both LOC and IC) and allow for the quantification of soil C priming. Soils were equilibrated with the biochars and carbonates at field moisture at 20°C for 50 days, during which time CO_2 and N_2O emissions were quantified after 0, 22, 30 and 44 days.

After the 50 day equilibration period, corn stover was mixed in at 0.5%, and fertilizer (87, 42 and 54 mg kg^{-1} of N, P and K, respectively) was added as NH_4NO_3 and K_2HPO_4. Soils were incubated for an additional 140 days following fertilization, during which time moisture was maintained at -1/3 bar equivalent (see Table S3.1). 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 this post-fertilization period. For each gas flux measurement, serum vials were sealed with a butyl septa and crimp cap, and 11.5 mL gas samples were collected using a syringe three times over the course of 2 to 24 hours, with longer gas accumulation times used when flux rates were low. Gas samples were stored in helium-flushed and evacuated airtight 6 mL Exetainer vials and analyzed for CO_2 and N_2O 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 (Iqbal et al. 2013).

Post-incubation soil analyses

Following the 190 day 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 at a 1:1 soil:solution ratio (Thomas, 1996). Plant available nutrients were extracted using the Mehlich 3 method and analyzed by inductively-coupled plasma optical emission spectroscopy (Thermo Scientific™ iCAP™ 7400 ICP-OES Analyzer). Soluble NH_4^+ and NO_3^- and DOC were extracted using 2 M KCl (5:1 solution:soil ratio). Samples were prepared with the Berthelot and Griess-Ilosvay reagents for analysis of NH_4^+ and NO_3^-, respectively (Hood-Nowotny et al. 2010), and the DOC in the extracts was quantified using the same method as BEOC. Total C and N were analyzed by combustion (Vario Microcube, Elementar). To assess the contribution of organic acids to soil DOC, organic anions were extracted using deionized water from control soils and

soils amended with two biochars at a solution:soil ratio of 5:1. Samples 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 analyses

Gas fluxes were calculated from the slope of the linear increase in gas concentrations over time, and any slopes with r^2 <0.5 were assumed to be zero (Iqbal et al 2013). Cumulative emissions were calculated by interpolating linearly between daily fluxes ("trapezoidal interpolation"). 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 CO_2-C). Average daily fluxes were compared using the PROC MIXED procedure and 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 p = 0.05 unless otherwise noted, and all analyses were conducted in SAS (v9.2).

Results and Discussion

Biochar soluble OC and IC

Biochar ASVM ranged from 12-56 mg g biochar^{-1}, and CS6s had the highest soluble OC, followed by MW6g, CS3s, HW5s, CS5f and CS5s (Table 3.1). Despite its high ASVM, CS6s had the lowest total VM (16%). BEOC ranged from 0.16-3.23 mg g biochar^{-1}, and CS5f had the highest BEOC, followed by CS3s, MW6g, HW5s, CS5s and CS6s. ASVM and BEOC were not correlated with each other (r^2 = 0.003).

Biochar IC ranged from 1.17-8.97 mg g biochar^{-1}, with MW6g having the highest IC followed by HW5s, CS6s, CS5f, CS5s and CS6s. IC was almost universally higher than BEOC, with the exception of CS3s, which had an IC of 1.17 mg g^{-1} and a BEOC of 2.44 mg g biochar^{-1}.

Table 3.1 Acid soluble volatile matter (ASVM), bicarbonate extractable organic carbon (BEOC) and inorganic carbon (IC), of biochars in mg per g of untreated biochar (± s.d.)

biochar	ASVM	BEOC	IC
CS3s	50 ±8	2.44 ±0.12	1.17 ±0.1
CS5f	36 ±4	3.23 ±0.09	4.51 ±0.11
CS5s	12 ±8	0.323 ±0.005	2.51 ±0.03
CS6s	61 ±6	0.16 ±0.08	5.51 ±0.13
HW5s	46 ±10	0.5 ±0.01	6.74 ±0.43
MW6g	56 ±3	0.64 ±0.11	8.98 ±0.24

Soil chemical properties

Total soil C and N

After the 190 day incubation percent total C (TC) and percent total N (TN) varied with respect to soil and biochar (Table 3.2). Soil A had higher TC and TN than Soil B across all treatments, and Soil B had universally higher C:N ratios. For both soils, biochar amendments significantly increased TC compared with the zero carbonate control (C0), but carbonate treatments (C1 and C2) did not increase TC. Relative to C0, biochar additions increased TC of Soil A and Soil B by 0.18-0.36% and 0.12-0.34%, respectively. No single biochar increased TC the most in both soils; rather, HW5s produced the highest increase of TC in Soil A, and CS5f produced the largest increase of TC in Soil B. However, HW5s increased TC more consistently than the other biochars. Neither soil exhibited a significant change in TN with biochar or carbonate amendment. Lastly, C:N ratios of biochar amended soils were universally higher than that of unamended soils (due to the increase in TC), and the C:N ratios of Soil B samples were universally higher than that of Soil A samples.

Mehlich-Extractable Nutrients

Nutrient contents of Soil A, Soil B and Quartz as determined by Mehlich 3 extraction are presented in Table 3.3 (Mehlich 1984). Biochars consistently increased extractable base cations from both soils, likely due to nutrients in the biochar. Indeed, the increase in extractable base cations in biochar-amended soils was largely in proportion to – but not always *equal* to – the quantity of base cations extracted from

the Quartz-biochar mixtures. However, biochar-amended Soil B samples had a disproportionately large extractable Fe, Mn, P and S contents compared with the controls that could not be accounted for by the quantity extractable from quartz-biochar mixtures. Soil A also exhibited moderately elevated Fe and Mn contents when amended with biochars, but no change in P and a decrease in S.

Soil and Quartz pH

Both soils and four out of six quartz-biochar mixtures exhibited a decrease in pH during the incubation from an initial pH of 6.3 to final pHs ranging from 5.1 to 6.1 (Figure 3.1). In agreement with previous studies, biochars and carbonate amendments increased the pH of Soil A relative to the unamended control (C0). However, while carbonate amendments increased the pH of Soil B, biochar amendments decreased soil pH relative to the zero carbonate Soil B control (B-C0) by 0.25-0.53 pH units. Additionally, quartz-biochar mixtures had final pHs ranging from 5.4 to 7.2. Only one quartz-biochar mixture, quartz-CS6s, exhibited an increase in pH relative to the starting quartz pH, most likely due to the high pH (10.3) of the biochar. The high K^+ concentration of CS6s, which probably increased alkali solubility, may have also contributed to the pH (see Ch1). Among biochar-amended soils and quartz-biochar mixtures, final pH was correlated with biochar alkalinity ($0.2< r^2 <0.8$) and with biochar carbonate alkalinity ($0.7< r^2 <0.9$) (Biochar total alkalinity is defined as the total H^+ neutralized by 0.05 M HCl, and carbonate alkalinity is defined as the total CO_2 evolved from the biochar when mixed with 0.05 M HCl; see Ch.1). Thus, biochar alkalis, including

carbonates, clearly impacted final pH, despite the lower pHs of biochar-amended Soil B samples.

Soil DOC

Soil A samples amended with all six biochars had lower KCl-extractable DOC relative to the controls, yet Soil B samples amended with biochars had higher DOC compared with controls (Figure 3.2). For Soil A, the addition of carbonate caused DOC to decrease consistently with increasing $CaCO_3$ added. After 190 days of incubation, Soil A controls A-C0, A-C1 and A-C2 had 169, 140, and 122 mg kg^{-1} DOC, respectively, whereas biochar-amended Soil A samples had about 50-80% lower DOC concentrations (35-58 mg kg^{-1}). Lower DOC concentrations in biochar-amended Soil A samples relative to controls may have been a result of DOC sorption to biochar.

Conversely, carbonate amendments did not have a consistent effect on the final DOC contents of Soil B samples; DOC increased in the order B-C5<B-C4<B-C0<B-C∼B-C2∼B-C3 (see Figure S3.1). Soil B controls B-C0, B-C1 and B-C2 contained 81, 90, and 90 mg kg^{-1} of KCl-extractable DOC, respectively, whereas biochar-amended Soil B samples contained approximately twice as much DOC as the controls (158-213 mg kg^{-1}). In summation, all six biochar amendments reduced the DOC of Soil A, but increased the DOC of Soil B; this biochar-soil interaction may be a product of the unique organic matter or mineral compositions of each soil.

NH_4^+ and NO_3^-

KCl-extractable NH_4^+ and NO_3^- measured at the end of the incubation varied greatly with respect to biochar and soil, with minimal variation among biochar-amended soils (Table 3.4). Total reactive nitrogen, Nr (Nr = NH_4^+ + NO_3^-) represented 41-76% of added fertilizer Nr. Soil A controls contained larger quantities of both NH_4^+ and NO_3^-, and had higher NH_4^+:NO_3^- ratios compared with Soil B controls. Carbonate addition did not cause significant changes in NH_4^+ or NO_3^- concentrations for either soil.

When amended to Soil A, the six biochars induced a ~50% decrease in NH_4^+ and a ~10-20% decrease in NO_3^- relative to the controls. Consequently, the total Nr was lower in biochar-amended Soil A samples relative to the controls, and NH_4^+:NO_3^- ratios were slightly lower in biochar-amended soils (0.46-0.56:1) compared with controls (0.56-0.66:1). By contrast, biochar treatments amended to Soil B induced a ~200% increase in NH_4^+ and a ~25-50% decrease in NO_3^- relative to the controls. Total Nr was consequently higher in biochar-amended Soil B relative to the controls, and NH_4^+:NO_3^- ratio was greatly elevated in Soil B samples that received biochar amendments, with ratios ranging from 3:1 to 4:1 compared with 0.84-0.96:1 in the Soil B controls.

Quartz-biochar mixtures exhibited much higher NO_3^- (97-119 mg kg^{-1}) and Nr concentrations (98-119 mg kg^{-1}), and lower NH_4^+ (0.003-0.006 mg kg^{-1}) than the soils, despite having received the same quantity of NH_4NO_3 fertilizer (Table 3.5). The NO_3^- concentrations were likely elevated in quartz-biochar mixtures because, in the absence

of soil organic matter or stover amendment, microbes may have used NH_4^+ in place of organic substrates as an electron source.

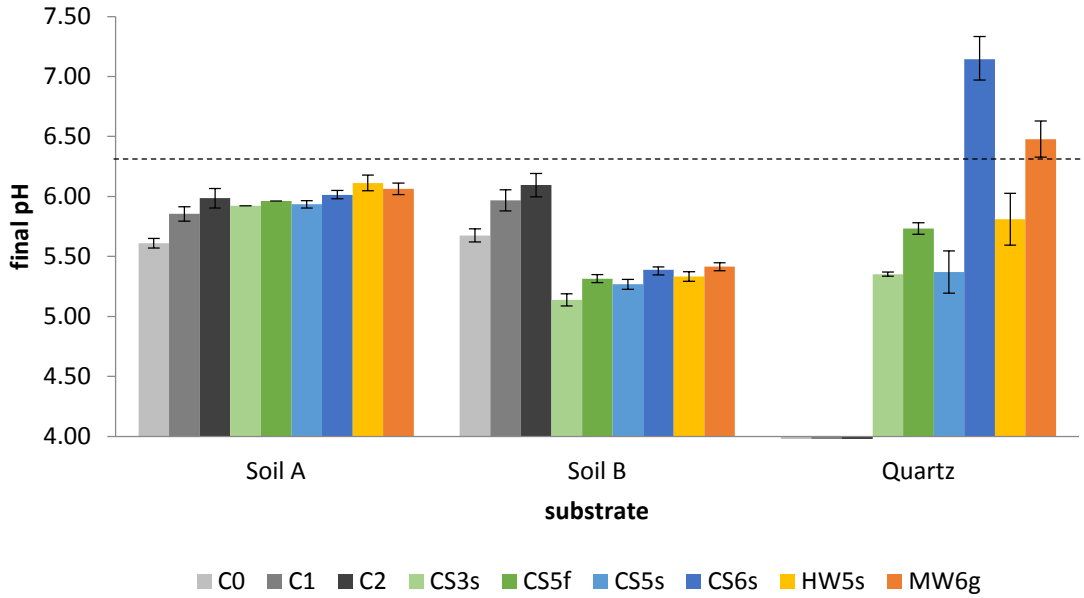

Figure 3.1. Final pH of soils and quartz-biochar mixtures after 190 d incubation. Dotted line shows initial pH of 6.3. Error bars represent one standard deviation.

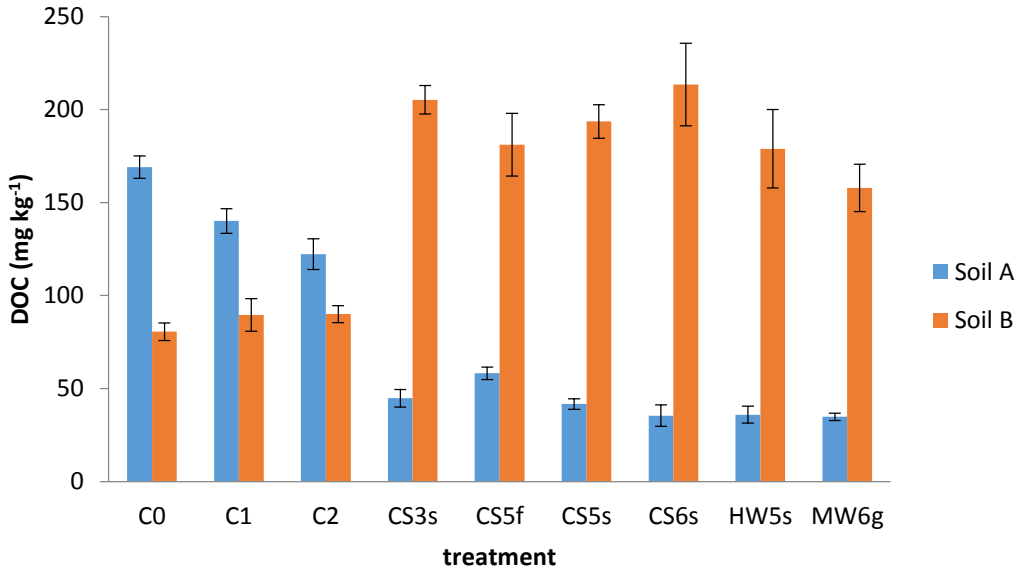

Figure 3.2. DOC in KCl extracts of soils that had been incubated with either $CaCO_3$ (C1 and C2) or biochar (C0 = control), in mg of C per kg of soil. (± s.d.)

Table 3.2. Final Total C (wt%), Total N (wt%) and C:N ratio by mass of soil after 190 day incubation (± s.d.)

	Total C (%)		**Total N (%)**		**C:N**	
treatment	Soil A	Soil B	Soil A	Soil B	Soil A	Soil B
C0	2.50 ±0.07	1.86 ±0.03	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

Table 3.3. Mehlich III-extractable nutrient content of Soil A, Soil B and quartz samples, in mg kg^{-1} (± s.d.)

				Soil A				
treatment	**Na**	**K**	**Mg**	**Ca**	**Mn**	**Fe**	**P**	**S**
C0	8.4 ±0.7	451 ±6	656 ±12	3949 ±134	242 ±5	316 ±7	297 ±8	84 ±4
C1	6.5 ±0.7	433 ±6	643 ±14	3996 ±130	235 ±3	308 ±5	289 ±6	73 ±3
C2	6.8 ±1.4	439 ±10	657 ±15	4194 ±83	228 ±13	306 ±8	282 ±5	67 ±1
CS3s	11.1 ±0.9	455 ±7	677 ±10	3949 ±71	241 ±5	323 ±4	282 ±7	53 ±2
CS5s	15.2 ±3.1	507 ±21	689 ±21	3946 ±114	243 ±4	335 ±15	292 ±20	56 ±4
CS6s	16.9 ±0.5	532 ±32	687 ±22	4003 ±98	243 ±3	338 ±1	288 ±6	54 ±3
CS5f	13.7 ±0.3	484 ±14	688 ±11	4108 ±118	256 ±3	341 ±7	289 ±10	57 ±1
HW5s	15.1 ±2.9	454 ±5	673 ±6	4092 ±140	250 ±7	340 ±8	275 ±7	56 ±1
MW6g	19.7 ±0.2	458 ±2	688 ±14	4039 ±114	245 ±7	325 ±5	284 ±11	56 ±3
				Soil B				
treatment	**Na**	**K**	**Mg**	**Ca**	**Mn**	**Fe**	**P**	**S**
C0	3.9 ±0.6	210 ±2	286 ±2	2543 ±18	164 ±1	247 ±6	84 ±5	48 ±2
C1	3.3 ±0.6	208 ±6	281 ±4	2713 ±47	166 ±3	241 ±6	84 ±3	50 ±3
C2	2.8 ±0.4	206 ±5	273 ±4	2802 ±56	160 ±5	231 ±7	87 ±5	51 ±1
CS3s	14.9 ±0.5	236 ±6	309 ±5	2685 ±52	221 ±4	360 ±11	182 ±7	103 ±5
CS5s	14.6 ±0.6	249 ±8	302 ±8	2638 ±74	212 ±8	344 ±8	178 ±8	99 ±4
CS6s	67.9 ±0.8	336 ±3	299 ±6	2700 ±49	215 ±6	344 ±9	170 ±5	98 ±5
CS5f	67 ±1	261 ±6	306 ±9	2726 ±71	213 ±7	329 ±12	171 ±7	96 ±3
HW5s	67 ±1.3	227 ±6	294 ±5	2871 ±72	211 ±7	325 ±17	158 ±7	96 ±5
MW6g	72.4 ±0.3	225 ±6	296 ±5	2816 ±116	209 ±3	325 ±4	150 ±10	96 ±2
				Quartz				
treatment	**Na**	**K**	**Mg**	**Ca**	**Mn**	**Fe**	**P**	**S**
CS3s	69 ±2	86 ±6	30 ±2	181 ±37	0.3 ±0.7	11 ±3	54 ±6	14 ±4
CS5s	77 ±1	138 ±10	35 ±2	207 ±27	0.7 ±0.4	19 ±2	45 ±3	15 ±2
CS6s	79 ±2	252 ±9	25 ±1	191 ±19	1.0 ±0.4	16 ±2	40 ±3	14 ±1
CS5f	79 ±1	122 ±4	34 ±1	223 ±25	0.5 ±0.5	13 ±4	44 ±9	18 ±4
HW5s	75 ±1	60 ±1	14 ±3	253 ±19	1.6 ±0.6	16 ±4	41 ±2	17 ±3
MW6g	77 ±2	62 ±1	21 ±0	301 ±24	1.1 ±0.2	21 ±3	44 ±3	20 ±2

Table 3.4. NH_4^+, NO_3^- , NH_4^+:NO_3^- ratio, and total Nr (NH_4^++NO_3^-) of Soil A, Soil B and quartz samples, in mg N kg^{-1} (± s.d.)

		Soil A		
treatment	**NH_4^+-N**	**NO_3^--N**	**NH_4^+:NO_3^-**	**Nr**
C0	23.0 ±0.5	35.3 ±3.4	0.66 ±0.07	58.3 ±3.2
C1	19.6 ±2.3	35.1 ±2.7	0.56 ±0.05	54.7 ±4.6
C2	18.3 ±2.6	32.0 ±4.1	0.57 ±0.04	50.4 ±6.5
CS3s	13.6 ±0.8	29.5 ±0.4	0.46 ±0.03	43.1 ±0.9
CS5f	13.8 ±0.9	27.0 ±1.7	0.51 ±0.05	40.8 ±2
CS5s	14.1 ±0.2	27.4 ±0.9	0.52 ±0.02	41.5 ±0.7
CS6s	13.3 ±0.6	27.5 ±2	0.48 ±0.04	40.7 ±2
HW5s	12.6 ±2.1	26.4 ±1.5	0.48 ±0.1	38.9 ±1
MW6g	13.5 ±0.4	24.1 ±1.2	0.56 ±0.04	37.5 ±0.9
		Soil B		
treatment	**NH_4^+-N**	**NO_3^--N**	**NH_4^+:NO_3^-**	**Nr**
C0	16.4 ±0.8	19.6 ±1.2	0.84 ±0.08	36.0 ±1
C1	18.2 ±2.3	21.6 ±0.4	0.84 ±0.09	39.8 ±2.6
C2	19.0 ±1.6	19.7 ±0.8	0.96 ±0.05	38.7 ±2.3
CS3s	47.4 ±3.6	10.2 ±1.4	4.70 ±0.59	57.6 ±4.5
CS5f	46.5 ±3.5	12.4 ±1	3.76 ±0.37	58.9 ±3.8
CS5s	42.0 ±3	10.0 ±1.6	4.25 ±0.46	52 .0 ±4.5
CS6s	44.5 ±2.2	10.9 ±1.1	4.12 ±0.6	55.5 ±1.6
HW5s	43.9 ±1.8	15.2 ±2.7	2.96 ±0.54	59.1 ±2.1
MW6g	52.6 ±4.1	13.5 ±1	3.91 ±0.54	66.1 ±3.5
		Quartz		
treatment	**NH_4^+-N**	**NO_3^--N**	**NH_4^+:NO_3^-**	**Nr**
CS3s	0.7 ±0.5	119 ±8	0.006 ±0.004	119 ±7
CS5f	0.3 ±0.2	109 ±4	0.003 ±0.001	110 ±4
CS5s	0.5 ±0.4	114 ±4	0.004 ±0.004	114 ±4
CS6s	0.46 ±0.04	97 ±7	0.0047 ±0.0001	98 ±7
HW5s	0.8 ±0.3	117 ±12	0.007 ±0.002	118 ±12
MW6g	0.3 ±0.2	102 ±6	0.003 ±0.002	102 ±6

Comparisons among chemical properties

Differences in pH among soil samples may partially explain differences in soil NH_4^+, NO_3^-, and DOC. Among controls receiving varying rates of $CaCO_3$, Soil A NH_4^+ was strongly negatively correlated with pH (r^2 = 0.50), and NO_3^- was weakly negatively correlated with pH (r^2 = 0.20). Among biochar-amended Soil A samples, soil NH_4^+ was not correlated with pH (r^2 = 0.05), but NO_3^- was strongly negatively correlated with pH (r^2 = 0.48). The NH_4^+ concentrations of Soil B controls were not correlated with pH, but NO_3^- concentrations in the controls were strongly negatively correlated with pH (r^2 = 0.40). Among biochar-amended Soil B samples, NO_3^- was weakly positively correlated with soil pH (r^2 = 0.20), but NH_4^+ was not significantly correlated with pH. However, the higher NH_4^+ concentrations of biochar-amended Soil B samples did correspond with lower pH values relative to controls.

Because bacterial nitrification is inhibited at low soil pH (Parton et al. 1996; Zheng et al. 2012), it is likely that biochars' pH effect is at least partially responsible for the positive correlation between NO_3^- content and pH among Soil B samples, and the negative correlation between NH_4^+ and pH among biochar-amended Soil A samples. However, because the NH_4^+ contents of biochar-amended Soil A and Soil B samples were not correlated with pH, and because NO_3^- and pH were negatively correlated among biochar-amended Soil A samples, an additional mechanism could have influenced observed differences in soil NH_4^+ and NO_3^- between biochar-amended and control soils. Since soil N transformations such as nitrification, denitrification and N

immobilization are tightly linked to C availability, this additional mechanism may have involved DOC.

Correlations between DOC and soil pH were consistently negative, and tended to be stronger among controls than biochar-amended soil samples. Among Soil A controls receiving varying rates of $CaCO_3$, DOC was very strongly negatively correlated with pH (r^2 = 0.94), and this correlation was weaker among biochar-amended Soil A samples (r^2 = 0.28). Among Soil B controls, DOC was moderately negatively correlated with soil pH (r^2 = 0.30), but this correlation was very weak among biochar-amended samples (r^2 = 0.10). Thus, the effect of biochar on soil DOC may have been related to soil pH for Soil A, but the cause of the increased DOC content of biochar-amended Soil B samples is not clear from these data alone.

To help elucidate the mechanism by which biochar amendment influenced pH and DOC differently for Soil A versus Soil B, water-extractable organic acid anions were quantified from a subset of treatments (C0, CS5s and HW5s) for each soil (Figure S3.2). It was found that organic acid concentrations increased with increasing DOC for both soils and biochars, and the ratio of organic acids to DOC was consistently higher among biochar-amended soils than unamended soils. Biochar amendment slightly decreased concentrations of the dominant organic acid anions (lactate, acetate, glycolate and formate) in Soil A, whereas biochar amendment more than doubled concentrations of these acids in Soil B. Therefore, biochar may have decreased the pH of Soil B by inducing microbial production of organic acids. Organic acids can be produced via various

microbial metabolic pathways such as mixed acid fermentation, which converts sugars to lactic, acetic and formic acids. Fermentation is an O_2-limited process, and thus an increase in fermentation in biochar-amended soil implies the formation of low-O_2 microsites in or near biochar particles. The observed increase of organic acids in biochar-amended Soil B is in agreement with the findings of Mitchell et al. (2015). The prevalence of anaerobic microsites would also explain the accumulation of NH_4^+ in biochar-amended Soil B samples, as nitrification is limited in low-O_2 conditions. Anarobic microsites may have become prevalent in biochar-amended Soil B but not Soil A due to differences in carbonate content, percent clay, or organic matter composition, but ascertaining the specific soil properties responsible for the contrasting response of soils A and B to biochar amendment will require further research.

CO_2 emissions

Equilibration period CO_2 emissions

Total accumulated CO_2 emissions from soils A and B during the 50 day pre-fertilization equilibration period are shown in Figure 3.4a. Emissions from quartz-biochar mixtures were negligible during this period (*data not shown*). Soil A initially had significantly lower CO_2 emissions than Soil B, but emission rates from Soil B samples decreased rapidly in the first 22 days of the equilibration period (Figure S3.3). Consequently, emission rates after 44 days were similar between the two soils. The large initial pulse of CO_2 emissions released from Soil B suggests that this soil had a significant fraction of protected soil organic matter that was exposed to O_2 upon sieving.

Furthermore, emissions from all biochar-amended Soil B samples were higher than that of B-C0 on day 0, and for all biochars except for MW6g, this difference was significant. The carbonate control B-C1 also had higher emissions than B-C0 on day 0, but the difference was not significant. Biochar amendments did not significantly increase CO_2 emissions from Soil A, however. Thus, initial CO_2 emissions results suggest that biochar amendments to Soil B may have resulted in hydrolysis of biochar carbonates, which temporarily increased emissions.

Biochar-amended and $CaCO_3$-amended Soil A samples had total equilibration period CO_2 emissions that did not differ significantly from that of A-C0. However, A-CS5s exhibited significantly lower total CO_2 emissions than A-C1, and A-CS6s exhibited significantly higher emissions than A-C1. In the case of Soil B, all biochar and $CaCO_3$ treatments increased CO_2 emissions relative to B-C0, but this difference was only significant for B-HW5s and B-MW6g. Total CO_2 evolved from biochar-amended Soil B samples was moderately positively correlated with biochar IC content (r^2 = 0.37), which combined with the elevated emissions of B-C1 and B-C2 suggest that biochar IC contributed to total pre-fertilization emissions from Soil B (Figure 3.6). However, no such correlation occurred for Soil A (possibly because Soil A contained free carbonates and Soil B did not), resulting in a larger IC impact on Soil B compared with Soil A.

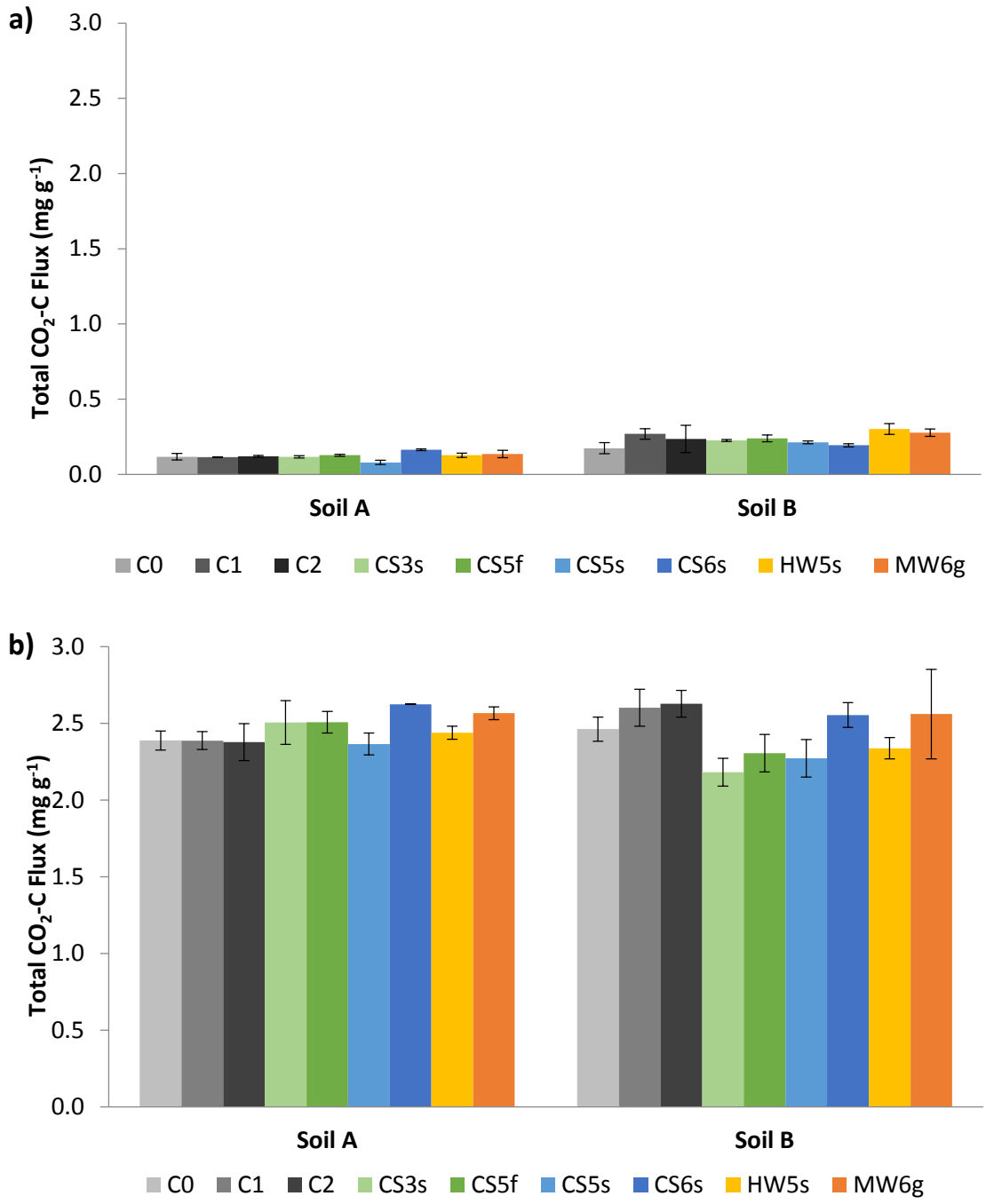

Figure 3.4. Total CO_2-C emissions during the (a) 50 day pre-fertilization equilibration period and (b) 140 day post-fertilization period, in mg C per g of soil. Error bars represent standard error.

Post-fertilization CO_2 emissions

Following the addition of NPK fertilizer, CO_2 emissions from both soils increased rapidly to >50 μg CO_2-C g^{-1} d^{-1} on day 0 (immediately following fertilization) and subsequently decreased to <8 μg CO_2-C g^{-1} d^{-1} over the 140 day post-fertilization incubation period (Figure S3.4). The soil*treatment*day interaction was significant on all days except for days 1 and 20. Soils A and B exhibited transient significant differences between biochar-amended samples and controls throughout the incubation. Correlations of CO_2 emissions from biochar-amended soils with biochar BEOC and IC were also transient, occurring only on specific days during the incubation, and alternating between positive and negative correlations. Thus, although the soil*treatment*day interaction was significant, biochar did not have a consistent effect on soil CO_2 emissions during the post-fertilization period.

After 140 days incubation following fertilization, total CO_2 evolved was similar between Soil A and Soil B (Figure 3.4), and biochar amendments only impacted emissions from Soil B (Figure 3.4b). No significant differences were observed between biochar treatments and controls for Soil A ($p > 0.05$). However, emissions from B-CS3s, B-CS5f, B-CS5s, and B-HW5s were significantly lower than B-C1 and B-C2 ($p < 0.05$). Of these, only B-CS3s also reduced CO_2 relative to B-C0 ($p < 0.05$). Thus, despite transient instances of higher emissions from biochar-amended soils, cumulative emissions showed that biochars did not significantly increase CO_2 emissions in over the 140 day incubation.

