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# Biochar Reduced Nitrous Oxide and Carbon Dioxide Emissions from Soil with Different Water and Temperature Cycles

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# Biochar Reduced Nitrous Oxide and Carbon Dioxide Emissions from Soil with Different Water and Temperature Cycles

Jiyul Chang, David E. Clay,\* Sharon A. Clay, Rajesh Chintala, Janet M. Miller, and Thomas Schumacher

## ABSTRACT

Interactions among biochar, respiration, nitrification, and soils can result in biochar increasing, decreasing, or not impacting greenhouse gas (GHG) emissions. This experiment determined the impact of water-filled porosity (WFP) and corn (*Zea mays* L.) stover biochar on CO<sub>2</sub> and N<sub>2</sub>O emissions in May (spring) and August (summer). The May experiment contained two N rates [0 and 224 kg Ca(NO<sub>3</sub>)<sub>2</sub>-N ha<sup>-1</sup>], whereas the August had three N rates [0, 224 kg Ca(NO<sub>3</sub>)<sub>2</sub>-N ha<sup>-1</sup>, and 224 kg (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-N ha<sup>-1</sup>]. The average temperatures in the May and Augusts 2014 experiments were 14 and 24°C, respectively. Biochar reduced CO<sub>2</sub>-C emissions in the high WFP Ca(NO<sub>3</sub>)<sub>2</sub> treatment in the May and August experiments 15.4 and 16.3 kg ha<sup>-1</sup>, respectively. Associated with the CO<sub>2</sub>-C decrease was a 15.7% reduction in the soil solution dissolved organic C. In addition, N<sub>2</sub>O-N and CO<sub>2</sub>-C emissions were not correlated in the May Ca(NO<sub>3</sub>)<sub>2</sub> ha<sup>-1</sup> treatment, whereas in the August experiment, N<sub>2</sub>O-N and CO<sub>2</sub>-C emissions were correlated ( $r^2 = 0.98$ ,  $P < 0.01$ ). In August, biochar increased the apparent nitrification from 16 to 25 kg NH<sub>4</sub>-N (ha × d)<sup>-1</sup> in the low WFP (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> treatment, and it did not influence the nitrification rate in the high WFP (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> treatment. In general, N<sub>2</sub>O-N emissions increased with WFP and N rate and were reduced 21.7% by biochar. The findings suggest that multiple mechanisms contributed to N<sub>2</sub>O emissions and seasonal differences in soil temperature could result in biochar having a mixed impact on GHG emissions.

## Core Ideas

- Biochar reduces CO<sub>2</sub> gas emission from soil in high soil temperature.
- Biochar reduces N<sub>2</sub>O gas emission from soil in high soil temperature.
- Biochar reduces N<sub>2</sub>O gas emission from high water-filled porosity condition.

**R**ESearch suggests that a large component of agriculture's carbon footprint is linked to nitrous oxide emission, which is a potent greenhouse gas (Wang et al., 2012b; Wilson et al., 2015). To reduce agricultural impact on the environment, GHG emissions need to be reduced and N fertilizer efficiency needs to be enhanced (McDonald et al., 2015; Mohammed et al., 2016). Traditional approaches to reduce GHG emissions include: (i) reducing the tillage intensity, (ii) reducing N fertilizer additions, (iii) adopting techniques, such as cover crops, that improve nutrient recycling; and (iv) splitting the N application (Clay et al., 2012, 2015; Reese et al., 2014; Kovács et al., 2015; Hooper et al., 2015). Unfortunately, some of techniques may increase labor and production costs while adversely effecting yield.

Biochar has been proposed as an alternative approach to reduce GHG emissions and enhance soil health (Mollinedo et al., 2015; Trippe et al., 2015; Cayuela et al., 2013,2014). However, the impact of biochar on the soil physical and biological properties has been mixed. For example, Chintala et al. (2014b) reported that biochar reduced CO<sub>2</sub> emission, whereas Fernández et al. (2014) and Wang et al. (2012a) reported that biochar did not influence or increased emission. Mixed results could be attributed to biochar having different impacts on different components of the N cycle, and that GHG emissions are impacted by the process used to produce biochar (Singh et al., 2010; Castaldi et al., 2011; Wang et al., 2011, 2012a, 2012b; Zimmerman et al., 2011; Zhang et al., 2012; Case et al., 2012; Kammann et al., 2012; Cayuela et al., 2013; Clough et al., 2013; Biederman and Harpole, 2012; Chintala et al., 2014a, 2014c; Fernández et al., 2014; Song et al., 2014; Prommer et al., 2014; Creamer et al., 2014; Nelissen et al., 2014; Mollinedo et al., 2015).

In soil, CO<sub>2</sub> and N<sub>2</sub>O emissions are the result of microbial respiration (denitrification/co-denitrification) and nitrification. In respiration, organic materials are oxidized to produce energy, and during the final stage of aerobic respiration, electrons are combined with O<sub>2</sub> to produce water. However, if O<sub>2</sub> becomes limited and nitrate is available, some microbes have the capacity to switch from O<sub>2</sub> to NO<sub>3</sub><sup>-</sup> as the terminal electron acceptor (Linn and Doran, 1984; Bateman and Baggs, 2005; Liu et al., 2007). This process is called denitrification, and the rate is indirectly related to the oxygen

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**Abbreviations:** GHG, greenhouse gas; WFP, water-filled porosity.

concentration, and directly related to soil temperature and organic C substrate availability. In the laboratory, Linn and Doran (1984) reported that denitrification was an important mechanism when WFP increases above 60%. Recent research suggests, that other mechanisms such as co-denitrification or dissimilatory nitrate reduction can also produce  $N_2O$  under  $O_2$  limited conditions (Spott et al., 2011). Under aerobic conditions,  $N_2O$  can be produced during the multistep process, called nitrification, where ammonium ( $NH_4$ ) is converted to nitrate ( $NO_3^-$ ) (Linn and Doran, 1984; Ulyett et al., 2014; Sanchez-Garcia et al., 2014).

Findings from these studies show that in many soils, the  $O_2$  concentration and the availability of the appropriate substrates influence if  $N_2O$  is