Quartz-biochar mixtures

Quartz-biochar mixtures exhibited CO_2 emission rates about one tenth of soil CO_2 emission rates during the post-fertilization period (Figure S3.5). Initially emission rates ranged from 2.5-5.5 μg CO_2-C g^{-1} d^{-1}, then rapidly decreased to <1.5 μg g^{-1} d^{-1} over the first 15 days. After 80 days, all quartz-biochar mixtures except for the CS6s-quartz mixture exhibited emissions of <0.25 μg CO_2-C g^{-1} d^{-1}, and CS6s continued to have emission rates higher than all other mixtures for the remainder of the incubation (0.8 μg CO_2-C g^{-1} d^{-1}). Total CO_2 emissions from quartz-biochar mixtures increased in the order CS3s~CS5s<CS5f<HW5s<MW6g~CS6s. Average total CO_2 emissions from quartz-biochar mixtures were strongly correlated with biochar IC (r^2 = 0.5), and this correlation became very strong when CS6s was treated as an outlier (r^2 = 0.91, slope = 1.2) (Figure 3.5). Average total CO_2 emissions from quartz-biochar mixtures were also very strongly correlated with final pH (r^2 = 0.78; includes all biochars). Furthermore, that the slope of the regression between CO_2 and carbonates was close to 1.0 suggests that a large fraction of CO_2 emissions originated directly from the hydrolysis of carbonates, although biologically-mediated pH effects and abiotic carbonate effects—which may both positively impact CO_2 emissions—cannot be fully distinguished here. The quartz-CS6s mixture may have also behaved differently from the other quartz-biochar mixtures due to its higher K content, which may have increased carbonate solubility or provided additional nutrients to microbes (see Ch.2). Nonetheless, biochar carbonates clearly contributed to CO_2 emissions from quartz-biochar mixtures.

Priming of soil C

Priming of soil C by biochar was negligible for Soil A, and significantly negative for Soil B for biochars CS3s, CS5f, CS5s and HW5s (Figure 3.6). Priming of C in Soil A was positive for CS3s, CS5f, CS6s and MW6g, and negative for CS5s and HW5s, but in all cases the magnitude of priming was <0.2 mg g^{-1}. The observed negative priming of Soil B's C reflects the suppressed emissions from B-CS3s, B-CS5f, B-CS5s and B-HW5s relative to B-C1, and suggest that these biochars helped stabilize added corn stover C and/or native SOC in Soil B. Added C may have been stabilized via sorption of labile C to biochar, or due to biochar-induced changes in the redox environment. For example, if biochar facilitated the formation of anerobic microsites, low oxygen would have limited the oxidation of DOC to CO_2, thereby supporting the observed higher DOC in biochar-amended soil B samples. The higher organic acid concentrations and lower pH in biochar-amended soil B may have also aided in the preservation of soil C.

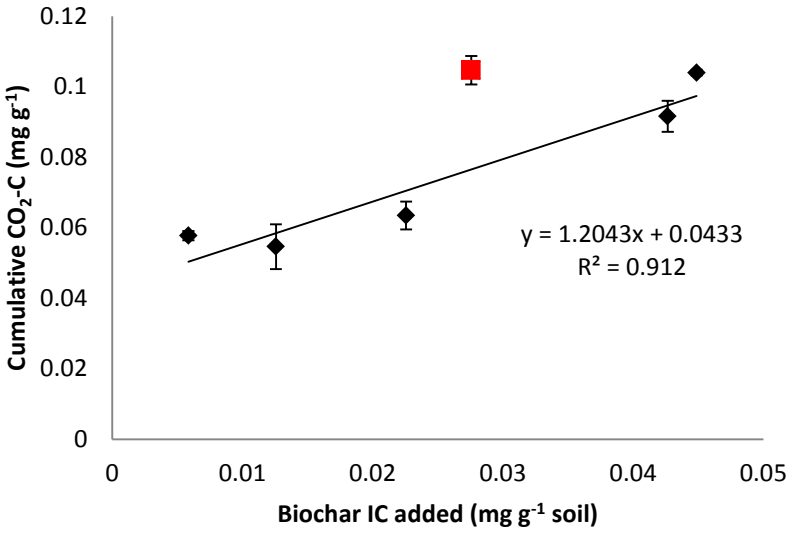

Figure 3.5. Correlation between cumulative CO_2 emissions from quartz-biochar mixtures and biochar IC (140 day period) (CS6s outlier shown in red square)

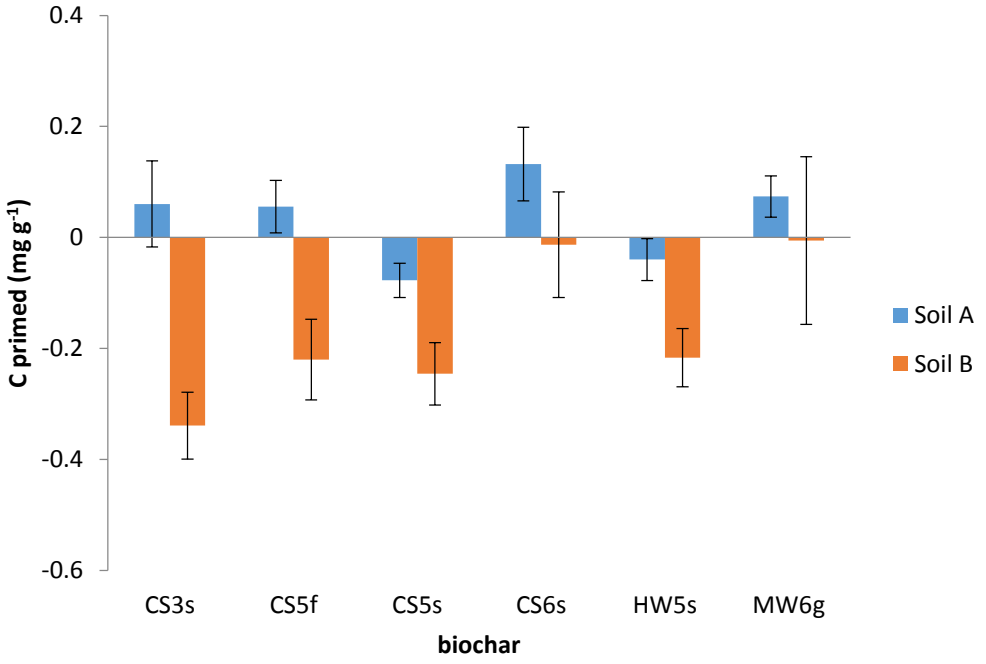

Figure 3.6. Soil and corn stover C primed by biochars, after fertilization. Primed C was calculated as the difference between the sum of the quartz-biochar mixture and C0 emissions, minus the emissions of the respective biochar-amended soils (in CO_2-C). Error bars represent standard errors (n = 4).

N_2O emissions

During the 50 day pre-fertilization equilibration, N_2O emissions from both soils and quartz-biochar mixtures were largely below the detection limit (<0.0003 μg N_2O-N g^{-1} d^{-1}) and averages within treatments approached zero (*data not shown*). Following fertilization, N_2O emissions from quartz-biochar mixtures remained below the detection limit, while N_2O emissions from the majority of soil samples rose above the detection limit during days 0-8, then fell below the detection limit after day 8 (Figure 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 ($p < 0.05$).

Total accumulated emissions from soils A and B during the first 8 days of the post-fertilization period are shown in Figure 3.7. Comparing total N_2O emissions over the 8 day period, A-C0 had significantly higher emissions than B-C0. Among Soil A samples, all biochars significantly suppressed emissions relative to all controls; among Soil B samples, CS3s significantly suppressed emissions relative to all controls, and MW6g significantly suppressed emissions relative to B-C1 and B-C2. Thus, all biochars suppressed N_2O emissions from soil A, but only CS3s and MW6g suppressed emissions from Soil B.

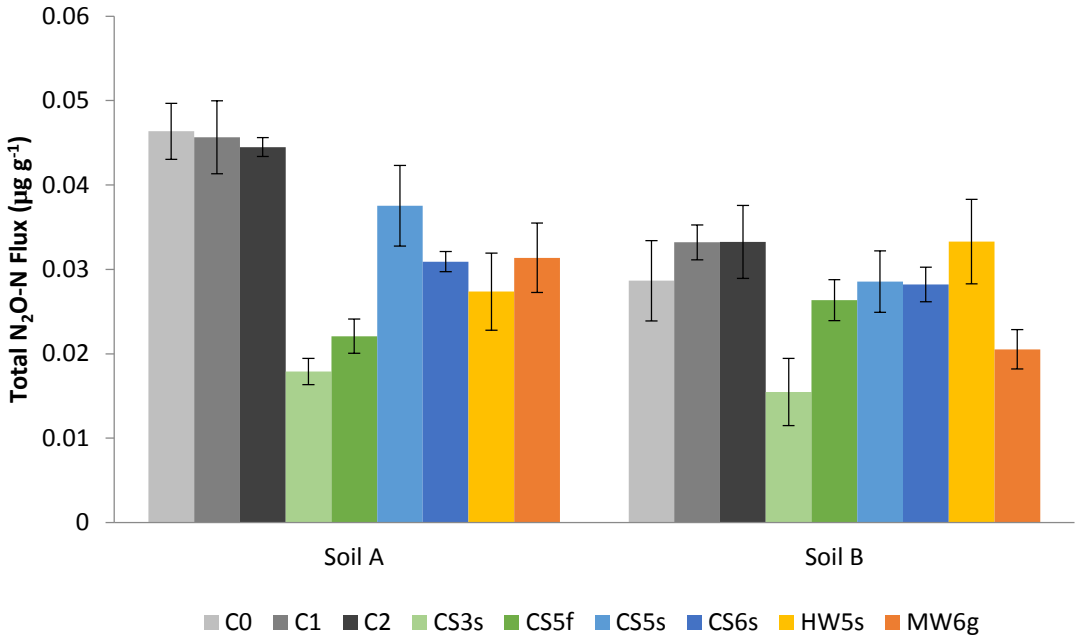

Figure 3.7. Total N_2O-N emissions over 8 days following fertilization, in µg N per g of soil (± s.e.) *(N_2O emissions were negligible after 8 days)*

Total N_2O emissions were not significantly correlated with biochar IC, ASVM, VM, FC or ash content for either soil. However, N_2O emissions from biochar-amended Soil A samples after 8 days were lowest from soil samples amended with high-BEOC biochars (CS3s and CS5f), suggesting that biochar BEOC (or a related biochar property) may have been partly responsible for the observed suppression of N_2O emissions in biochar-amended Soil A. With regards to soil chemical properties, N_2O emissions of biochar-amended Soil A samples were moderately positively correlated with soil pH (r^2 = 0.33), but correlations with all other soil properties were weak (r^2 <0.3). The lower N_2O emissions of Soil A biochar-amended samples relative to control samples corresponded

to a decrease in both NO_3^- and DOC, suggesting that biochar may have suppressed N_2O emissions from Soil A by reducing NO_3^- and/or DOC availability. In the case of Soil B, N_2O emissions of biochar-amended samples were not correlated with BEOC, and correlations with soil chemical properties were weak (r^2 <0.3).

Similarly to Soil A, biochar-amended Soil B samples contained less NO_3^- than control samples, but lower NO_3^- concentrations correspond with lower N_2O emissions only for B-CS3s and B-MW6g. In contrast to Soil A, biochar-amended Soil B samples contained higher DOC concentrations and lower pHs than controls, and N_2O emissions in biochar-amended samples were either equal to or lower than that of controls. Thus, elevated DOC in biochar-amended Soil B samples did not correspond with higher N_2O emissions. Although denitrification rates normally increase with increased available C, the acidic nature of the DOC in biochar-amended Soil B samples may have prevented its consumption by denitrifying bacteria. Additionally, organic acid concentrations may have been higher in biochar-amended Soil B samples because biochar induced the formation of anaerobic microsites (see *Comparisons among chemical properties*), and these anaerobic microsites may have facilitated the reduction of N_2O to N_2.

Conclusions

The results showed that, as hypothesized, (1) biochar IC contributed to short-term CO_2 emissions, (2) positive priming of soil OC by biochar was negligible when biochar IC and soluble OC were accounted for, and (3) reductions in N_2O emissions from

biochar amended soil corresponded with biochar properties and N cycle perturbations. In agreement with Jones et al, who showed that carbonates contribute significantly to short-term CO_2 emissions, cumulative CO_2 emissions from biochar-amended substrates without free carbonates (Soil B and quartz) were positively correlated with biochar carbonate content. Reduced emissions from biochar-amended Soil B samples relative to controls – likely due to the prevalence of anerobic microsites and resultant decrease in pH – resulted in negative priming. All six biochars reduced N_2O emissions significantly from Soil A and two biochars (CS3s and MW6g) suppressed emissions significantly from Soil B. The magnitude of emissions suppression varied widely, ranging from 18-61% and 0-53% for soils A and B, respectively. Biochar amendment consistently resulted in reduced NO_3^- concentrations in both soils, suggesting that the biochars studied here may suppress N_2O emissions by reducing the availability of NO_3^-. Furthermore, the biochars most effective at suppressing N_2O emissions from Soil A also had the highest BEOC, suggesting that this biochar fraction, or a related fraction, may have played a role in suppressing N_2O emissions from biochar-amended soil. Thus multiple mechanisms may be responsible for the effect of biochars on soil N_2O emissions, and the dominant mechanisms may vary from soil to soil. Because no significant increases in CO_2 or N_2O emissions were observed following biochar amendment, the lignocellulosic biochars studied here are therefore unlikely to increase emissions under similar contexts. However, further research is necessary to understand the mechanisms governing interactions between biochar properties and soil properties that result in divergent soil responses to biochar amendment.

APPENDIX B. SUPPLEMENTARY INFORMATION FOR CHAPTER 3

Table S3.1. Percent moisture (wt%) of soils, soil-biochar mixtures, quartz, and quartz-biochar mixtures at -1/3 bar matric potential (± s.d.)

biochar	**Soil A**	**Soil B**	**Quartz**
none	29.0 ±0.1	19.5 ±0.1	18.8 ±0.5
CS3s	32.2 ±0.1	20.5 ±0.5	19.6 ±0.5
CS5f	30.6 ±0.2	20.7 ±0.3	20.4 ±0.2
CS5s	31.2 ±0.1	20.4 ±0.2	20.4 ±0.6
CS6s	30.5 ±0.2	20.7 ±0.1	20.6 ±0.5
HW5s	29.4 ±0.1	20.9 ±0.7	18.3 ±0.5
MW6g	28.8 ±0.8	20.4 ±0.2	18.2 ±0.1

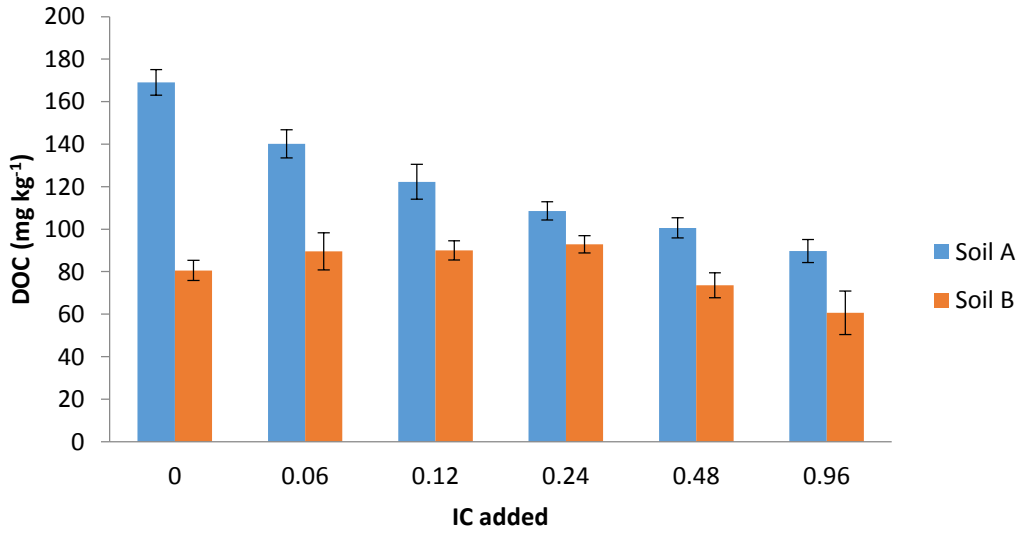

Figure S3.1. KCl-extractable DOC and mg of IC per kg of soil added as Na_2CO_3 to soils A and B (± s.d.)

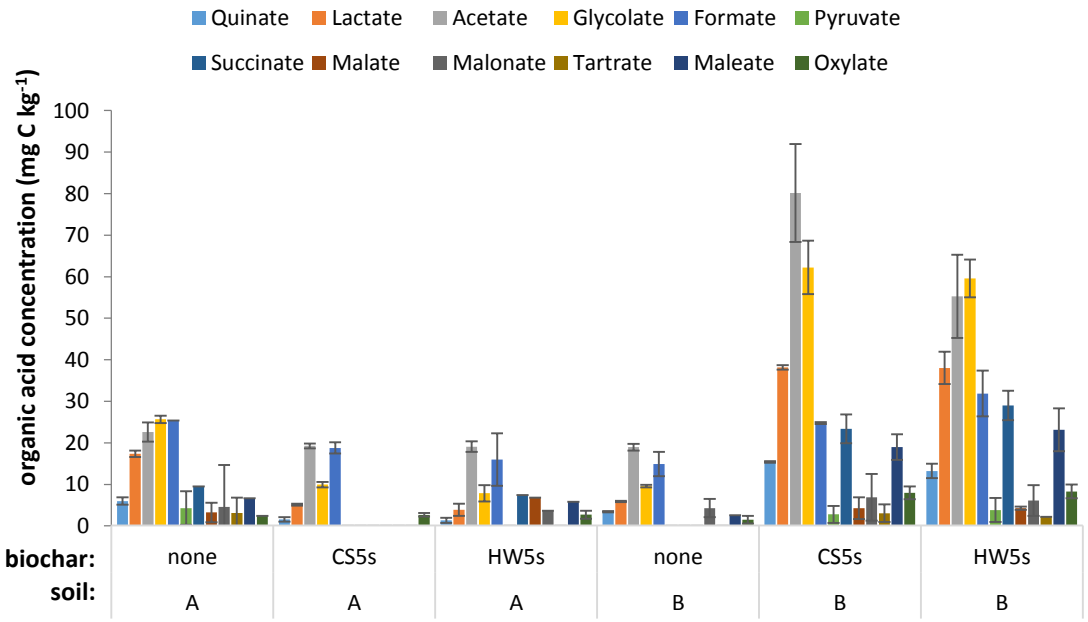

Figure S3.2. Concentrations, in mg C per g of soil, of water-soluble organic acids in soils A and B incubated for 190 days with and without CS5s and HW5s slow pyrolysis biochars, measured in duplicate (± s.d.)

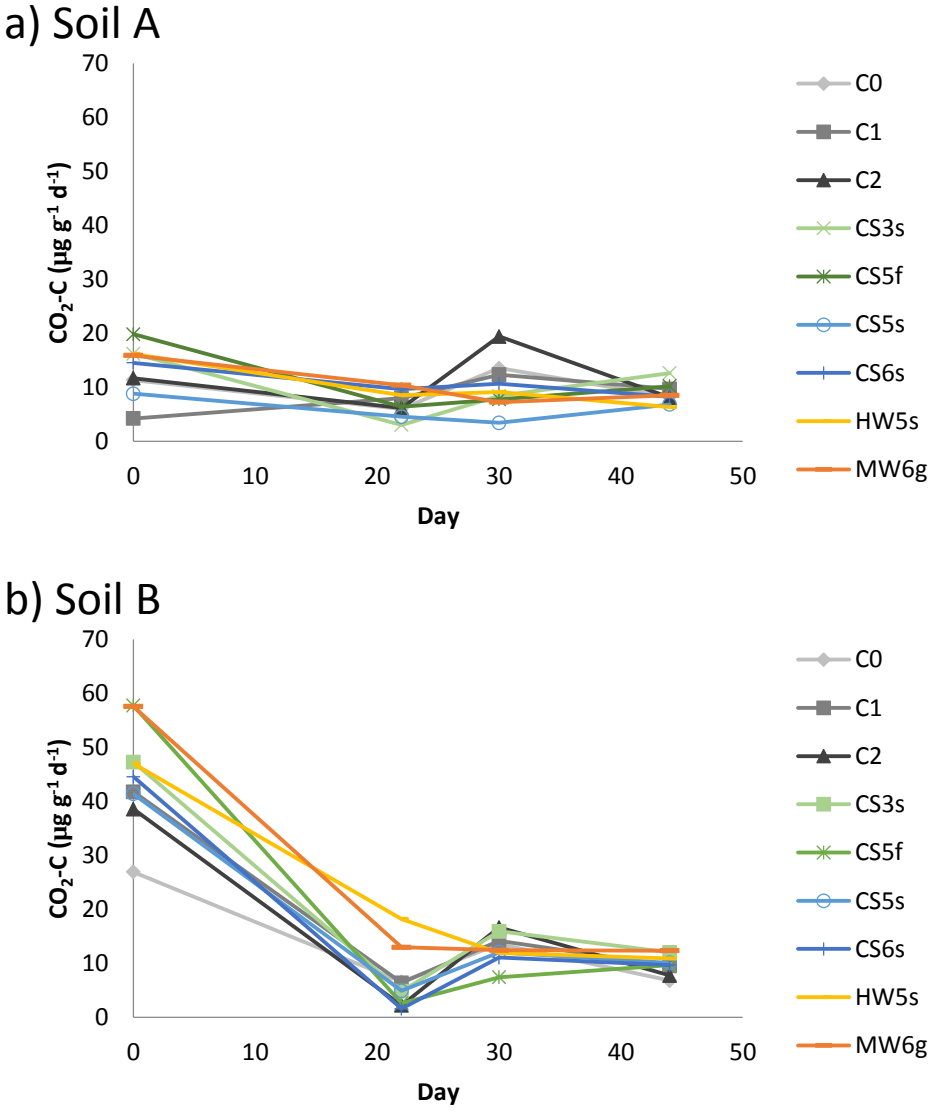

Figure S3.3. Total CO_2 emissions during the 50 day pre-fertilization equilibration period from (a) Soil A and (b) soil B, mg CO_2 per g of soil

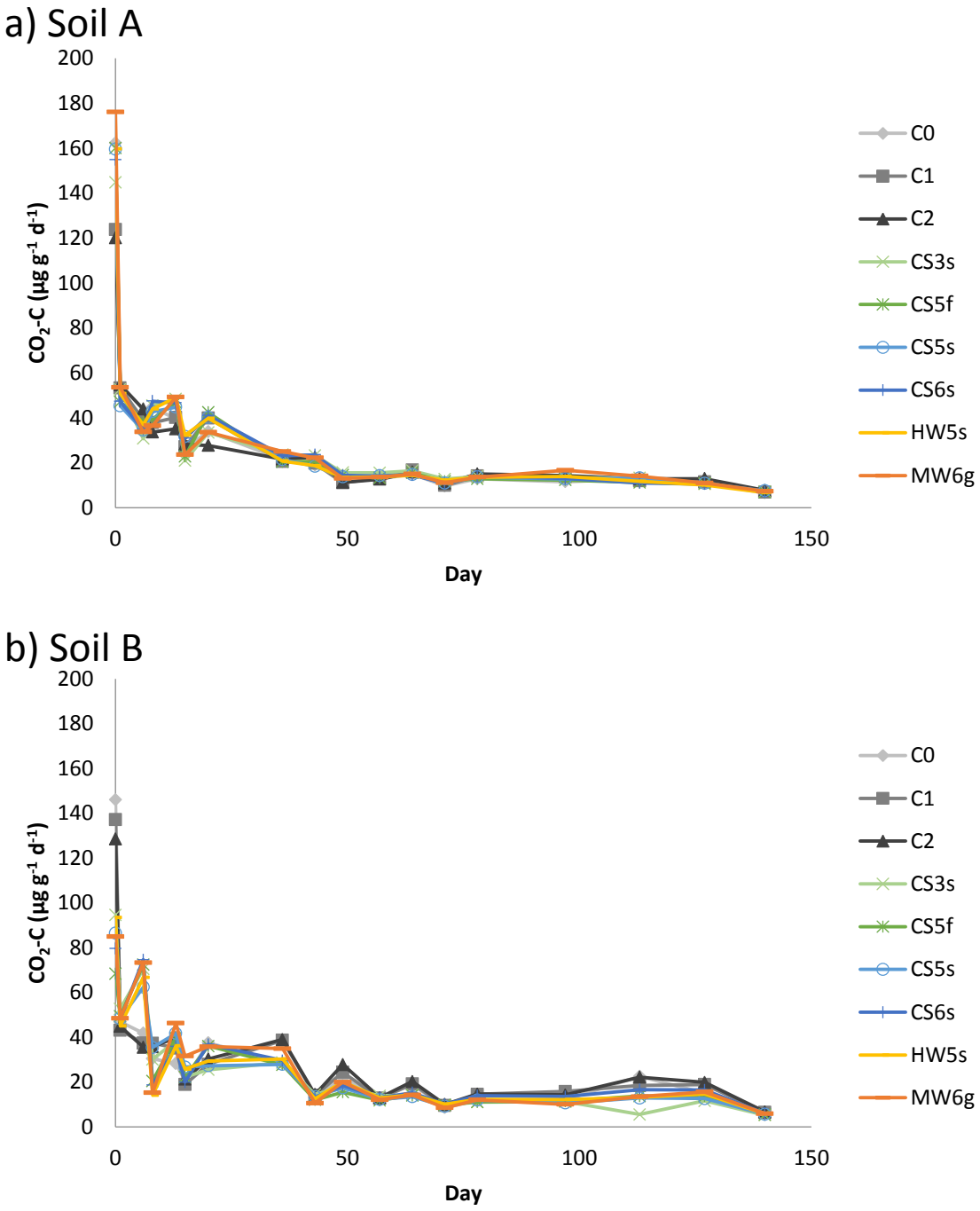

Figure S3.4. Daily CO_2-C emissions from Soil A (a) and Soil B (b) following fertilization, in µg of C per g of soil per day.

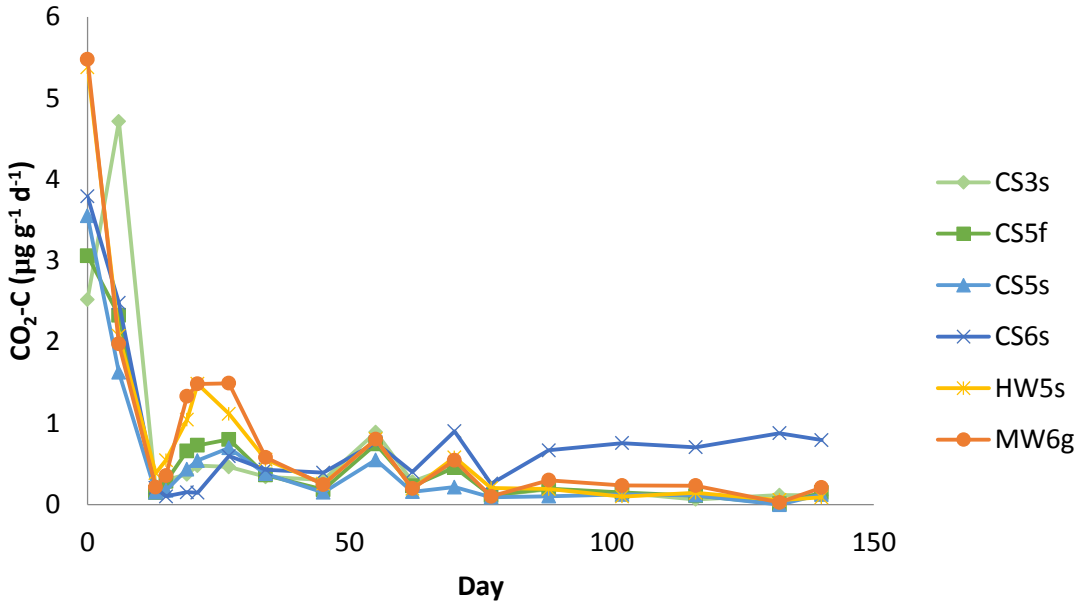

Figure S3.5. Daily CO_2 emissions of quartz-biochar mixtures over 140 days following fertilization, in µg C per g of mixture

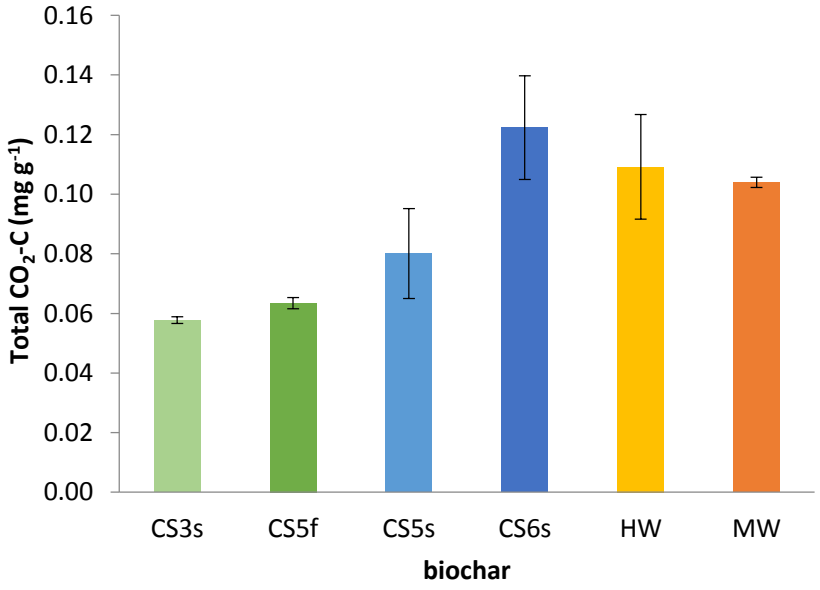

Figure S3.6. Total accumulated CO_2-C emissions of quartz-biochar mixtures over 140 days following fertilization, in mg C per g of mixture

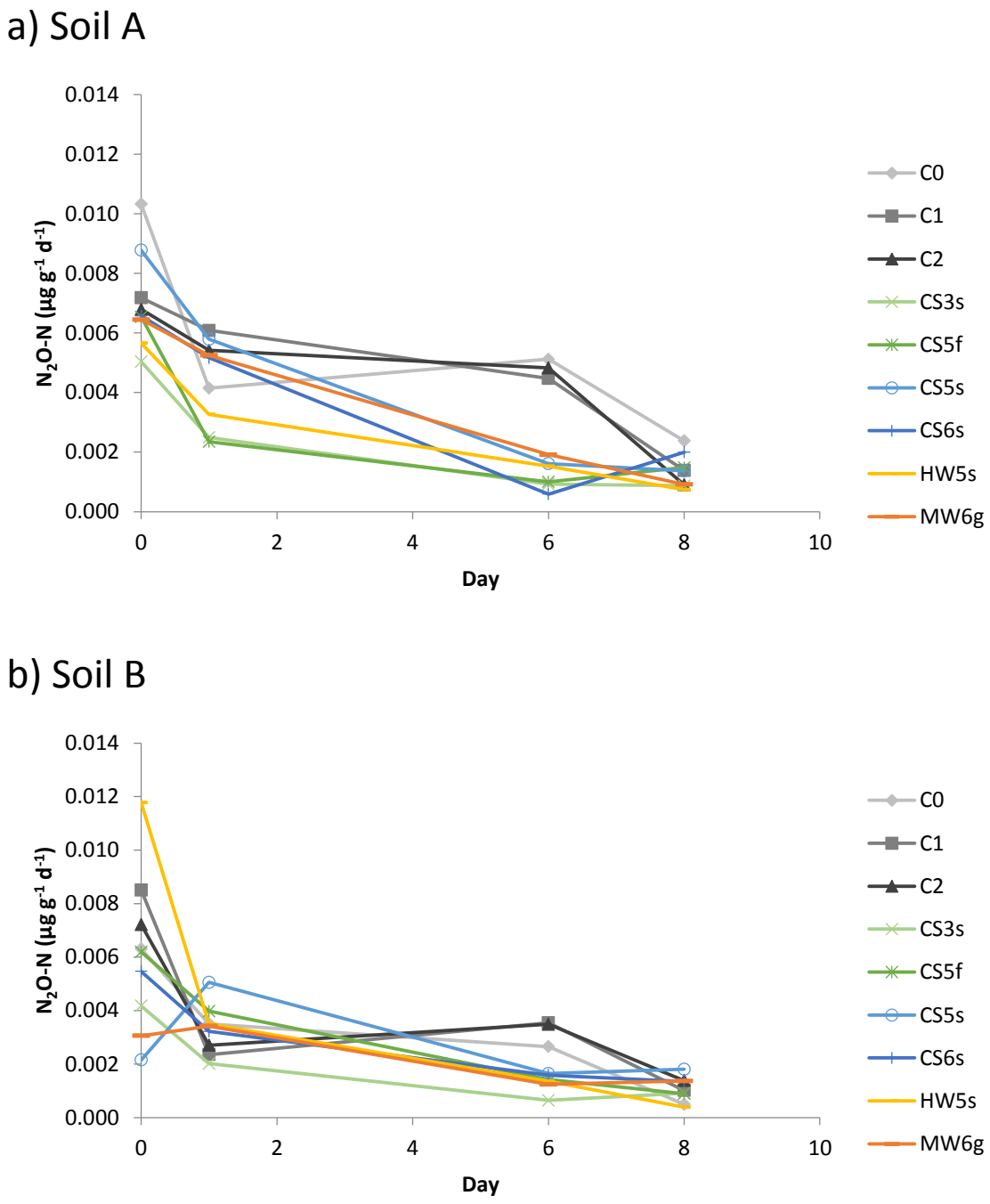

Figure S3.7. Daily N_2O-N emissions from Soil A (a) and Soil B (b) over 8 days following fertilization, in µg N per g of soil per day *(N_2O emissions were negligible after 8 days)*

CHAPTER 4. IMPACT OF BIOCHAR FRACTIONS ON SOIL CO_2 AND N_2O EMISSIONS

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A paper to be submitted to *Agriculture, Ecosystems and the Environment*

Abstract

Biochar has been shown to influence short-term (≤1 month after application) soil CO_2 and N_2O emissions, but the specific biochar fractions that influence emissions, and the amount of time biochar fractions can continue to influence emissions are not well established. Here we assess the impact of the carbonate, acid-extractable, bicarbonate-extractable, acid-insoluble and residual fractions of two biochars on greenhouse gas emissions. We hypothesized that both labile organic carbon (LOC) and inorganic carbon (IC) associated with biochar will contribute significantly to CO_2 emissions. We also hypothesized that (1) LOC and IC will have a negligible impact on N_2O emissions, (2) all biochar fractions will influence soil NH_4^+ and NO_3^- pools, and (3) changes in soil N pools will impact soil N_2O emissions. We found that all biochar fractions as well as untreated biochars increased CO_2 emissions in the very short term (24-48 h), but with the exception of the bicarbonate-soluble fraction, these effects did not influence cumulative emissions from the 140 day incubation. Both short and long-term results suggest that biochar fractions may behave differently when applied as whole untreated biochar than when applied as isolated biochar fractions. Furthermore, all fractions of a mixed wood biochar increased NO_3^- concentrations, and all fractions of both mixed wood and corn

stover biochars reduced NH_4^+ concentrations, but neither untreated nor fractionated biochar had a consistent impact on N_2O emissions.

Introduction

Biochar is the solid co-product of biomass pyrolysis suitable for soil application (Sohi et al. 2010). Due to the long residence time of biochar in soils, the pyrolysis of biomass feedstocks and subsequent application of the biochar co-product to soil has been proposed as a means for carbon-negative energy generation and carbon (C) sequestration (Lehmann et al. 2006). Biochar has also been shown to improve soil quality and thereby to increase net primary productivity and provide a secondary means for C sequestration (Woolf et al. 2010; Biederman and Harpole 2013). It is estimated that using pyrolysis to generate energy coupled with biochar application to soil could offset up to about 12% of anthropogenic greenhouse gas (GHG) emissions (Woolf et al. 2010). However, biochars are a diverse suite of materials, and the net effect of biochar amendment on GHG emissions offsets is highly sensitive to biochar properties and their impact on biochar mineralization and soil GHG emissions (Spokas and Reicosky 2009).

Biochar-induced changes in soil CO_2 and N_2O emissions are a critical component in biochar life cycle analysis models, as changes in emissions can either enhance or diminish biochar's potential to offset anthropogenic GHG emissions (Whitman et al. 2010; Woolf et al. 2010). While it is clear that both biochar and soil properties influence biochar-induced changes in soil GHG emissions, it has yet to be determined which biochar fraction(s) have the largest impact on emissions, and whether these fraction(s)

have a short-term or a long-term influence on emissions. Most biochars are predominately comprised of a highly condensed aromatic C framework which is responsible for their chemical and biological recalcitrance (Schimmelpfennig and Glaser 2012). During pyrolysis, 20-50% of the feedstock carbon (C), which was removed from the atmosphere by photosynthesis prior to biomass harvest, is converted into condensed aromatic C in biochar. In biochars produced at moderate to high temperatures (400-700°C), this condensed aromatic C constitutes the majority of biochar C (Keiluweit et al. 2010). Biochar also contains two smaller but more labile fractions: (1) a labile organic carbon (LOC) fraction consisting of low molecular weight volatile compounds that precipitated into the biochar during pyrolysis and incompletely pyrolyzed biomolecules, and (2) an inorganic C (IC) fraction consisting of carbonates associated with base cations. Labile fractions can have a disproportionately large impact on soil-biochar interactions in the short term, whereas recalcitrant fractions are of greater importance in the long term (Joseph et al. 2010; Jones et al. 2011).

Identification of labile biochar fractions and quantification of their impact on soil GHG emissions is necessary to help distinguish short-term impacts from long-term impacts. Failure to identify these fractions may lead to erroneous estimates of biochar impacts when short-term (<6 mo) studies are used to extrapolate impacts into the long term (1-10 yrs). Once influential labile fractions have been identified, efforts can be made to account for their effects, such as pre-treating biochars to remove labile fractions as a negative control, or adding isolated labile fractions to untreated soil as a

positive control (Jeffery et al. 2015). Distinguishing the influence of labile fractions will furthermore facilitate understanding of more recalcitrant fractions by difference, and aid in the parameterization of soil organic carbon cycling models for predicting the long-term impact of biochar.

Few studies have directly quantified biochar LOC or IC to inform incubation experiment results (Calvelo Pereira et al. 2011), and even fewer have used positive or negative controls to assess interactions of these fractions with the biochar matrix (Jones et al. 2011). Some studies have quantified LOC directly by using wet chemical analyses (Calvelo Pereira et al. 2011; Lin et al. 2012), while others quantify "effective" biochar LOC via C mineralization using incubations of biochar with sand (Cross and Sohi 2011; Zimmerman et al. 2011) or isotopically labeled biochar with soil (Farrell et al. 2013; Fang et al. 2014). Pereira et al. (2011) quantified LOC using potassium permanganate oxidation of several biochars and conducted an incubation experiment, but they were unable to compare CO_2 evolution with LOC because biochar-derived and soil-derived CO_2 were not distinguished. The influence of biochar IC on CO_2 or N_2O emissions has been reported by just three publications, none of which considered simultaneous emissions of both gases (Jones et al. 2011; Cayuela et al. 2013a; Bruun et al. 2014). Bruun et al. (2014) quantified the amount of ^{14}C in the carbonate fraction of ^{14}C-labeled biochar and then measured $^{14}CO_2$ emissions from biochar-amended soil. They found that 0.2-0.4% of biochar C was mineralized, but they were not able to distinguish between emissions attributed to IC or LOC. Cayuela et al. (2013) compared N_2O

emissions from soil amended with untreated biochars and biochars adjusted to the pH of the soil. But they did not document whether carbonates were completely removed; therefore the study constitutes only an indirect assessment of the biochar IC's influence on N_2O emissions.

Jones et al. (2011) are, to our knowledge, the only authors to have investigated the contribution of both biochar LOC and IC to soil CO_2 emissions. The authors quantified water-soluble OC of a slow pyrolysis hardwood biochar, and they compared emissions from soil amended with untreated biochar, water-washed biochar, and biochar water extracts. They found that water-washing the biochar resulted in an about 50% reduction of CO_2 emissions during the first 36 hours of the experiment, and that mineralizable OC represented about 0.1% of untreated biochar C. However, as noted by the authors, a single water wash may not have been sufficient to isolate all of the biochar's water-soluble OC. Furthermore, the method does not account for differences in the pH of water-biochar slurries among different biochars. Since pH affects solubility, the use of water-extractable OC as an index of biochar LOC may be problematic. IC was not removed from the water extracts, and thus the influence of water-soluble IC and OC was not distinguishable. The authors incubated acid-washed biochar with soil to assess the effects of biochar IC by difference, and found that acid-washing resulted in a 60-70% reduction in CO_2 evolution 48 hours following biochar addition. However, any DOC that may have been removed during the acid washing procedure was not distinguished from IC. Overall, it was shown that both biochar LOC and IC contributed significantly to short-

term CO_2 emissions, but their contributions could not be completely distinguished. No previous study has investigated the impact of both biochar LOC and IC on N_2O emissions from biochar-amended soil.

Here, we assess the relative impacts of biochar LOC and IC on soil CO_2 and N_2O emissions from soil amended with two untreated biochars and their acid-soluble, bicarbonate-soluble, acid-insoluble, and residual fractions. We hypothesized that both labile organic carbon (LOC) and inorganic carbon (IC) associated with biochar will contribute significantly to CO_2 emissions. We also hypothesized that (1) LOC and IC will have a negligible impact on N_2O emissions, (2) all biochar fractions will influence soil NH_4^+ and NO_3^- pools, and (3) changes in soil N pools will impact soil N_2O emissions.

Methods

Biochar preparation

A corn stover biochar produced by fast pyrolysis at 500°C (Cs) and a mixed wood biochar produced by gasification at 600°C (Mw) were chosen for their contrasting properties as illustrated by bicarbonate-extractable organic carbon (BEOC) and IC (see Ch. 3). Cs contained more BEOC (3.2 mg C g^{-1}) than Mw (0.64 mg C g^{-1}), whereas Mw contained more IC (9.0 mg C g^{-1}) than Cs (4.5 mg C g^{-1}). Two broad types of biochar fractions were isolated: washed biochars (solid), and biochar extracts (aqueous). Each biochar was ground and sieved to <0.5 mm, and it was then subjected to three treatments: untreated (no treatment), acid washing, or bicarbonate washing followed

by acid washing. The acid washing treatment consisted of shaking the biochars with 0.05 M HCl for 24 h to remove the acid-soluble biochar fraction (primarily alkalis and low molecular weight organic compounds), followed by washing with 1 M $CaCl_2$ twice and deionized water four times for 15 min each to remove excess HCl. The bicarbonate washing treatment consisted of shaking each biochar with 0.05 M $NaHCO_3$ for 24 h, and this treatment was followed by the acid-washing treatment (including $CaCl_2$ and water washes) to remove excess $NaHCO_3$ as well as the acid-soluble biochar fraction. A 50:1 solution-to-biochar ratio (mL:g) was used for all washes, and samples were vacuum filtered using 0.45 µm nitrocellulose filter paper following each wash. After the final wash, the treated biochars were dried at 60°C for 72 hours. Acid and bicarbonate extracts from the initial HCl and $NaHCO_3$ washes were conserved, and their pHs were adjusted to the soil pH (6.3) by adding 1 M NaOH, 1 M HCl, or 6 M HCl, drop-wise as appropriate. The pH-adjusted bicarbonate extracts were sparged with N_2 for 2 h to remove excess bicarbonate. According to Le Chatelier's Principle, forcing CO_2 out of solution would have facilitated the conversion of bicarbonate to CO_2, which would be subsequently removed. The acid-washed biochars are hereafter referred to as the "acid-insoluble" biochar fraction, and the bicarbonate and acid-washed biochars are considered the "residual" biochar fraction; acid and bicarbonate extracts are considered the "acid-soluble" and "bicarbonate-soluble" fractions, respectively. Soluble OC and IC added in each treatment are shown in Table 4.1, and a graphic representation of the biochar fractionation methods is shown in Figure 4.1.

Table 4.1. Treatment names, corresponding biochar feedstocks, and biochar fractions. Inorganic C (IC) applied in extracts, biochar, or carbonate control treatments; soluble OC applied in extracts, in mg of C per g of soil (solid biochar fractions applied at 0.5 wt% (g per g of soil); aqueous extracts applied at 0.5 wt% equivalent; IC added to C1 and C2 as Na_2CO_3)

Treatment	Biochar Feedstock	biochar fraction	IC (mg C g^{-1} soil)	Soluble OC (mg C g^{-1} soil)
C0	none	none	none	none
C1	none	none	0.023	none
C2	none	none	0.045	none
CsEa	corn stover	acid-soluble	0.0023	0.0014
CsEb	corn stover	bicarbonate-soluble	0.0016	0.0102
CsWu	corn stover	untreated (whole)	0.023	none
CsWa	corn stover	acid-insoluble	none	none
CsWb	corn stover	residual	none	none
MwEa	mixed wood	acid-soluble	0.0001	0.0011
MwEb	mixed wood	bicarbonate-soluble	0.0087	0.0057
MwWu	mixed wood	untreated (whole)	0.045	none
MwWa	mixed wood	acid-insoluble	none	none
MwWb	mixed wood	residual	none	none

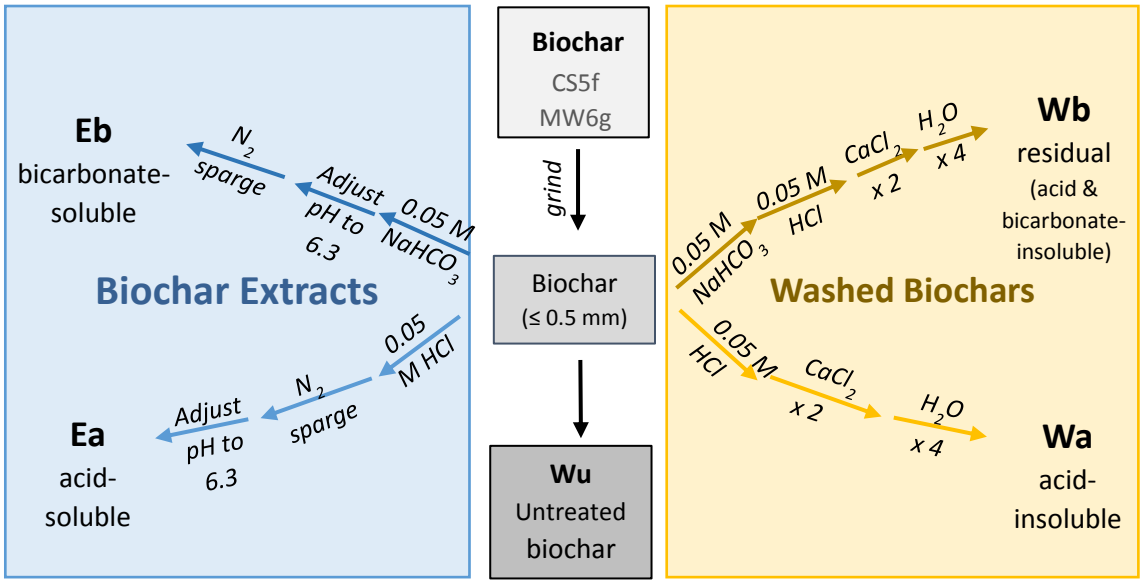

Figure 4.1. Graphic representation of biochar fractionation methods.

Incubation with soil

The soil used for incubation was sampled from the Iowa State University Armstrong Memorial Research and Demonstration Farm, and is an Exira silt loam (Fine-silty, mixed, superactive, mesic Typic Hapludoll) located on an eroded hillslope with 15% sand, 80% silt and 5% clay. Soil material was collected from the top 5 cm of the A horizon prior to fertilizer application in the spring, kept frozen for 6 months, and finally thawed and sieved to <4 mm before use. Visible crop residues not removed by sieve were removed by hand. Moisture was determined on an oven dry basis, and 10 g of oven-dry-weight-equivalent soil was weighed into 150 mL glass serum vials. The 13 treatments used (5 replicates of each) are described in Table 4.1. For the C1 and C2 treatments, 2.5 mL of Na_2CO_3 solution was added in concentrations selected to match the IC in the Cs and Mw biochars, respectively. Acid and bicarbonate-soluble fractions were added as 2.5 mL of acid and bicarbonate extracts, respectively. All biochar treatments were applied as 0.5% (wt/wt) of untreated or washed biochars. This application rate is equivalent to rates used in previous incubations and field studies (Rogovska et al. 2014; Cayuela et al. 2015). Sodium (Na) content of all samples was adjusted to 0.0125 mmol g^{-1} using NaCl, and all samples received a total of 2.5 mL of aqueous solution (as acidic biochar extract or a mixture of Na_2CO_3 and NaCl, or NaCl only) to ensure equivalent moisture contents. All samples were mixed gently prior to application of aqueous solutions to mix in biochar amendments and to physically disturb all samples equally.

Soil samples were equilibrated for 60 days at 20°C, during which time CO_2 and N_2O emissions were measured on days 0, 1, 4, 7, 13, 21, 32, 42 and 48, and soil was gradually allowed to dry to -1/3 bar matric potential (29-31% moisture on a dry weight basis) by periodically opening the vials. After 60 days, air dried and ground (<0.5 mm) corn stover was mixed in at 0.5% (wt/wt), and fertilizer was added as NH_4NO_3 and K_2HPO_4 at a rate equivalent to 72, 42 and 54 mg kg^{-1} of N, P and K, respectively. The samples were incubated for an additional 80 days following fertilization at 20°C, during which time moisture was maintained at -1/3 bar matric potential equivalent, and emissions were quantified on days 0, 1, 2, 3, 4, 8, 9, 18, 25, 31, 52, 66, and 80. Between gas flux measurements, samples were kept covered with grey butyl septa (no crimp cap). For each gas flux measurement, serum vials were sealed with butyl septa and crimp caps, and 11.5 mL gas samples were collected using a syringe three times over the course of 16 to 48 hours, as appropriate for the flux rate. Gas samples were stored in helium-flushed and evacuated airtight 6 mL Exetainer vials and analyzed for CO_2 and N_2O using a gas chromatograph equipped with a methanizer-flame ionization detector and an electron capture detector. Concentrations were measured by volume and converted to mass units using the ideal gas law.

Chemical analysis of soil

Following the incubation, a sub-sample of each soil was oven-dried at 105°C and conserved for analysis of pH, total C, and total N. Soil pH was measured in deionized water at a 1:1 solution:soil ratio (Thomas, 1996). Soluble NH_4^+ and NO_3^- were extracted

using 2 M KCl (5:1 solution:soil ratio). Samples were prepared with the Berthelot and Griess-Ilosvay reagents for analysis of NH_4^+ and NO_3^-, respectively (Hood-Nowotny et al. 2010) and analyzed using a microplate spectrophotometer. Total C and N were analyzed via combustion (Vario Microcube, Elementar).

Calculations and statistical analyses

Gas flux rates were calculated from the slope of the linear increase in gas concentrations over time (Iqbal et al 2013), and any slopes with $r^2 < 0.5$ were assumed to be zero. Average daily flux rates were compared using the PROC MIXED procedure and 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 $p = 0.05$, and all analyses were conducted in SAS (v9.2).

Results and Discussion

Soil chemical analysis

Total C, total N and C:N ratios of soil and soil amendments measured prior to the incubation and following the 140 day incubation period are shown in Tables 4.2 and 4.3, respectively. Biochar amendments, but not biochar extracts or carbonate additions, consistently increased final total C relative to the C0 (zero carbonate) control. Increases in final total C were significant for treatments CsWa, CsWb and MwWb only. No consistent effects on final total N were observed, although CsWa and MwEb both

slightly but significantly reduced total N relative to C0. Biochar amendments also consistently increased C/N ratios, and this increase was significant for the CsWu, CsWa, CsWb, MwWu, and MwWb treatments. In contrast, the sodium carbonate and biochar extract amendments did not significantly increase C/N ratios.

Final soil pHs are shown in Figure 4.2. The pH of the zero carbonate control, C0, fell from an initial pH of 6.30 to a final pH of 5.95 by the termination of the incubation. Relative to C0, all other treatments increased the final soil pH. Both untreated biochars, CsWu and MwWu, increased soil pH relative to their respective carbonate controls, C1 and C2. Even biochar extracts and washed biochars – which had negligible quantities of carbonate – increased soil pH relative to C0, and in many cases increased pH relative to C1 and C2 as well. This liming effect, irrespective of biochar fraction added, suggests that all four biochar fractions contain significant quantities of pH-buffering moieties.

Concentrations of extractable soil NH_4^+-N and NO_3^--N, measured at the end of the incubation, are shown in Figure 4.3. Most treatments significantly impacted concentrations of NH_4^+, NO_3^- or both. All treatments significantly reduced extractable NH_4^+ concentrations relative to the C0 control. The C1 and C2 treatments reduced extractable NH_4^+ by 3 mg N kg^{-1}, and all other treatments reduced extractable NH_4^+ by 6 to 13 mg N kg^{-1}. In general, Mw treatments reduced NH_4^+ more than did Cs treatments, although CsWb-treated soil had similar extractable NH_4^+-N to MwWu-treated soil. Among Mw treatments, the MwWa and MwWb treatments had the lowest NH_4^+ concentrations. With regards to NO_3^-, carbonate additions did not have a significant

effect, but CsEb, CsWb, and all Mw treatments increased NO_3^- significantly by 1 to 7 mg N kg^{-1} relative to C0 and C2, and 3 to 9 mg N kg^{-1} relative to C1. Several treatments reduced NH_4^+: NO_3^- ratios: carbonate controls had ratios of 2.2-2.6, whereas Cs treatments had ratios of 1.2-2.2 and Mw treatments had ratios of 0.9-1.3. Because acid and bicarbonate-soluble fractions and untreated biochars decreased the NH_4^+:NO_3^- ratios to a similar degree, this effect cannot be attributed entirely to adsorption of NH_4^+ or NO_3^- to biochar.

Although the cause of changes in extractable NH_4^+ and NO_3^- cannot be confirmed from these data alone, NO_3^- tended to increase with increasing pH among biochar and biochar extract treatments, and NH_4^+ tended to decrease with increasing pH among all treatments, suggesting that changes in N speciation may have been tied to pH. This observation was confirmed by a significant positive correlation between soil pH and extractable NO_3^- (r^2 = 0.54) among biochar and biochar extract treatments, and a significant ($p < 0.05$) negative correlation between soil pH and extractable NH_4^+-N among carbonate controls (r^2 = 0.62) and among unwashed biochar and biochar fraction treatments (r^2 = 0.22) (Figures S4.1 and S4.2). As nitrification rates are inhibited at low pHs (Parton et al. 1996; Zheng et al. 2012), the apparent impact of pH suggests that biochar alkalis may have accelerated nitrification. Additionally, NO_3^- concentrations were slightly but significantly higher in soils amended with mixed wood biochar (both untreated and fractionated) than in the C2 control, suggesting that biochar alkalis or

perhaps biochar OC were more effective than carbonate alone at increasing soil nitrification rates.

Table 4.2. Percent total carbon (TC) and total nitrogen (TN) of soil, corn stover, untreated biochar and acid-washed biochar measured prior to incubation, in wt% ± s.d (n=3). Acid-washed and bicarbonate-washed biochars are assumed to have equivalent TC and TN.

	TC (%)	TN (%)
Soil	2.93 ±0.04	0.305 ±0.004
Corn stover	40 ± 2	0.82 ±0.1
CsWu	52 ±2	0.58 ±0.04
CSWa & CsWb	52 ±2	0.58 ±0.02
MwWu	63 ±1	0.58 ±0.09
MwWa & MwWb	70.0 ±0.4	0.576 ±0.006

Table 4.3. Total C, total N and C:N ratios of soil following 140d incubation period, in wt% ± s.d (n=5).

treatment	TC (%)	TN (%)	C:N
C0	3.0 ± 0.1	0.33 ± 0.01	8.8 ± 0.3
C1	2.8 ± 0.3	0.31 ± 0.03	8.9 ± 0.5
C2	2.9 ± 0.1	0.33 ± 0.01	8.7 ± 0.3
CsEa	2.86 ± 0.06	0.32 ± 0.01	8.8 ± 0.2
CsEb	2.9 ± 0.2	0.33 ± 0.02	8.8 ± 0.4
CsWu	3.2 ± 0.3	0.32 ± 0.02	9.9 ± 0.4*
CsWa	3.17 ± 0.04*	0.31 ± 0.01*	10.1 ± 0.2*
CsWb	3.2 ± 0.2*	0.32 ± 0.01	10.1 ± 0.3*
MwEa	3.2 ± 0.3	0.34 ± 0.03	9.3 ± 0.5
MwEb	2.9 ± 0.1	0.32 ± 0.01*	9.0 ± 0.2
MwWu	3.1 ± 0.4	0.32 ± 0.04	9.6 ± 0.5*
MwWa	3 ± 1	0.3 ± 0.1	9.8 ± 0.8
MwWb	3.2 ± 0.2*	0.33 ± 0.02	9.8 ± 0.3*

*Significant difference from control at $p<0.05$

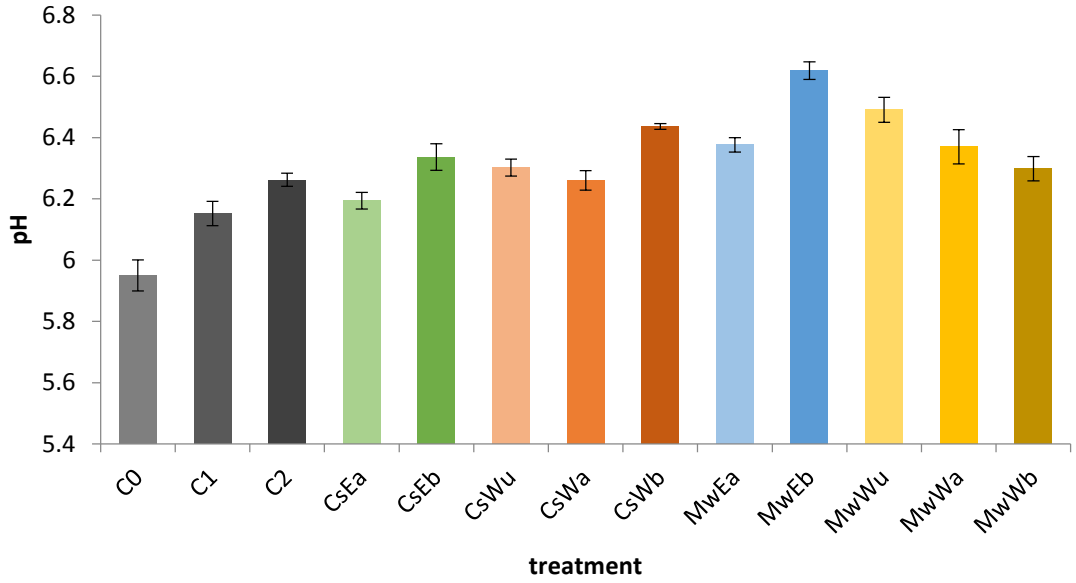

Figure 4.2. Final pH of air dry soil samples incubated for 140 days. Error bars represent standard deviations of five replicates. (C = carbonate treatment (no biochar), Cs = corn stover biochar, Mw = mixed wood biochar, Ea = acid extract, Eb = bicarbonate extract, Wu = untreated biochar, Wa = acid washed, Wb = bicarbonate washed)

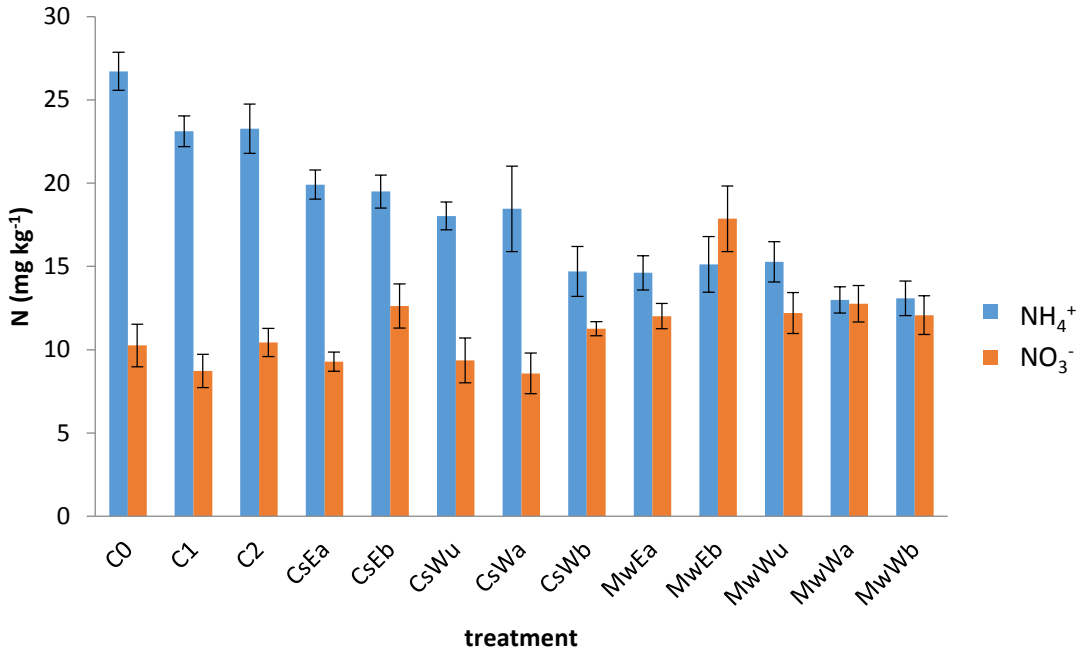

Figure 4.3. NH_4^+ and NO_3^- concentrations, in mg N per kg air dry soil, following 140 day incubation period. Error bars represent standard deviations of five replicates.

Equilibration period soil CO_2 emissions

CO_2 emissions exhibited an initial "flush" in the first few days of the equilibration period, during which all soil samples exhibited their highest emission rates, and differences between control soil and soil receiving amendments were greatest (Figure 4.4). The main effects of treatment and day, and the treatment*day interaction were all significant. Because of interactions, attempts to isolate and separately quantify CO_2 emissions from various biochar fractions may not accurately reflect emissions coming from whole biochars. We therefore did not attempt to quantify the contribution of discrete fractions to the emissions from the whole biochar here, but rather we compared the emissions induced by amendment of each fraction to qualitatively index the amount of labile or hydrolyzable C therein.

Most significant differences observed on the first few days of the incubation did not persist through the end of the equilibration period, but these initial emissions are indicative of the short term lability of biochar fractions. After days 0 and 1, CO_2 emissions for most treatments were not significantly different from the C0 control. On day 0, both CsWu and MwWu had significantly lower initial emission rates than their respective carbonate controls, C1 and C2. Emissions from acid-washed fractions (CsWa and MwWa) were similar to emissions from C0, suggesting that acid washing removed most of the rapidly mineralizeable or hydrolyzable C from the biochars. The magnitude of the difference in day-0 emissions between C0 and C1 (4.1 μg CO_2-C $g^{-1}d^{-1}$) was similar to that between CsWa and CsWu (4.4 μg CO_2 $g^{-1}d^{-}$), suggesting that carbonates

accounted for the majority of CO_2 emitted from acid-soluble biochar C. However, the difference between C0 and C2 (10 μg CO_2-C $g^{-1}d^{-1}$) was larger than between MwWa and MwWu (3 μg CO_2-C $g^{-1}d^{-1}$), suggesting that the carbonates in Mw were less susceptible to hydrolysis than reagent-grade sodium carbonate applied separately, perhaps due to adsorption of the carbonates to the biochar (or occlusion within). Overall, the initial CO_2 flush induced by biochar fractions and carbonate amendments during the first few days of the equilibration period suggests that all biochar fractions contribute to very short-term (≤48 h) CO_2 emissions, and labile biochar IC and OC – as represented by biochar extracts and carbonate amendments – had the largest impact on emissions.

The elevated CO_2 emissions from soil amended with the bicarbonate-soluble biochar fractions of both biochars suggests that this fraction is the most labile and readily bioavailable of all the biochar fractions studied here. Emissions from soil amended with bicarbonate-soluble biochar fractions also were 10 to 28 times higher than the amount of OC added, indicative of a large positive priming effect of this fraction. Moreover, the bicarbonate-soluble fraction was the only amendment to result in persistently elevated emissions (relative to C0) for the entirety of the equilibration period. The relatively small initial CO_2 flux from soils amended with untreated biochar compared with the large flux from soils amended with the bicarbonate biochar extract further suggests that this labile fraction is less bioavailable when associated with the whole biochar. This phenomenon may be due to strong adsorption of the bicarbonate-soluble OC to the biochar aromatic C framework in the whole, untreated, biochar, and

more generally suggests that adsorption of labile organic compounds to biochar's recalcitrant aromatic framework may prevent positive priming that would occur if labile compounds were added to soils separately.

The carbonate control results also provided strong evidence that carbonates contribute to CO_2 emissions primarily in the short term. Emissions from carbonate controls during day 0 increased with increasing carbonate added (C0≤C1<C2). Carbonates in the C1 and C2 carbonate controls and untreated biochars likely contributed to CO_2 emissions by the uptake of H^+ from the solution and the hydrolysis of the carbonates to CO_2. Carbonates may have also indirectly increased CO_2 emissions by increasing the soil pH to levels more favorable for mineralization of SOC by bacteria. However, the elevated CO_2 emissions did not persist past the first few days of the incubation, whereas pHs were elevated all the way to the end of the experiment; hence it is more likely that carbonates contributed to CO_2 emissions by direct hydrolysis rather than indirectly through increasing pH. The initially elevated CO_2 emissions from soils amended with the acid-soluble biochar extracts and acid-insoluble and residual biochar did not significantly contribute to the total CO_2 emissions during the equilibration period. Overall, the CO_2 emissions suggest that while all biochar fractions promote CO_2 emission, these impacts are largely limited to the short term (<50 days), and may be mitigated or slowed by adsorption of the labile fractions to the biochar matrix.

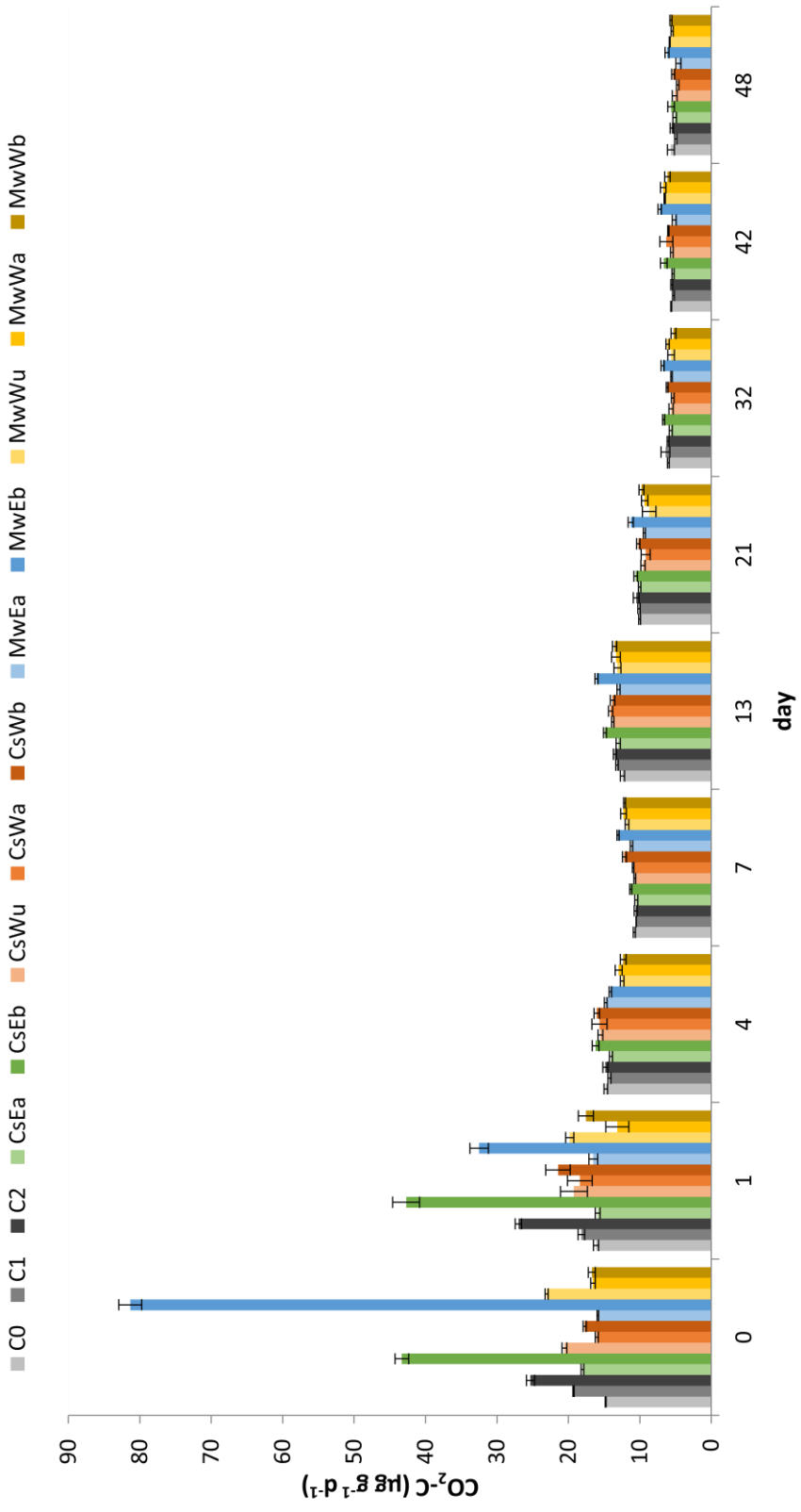

Figure 4.4. Average CO_2-C fluxes during equilibration period from soil amended with carbonate and biochar. Error bars represent standard errors of five replicates.

Post-fertilization soil CO_2 emissions

Following addition of corn stover and fertilizer, emissions increased rapidly from about 5 µg CO_2-C g^{-1} d^{-1} to 100-120 µg CO_2-C g^{-1} d^{-1} (Figures 4.4 and S4.3). After day 0 of the post-fertilization period, emissions declined rapidly, and by day 80 they had reached about 10 µg CO_2-C g^{-1} d^{-1} (Figure S4.3). The treatment, day, and treatment*day interaction effects were significant, as evaluated using repeated-measures analysis. Total accumulated emissions during the post-fertilization period were highest for soils amended with the bicarbonate-extractable fractions of both biochars (both CsEb and MwEb), which had significantly higher emissions than unamended soil and soil amended with untreated biochars (Figure 4.5). Sodium carbonate-amended soil had lower emissions than unamended soil, but the difference was not significant. The acid-insoluble fraction of Cs (CsWa) and the untreated Cs biochar (CsWu) significantly reduced emissions relative to the C0 control, but not relative to the other controls. Furthermore, emissions from the CsWb-treated soil exceeded that of CsWu-treated soil, but they did not exceed emissions from the control soil; this may have occurred due to the presence of emission-suppressing compounds in the untreated and acid-washed biochar that were removed during bicarbonate-washing (Buss et al 2014; Deenik et al 2010; Spokas et al 2010; Spokas et al., 2011). CsEb-treated soil had emissions that were 0.2 mg CO_2-C g^{-1} higher than C0, and this treatment also exhibited significantly higher CO_2 emissions than all other treatments except for MwEb. Thus, with the exception of

the bicarbonate-soluble fractions, the effects of biochar fractions that were observed in the equilibration period overall did not persist into the post-fertilization period.

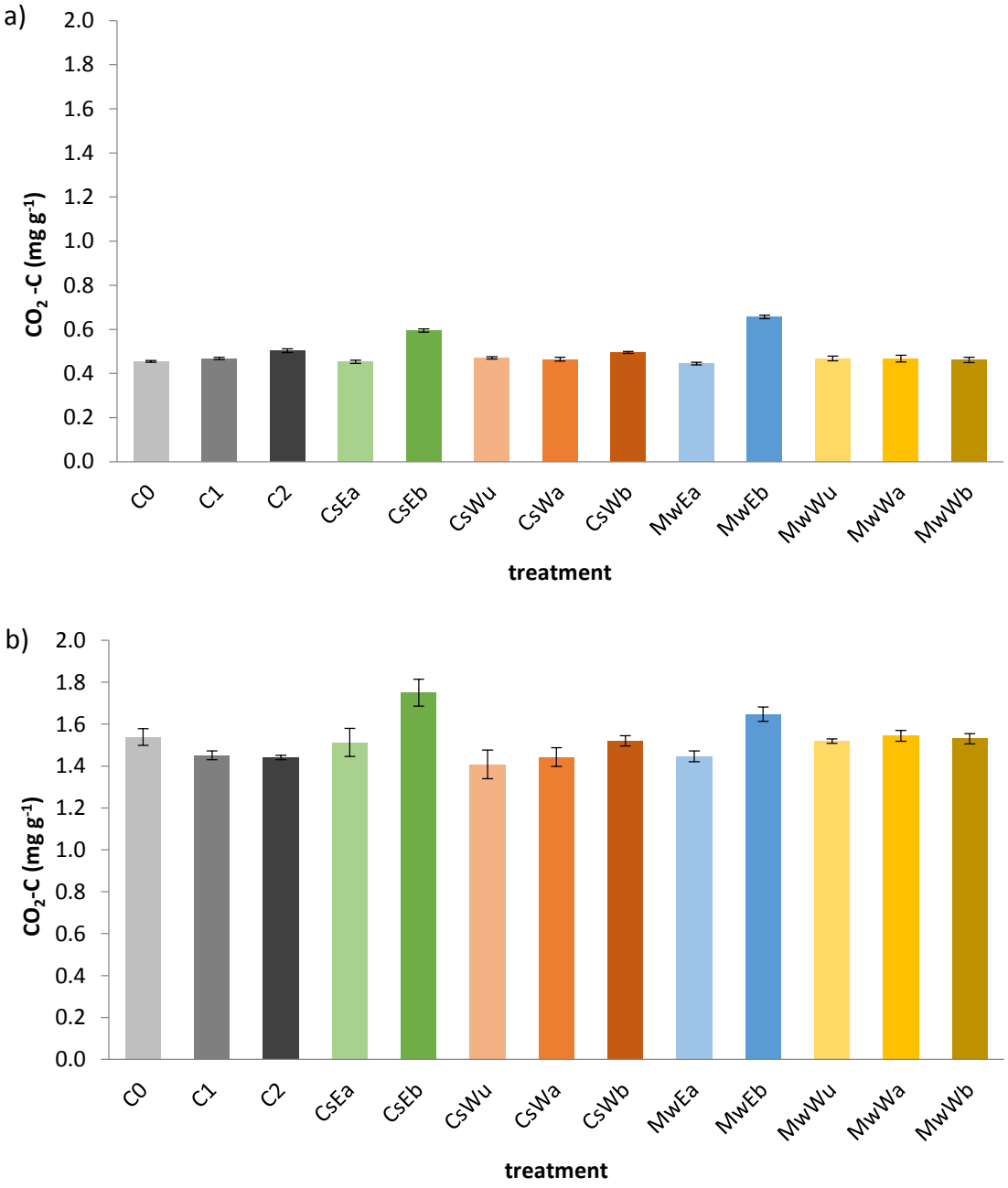

Figure 4.5. Cumulative CO_2-C emissions during (a) 60-day equilibration period, and (b) 80-day post-fertilization period. Error bars represent standard errors of five replicates.

Soil N_2O emissions

Before the fertilizer and corn stover amendments, N_2O emissions from the majority of samples were below the detection limit (*data not shown*). Following fertilization, the main effect of treatment was not significant but the effect of day and the treatment*day interaction were both significant. However, the effects of treatments on soil N_2O emissions were transient over time (Figure S4.4). After 9 days, N_2O flux rates were below the detection limit for over half of the samples, and therefore only the first 9 days of flux measurements following fertilization are discussed here.

Total N_2O emissions during the first 9 days following fertilization are shown in Figure 4.6. Total N_2O emitted ranged from 0.025 to 0.055 μg N_2O-N g^{-1} soil. The effect of treatment on total N_2O emissions was not significant, and no significant differences were observed between biochar treatments and their respective carbonate controls (C0 = control for washed biochars and extracts; C1 = control for CsWu; C2 = control for MwWu). Furthermore, N_2O emissions did not show a consistent trend with increasing carbonate amendment (C1<C2<C0), and no significant differences were observed among carbonate controls. Thus, biochar fractions did not have a lasting effect on N_2O emissions relative to their respective controls beyond initial (≤24 h) impacts. These findings are in agreement with those of Cayuela et al. (2014), who surveyed the biochar N_2O literature and found that studies using ≤1% biochar amendment rates or NH_4NO_3 fertilizer were less likely to report significant reductions in N_2O emissions following

biochar addition compared with studies using higher amendment rates and different fertilizers.

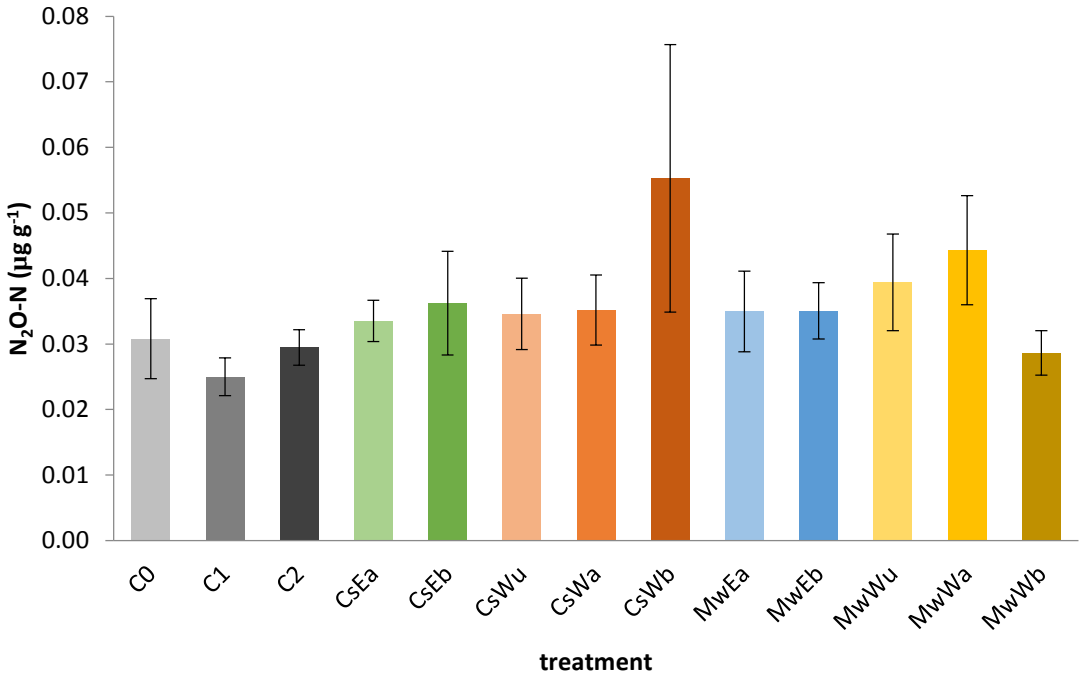

Figure 4.6. Cumulative N_2O-N emissions during the first 9 days following fertilization. Error bars represent standard errors of five replicates *(N_2O emissions were below the detection limit for most samples after 9 days).*

Conclusions

All biochar fractions investigated here contributed to very short-term (24-48h) soil CO_2 emissions. Biochar IC, acid-soluble OC, and acid-insoluble biochar fractions were all shown to promote CO_2 emission immediately following biochar application (≤24 h). We therefore caution against the use of acid-washing biochars as a means to quantify the contribution of IC to CO_2 emissions by difference. The untreated biochar amendments did not have a significant impact on emissions after the 21 days following amendment, suggesting that a 20-30 day equilibration period is sufficient to minimize the impact of labile biochar C when the biochar has not been fractionated. However, the bicarbonate-soluble fraction of both biochars – applied as an aqueous extract – significantly increased CO_2 emissions throughout the incubation (140 days) relative to the controls and untreated biochars, suggesting a positive priming effect. Furthermore, CO_2 emissions from biochar-amended soil were often equivalent to or less than emissions from their respective carbonate-amended controls. Thus the carbonate-C and bicarbonate-extractable organic C were much more labile when extracted and applied separately to soil than when applied together as untreated biochar, suggesting that the labile organic and inorganic fractions were stabilized by interaction with the recalcitrant biochar matrix. Lastly, no effect of biochar or biochar fraction amendment on N_2O emissions was observed; this supports the observation of Cayuela et al (2014) that biochars do not consistently reduce N_2O emissions when applied at rates ≤1% or with NH_4NO_3 fertilizer. More research will be needed to determine if the impacts of acid and

bicarbonate-fractionated biochar observed here apply in other contexts where other biochars, soils, and fertilizers are used.

APPENDIX C. SUPPLEMENTARY INFORMATION FOR CHAPTER 4

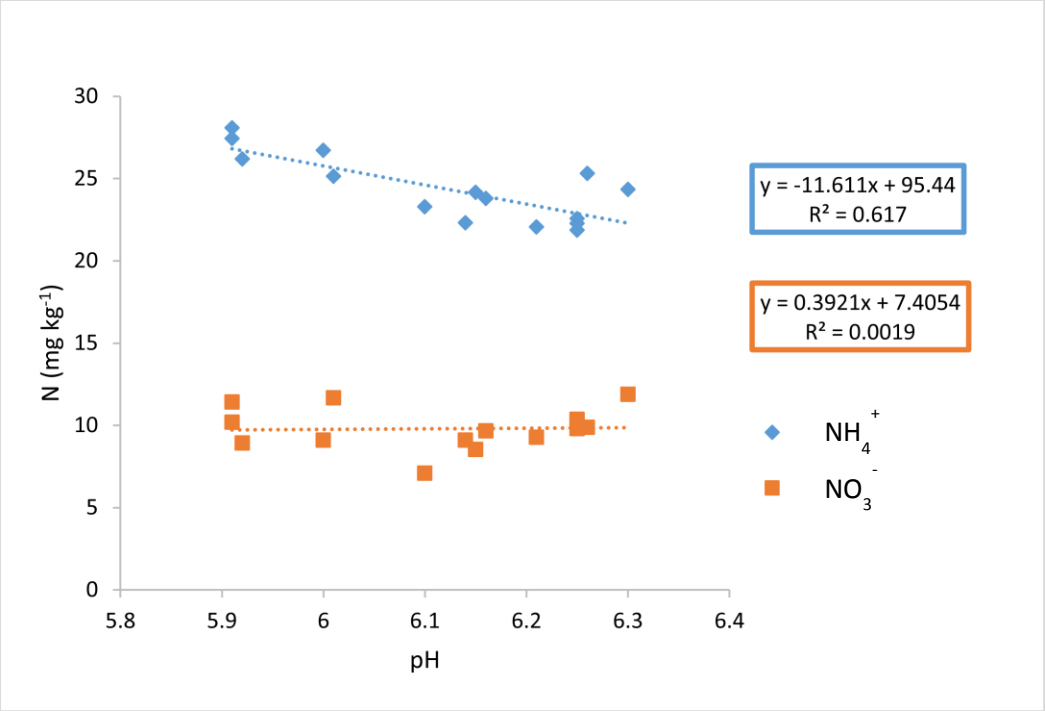

Figure S4.1. Correlations between NH_4^+-N, NO_3^--N and pH among carbonate controls (C0, C1 and C2).

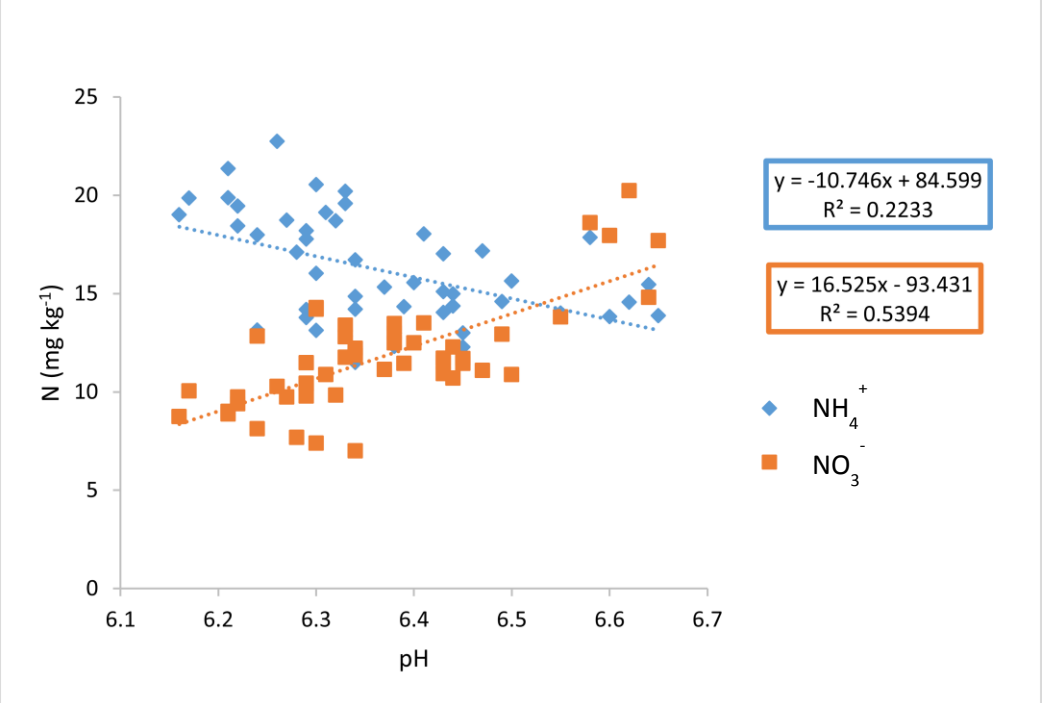

Figure S4.2. Correlations between NH_4^+-N, NO_3^--N and pH among soil samples amended with biochar fractions.

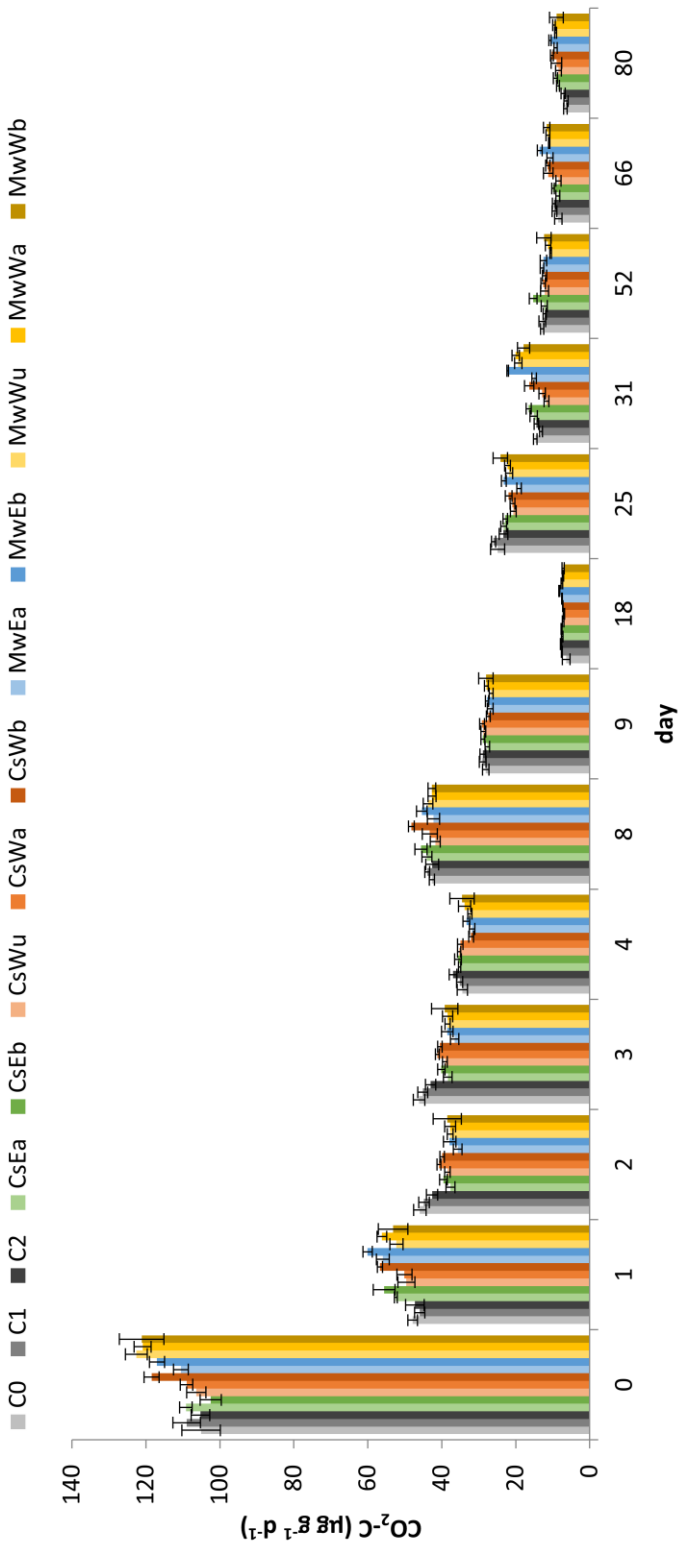

Figure S4.3. Daily CO_2-C emissions during post-fertilization period, in µg of CO_2 per g of soil per day. Error bars represent the standard error of five replicates

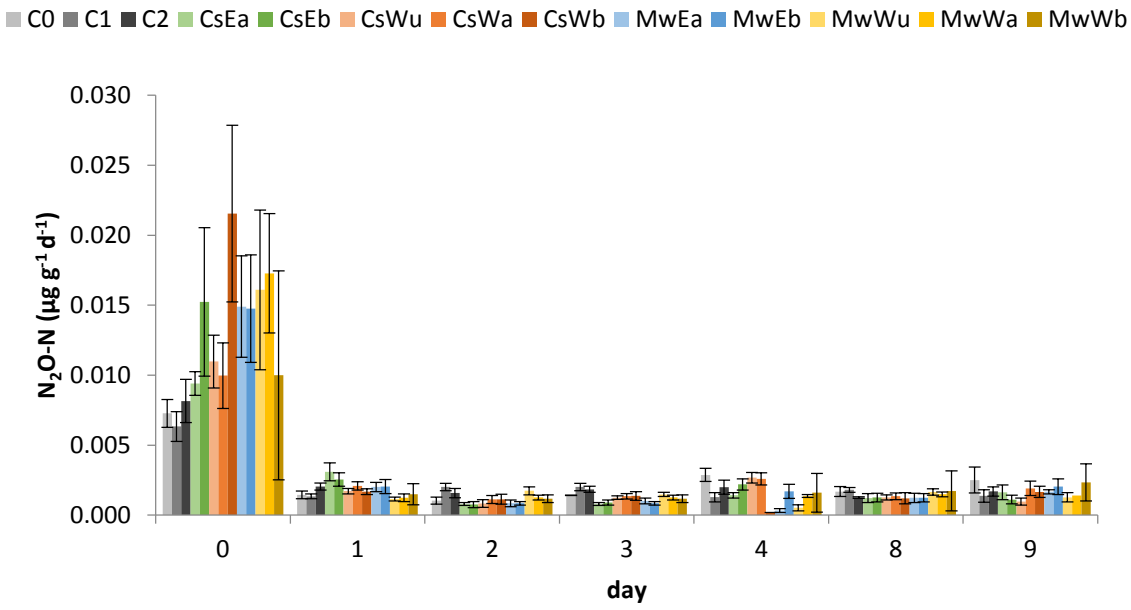

Figure S4.4. Daily N_2O-N emissions during the first 9 days following fertilization. Error bars represent the standard error of five replicates.

Table S4.5. Cumulative C lost as CO_2 during 140 day incubation (± s.e.)

treatment	**Total CO_2-C lost** (mg C g^{-1})
C0	1.99 ± 0.04
C1	1.92 ± 0.02
C2	1.94 ± 0.01
CsEa	1.96 ± 0.07
CsEb	2.34 ± 0.06
CsWu	1.88 ± 0.07
CsWa	1.91 ± 0.05
CsWb	2.02 ± 0.02
MwEa	1.89 ± 0.03
MwEb	2.30 ± 0.03
MwWu	1.99 ± 0.01
MwWa	2.01 ± 0.03
MwWb	1.99 ± 0.03

CHAPTER 5. EFFECT OF BIOCHAR ON SOIL GREENHOUSE GAS EMISSIONS AT THE LABORATORY AND FIELD SCALES

Rivka Fidel, David Laird, and Tim Parkin

A paper to be submitted to *Global Change Biology Bioenergy*

Abstract

Biochar application to soil has been proposed as a means for greenhouse gas emissions mitigation, due to both the removal of CO_2 from the atmosphere through biochar production and soil application and potentially through reduction of soil greenhouse gas emissions. The effects, however, of interactions between biochar, moisture and temperature on soil CO_2 and N_2O emissions, and the applicability of lab scale observation, remain poorly understood. Here we compare the impact of a mixed wood gasification biochar on CO_2 and N_2O emissions under controlled laboratory incubation at three moistures and temperatures, with emissions from field soils under four cropping systems. Biochar reduced N_2O emissions under specific temperatures and moistures in the laboratory and in the continuous corn cropping system in the field. However, the effect of biochar on N_2O emissions was only significant in the field, and no effect on cumulative CO_2 emissions was observed. Results were consistent with previous studies showing that, while biochar can be used to reduce soil N_2O emissions under specific conditions, its efficacy is context-dependent. The disparity in N_2O emission responses at the lab and field scales suggests that laboratory incubation experiments may not always accurately predict the impact of biochar at the field scale.

Introduction

The production and application of biochar – a carbon-rich material produced during the pyrolysis of biomass – to soil has been proposed as a means for mitigating anthropogenic greenhouse gas (GHG) emissions (Lehmann et al. 2006). Emissions mitigation occurs first when atmospheric CO_2 is biologically reduced to biomass carbon (C) and then thermochemically converted into recalcitrant condensed aromatic C, which may be sequestered for long periods of time by soil application. Emissions of GHG may be further mitigated if soil biochar applications enhance crop biomass production, or reduce soil GHG emissions. The first mitigation pathway has been well documented, and studies show that – when produced under appropriate conditions – biochar conserves 10-50% of biomass C and persists in soil for hundreds to thousands of years (Lehmann et al. 2006; Lehmann 2007; Laird 2008; Roberts et al. 2010; Kauffman et al. 2014). The second mitigation pathway is less well-studied, but under most circumstances biochar either increases or has no impact on crop biomass produced, and on average tends to increase crop yields (Woolf et al. 2010; Biederman and Harpole 2013). The third potential pathway – for soil biochar applications to reduce GHG emissions by suppressing the release of CO_2 and N_2O from soils – remains the most uncertain, due to the complex soil biochemical processes and in part due to the myriad challenges inherent to measuring soil GHG emissions. Yet understanding the impact of biochar on soil microbial processes and GHG emissions is of critical importance for modeling climate impacts of biochar (Woolf et al. 2010; Cayuela et al. 2013b).

Consensus as to how biochar affects soil N_2O and CO_2 emissions in the literature is lacking, with some biochar-soil combinations resulting in increased emissions and others resulting in decreased emissions (Spokas and Reicosky 2009; Cayuela et al. 2013a; Cayuela et al. 2013b). Biochar's impact on soil GHG emissions has been shown to depend on both biochar and soil properties, but the mechanisms of interaction and the effects of soil moisture, temperature, and cropping system on emissions remain unclear. Short-term CO_2 emissions (< 1 yr) have been shown to be influenced by both inorganic C and labile organic C in biochar (Cross and Sohi 2011; Jones et al. 2011), whereas there is a lack of consensus regarding long-term (>2 yr) impacts on CO_2 emissions (Smith et al. 2010; Zimmerman et al. 2011; Farrell et al. 2013; Bruun et al. 2014; Fang et al. 2014; Lu et al. 2014; Lin et al. 2015). Most studies reporting decreased CO_2 emissions, increased microbial C use efficiency, or increased stabilization of C inputs after ≥30 days following biochar application were conducted at the laboratory scale and did not continue observations for longer than one year (Smith et al. 2010; Keith et al. 2011; Watzinger et al. 2014; Whitman et al. 2014). A meta-analysis of biochar's effect on N_2O emissions, which included both long and short-term studies, revealed that biochar feedstock, pyrolysis conditions, biochar C:N and H:C ratios, biochar application rate, soil pH, and soil texture all influence soil N_2O emissions (Cayuela et al. 2013b; Cayuela et al. 2015). The impact of soil texture was additionally shown to be moisture-dependent: under low moisture conditions (<80% WFPS), biochar reduced N_2O emissions from coarse to medium-textured soils, while under high moisture conditions (>80% WFPS) biochar reduced emissions from medium to fine-textured soils. The effects of temperature and

cropping system were not addressed, likely due to the lack of studies addressing these issues (Cayuela et al. 2013b). Overall biochar was shown to reduce N_2O emissions on by 54 ±3% at the lab scale, and 28 ±16% at the field scale (Cayuela et al. 2015). The higher variability of the field scale estimate reflects the wide distribution of climates and cropping systems encompassed in the sixteen field-scale studies incorporated in the meta-analysis, while the lower magnitude reflects the lower biochar application rates used in the field. Of these studies, ten reported significant reductions in N_2O emissions at the field scale, five reported no significant impact, and one reported a significant increase. Furthermore, all but two of the cited studies reporting significant changes in N_2O emissions measured ≤12 months after biochar application, and all studies focused on a single cropping system (Zhang et al., 2010, 2012, 2013; Scheer et al., 2011; Liu et al., 2012; Bian et al., 2014; Felber et al., 2014; Pandey et al., 2014; Shen et al., 2014; Van Zwieten et al., 2014; Case et al., 2014). Recent evidence suggests that biochar's impact on N_2O emissions may change over time as biochar ages, but these findings have not yet been verified at the field scale (Spokas 2013). Thus, there is a clear need for more studies incorporating the effects of biochar aging, moisture, temperature and cropping system on biochar CO_2 and N_2O emission impacts at the field and laboratory scales.

Here we analyze the impact of a mixed wood gasification biochar on soil CO_2 and N_2O emissions in (1) a controlled laboratory incubation at three moistures and temperatures, and (2) a field study under four cropping systems. We hypothesize that (1) biochar's impact on CO_2 and N_2O emissions will depend on soil moisture, temperature and cropping system, (2) biochar suppression of N_2O emissions will be

greater in cropping systems with higher average N_2O emissions, and (3) biochar amendment will have a similar effect on N_2O emissions at the laboratory and field scales.

Methods

Biochar and field study site

The biochar in this study is the same mixed wood biochar as was used in three previous studies (see Ch. 1-3). Briefly, the biochar was produced by ICM, Inc. from a mixture of hardwood and soft wood feedstocks via gasification in an auger bed system at 550-650°C. The biochar had a pH of 8.8 and was comprised of 54% fixed carbon, 16% volatile matter, and 28% ash, with molar H:C and C:N ratios of 0.35 and 151, respectively.

The field site used for this study was located at the Iowa State University Armstrong Research Farm in southwest Iowa. The soils at this site were loess-derived Mollisols (Ackmore-Colo-Judson, Clarinda, Exira, and Marshall), and varied widely with respect to drainage class and slope. Biochar was applied in the fall of 2011, and four cropping systems were established in 50 x 68 m plots the spring of 2012: continuous corn (CC), switchgrass (SG), low-diversity grass mix (LD), and high-diversity grass and forb mix (HD). The cropping systems were chosen to compare a variety of both grain-based and cellulosic feedstocks for use in bioenergy systems. Plots were arranged in a completely randomized split plot design with four replicates for each cropping system. A split plot designed was used wherein half of each plot receiving no biochar (control)

and the other half receiving 9.3 Mg ha^{-1} (dry weight equivalent) biochar amendment of moist (~50% water by mass) biochar incorporated to a depth of 15 cm. Since the incorporation of the biochar in 2011, the plots have been in perennial biomass crops or managed for continuous no-till maize production. Because 2012 was a drought year, the switchgrass plots were not properly established, and needed to be re-planted in the spring of 2013. Thus the 2014 growing season can be considered two years after the establishment of the grass mixes and one year after the establishment of SG. Fertilizer was applied to corn plots (CC) at 224 kg N ha^{-1} as urea ammonium nitrate (knife application) on May 4th and to grass plots (SG and LD) at 56 kg N ha^{-1} as urea (broadcasted application) on May 2nd. Corn was planted on May 8th.

Incubation study

Soil was incubated with and without biochar at three soil moistures and temperatures for a total of 140 days in a full factorial design with five replicates. Soil for the incubation study was collected from the top 5 cm of the control (biochar-unamended) portion of a continuous corn (CC) plot at the Armstrong study site (Exira soil with 15% sand, 80% silt and 5% clay) prior to fertilization in the spring, and frozen for 6 months. Prior to use, soil was thawed and sieved to <4 mm. Gravimetric moisture content was determined by oven-drying (10 g for 2 hrs at 105^{o}C), biochar was amended at a 0.5% (wt/wt) rate, and all samples were stirred thoroughly. Moisture content of control and biochar-amended soil at -1, -1/3 and -1/10 bar pressure was determined using a pressure plate prior to the initiation of the study, and it was determined that the

biochar did not significantly affect soil moisture at these matric potentials. For the incubations, 10 g (dry weight equivalent) of sieved (<0.4 mm) field-moist soil was weighed into 150 mL glass serum vials. Control and biochar-amended samples were pre-equilibrated for 60 days, and during the first two weeks of this period soil moistures were gradually adjusted to the target -1, -1/3 and -1/10 bar matric potential valued (equivalent to 27, 31 and 35% wt/wt moisture). The moisture adjusted samples were incubated at 10, 20 and 30°C in a full factorial design with 5 replications. CO_2 and N_2O emissions were quantified on days 0, 2, 6, 9, 20, 23, 30, 36, and 56. On day 60 of the pre-equilibration period, corn stover was mixed in at 0.5% (wt/wt), and fertilizer was added as NH_4NO_3 and K_2HPO_4 at a rate equivalent to 72, 42 and 54 mg/kg of N, P and K, respectively. Soils were incubated for an additional 80 days following fertilization, during which time emissions were quantified on days 0, 1, 2, 3, 7, 10, 16, 38, 52, 62 and 80. For each gas flux measurement, serum vials were sealed with a crimp cap with a butyl septa, and 11.5 mL gas samples were collected via syringe three times over the course of 16 to 48 hours, with longer gas accumulation times used when flux rates were low. Gas samples were stored in helium-flushed and evacuated airtight 6 mL Exetainer vials, and then analyzed for CO_2 and N_2O using a gas chromatograph equipped with a Methanizer (SRI Instruments) flame ionization detector and an electron capture detector. Concentrations were measured by volume and converted to mass units using the ideal gas law. Following termination of the incubation, soil samples were oven dried and analyzed for total C and total N using a combustion analyzer (Vario Microcube, Elementar).

Field study

Emission rates of CO_2 and N_2O from soil under four cropping systems with and without biochar were quantified during the 2014 growing season at the Armstrong field site. Prior to fertilization and corn planting, two stainless steel pans (49 x 29 cm) were installed in each split plot (4 per plot). Pans within each split plot were 17 m apart and 13.2 m from the boundary between split plots. Pans in CC plots were removed prior to fertilization and planting and re-installed afterwards; care was taken to avoid installing pans in disturbed soil. Following fertilizer application, all pans in CC plots were placed along the fertilized band, such that the longer edge of each pan was parallel to the strip of fertilized soil located within the pan. To minimize the effect of root respiration and root exudates, soil within each pan and in a 50 cm radius around each pan was kept free of plants using a combination of gentle hand-weeding and hand-spraying with both pre- and post-emergent herbicides. Care was taken not to disturb soil within the pans. Following pan installation, greenhouse gas emissions were measured regularly from April 21st to September 16th (days 0, 14, 22, 29, 38, 45, 50, 60, 72, 79, 86, 93, 109, 120, and 148), with more frequent measurements during periods of expected high emission rates. Gas sampling dates were chosen so as to capture post-rainfall fluxes while avoiding saturated soil conditions. Plots – arranged spatially at random – were organized into four temporal blocks, with one plot of each cropping system in each block, and gas samples from every plot within a block were taken within one hour of each other to minimize diurnal temperature variability within each block. Soil moisture (% by volume) and temperature (°C) were measured at each pan location at 5 cm depth

concurrently with gas sampling. To quantify emission rates, pans were covered with an insulated pan lid and clamped down to form an airtight seal, then gas samples were collected at four times with a syringe through a grey butyl septa installed in the lid of the pan, and average gas accumulation time was 30-90 minutes (longer times were used on days with lower expected emission rates). Gas samples were stored and analyzed in the same manner as in the incubation study (see above). At the conclusion of the field study, soil was destructively harvested from within the GHG sampling pans and analyzed for total C and N (Vario Microcube).

Calculations and statistical analyses

Gas fluxes were calculated from the slope of the linear increase in gas concentrations over time, and any slopes with r^2 <0.5 were assumed to be zero (Iqbal et al 2013). Cumulative emissions were calculated by interpolating linearly between daily fluxes ("trapezoidal interpolation"). All statistical analyses were conducted using SAS (v. 9.2). Daily gas fluxes (field and laboratory), soil moistures (field only), and soil temperatures (field only) were compared using repeated measures (ante-dependence and compound symmetry models, as appropriate). For the statistical analysis, plots were divided into four blocks based on in-field sampling times to reduce variance due to diurnal temperature fluctuations. Accumulated gas fluxes measured over the entire season or incubation period were compared using ANOVA. Significance was evaluated at p = 0.05.

Results

Soil laboratory incubation

Emission rates of CO_2 during the 60 day pre-equilibration period varied over time and did not consistently correspond to biochar and moisture treatments, but CO_2 emissions did exhibit a significant increase with increasing temperature. The majority of soil sample N_2O emissions were not significantly different from zero during the pre-equilibration period (Figure S5.1).

Over the course of the 80 day post-fertilization incubation, daily CO_2 emissions increased significantly with increasing temperature (10°C < 20°C < 30°C), and this effect was consistent over time and among different moisture and biochar treatments (Table 5.1, Figure 5.1). By contrast, the effects of soil moisture treatments on CO_2 emissions were variable: on some days emissions increased with moisture and on other days they decreased, and the response to moisture was inconsistent across temperature and biochar treatments. The lack of a consistent moisture response may have been in part due to the small difference in percent moisture (8%) between the highest and lowest moisture treatments. In spite of the variability in daily emissions, both temperature and moisture had positive effects on cumulative CO_2 emissions, and the temperature*moisture interaction was significant. Biochar and interactions thereof, however, had no significant effect on cumulative CO_2 emissions. These data support previous research showing that long-term soil CO_2 emissions are often unaffected by biochar amendments (Spokas and Reicosky 2009; Jones et al. 2011; Thomazini et al. 2015).

Nitrous oxide emissions were significantly greater than zero during the first 10 days of the incubation after fertilization; however after day 10, N_2O emissions fell below the pre-fertilization emission rates. Hence, only emissions during the first 10 days following fertilization are presented in Figure 5.2 and considered in the statistical analysis of N_2O emissions. Temperature, moisture, and day significantly affected daily N_2O emissions following fertilization, but biochar amendment did not have a significant impact. Similar to daily emissions, the main effects of temperature and moisture as well

as the temperature*moisture interaction on N_2O accumulated over the first 10 days were all significant, and the main effect of biochar was not significant ($p > 0.05$; Table 5.1). The biochar*temperature*moisture, biochar*temperature, and biochar*moisture interactions were also non-significant. Although the effect wasn't significant, biochar amendment did reduce total N_2O emissions by 50% at 20°C and 31% moisture, a result consistent with a previous study using the same biochar and soil (see Ch. 3). Thus, both moisture and temperature significantly affected CO_2 and N_2O emissions; overall results suggest that biochar amendment presents a minimum potential for increasing CO_2 and N_2O emissions, and may reduce N_2O emissions under specific contexts including moderate temperatures and moistures.

Table 5.1. Cumulative total CO_2 and N_2O emissions from biochar-amended and control soil measured over 80 days and 10 days, respectively, following fertilization during laboratory incubation (± se). No significant effects of biochar amendment were observed.

Temperature	**Moisture (%)**	**CO_2** (mg CO_2 g^{-1})		**N_2O** (µg N_2O g^{-1})	
		control	biochar	control	biochar
10°C	**27**	2.33 ±0.05	2.1 ±0.1	0.043 ±0.003	0.047 ±0.002
	31	2.21 ±0.09	2.4 ±0.1	0.041 ±0.006	0.029 ±0.006
	35	2.6 ±0.1	2.9 ±0.1	0.1 ±0.05	0.10 ±0.07
20°C	**27**	5.7 ±0.4	5.4 ±0.4	0.15 ±0.02	0.09 ±0.01
	31	5.8 ±0.5	6.2 ±0.5	0.34 ±0.1	0.15 ±0.02
	35	5.9 ±0.2	6.6 ±0.6	3.2 ±0.4	4.3 ±0.9
30°C	**27**	8.0 ±0.5	7.9 ±0.4	0.4 ±0.05	0.4 ±0.07
	31	8.4 ±0.5	8.2 ±0.6	1.6 ±0.5	1.6 ±0.5
	35	7.5 ±0.1	7.2 ±0.3	7 ±2	10 ±2

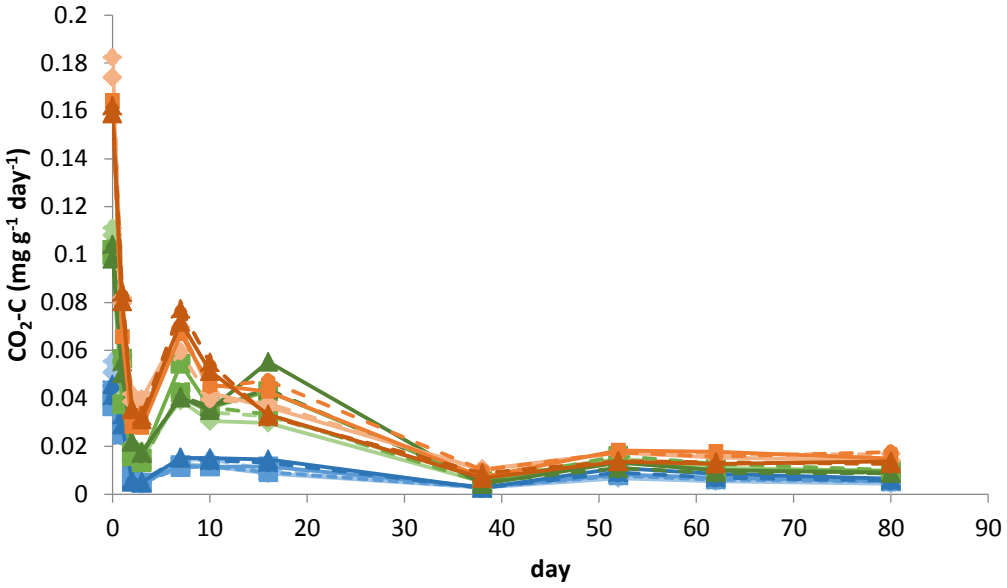

Figure 5.1. Daily CO_2-C emissions, in mg of CO_2-C per gram of soil per day (5 replicates) (blue = 10°C, green = 20°C, orange = 30°C; diamonds = 27%, squares = 31%, and triangles = 35% moisture; dashes = controls, solid lines = biochar)

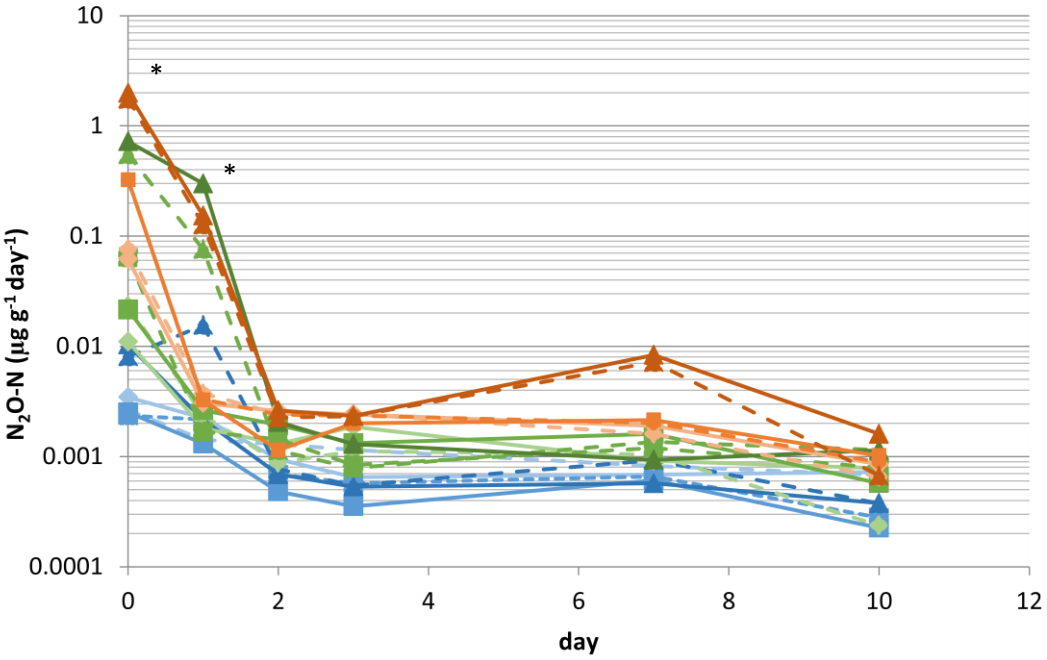

Figure 5.2. Daily N_2O-N emissions during the first 10 days following fertilization, in µg of N_2O-N per gram of soil per day *(emissions below detection limit after day 10)* (5 replicates)
(blue = 10°C, green = 20°C, orange = 30°C; diamonds = 27%, squares = 31%, and triangles = 35% moisture; dashes = controls, solid lines = biochar)
*interaction of biochar*moisture*temperature*day significant ($p < 0.05$)

Field study: total C and N, precipitation, temperature and moisture

Average total C and N of soil collected from within the GHG sampling pans (5 cm depth) are shown in Table 5.2. Biochar consistently increased total C but did not affect total N. Cropping system also had a minimal impact on total C and N, although the soil from switchgrass (SG) plots did have slightly higher total C than soil from continuous corn (CC) plots.

Daily rainfall data are presented in Figure 5.3. Total rainfall for the study period was 726 mm. Total rainfall from April through September was 949 mm, higher than the 624 mm 30-year average rainfall of the field site for this period. Major rainfall events (>40 mm) occurred on days 21, 44, 129 and 142.

Daily soil temperature data (5 cm depth) are presented in Figure 5.4. The main effects of crop, day, and block (representing plot location and sampling time group) on daily soil temperature were all significant, but the effect of biochar was not significant. Average soil temperatures were highest in the high diversity (HD) plots, followed by low diversity (LD), SG and CC. The differences in temperatures among cropping systems may be attributed to differences in canopy cover. Grass plots had less canopy cover because grasses were slower growing than corn, and the LD and HD plots contained weedy areas with less canopy cover near the gas sampling pans. In addition, the HD plots were mowed on day 120 to minimize weed proliferation, which further decreased canopy

cover. By contrast, the average difference in temperature between the biochar-amended and unamended soils was negligible (<0.1°C).

Daily soil moisture data (5 cm depth) are presented in Figure 5.5. The main effects of crop, biochar, day, and block on moisture were all significant ($p < 0.05$). The block*biochar, block*crop and block*day interactions were significant, but the block*crop*char*day interaction was not significant. The crop*biochar*day interaction was significant on eight out of 15 measurement days. Soil moisture tended to be highest in SG plots and lowest in HD plots, but the effect of cropping system was somewhat variable over time. Differences in soil moisture between biochar-amended and control soils were generally greater on days with lower average soil moisture, and biochar increased soil moisture for at least three out of four cropping systems on all days with an average soil moisture of ≤30%. The LD plots exhibited the greatest increase in moisture with biochar amendment, followed by SG, CC and HD; when moistures were averaged over all measurement dates, biochar increased average moisture by 1-3% within each cropping system. Thus results show that biochar consistently increased average soil moisture, and the exact magnitude of this effect was dependent on cropping system.

Table 5.2. Total %C and %N (weight basis) of soil within greenhouse gas monitoring pans (±se) (CC = continuous corn, SG = switchgrass, LD = low diversity grass mix, HD = high diversity grass and forb mix)

	%C		%N	
crop	control	biochar	control	biochar
CC	2.5 ± 0.3	2.9 ± 0.2	0.26 ± 0.01	0.26 ± 0.02
SG	2.5 ± 0.1	3.2 ± 0.3	0.26 ± 0.01	0.26 ± 0.01
LD	2.2 ± 0.1	3.4 ± 0.2	0.24 ± 0.01	0.27 ± 0.01
HD	2.9 ± 0.3	3.2 ± 0.3	0.27 ± 0.01	0.27 ± 0.02

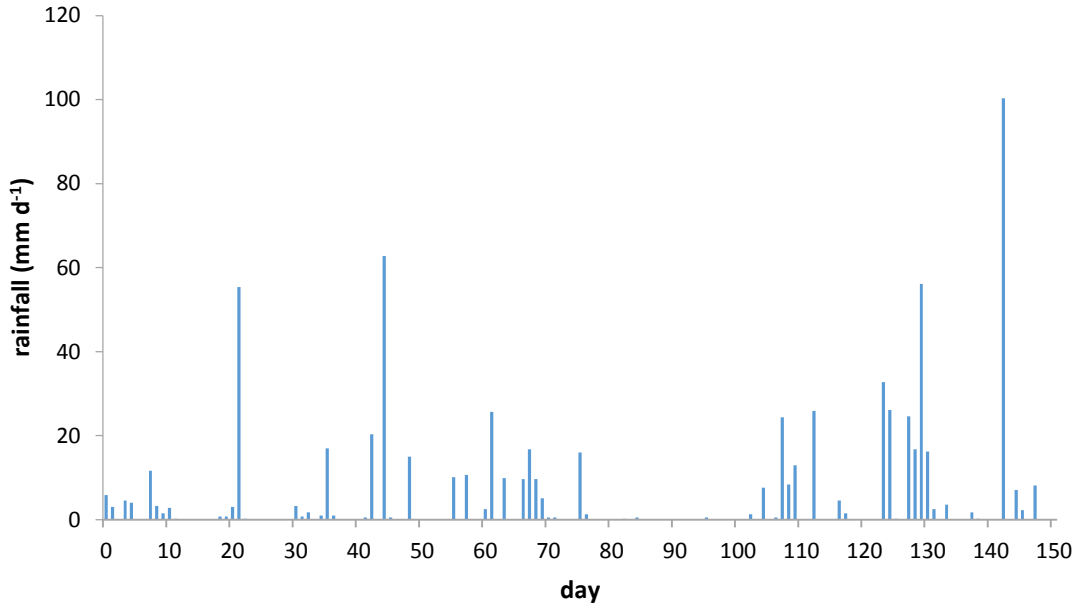

Figure 5.3. Daily precipitation in mm of rainfall per day during the 148 day field experiment (April 21st – September 16th), where the beginning of the experiment (April 21st) is defined as

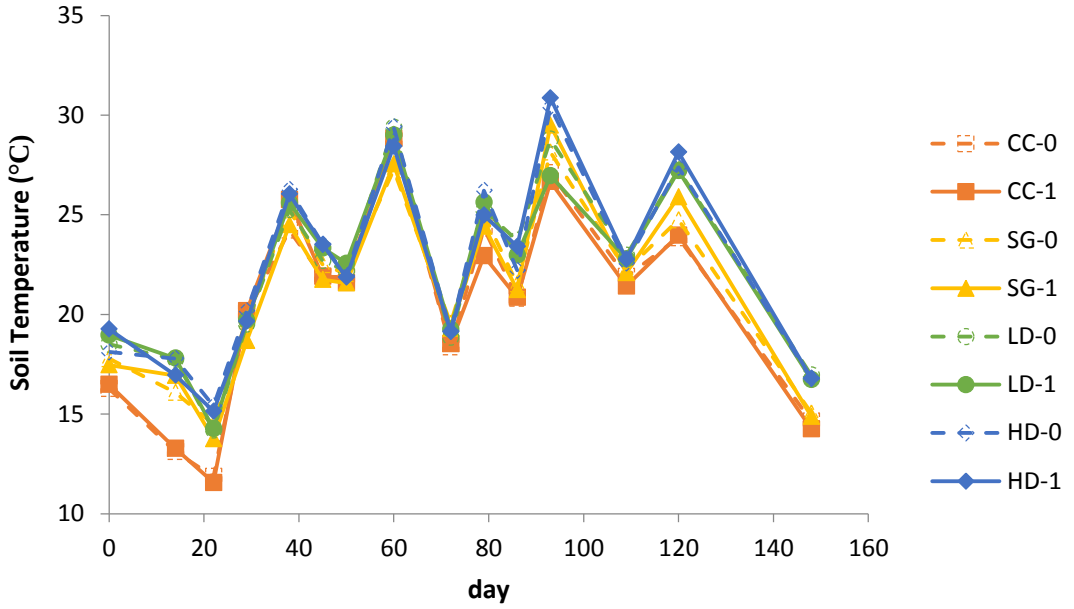

Figure 5.4. Average soil temperatures measured for each cropping system with biochar and without biochar on each day that GHG emissions were measured (CC = continuous corn, SG = switchgrass, LD = low diversity grass mix, HD = high diversity grass and forb mix; 0 = control, 1 = biochar-amended).

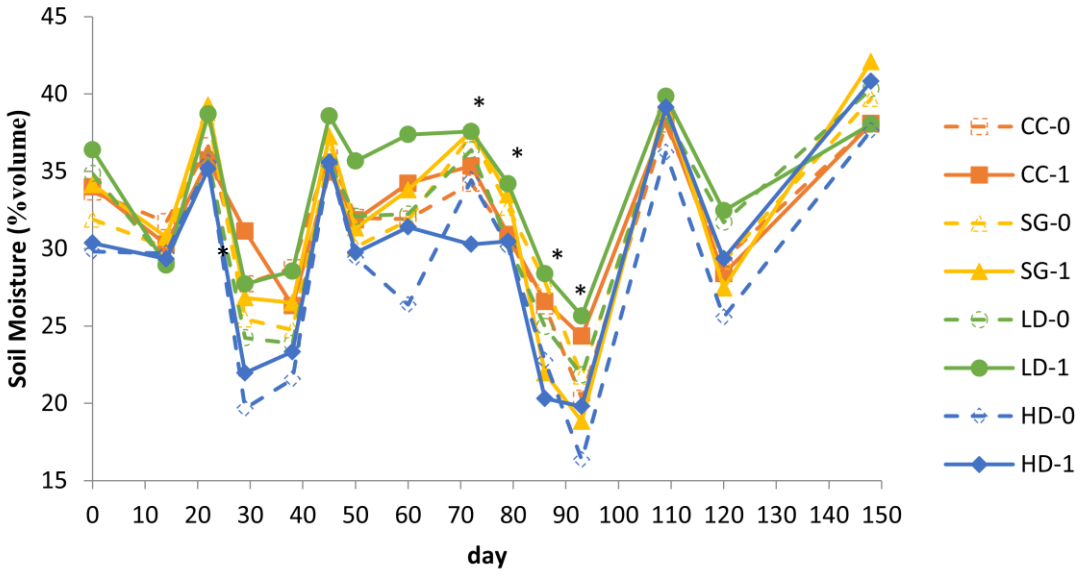

Figure 5.5. Average soil moistures measured from each cropping system, with biochar and without biochar (n=8) on each day that GHG emissions were measured.
*significant effect of biochar within at least one cropping system ($p < 0.05$)

Field study: CO_2 emissions

Daily soil CO_2 emissions were significantly affected by day, crop and block (spatial and diurnal variability), but not by biochar (Figure 5.6). Transient significant differences were observed between biochar amended and control soils within the SG and LD cropping systems, but these effects were inconsistent. Compared with controls, significantly higher CO_2 emissions were measured from biochar-amended soils on some days, and on other days emissions from biochar-amended soils were lower than controls. On average, daily CO_2 emissions increased in the order CC<HD∼LD<SG (Figure 5.6), and this trend was reflected in the cumulative total CO_2 emissions (Figure 5.7).

Similar to daily emissions, the effects of block and crop on cumulative total CO_2 emissions over the 148 day gas monitoring period were significant, but the effect of biochar was not significant. However, the block*biochar interaction was marginally significant (Table 5.4). Differences in CO_2 emissions among cropping systems were likely due to differences in root or biomass density and resultant differences in labile soil organic carbon. Thus, overall, CO_2 emissions were significantly affected by cropping system as well as spatial (block) and temporal (block and day) variables, but not by biochar.

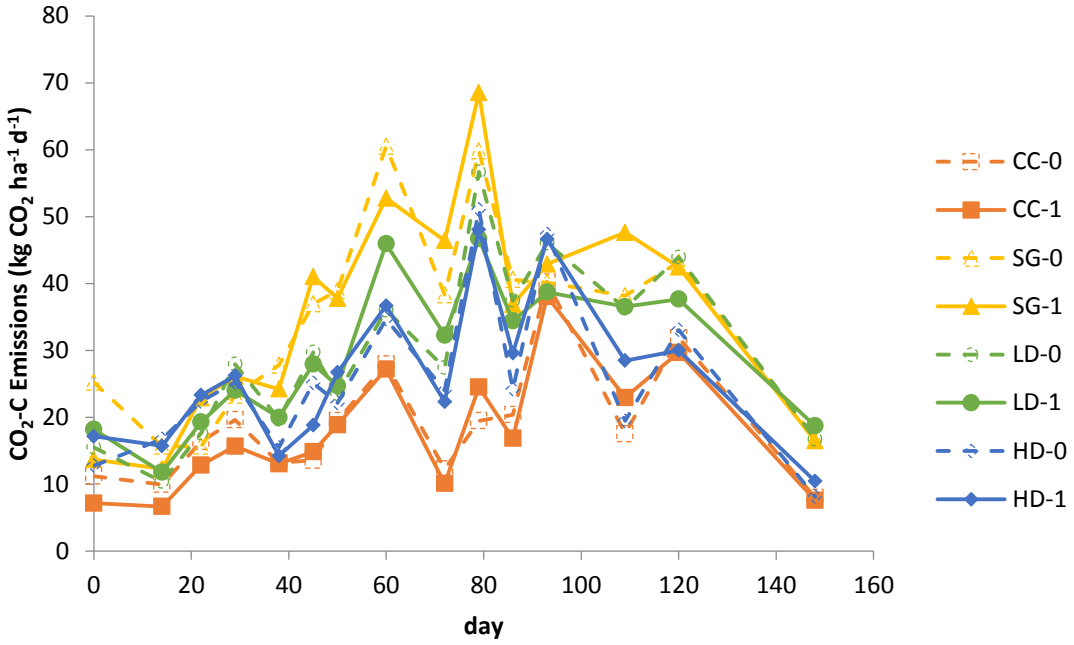

Figure 5.6. Average daily soil CO_2-C emissions measured for each treatment.

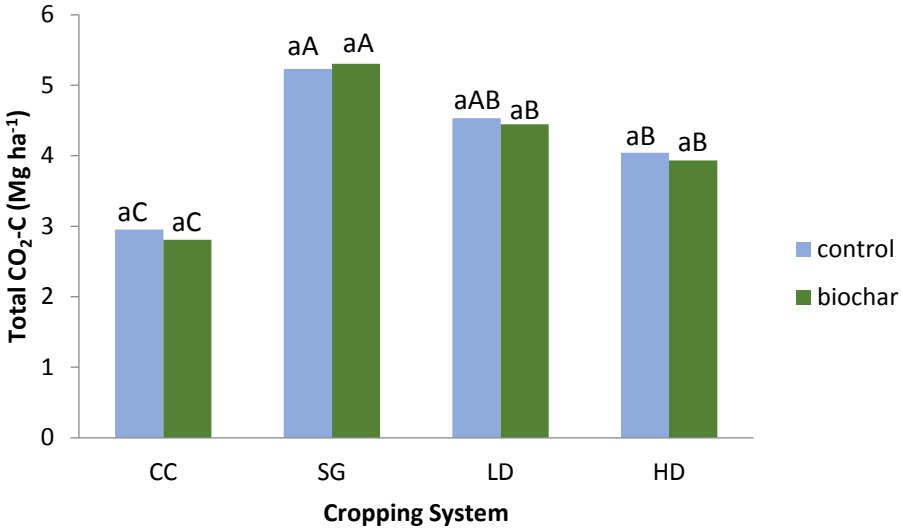

Figure 5.7. Accumulated total soil CO_2-C emissions from the four cropping systems, with and without biochar. Lowercase letters indicate significant differences within each cropping system; uppercase letters indicate significant differences between cropping systems.

Field study: N_2O emissions

Daily soil N_2O emissions were significantly affected by day, crop, and block (spatial and diurnal variability), but not by biochar (Figure 5.8). The block*day*biochar interaction, however, was significant. Emission rates from CC soils were highest directly following fertilizer application (day 22) and decreased rapidly thereafter, whereas emissions from grass system soils were elevated for a longer period (days 38-60). Differences in observed fertilizer response time are likely due to respective differences in fertilizer type (UAN vs. urea) and/or application rate (224 vs. 50 kg N ha^{-1}). On three peak emission days following fertilization, biochar-amended CC soils had significantly lower N_2O emissions (0.04-0.06 kg N_2O ha^{-1}) than control CC soils (0.06-0.1 kg N_2O ha^{-1}). Similarly, biochar-amended SG soils had significantly lower N_2O emissions than control SG soils on days when emissions from SG plots were elevated (0.04-0.06 kg N_2O ha^{-1}). The ~40% suppression of N_2O emissions from biochar-amended CC soils on days of elevated emissions (>0.04 kg N_2O ha^{-1}) often occurred despite higher soil moistures in biochar-amended soils relative to control soils. Daily N_2O emissions also tended to increase in the order HD<LD<SG<CC prior to day 80, but after day 100, SG soils tended to emit more N_2O than CC soils. Overall, biochar amendment consistently suppressed short-term N_2O emissions from CC cropping systems, and less consistently suppressed emissions from SG cropping systems.

With regards to total N_2O emissions accumulated over the 148 day emission monitoring period, the main effects of crop, block and day were significant, but the

main effect of biochar was not significant (Table 5.4, Figure 5.9). Total N_2O emissions increased with increasing biomass production and increasing fertilizer application rate (HD≤LD<SG≤CC). Biochar did significantly reduce N_2O emissions from CC soils by 27%, a reduction of nearly the same magnitude as the field study literature average of 28% reported by Cayuela et al. This suppression of N_2O emissions caused the biochar-amended CC soils to have N_2O emissions of a similar magnitude to SG systems. Thus overall the N_2O results suggest that biochar did not impact N_2O emissions from grass systems studied here, but was effective at reducing emissions from the continuous corn systems.

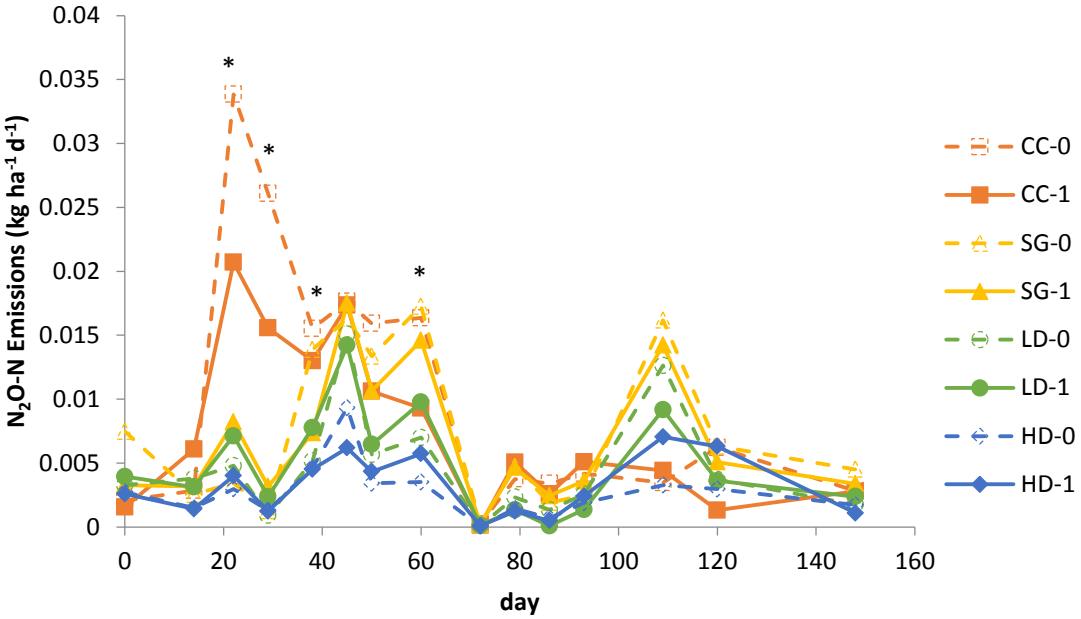

Figure 5.8. Average daily soil N_2O-N emissions measured for each treatment. *significant effect of biochar within at least one cropping system ($p < 0.05$)

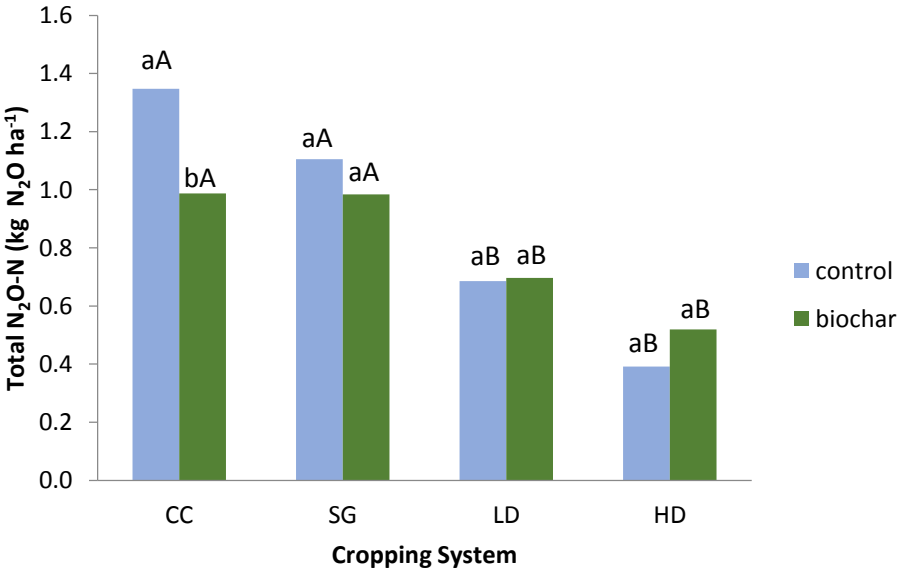

Figure 5.9. Accumulated total soil N_2O-N emissions from the four cropping systems, with and without biochar. Lowercase letters indicate significant differences within each cropping system; uppercase letters indicate significant differences between cropping systems.

Discussion

Among the laboratory-incubated soils and field-scale cropping system soils investigated here, biochar had no significant effect on cumulative soil CO_2 emissions, but did reduce N_2O emissions from a continuous corn cropping system. The lack of effect on soil CO_2 emissions supports previous observations that the impact of biochar on CO_2 emissions is largely restricted to the short term (<1 month) (See Ch. 4). Also consistent with previous laboratory results, biochar reduced N_2O emissions at 20°C with 27% and 31% moisture. Although non-significant, the magnitude of reduction (38-56%) was in agreement with literature values (Cayuela et al. 2015). In the field study, biochar reduced N_2O emissions from soils under continuous corn by an average of 27%, and this magnitude of suppression was remarkably consistent with the reported literature average of 28% (Cayuela et al. 2015).

Despite significant suppression of N_2O emissions in biochar-amended soil at the field scale, the emissions measured at the laboratory scale did not show emission suppression at all temperatures and moistures. It cannot be proven from these data alone exactly why lab-scale and field-scale studies did not produce consistent N_2O emissions results. Apparent differences in results may have arisen from slight differences in study design between the lab and field, such as (a) environmental factors present in the field only, including temperature and moisture fluctuations and biological inputs, (b) differences in fertilizer distribution in the soil between the lab and field, (c) differences in soil properties between the soil stored in the lab for incubation and the field soil, and (d) differences in properties between the fresh biochar added in the

incubation compared with the aged biochar present in the field study. Furthermore, due to the inherent variability of N_2O emissions, the possibility that type II statistical errors obscured significant differences in the incubation study cannot be excluded. Moreover, Cayuela et al found that, in studies using biochar application rates of less than 1% and ammonium nitrate fertilizer, biochar was less likely to significantly suppress N_2O emissions compared with studies using higher biochar application rates; thus a lack of significant N_2O suppression in the laboratory experiment may be attributed in part to either of these factors.

Cumulative cropping system N_2O emissions were, as expected, highest for continuous corn – the system receiving the most fertilizer – and tended to decrease with increasing plant species diversity (HD≤LD<SG≤CC). Grass-based cropping systems tended to have lower N_2O emissions than continuous corn cropping systems, most likely due to the lower fertilizer application rate (56 kg N ha^{-1}) for the grass plots compared with the corn plots (224 kg N ha^{-1}). Lower emissions in the grass mix plots (LD and HD) compared with switchgrass may have been a result of mixed grasses and forbs taking up N over a broader range of the growing season than switchgrass alone. Alternatively, lower labile organic carbon inputs to the soil (due to lower crop biomass production) in the LD and HD grass plots may have decreased the supply of carbon to N_2O-producing microbes (Velthof et al. 2002). Lastly, lower N_2O emissions from grass system soils might have been influenced by lateral movement of inorganic nitrogen from within the gas measurement pans to the roots of plants growing >50 cm away from the pans, but this possibility cannot be confirmed with available data.

Biochar either reduced or did not affect soil N_2O emissions at the field scale in spite of the increased soil moisture of biochar-amended soils. Compared with controls, soil moisture averaged over the whole growing season was consistently 1-3% higher in biochar-amended soils for all cropping systems, and this finding supported previous laboratory and field-scale evidence for biochar increasing soil moisture (Karhu et al. 2011; Novak et al. 2012; Ulyett et al. 2014). Increased soil moisture is known to contribute to elevated soil N_2O emissions (Bateman and Baggs 2005; Castellano et al. 2010), and increased N_2O emissions with increasing soil moisture was also observed in the laboratory incubation conducted here. Biochar may have reduced N_2O emissions in part by reducing the moisture sensitivity – the degree to which emissions increase with increasing moisture – of soil N_2O production. Indeed, Deng et al (2015) observed that biochar amendment reduced the moisture sensitivity of soil N_2O emissions, and posited that reduced moisture sensitivity arises from the synergistic impact of multiple soil properties influenced by biochar. For example, biochar may have decreased soil bulk density and increased the absorption capacity for organic molecules and nutrients, thereby simultaneously increasing oxygen availability and reducing the availability of substrates to nitrate and nitrite-reducing microbes – two conditions that could reduce the sensitivity of N_2O emissions to changes in moisture.

In summation, the results partially supported the hypothesis that biochar would affect CO_2 and N_2O emissions, and fully supported the hypothesis that biochar's suppression of N_2O emissions would be greatest in continuous corn cropping systems; however, the results did not support the hypothesis that laboratory studies could

predict the impact of biochar on field-scale soil N_2O emissions. These findings also support previous observations of reduced moisture sensitivity of N_2O emissions in biochar-amended soil (Deng et al. 2015). More research is needed to determine the mechanism(s) by which biochar reduced N_2O emissions under field and laboratory conditions, and how or if emissions impacts depend on additional contextual factors not examined here.

APPENDIX D. SUPPLEMENTARY INFORMATION FOR CHAPTER 5

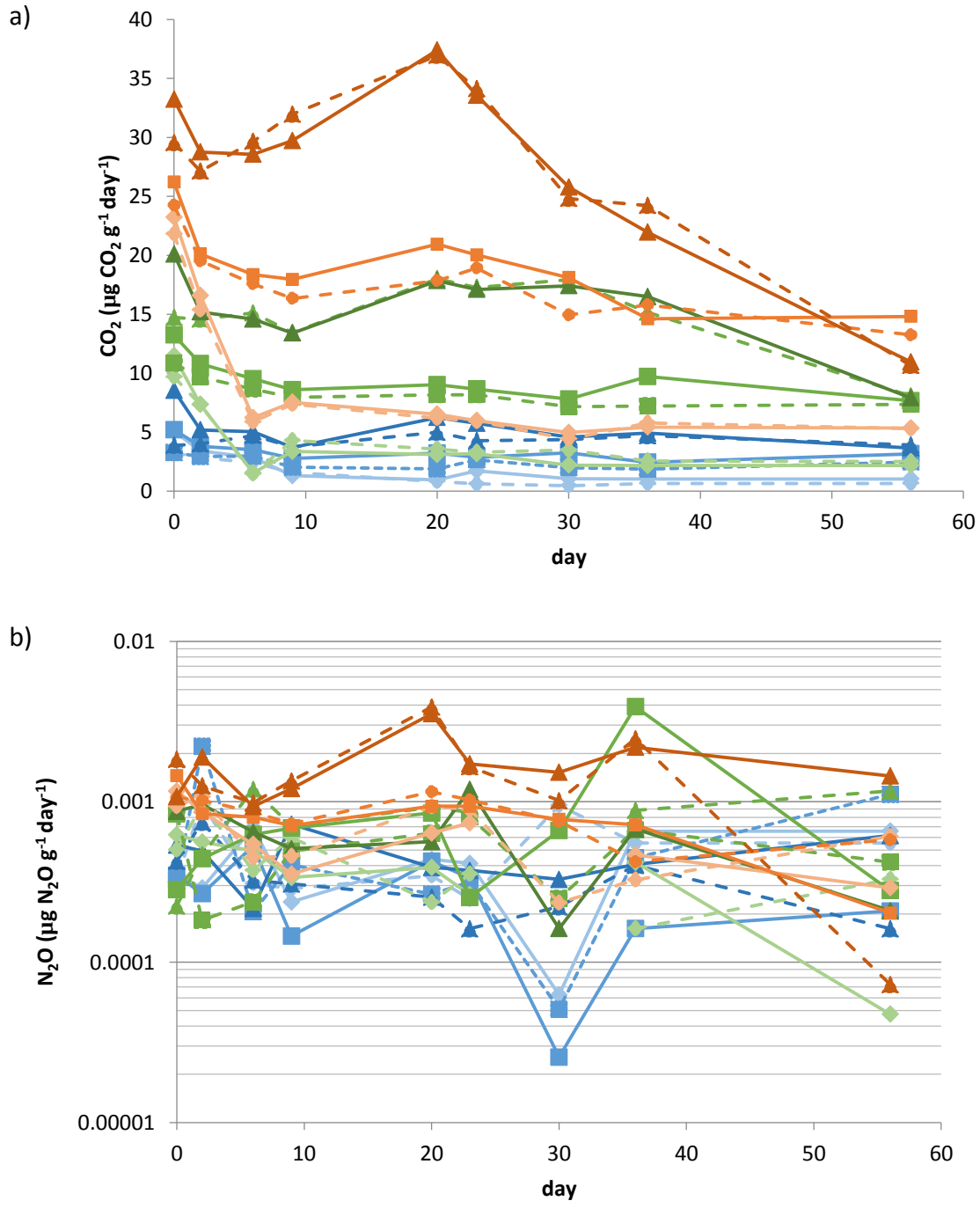

Figure S5.1. Daily soil (a) CO_2 and (b) N_2O emissions measured during the equilibration period of the laboratory incubation study (prior to the addition of fertilizer).
(blue = 10°C, green = 20°C, orange = 30°C; diamonds = 27%, squares = 31%, and triangles = 35% moisture; dashes = controls, solid lines = biochar)

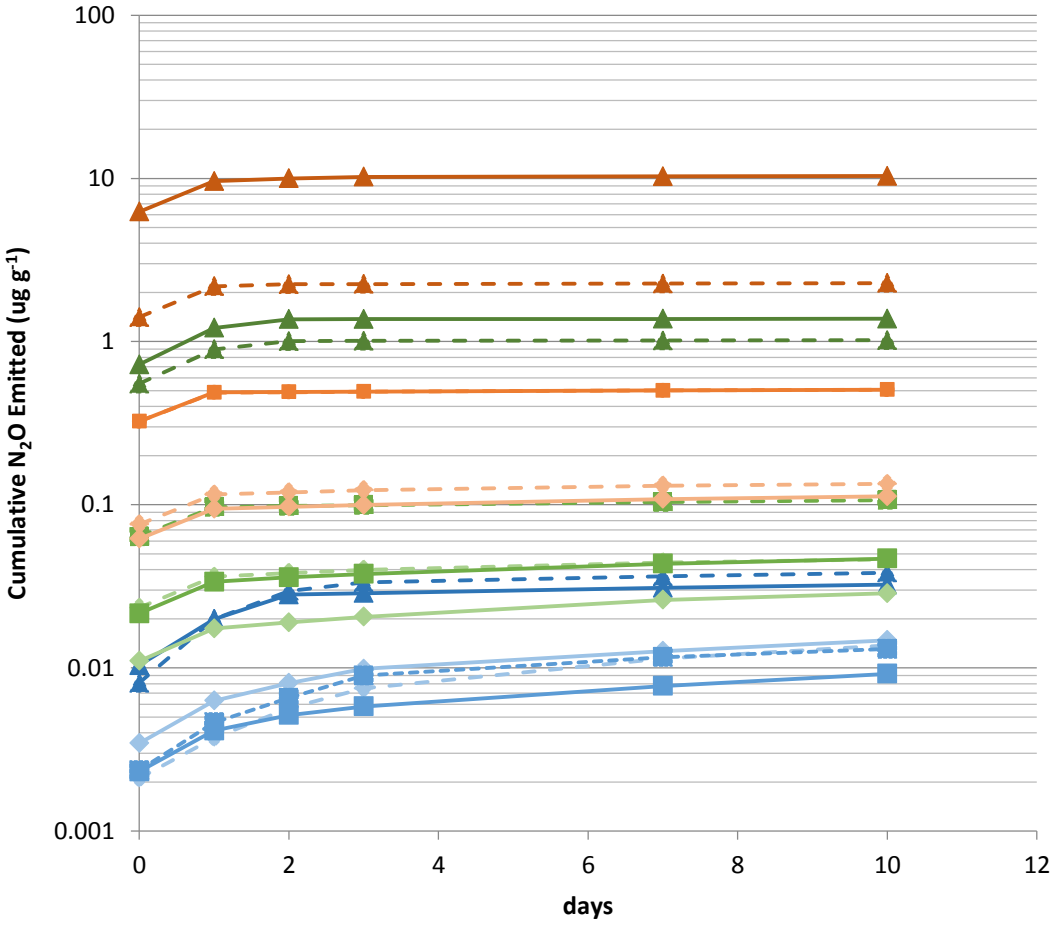

Figure S5.2. Cumulative N_2O emissions measured following the addition of fertilizer during the incubation study. (blue = 10°C, green = 20°C, orange = 30°C; diamonds = 27%, squares = 31%, and triangles = 35% moisture; dashes = controls, solid lines = biochar)

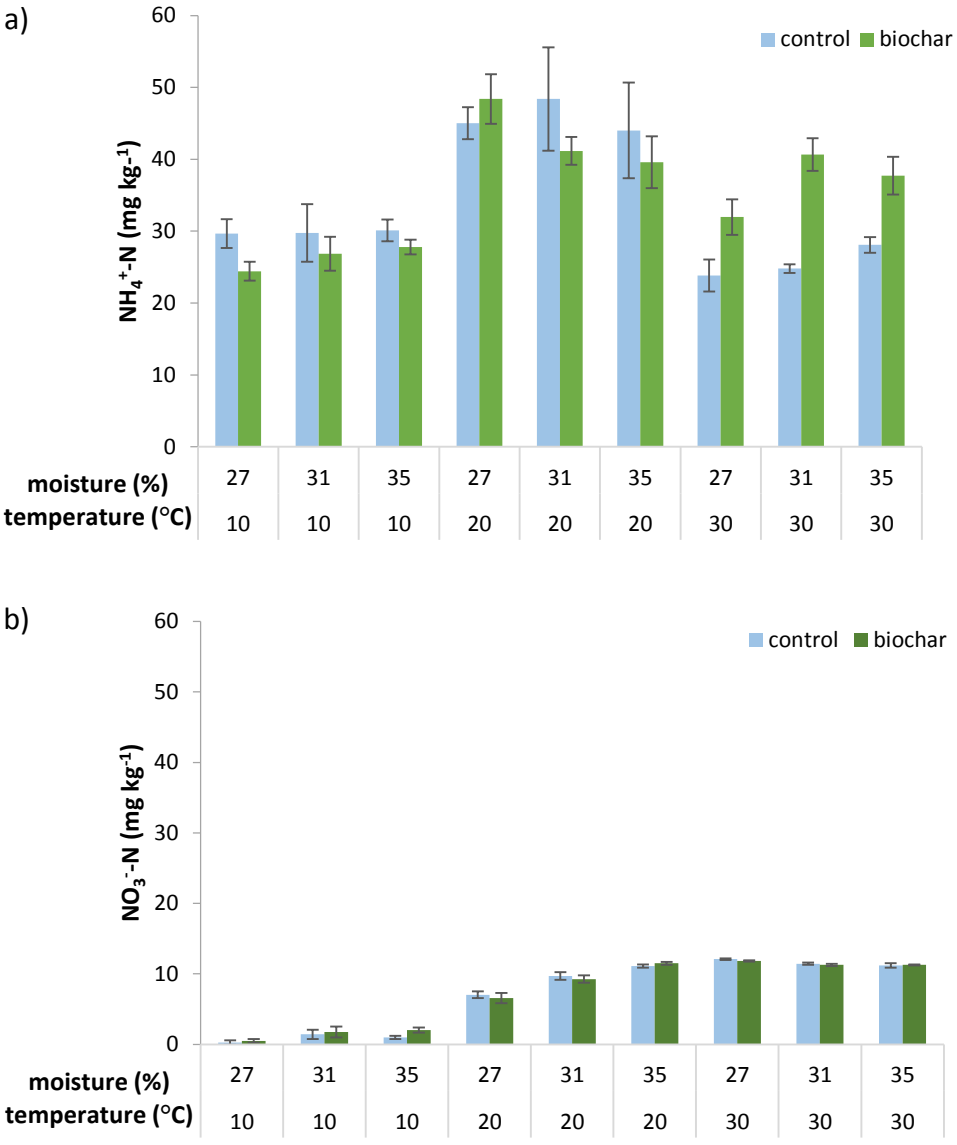

Figure S5.3. Final (a) NH_4^+ and (b) NO_3^- concentrations, in mg of N per kg soil, after 140 day incubation at three temperatures and moistures, with and without biochar.

CHAPTER 6. IMPACT OF BIOCHAR ON CO_2 AND N_2O EMISSIONS: ASSESSING TRADE-OFFS IN THE GREENHOUSE AND FIELD

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A paper to be submitted to *Geoderma*

Abstract

Biochar application to soil has been shown to decrease N leaching and N_2O emissions from soil while increasing soil moisture. However, few studies have examined potential trade-offs among these benefits. Here we examine trade-offs resulting from application of mixed wood biochar to soil on CO_2 and N_2O emissions, soil moisture and N leaching in parallel greenhouse and field studies. In the greenhouse column study, decreased NO_3^- leaching in soil columns amended with fresh biochar corresponded with higher N_2O emissions, and higher soil moisture in both fresh and aged biochar-amended columns corresponded with higher N_2O emissions. Biochar also slightly increased soil moisture and N_2O emissions in the field study, but the effect of biochar on CO_2 and N_2O emissions was not significant. Overall the results suggest that application of biochar to agricultural soils may involve trade-offs among soil water retention, NO_3^- leaching, and N_2O emission. Further research is needed to determine whether these trade-offs are significant at the field scale, under what conditions they occur, and whether there are management options that mitigate any adverse effects.

Introduction

Amendment of soil with biochar has been proposed as a means for improving soil quality while mitigating adverse environmental impacts of agriculture and energy production systems. Potential benefits to soils include increased plant-available water, cation exchange capacity, and soil pH, as well as decreased bulk density and penetration resistance (Joseph et al. 2010; Laird et al. 2010a; Rogovska et al. 2014). Biochar has also been shown to reduce leaching of NH_4^+, NO_3^- and other nutrients and to suppress emissions of CO_2, CH_4 and N_2O from soils. Most research addressing the impact of biochar applications have examined these environmental and agronomic benefits individually or in pairs, with each study conducted under unique conditions, hence little information regarding trade-offs among agronomic and environmental benefits is available. Unfortunately, it may not be possible to simultaneously maximize all benefits, hence understanding potential trade-offs among environmental and agronomic benefits is imperative to successful biochar implementation. To accurately predict trade-offs among biochar benefits in specific contexts, both a mechanistic (i.e. reductionist) and a systems (i.e. holistic) understanding of how these benefits arise is needed (Jeffery et al. 2015).

There is potential for trade-offs to occur among three of the most prominent benefits attributed to biochar applications: (1) decreased soil greenhouse gas (GHG) emissions, (2) decreased nitrogen (as ammonium [NH_4^+] and nitrate [NO_3^-]) leaching, and (3) increased retention of plant-available water (Novotny et al. 2015; Jeffery et al.

2015). Biochar has been shown to decrease soil GHG emissions, but emission rates are sensitive to NH_4^+, NO_3^-, and moisture concentrations in soils. Nitrous oxide (N_2O) emission rates are especially dependent on these variables, and can increase exponentially with increasing soil moisture and NO_3^- concentrations in unsaturated soils (Bateman and Baggs 2005; Castellano et al. 2010). Thus biochars that are more effective at decreasing nitrogen leaching or increasing soil moisture might be less effective at reducing N_2O emissions, or even cause an increase in N_2O emissions. Conversely, biochars that are effective at reducing GHG emissions might not be as effective at decreasing nitrogen leaching or increasing soil water retention. Studies examining all three of these variables concurrently are relatively few in number (Rogovska et al. 2011; Angst et al. 2013), and do not address trade-offs between benefits. Moreover, studies examining all three variables have done so using fresh biochar not previously exposed to soil, and evidence suggests that weathering of biochar in the field may influence biochar's impact on GHG emissions (Spokas 2013). By contrast, a plethora of studies have examined the impact of fresh biochars on soil GHG emissions (Spokas and Reicosky 2009; Taghizadeh-Toosi et al. 2011; Augustenborg et al. 2012; Wang et al. 2012; Zheng et al. 2012; Cayuela et al. 2013a; Suddick and Six 2013; Bruun et al. 2014; Case et al. 2014; Wells and Baggs 2014; Angst et al. 2014), on nitrogen leaching (Ding et al. 2010; Laird et al. 2010b; Zheng et al. 2012; Hollister et al. 2013; Sika and Hardie 2014), and on soil moisture (Laird et al. 2010a; Basso et al. 2013; Abel et al. 2013; Ulyett et al. 2014), clearly demonstrating an effect of biochar on each of these variables. In most studies, N_2O and CH_4 emissions decreased or did not change following biochar amendment,

whereas the reported impact of biochar amendments on CO_2 emissions is variable. However, an increase in emissions of each of the three major GHGs (N_2O, CH_4 or CO_2) following biochar amendments has been reported at least once (Spokas and Reicosky 2009; Cayuela et al. 2013b; Shen et al. 2014). This lack of consensus among studies may be due to the diverse properties of biochars and soils, and context-specific interactions. The impact of biochar on GHG emissions could, for example, be dependent on biochar's impact on soil nitrogen (N) and water retention, and consequently variability among studies may be due to trade-offs among benefits related to water or N cycling. Thus a more thorough investigation into potential trade-offs between reduced GHG emissions and other benefits is needed in order to predict under what circumstances, if any, such trade-offs may occur.

Here we examine the impact of a mixed wood gasification biochar on soil CO_2 and N_2O emissions, on NH_4^+ and NO_3^- leaching, and soil water retention using both greenhouse and field studies, and compare the effects of fresh and aged biochar in the greenhouse column study. We hypothesize that (1) fresh biochar will significantly reduce N_2O emissions, (2) soil amended with aged biochar will have higher N_2O and CO_2 emissions than soil amended with fresh biochar, and (3) biochar effects on leaching of NH_4^+ and NO_3^- or soil moisture concentrations will have subsequent effects on CO_2 and N_2O emissions due to benefit trade-offs.

Methods

Biochar preparation

The biochar used in this study was produced from a mixed wood feedstock at 600°C using an auger gasifier (ICM Inc). This biochar was previously characterized and used in three laboratory incubation studies and a field study (Ch. 1-4). Proximate analysis results showed that the biochar contained 55% fixed carbon, 16% volatile matter and 29% ash. Other metrics of interest include biochar pH (8.8), H:C ratio (0.03), total alkalinity (2.69 meq g^{-1}), and carbonate alkalinity (1.50 meq g^{-1}). In a laboratory incubation study, application of this biochar (0.5% dry wt) reduced N_2O emissions by ~30% from a loess-derived silt loam soil (Exira) and from a glacial-till derived loam soil (Clarion) (see Ch. 1; Fidel et al. *in preparation*).

Field site

The field experiment was located on the Iowa State University Boyd Farm in Boone, IA. The dominant soil on the site is a Clarion loam (fine-loamy, mixed, superactive, Mesic Typic Hapludolls) although there is soil variability due primarily to a history of erosion (3-10% clay and 44-65% sand). Moist biochar (~45% water) had been applied to 18 small plots (23.7 m^2) in an incomplete Latin Square design at 6 rates (0, 19.2, 38.3, 57.5, 76.6, and 95.8 Mg ha^{-1} oven dry weight equivalent) with 3 replications in October, 2010. The biochar was incorporated to 30 cm depth by a combination of rototillage and moldboard plow tillage. Thereafter the site was managed with continuous no-till maize. More details of experimental design and analysis of biochar

impacts on crop yields and soil quality are available in Rogovska et al. (2014). Here our focus is on soil moisture and emissions of CO_2 and N_2O from the plots.

Greenhouse soil microcosm experiment

A six month soil microcosm experiment was conducted in a greenhouse at Iowa State University from July 2013 through January 2014 (Huang et al, *in preparation*). The overall experiment aimed to assess the impact of both aged and fresh biochar on water and nitrogen dynamics in a typical Midwestern soil. Here we focus on the GHG emissions component of the study and how N_2O and CO_2 emissions relate to water, NH_4^+, and NO_3^- leaching and retention. Soil containing no biochar and aged biochar was collected from site of the field study described above prior to fertilizer application in the spring of 2013. For fresh biochar treatments, soil was collected from several locations within an unamended buffer strip between the plots, sieved (<4 mm), mixed thoroughly using a cement mixer, then fresh biochar (the same biochar as used in the field study except that the biochar was sieved <4 mm) was added to subsamples of the composite soil at rates equivalent to those applied in the field plots. For aged biochar treatments, soil was collected from a single row (block) of biochar-amended plots (including a control plot) and sieved (<4 mm). Any biochar particles larger than 4 mm were hand-crushed to pass through the 4 mm sieve with the soil.

To prepare the soil microcosms, PVC columns (7.7 cm id by 25 cm length = 1164 cm^3 volume) were prepared by first placing 100 g of coarse sand (2–5 mm) in the bottom of each column and then 1.2 kg of soil (either unamended, or amended with

fresh or aged biochar) was added and compacted as the column was being filled to achieve a uniform bulk density of 1.3 g cm^{-3}. Plastic Tygon tubing was attached to a drain hole in the bottom of each column using a brass fitting to allow free drainage of water. The microcosms were leached with 200-250 mL of 0.005 M $CaCl_2$ several times, and leachate was collected and analyzed for NH_4^+ and NO_3^- using the steam distillation method. After the first 3 leaching events, 50 mL of fertilizer solution (equivalent to 100 kg N ha^{-1}, 56 kg P_2O_5 ha^{-1}, and 56 kg K_2O ha^{-1}) was applied. Nitrogen fertilizer was applied as either NH_4^+ or NO_3^-. All treatments received equal amounts of P and K fertilizer. Thus the overall experiment included two N fertilizer treatments, two ages of biochar, 6 rates of biochar application, and 6 replications in full factorial design (144 columns total). Two sets of controls were included, one representing the mixed composite soil that was amended with fresh biochar and one from a control plot in the field study.

Gravity drained moisture content was determined by weighing the columns 24 h after each of 3 leaching events and subtracting the tare weight of the column and the oven dry weight of the added sand and soil. Gravimetric water content was converted to volumetric water content using the density of water, the original mass of added soil, and the measured bulk density of soil in each column. Soil moisture was multiplied by particle density and divided by bulk density to calculate water filled pore space (WFPS). Here we relate CO_2 and N_2O emissions to WFPS; effects of biochar on soil moisture retention and bulk density are reported elsewhere (Huang et al. *in preparation*).

Emissions (CO_2 and N_2O) were monitored for four weeks. Columns were leached three times prior to the initiation of gas sampling. The first gas sampling date (day 1) was one week following the third leaching event. The second gas sampling date (day 4) occurred 24 hours following the application of fertilizer, and the soil was leached three additional times over the course of the emission monitoring period (24 hours prior to gas sampling days 8, 16 and 22). To collect gas samples, columns were covered with PVC caps fitted with grey butyl septa, and gas samples were collected from the column headspace after approximately 5, 10 and 15 min. An ambient air sample was used to establish initial concentrations of CO_2 and N_2O. Gas samples were stored in helium-flushed an evacuated airtight 6 mL Exetainer vials and analyzed for CO_2 and N_2O using a gas chromatograph (see Ch. 2; Fidel et al. *in preparation*). Concentrations were measured by volume and converted to mass units using the ideal gas law. Flux rates were calculated using the rate of concentration change over time.

After the leaching events, wheat was planted in the columns and grown for 3 months to assess the effect of biochar on plant-available N. Following wheat harvest, soil columns were divided into 3 depths (0-6 cm, 6-12 cm, and >12 cm), and soil within each depth was homogenized. From each column and depth, 4 g air dry soil samples were extracted with 25 mL of 2 M KCl and analyzed for NH_4^+ and NO_3^- (Hood-Nowtony et al., 2010).

Field experiment

The field experiment was conducted at the same site from which soil samples were collected for the column experiment (see above). Measurement of CO_2 and N_2O emissions was confined to the 0, 10, 20 and 58 Mg ha^{-1} biochar application rates to reduce the amount of time needed to complete a measurement and thereby minimize the effects of diurnal temperature fluctuations (n = 3 plots per treatment). Within each plot, two stainless steel gas sampling pans (49x29 cm) were installed, one in the row and one in the inter-row. Pans in the inter-row were installed ten days prior to the application of fertilizer, whereas pans in the row were installed directly after fertilizer was applied as a sidedress of urea ammonium nitrate (190 kg N ha^{-1}) on June 11^{th} (day 10). Each row pan contained 3 corn plants and 3 fertilizer injection points; inter-row pans did not overlap with fertilizer injection points. Emission rates of CO_2 and N_2O were quantified from the inter-row only on days 0, 4, and 6, and from both the row and inter-row on days 15, 18, 29, 41, 56, 63, 100 and 116. Soil moisture (Delta-T ThetaProbe) and temperature of soil directly adjacent to the pans were measured concurrently with emission rates. When the corn plants became too large to fit under the pan lid, they were severed at the base of the stem and aboveground biomass was removed from the pans. To quantify gas emission rates, pans were covered with an insulated pan lid connected to a photoacoustic gas analyzer and clamped down to form an airtight seal (Iqbal et al. 2013). A minimum of four concentrations were recorded for each pan on each sampling date, over a period of 10-30 min, with longer gas accumulation times for lower flux rates. Concentrations were measured by volume, then converted to mass

units using the ideal gas law, and flux rates were calculated using the rate of concentration change over time (Iqbal et al 2013).

Statistical analyses

All statistical analyses were conducted using SAS (v. 9.2). Daily CO_2 and N_2O emission rates from the greenhouse and field experiments were compared using repeated measures (compound-symmetry, Toeplitz and heterogeneous autoregressive models, as appropriate). Accumulated gas fluxes, soil moisture, NH_4^+ and NO_3^- (in leachate and soil extracts) were compared using ANOVA and subsequent Tukey's test. Significance was evaluated at $p = 0.05$.

Results and Discussion

Soil column experiment

Water-filled pore space

Soil WFPS measured 24 h after a leaching event (Figure 6.1) was positively linearly correlated with fresh biochar application rates ($r^2 = 0.80$). Relative to the WFPS of the control columns, columns receiving 96 Mg ha^{-1} of fresh biochar had a 6% increase, those receiving 77 Mg ha^{-1} had a 5% increase, and those receiving 58 Mg ha^{-1} had a 2% increase in WFPS. Amendment with 19 and 38 Mg ha^{-1} of fresh biochar did not significantly increase WFPS. By contrast, the relationship between WFPS and application rate for aged biochar followed a parabola ($r^2 = 0.94$). Amendment of soil with 19 and 38 Mg ha^{-1} aged biochar increased WFPS by 23% and 35%, respectively, relative to the

control soils. Aged biochar amendment rates of 56 and 77 Mg ha^{-1} resulted in no further increased WFPS, and WFPS for soils receiving the highest application rate of aged biochar (96 Mg ha^{-1}) was only 5% greater than WFPS of the control soils. The results indicate that biochar applications increase water retention and decrease air filled porosity when measured 24 h after a leaching event. Furthermore, the results indicate that aged biochar may have a very different influence on soil water retention, WFPS and aeration than fresh biochar.

Soil amended at the highest biochar application rate (96 Mg ha^{-1}) may have had low WFPS relative to soil receiving less biochar due to spatial heterogeneity in the soil collected for the experiment. Indeed, in a previous study involving the same field plots, surface soil bulk density was shown to decrease and soil moisture was shown to increase consistently with increasing biochar application rate both in the field and under controlled laboratory conditions (-10 and -33 kPa) (Rogovska et al. 2014). The relatively low WFPS for soils given the 96 Mg ha^{-1} aged biochar treatment may also have been a result of unique soil physical phenomenon occurring at very high (≥5% wt) biochar application rates, such as improved soil drainage. The exact mechanism by which the 96 Mg ha^{-1} aged biochar treatment influenced soil moisture, bulk density, and WFPS cannot, however, be determined from these data alone.

Overall the results indicate that both fresh and aged biochar increased gravity drained water content, and aged biochar increased water content more than fresh biochar. These results are in agreement with literature data showing that fresh biochar

amendment increases soil moisture and decreases bulk density (Laird et al. 2010a; Basso et al. 2013; Ulyett et al. 2014), while further indicating that biochar age influences its ability to retain water in soil.

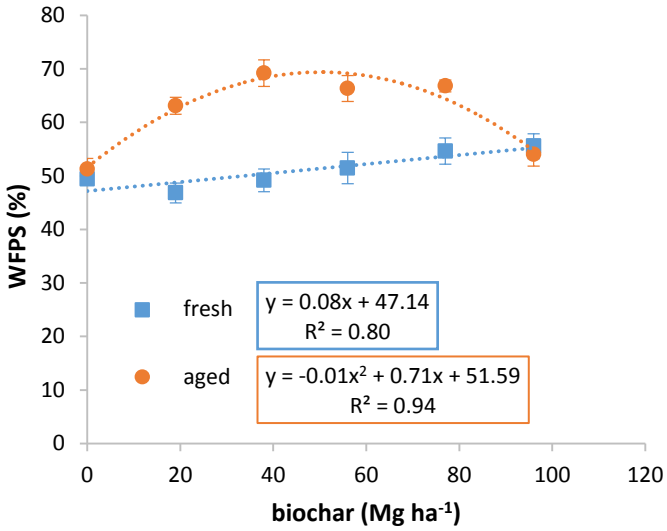


Figure 6.1. Average percent water-filled pore space (WFPS%) averaged over one month greenhouse gas measurement period for soil columns amended with 0-96 Mg ha^{-1} dry weight equivalent of biochar. Error bars indicate standard error of six replicates.

<u>CO_2 emissions for the greenhouse column study</u>

The main effects of fertilizer type (NH_4^+ or NO_3^-) and biochar application rate on daily CO_2 emissions were not significant ($p > 0.05$) (Figures S6.1 and S6.2). The effect of day and the day*fertilizer interaction on daily CO_2 emissions were significant for both fresh and aged biochar treatments, but the biochar*day interaction was only significant for aged biochar ($p < 0.05$) (Figure S2). The effect of aged biochar amendments on CO_2 emissions was inconsistent and only significant for day 3, when the 38 Mg ha^{-1} and 77

Mg ha^{-1} aged biochar treatments significantly increased CO_2 emissions relative to the 0 Mg ha^{-1} treatments. Total CO_2 emissions accumulated over the 1 month experiment (Figure 6.2) were not significantly influenced by fertilizer type ($p > 0.05$) or biochar application rate ($p > 0.05$).

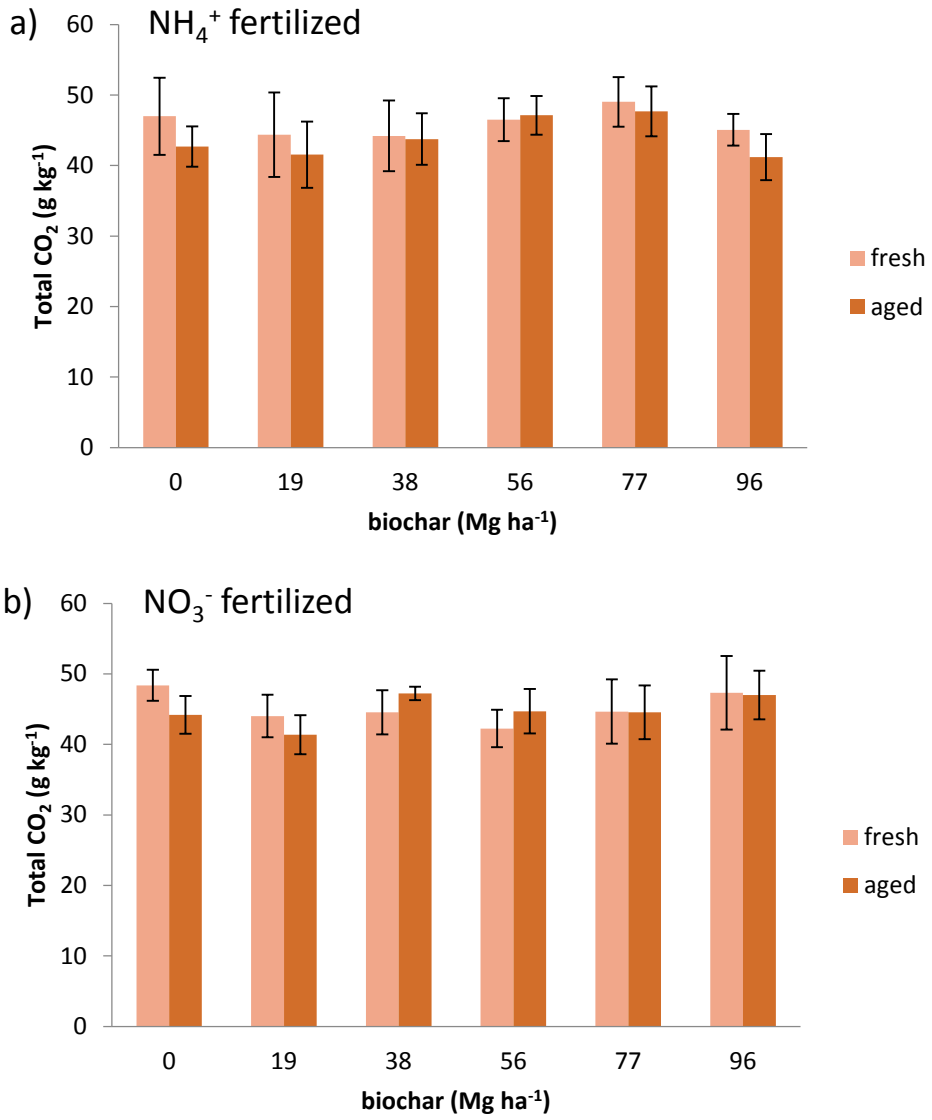


Figure 6.2. Accumulated total CO_2-C emissions from columns amended with different rates of aged and fresh biochar, and fertilized with (a) NH_4^+ and (b) NO_3^-. Error bars represent the standard error of six replicates.

N_2O Emissions from soil columns in the greenhouse study

The main effects of day, biochar application rate, and fertilizer type (NH_4^+ or NO_3^-) and the day*fertilizer and day*biochar interaction effects on daily N_2O emissions were all significant ($p < 0.05$; Figures S6.3 and S6.4). Biochar amendments tended to increase daily N_2O emissions relative to controls, but these effects weren't always significant, and few biochar treatments consistently influenced N_2O emissions on multiple measurement dates. Among columns fertilized with NH_4^+, emissions from soil columns amended with 96 Mg ha^{-1} of biochar increased emissions on days 7, 10, 15 and 22. Columns amended with lower biochar application rates also had higher emission rates than controls, but the difference was not always significant. Among columns fertilized with NO_3^-, emissions from soil columns amended with the highest biochar application rate (96 Mg ha^{-1}) were higher than controls on days 0, 7, 10, and 15.

The main effects of fertilizer type, biochar application rate, and biochar age as well as the biochar rate*age interaction all significantly ($p < 0.05$) affected cumulative total N_2O emissions (Figure 6.3). Among columns fertilized with NH_4^+, columns receiving 19 Mg ha^{-1} of fresh biochar had lower N_2O emissions relative to controls, but N_2O emissions increased with increasing fresh biochar application rates above 19 Mg ha^{-1}. By contrast, columns with aged biochar receiving NH_4^+ had higher N_2O emissions than no-biochar control columns also receiving NH_4^+. However, N_2O emissions did not increase consistently with increasing biochar application rate. Among columns fertilized with NO_3^-, application of fresh biochar slightly increased N_2O emissions, and N_2O emissions

increased with increasing fresh biochar application rates. By contrast, columns receiving NO_3^- and aged biochar had higher N_2O emission rates only when biochar was applied at 19, 38 and 96 Mg ha^{-1}. Inconsistent increases in N_2O emission rates with increasing aged biochar application rate suggest that either a) multiple mechanisms were responsible for biochar's effect on N_2O emissions, or b) soil textural differences arising from field site heterogeneity confounded the analysis of biochar application rate effects. Overall emissions from soil columns receiving NH_4^+ were higher than emissions from columns receiving NO_3^-, emissions from biochar-amended soils were higher than emissions from control soils, and aged biochar-amended soil tended to have higher N_2O emissions than fresh biochar-amended soil.

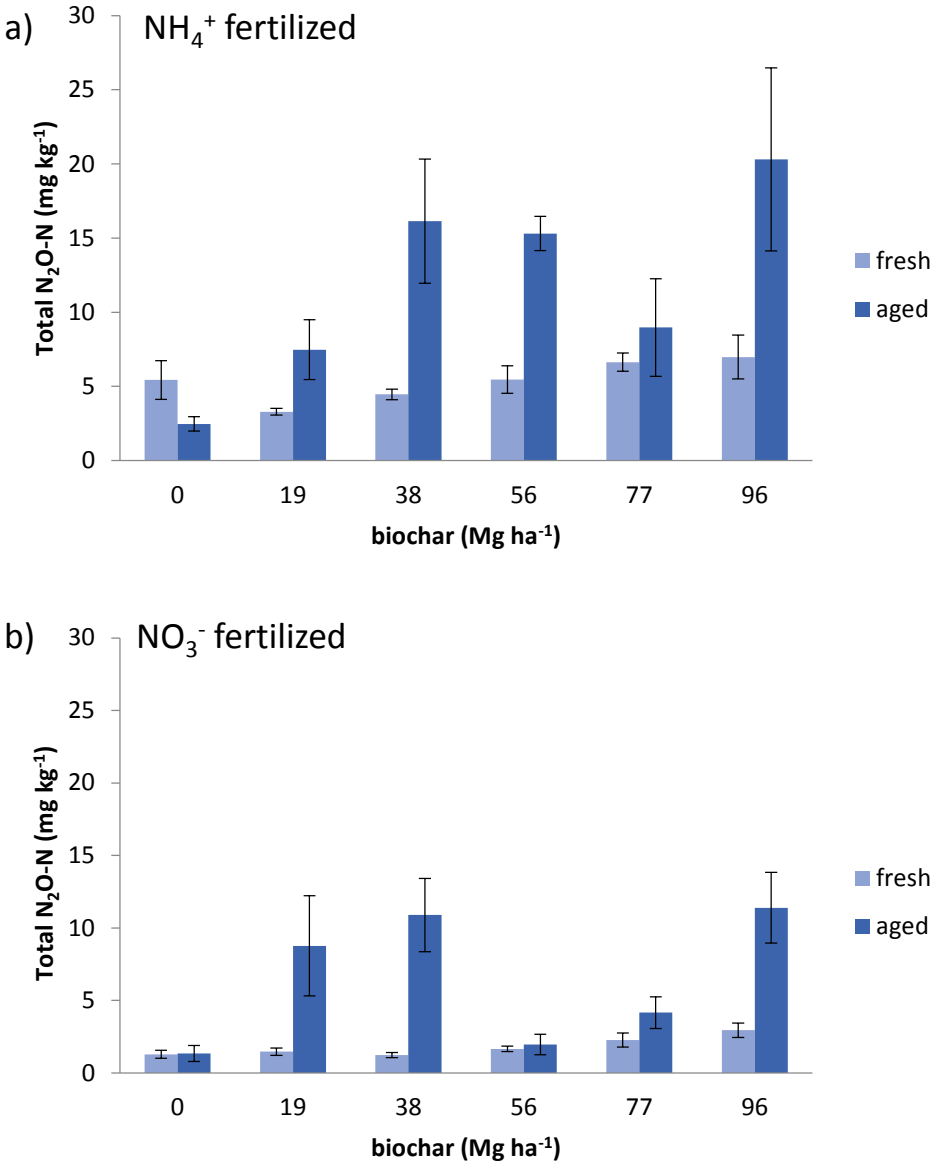


Figure 6.3. Accumulated total soil N_2O-N emissions from columns amended with different rates of aged and fresh biochar, and fertilized with (a) NH_4^+ and (b) NO_3^-. Error bars represent the standard error of six replicates.

N_2O emissions, WFPS, and NO_3^- leaching

Soil column N_2O emission rates were compared with WFPS and NO_3^- leaching to assess the possibility of trade-offs among emissions, moisture, and NO_3^- retention benefits. The main effect of biochar application rate was significant for both WFPS and NO_3^- leaching ($p < 0.05$). When fresh biochar amended columns were grouped according to fertilizer type (NH_4^+ or NO_3^-), total accumulated N_2O emissions within each category were positively correlated with WFPS (r^2 = 0.33 to 0.45) (Figures 6.4a and 6.4b), suggesting that fresh biochar may have increased N_2O emissions by increasing WFPS. With the exception of the 96 Mg ha^{-1} aged biochar treatment, N_2O emissions from soil amended with aged biochar also tended to increase with increasing WFPS. When the 96 Mg ha^{-1} aged biochar treatment was included, N_2O emissions were not significantly correlated with WFPS for either fertilizer type ($p > 0.05$; $r^2 < 0.1$; Figure S5). However, when this high application rate was excluded, N_2O emissions were significantly positively correlated with WFPS (r^2 = 0.28 to 0.42) (Figures 6.4c and 6.4d). Unlike the 19-77 Mg ha^{-1} aged biochar treatments, which had higher N_2O emissions and higher WFPS compared with the 0 Mg ha^{-1} aged biochar control, the 96 Mg ha^{-1} aged biochar treatment had higher N_2O emissions and lower WFPS compared with the control. Thus at the highest biochar application rate, N_2O emissions became divorced from WFPS, perhaps due to the influence of other factors not measured here. Nonetheless, the consistently positive correlations between N_2O and WFPS suggest that trade-offs between soil moisture and N_2O emissions can occur for both fresh and aged biochar amendments.

Total N_2O emitted from fresh biochar-amended columns and respective controls were negatively correlated with total NO_3^- leached (r^2 = 0.46), but this correlation did not occur among columns amended with aged biochar and their respective controls (r^2 = 0.04). Among columns treated with fresh biochar (and controls), N_2O emitted and NO_3^- leached formed two distinct clusters (Figure 6.5a). Columns fertilized with NH_4^+ had both higher N_2O emissions and lower total NO_3^- leached compared with columns fertilized with NO_3^-, perhaps due to greater sorption of NH_4^+ to the soil and a delay between when the NH_4^+ was added and when it was mineralized to NO_3^-. Average N_2O emissions for each biochar treatment were strongly negatively correlated with NO_3^- leached among both NH_4^+ (r^2 = 0.65) and NO_3^- (r^2 = 0.82) treated soil columns when fresh biochar-amended columns were grouped by fertilizer type and N_2O emissions from each biochar rate (including the 0 Mg ha^{-1} control) were averaged (Figure 6.5b). Therefore, fresh biochar amendment may have increased N_2O emissions by reducing NO_3^- leaching and thereby increasing the amount of available NO_3^- remaining in the soil.

Using a multiple linear regression, N_2O emissions were compared with WFPS and NO_3^- leached. Together WFPS and NO_3^- leached explained 34% of the variability in N_2O emissions from columns amended with fresh biochar. However, N_2O emissions from columns amended with aged biochar were not significantly correlated with WFPS or NO_3^- leached ($p > 0.05$). Overall the results suggest that benefit trade-offs may have occurred, but more research will be needed to see if such trade-offs can occur under different conditions – such as under different moisture or temperature regimes, or with different biochars or soils.

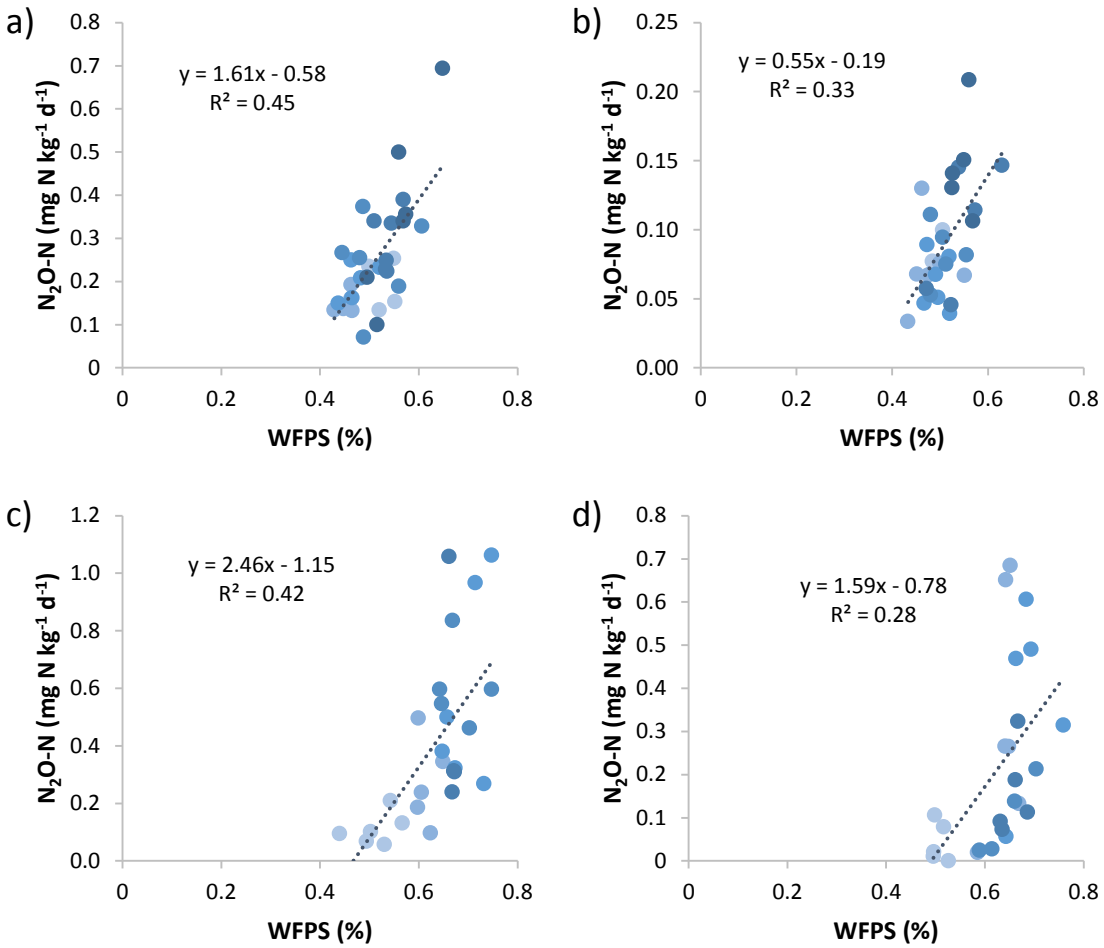


Figure 6.4. Daily soil N_2O emissions and WFPS (% volume) averaged over all sampling dates from columns with a) fresh biochar and NH_4^+ fertilizer, b) fresh biochar and NO_3^- fertilizer, c) aged biochar and NH_4^+ fertilizer, and d) aged biochar and NO_3^- fertilizer, in mg of N per kg of soil (96 Mg ha^{-1} aged biochar application rate excluded; see Figure S6.1) (*not to same scale*).

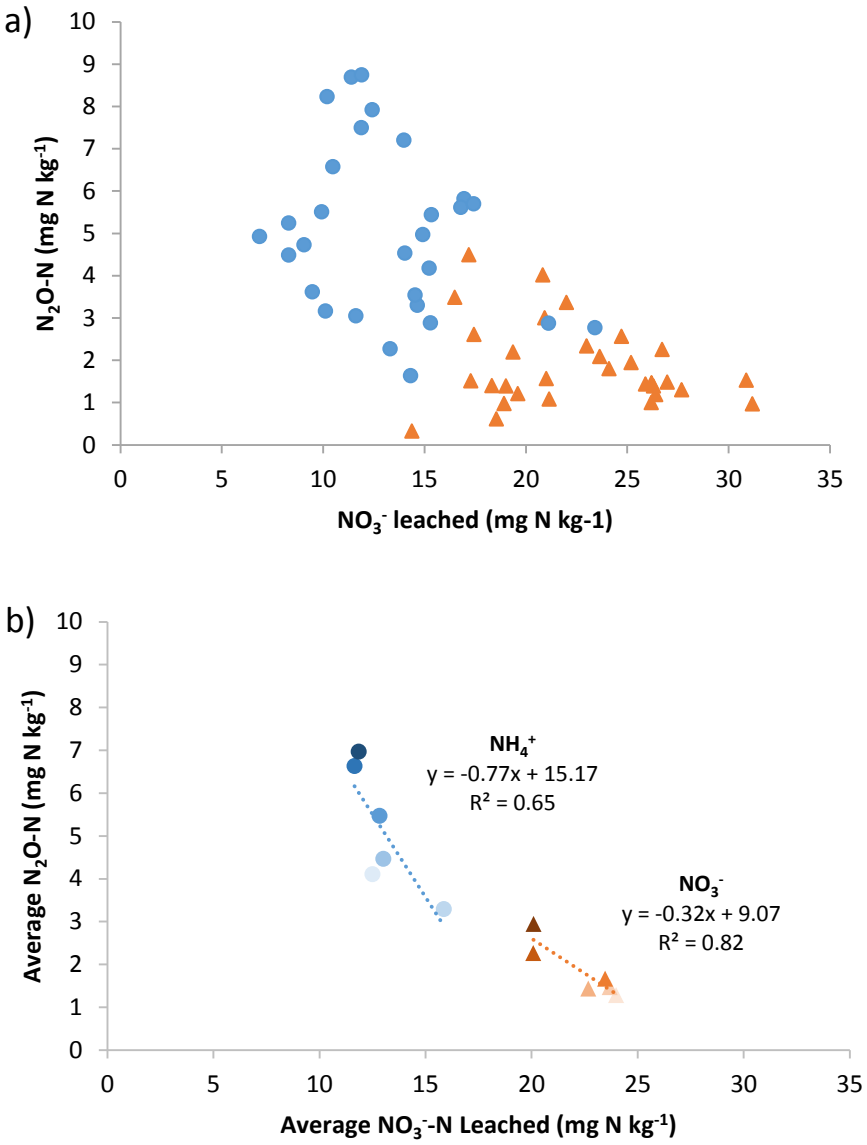


Figure 6.5. Total N_2O emissions and NO_3^- leached from control and fresh biochar-amended columns, shown a) with all data points, and b) with N_2O emitted and NO_3^- leached averaged for each fresh biochar application rate (darker points represent higher biochar application rates; NH_4^+ fertilized treatments shown in blue circles and NO_3^- fertilized treatments shown in orange triangles) (n = 6).

Field experiment

Soil moisture

The main effect of biochar application rate was significant for both daily soil moisture and average soil moisture ($p < 0.05$). Soil amended at all biochar application rates (19, 38 and 58 Mg ha^{-1}) increased moisture by 1-3% relative to the control, but the increase was significant only at the 19 and 58 Mg ha^{-1} application rates (Figure 6.6). When pooled together, all biochar-amended soils had 2.0 ±0.6% higher moisture on average compared with controls ($p < 0.05$). Thus biochar application was shown to slightly but significantly increase soil moisture in the field, and this observation was supported by column study results as well as previous results from the same field site (Rogovska et al. 2014).

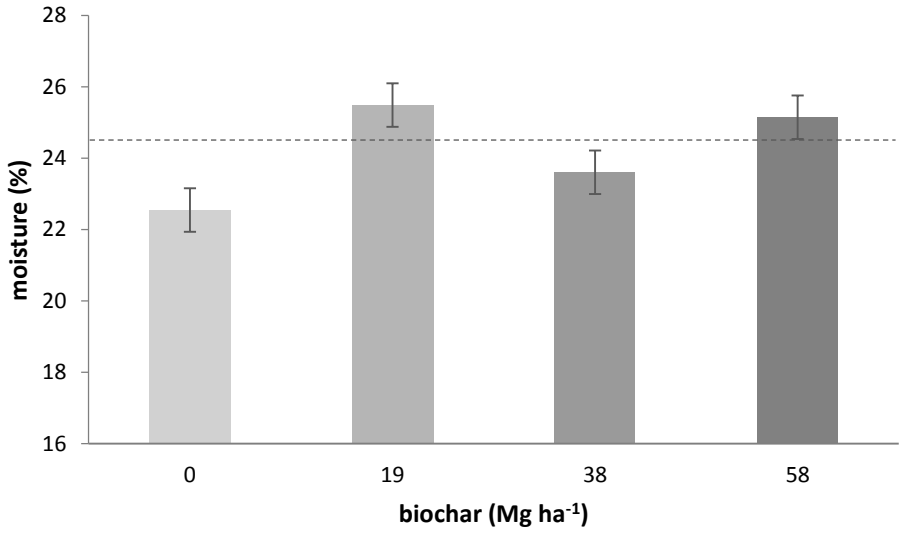


Figure 6.6. Soil moisture measured concurrently with CO_2 and N_2O emissions during the field study and averaged over the entire study. Dotted line represents average moisture of all (19-56 Mg ha^{-1}) biochar-amended plots (±s.e.; n = 3)

CO_2 emissions

Biochar application rate did not significantly affect daily emission rates or total soil CO_2 emissions in crop rows receiving fertilizer or in the inter-row (unfertilized) area ($p > 0.05$) (Figure 7a). The effect of day on daily CO_2 emissions (*data not shown*) was significant ($p < 0.0001$), but the day*biochar interaction was not significant. Emissions from the row soil were consistently higher than from the inter-row soil. Total accumulated CO_2-C emissions ranged from 2.1-2.4 Mg C ha^{-1} and 3.5-4.5 Mg C ha^{-1} from the inter-row and row, respectively. Thus, consistent with the column study results, biochar application did not affect CO_2 emissions in the field study.

N_2O emissions

Biochar application rate did not significantly affect daily or total soil N_2O emission rates in crop rows receiving fertilizer or in the inter-row (unfertilized) area ($p > 0.05$) (Figure 6.7b). The effect of day on daily N_2O emissions (*data not shown*) was significant ($p < 0.0001$), but the day*biochar interaction was not significant ($p = 0.078$). Consistent with the column study results, the biochar treatments with the highest average moistures (19 Mg ha^{-1} and 56 Mg ha^{-1}) also had the highest N_2O emissions. However, unlike the column study, cumulative N_2O emissions in the field did not exhibit significant differences. This lack of impact on N_2O emissions occurred in spite of a significant increase in soil moisture (1-3%) due to the biochar amendments. Biochar may

have failed to significantly impact N_2O emissions in the field study due to a) the small magnitude of impact on soil moisture at the time that GHG emissions were measured (relative to the columns), b) lack of heavy leaching events, c) removal of N by plant roots, or d) variability introduced by field-scale heterogeneity and/or moisture and temperature fluctuations. Thus biochar had similar effects on soil moisture in the greenhouse and in the field but the effect of biochar on N_2O emissions was not significant in the field study.

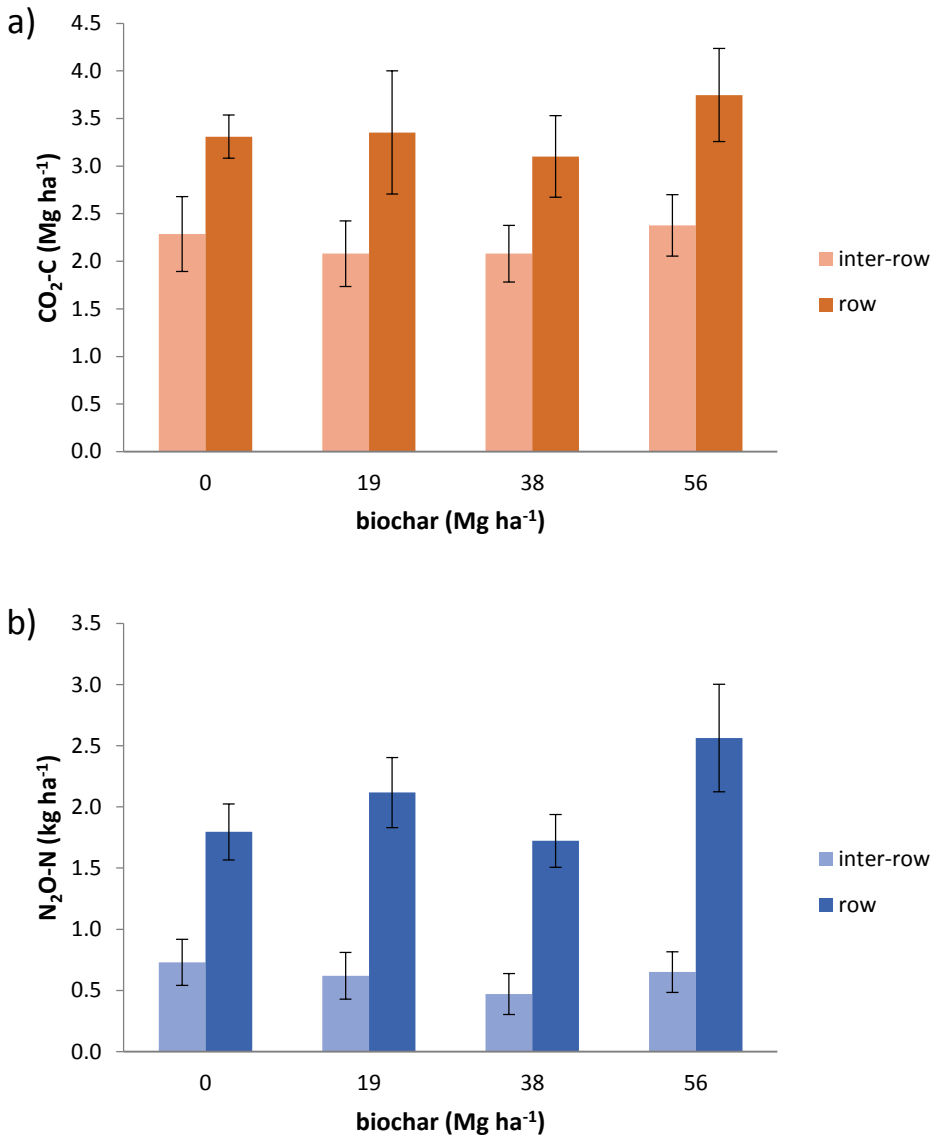


Figure 6.7. Accumulated total soil (a) CO_2-C (b) N_2O-N emissions per hectare from the field site measured during the 4 month growing season. (±s.e.; n = 3)

Conclusions

Based on the preponderance of literature evidence and the results of our previous study (see Ch. 3) we hypothesized that both aged and fresh biochar would decrease N_2O emissions from soils. However, our results from soil microcosms studied in the greenhouse and small field plots indicate that biochar applications increased soil moisture retention and N_2O emissions. The increase in N_2O emissions with increasing biochar applications was significant for the greenhouse study but not for the field study. The greenhouse study also indicated that aged biochar increased N_2O emissions to a greater extent than fresh biochar, and that fresh biochar - but not aged biochar - decreased NO_3^- leaching. Biochar amendment did not affect CO_2 emissions in the greenhouse or field studies. Greenhouse study results suggest that under the heavy leaching conditions used in this experiment, trade-offs (or "pollution trading") between N_2O emissions and NO_3^- leaching or gravity-drained soil moisture may have occurred in columns amended with fresh biochar, but it is less clear whether this trade-off occurred in columns amended with aged biochar or in the field. The slight elevation in N_2O emissions from fresh biochar-amended soil observed here contradicts the previous observation of reduced N_2O emissions from the same soil and biochar when measured in a laboratory incubation (see Ch. 3). This difference between study results may reflect the use of relatively large (1.2 kg of soil), freely drained soil microcosms and heavy leaching conditions in this study in contrast with the use of small (10 g) closed, non-draining system in the previous study. The results call attention to the need for studies conducted at scales large enough to encompass complexities and trade-offs found

under field conditions. Thus overall results suggest that trade-offs among NO_3^- leaching, soil moisture and N_2O emissions are possible in specific contexts, but more research is needed to determine when these trade-offs can occur at the field scale.

APPENDIX E. SUPPLEMENTARY INFORMATION FOR CHAPTER 6

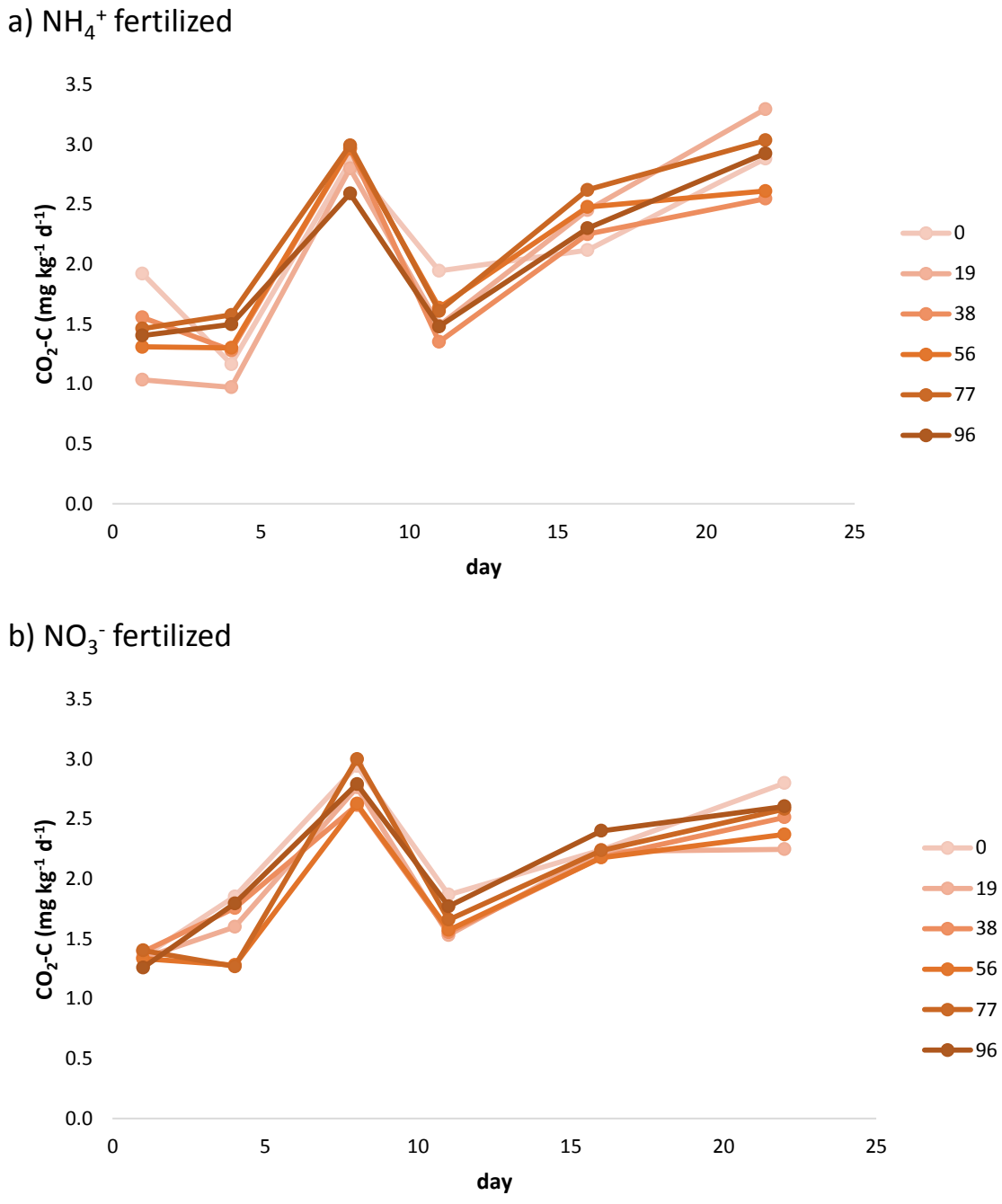
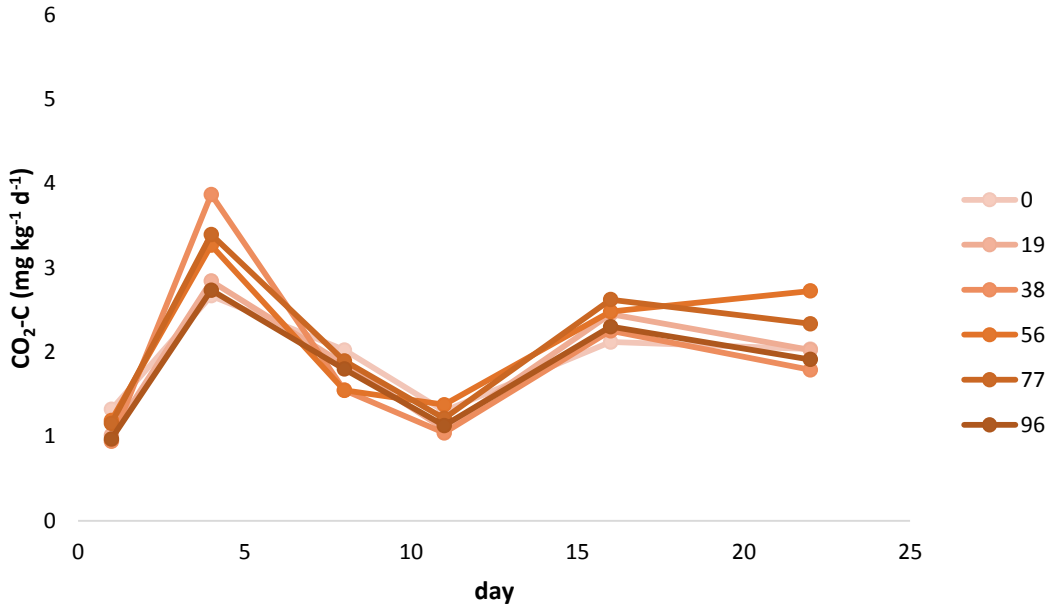


Figure S6.1. Daily CO_2-C emissions from soil columns amended with 0-96 Mg ha^{-1} fresh biochar and fertilized with a) NH_4^+ and b) NO_3^-.

a) NH_4^+ fertilized



b) NO_3^- fertilized

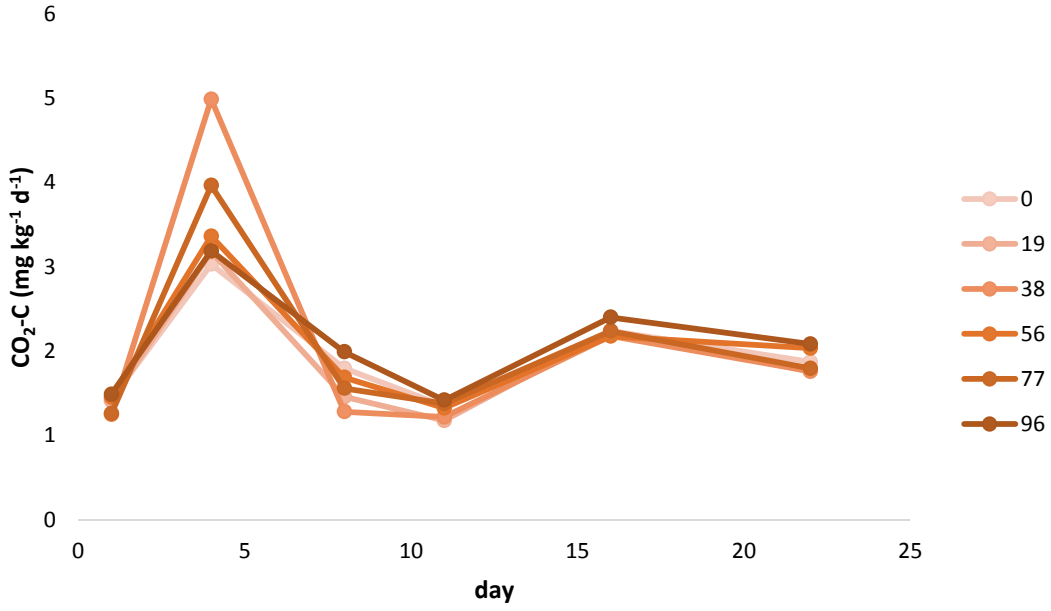


Figure S6.2. Daily CO_2-C emissions from soil columns amended with 0-96 Mg ha^{-1} aged biochar and fertilized with a) NH_4^+ and b) NO_3^-.

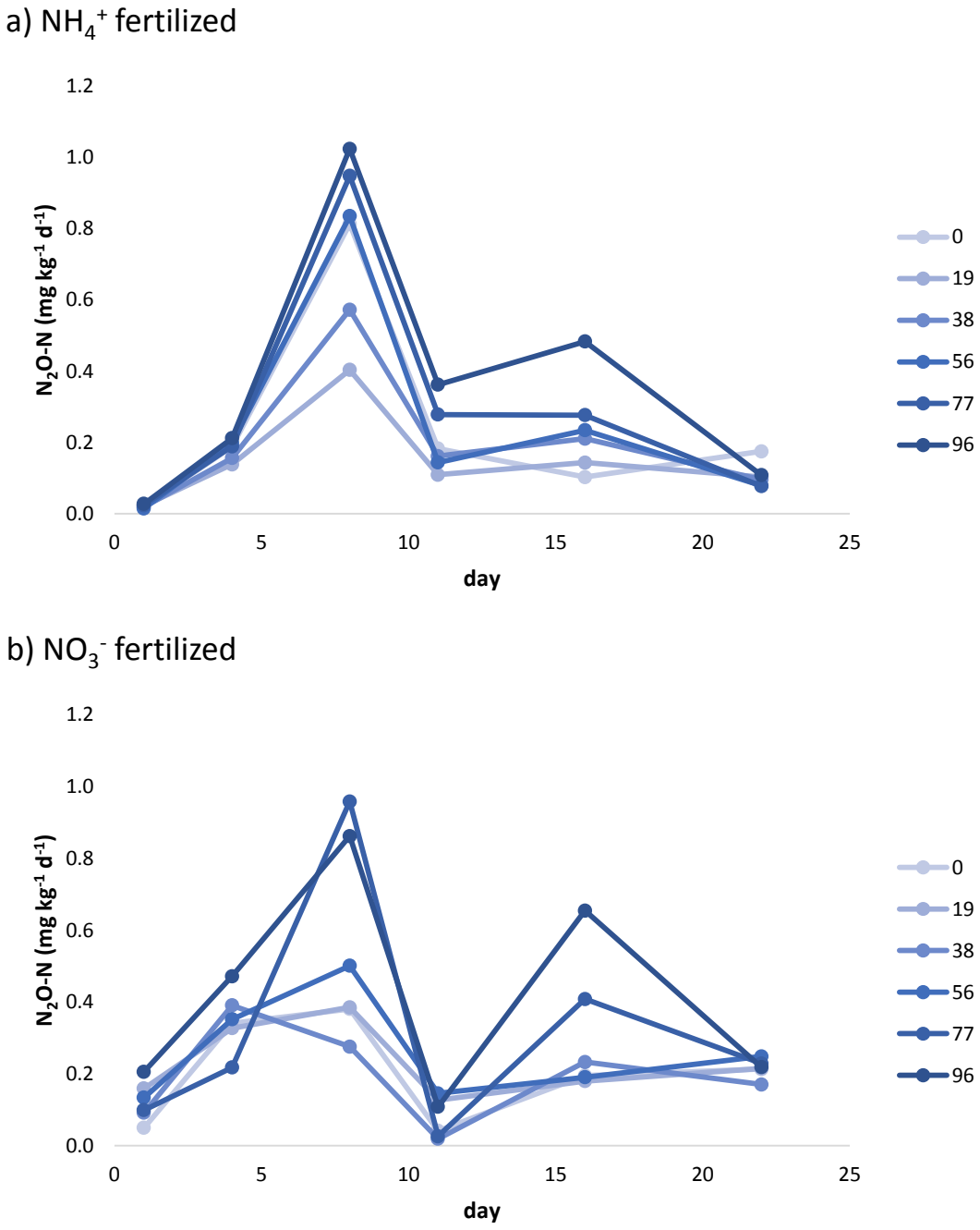


Figure S6.3. Daily N_2O-N emissions from soil columns amended with 0-96 Mg ha^{-1} fresh biochar and fertilized with a) NH_4^+ and b) NO_3^-.

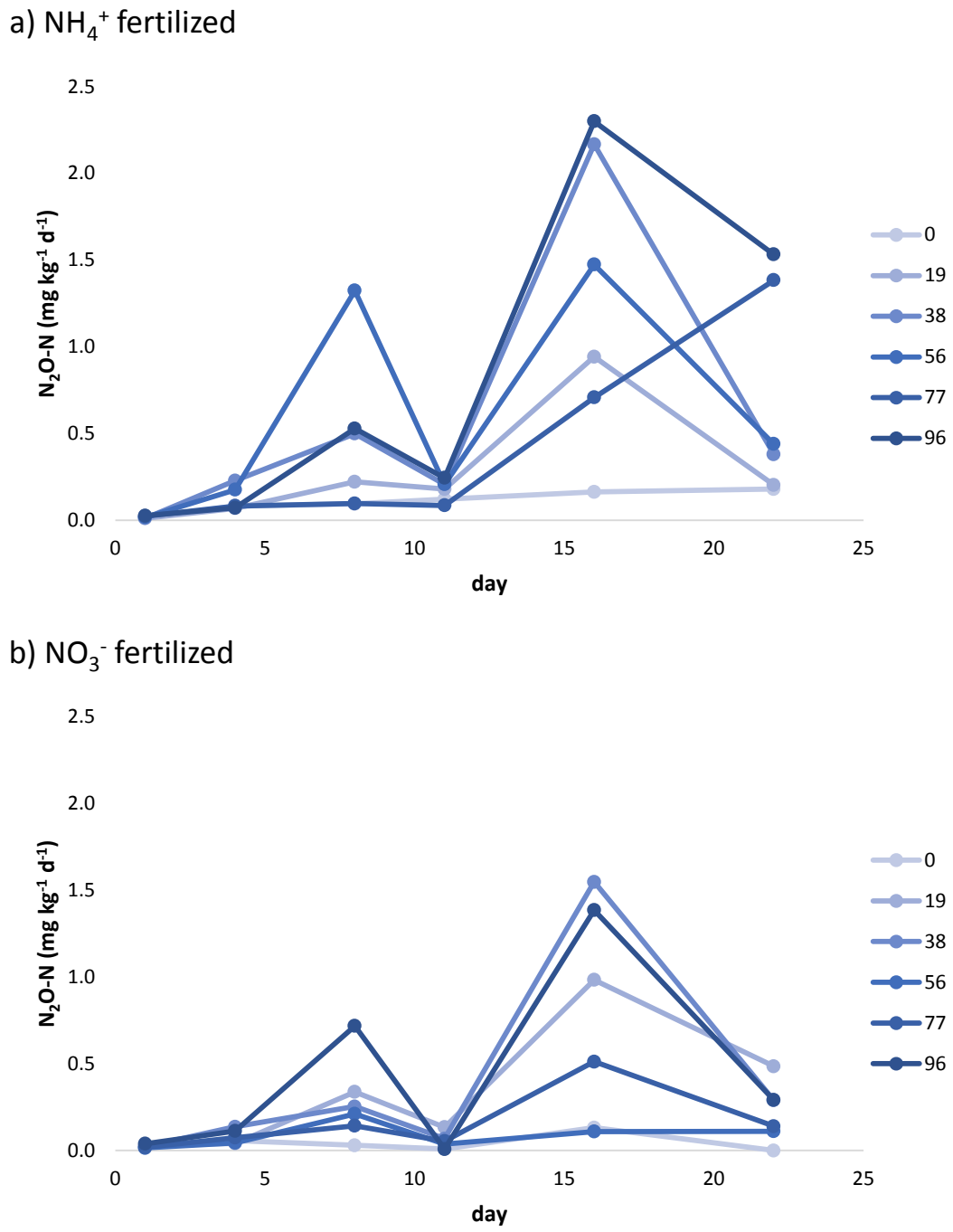


Figure S6.4. Daily N_2O-N emissions from soil columns amended with 0-96 Mg ha^{-1} aged biochar and fertilized with a) NH_4^+ and b) NO_3^-.

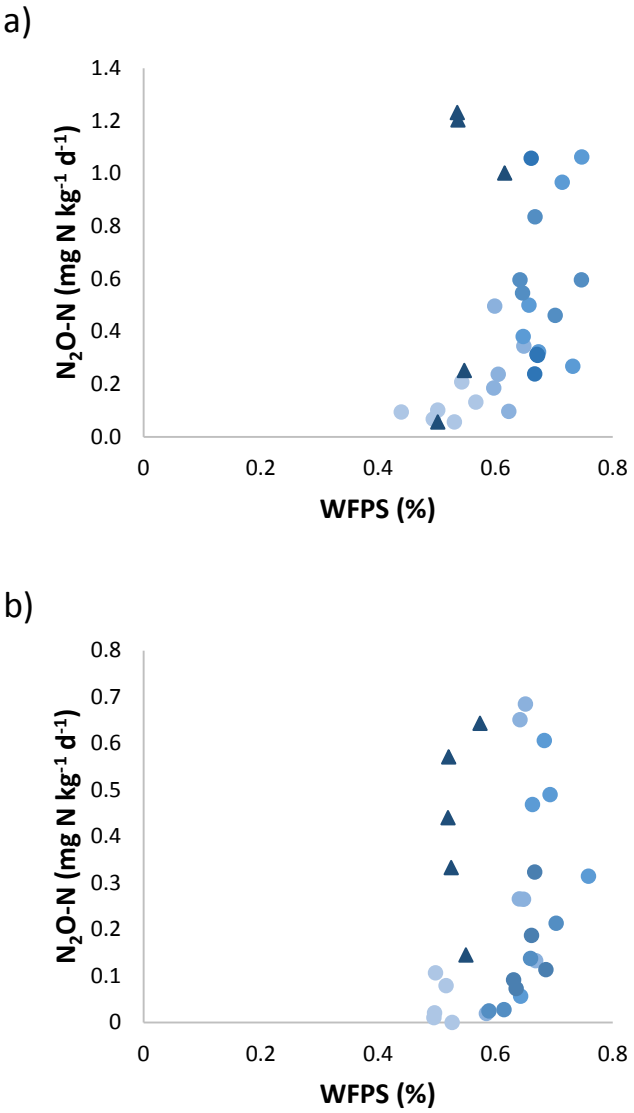


Figure S6.5. Daily N_2O emissions and WFPS of aged biochar treatments (0-96 Mg ha^{-1}) averaged over the measurement period, fertilized with a) NH_4^+ and b) NO_3^-. Darker points represent higher biochar amendment rates; 0-77 Mg ha^{-1} application rate shown in circles; 96 Mg ha^{-1} application rate shown in triangles (*not to same scale*).

CHAPTER 7. GENERAL CONCLUSIONS

Biochar production and application to soil is considered a promising potential tool for mitigating anthropogenic greenhouse gas emissions, but biochars are diverse materials and their effects on soil greenhouse gas emissions remain poorly understood. Here laboratory, greenhouse and field experiments were conducted using a diverse suite of biochars and two soils to (1) quantify the organic and inorganic alkalis of several biochars, (2) quantify the impact of biochars on GHG emissions from diverse soils, and (3) identify mechanisms by which biochars influence GHG emissions from soils.

Quantification of low-pK_a structural, other organic, carbonate, and other inorganic biochar alkalis revealed that both total alkalinity and relative quantities of alkalis varied widely with respect to feedstock and pyrolysis conditions among the eight lignocellulosic biochars studied. Corn stover biochars tended to have higher low-pK_a structural alkalinity and other inorganic alkalinity compared with wood biochars produced under similar conditions, while wood biochars tended to contain more carbonates. However, total biochar alkalinity did not correspond consistently with biochar production parameters or thermogravimetric properties, suggesting that biochar alkalinity may arise from biochar production parameters in a complex-interactive manner.

Carbon dioxide emissions results showed significant effects of biochar labile fractions in the short term, but no significant long term effects. When six biochars were incubated with two soils and quartz (50/50 silt and sand sized), pre-fertilization CO_2

emissions from one of the soils and post-fertilization CO_2 emissions from the quartz were positively correlated with biochar carbonate content (Ch. 3). The positive effect of biochar carbonates on CO_2 emissions was confirmed when amendment of CO_3^{2-} and untreated biochars to soil resulted in elevated CO_2 emissions in the very short term (<48 h) relative to soil amended with acid-washed biochar and controls receiving no amendment (Ch. 4). Application of bicarbonate extracts of biochars also revealed the presence of a labile, alkali-soluble OC fraction which greatly increased emissions relative to controls and soil amended with untreated biochar. This fraction continued to increase CO_2 emissions throughout the experiment, but untreated biochars did not increase CO_2 emissions after 30 days of equilibration with soil. These findings are in agreement with Jones et al. (2011), who cited carbonates as a primary source of short-term CO_2 emissions, in addition to a small but labile OC fraction. However, we also found evidence for OC in the acid-soluble fraction of biochar, and therefore caution against the use of acid washing to determine the effect of biochar carbonates by difference as was done by Jones et al. The brevity of biochar's effect on CO_2 emissions was confirmed by field and greenhouse study results, which showed that field-aged biochar did not significantly increase CO_2 emissions in continuous corn, switchgrass, low diversity grass mix, or high diversity grass-forb mix cropping systems (Ch. 5-6). Thus, biochar was shown to primarily influence CO_2 emissions through the mineralization and hydrolyzation of labile OC and IC, and these effects were largely restricted to the short term (<30 days). We therefore expect the risk of lignocelluosic biochars increasing long-term CO_2 emissions of the Typic Hapludols studied here to be minimal.

The sensitivity of soil N_2O emissions to temporally and spatially variable environmental conditions has long challenged the assessment of N_2O management practices, including the application of biochar. The varying responses of N_2O emissions to biochar amendment in the lab, greenhouse and field studies conducted here reflect this sensitivity, but results can still provide valuable insights into possible underlying mechanisms. Suppression of soil N_2O emissions observed for most soil-biochar combinations (Ch. 3) coincided with reduced soil NO_3^- concentrations, suggesting that biochar may have reduced N_2O emissions by reducing NO_3^- availability. Multiple biochars also reduced NH_4^+ from the Exira silty clay loam soil, implying that NH_4^+ may also have been involved in the suppression of N_2O emissions. Furthermore, amendment of carbonate controls did not affect N_2O emissions in either incubation, suggesting that the effect of biochar on N_2O emissions is not solely due to carbonates and/or pH. Field study results paralleled the lab, with only the continuous corn cropping system from the Armstrong field site (silty clay loam soils, including Soil A) exhibiting a suppression of N_2O emissions with biochar application. Inconsistencies among incubations and field study results, which did not all show a reduction in N_2O emissions with biochar application, likely reflect the context-sensitive nature of N_2O emissions. Indeed, the meta-analysis conducted by Cayuela et al (2014) showed that the suppression of soil N_2O emissions following biochar amendment was smaller in magnitude and more variable when biochar was amended at <1% by weight and when NH_4NO_3 fertilizer was used compared with higher biochar application rates and other fertilizers (nitrate, urea, or no fertilizer). Under the conditions studied here, it is likely that very slight differences

in incubation design – such as equilibration period length, amount of time soil was stored for, and exact amount of fertilizer added – or simply the small magnitude of emission rates relative to noise could have resulted in the observation of significant differences in some incubations and not others. Overall the laboratory and field experiment results showed that biochar affects N_2O emissions in a context-specific manner dependent on both biochar and soil properties, and likely involving soil inorganic N transformations.

The greenhouse study – the only experiment presented here to feature heavily leached, free-draining columns – presented a unique set of circumstances in which biochar increased N_2O emissions. Increased N_2O emissions from both fresh and field-aged biochar-amended soils relative to controls corresponded with higher WFPS and lower NO_3^- leaching, suggesting that retention of water and NO_3^- in biochar-amended soil – due to the porous nature and sorptive capacity of the biochar –may have been responsible for increased N_2O emissions. The amount of water added to the columns was much higher than the amount of precipitation that occurred during the parallel field experiment, which may explain why no increases in N_2O emissions were observed in the field. Despite a predominance of evidence for biochar-induced N_2O suppression in the literature, the greenhouse study findings are not completely unprecedented. Rather, the observed increase in N_2O emissions with increasing biochar amendment rate supports previous findings of elevated N_2O emissions in soils amended with aged biochar relative to fresh biochar in a closed system study design (Spokas 2013) and increased N_2O emissions from fresh biochar-amended soil (Wells and Baggs 2014). Increased N

retention in the field (Güereña et al. 2013), increased water retention (Novak et al. 2009; Novak et al. 2012; Ulyett et al. 2014), and decreased inorganic N leaching in free-draining microcosm studies (Singh et al. 2010b; Zheng et al. 2012) have been reported elsewhere. Only in this study have increased N_2O emissions, N retention, and water retention coincided, and thereby provided evidence for potential benefit trade-offs. Additional trade-offs among biochar benefits which were not within the scope of this study may also occur, such as between C sequestration and crop yields, or between nitrification and N losses. Thus the greenhouse study identified N and water retention as possible mechanisms by which biochar affects N_2O emissions of free-draining systems, and highlighted the need for studies investigating trade-offs under multiple contexts.

In summation, the lab, greenhouse and field results together indicated that it is possible to use biochar as a CO_2 and N_2O emission mitigation tool, but multiple mechanisms likely govern how biochar influences emissions. Here we highlight labile IC and OC release, perturbation of N transformations, direct sorption of N, and enhanced water retention as potential key mechanisms of biochar-soil interaction, and emphasize that some mechanisms may become dominant over others depending on how and when biochar is applied. Consequently, the efficacy of biochar for reducing soil GHG emissions will depend on which mechanisms dominate under the specific conditions in question. Thus further research testing multiple mechanisms under varying contexts is imperative to optimizing the use of biochar as a GHG mitigation tool.

FUTURE WORK

This dissertation has highlighted potential underlying mechanisms of biochar's influence on N_2O emissions which would require further research to verify. For this purpose, a postdoctoral research project was proposed investigating if the observed changes in soil N_2O emissions following biochar application are due to perturbations of the N cycle via one or more of the following mechanisms: (1) organic and inorganic alkalis in biochar increase or buffer soil pH and create high-pH microsites adjacent to char particles ("*alkali*" mechanism), (2) labile C in biochar provides substrate for microbes ("*substrate*" mechanism), (3) toxic compounds in biochar or derived from biochar inhibiting microbial activity ("*inhibition*" mechanism), (4) sorption of soluble C and N to biochar may alter their accessibility to microbes ("*sorption*" mechanism), (5) biochar provides microbial habitat due to its porous structure and high surface area, thereby influencing microbial abundance or community composition ("*habitat*" mechanism), (6) biochar increases soil microporosity, thereby influencing soil water dynamics and making water and/or oxygen more available to microbes ("*porosity*" mechanism), and (7) biochar acts as an "electron shuttle," thereby catalyzing biochemical and abiotic redox reactions ("*electron shuttle*" mechanism). The goals of the proposed study are to (1) determine the mechanistic pathways of the observed N_2O emission supression in biochar-amended soils and (2) assess if mechanism dominance is soil-dependent. These goals will be achieved by fulfilling the following objectives: (1) assess the influence of soluble and gaseous components of biochar on N_2O emissions, (2) determine if biochar influences N_2O emissions by providing surfaces suitable for

substrate adsorption and/or microbial habitat, and (3) determine if biochar influences N_2O emissions by altering the redox environment of soil.

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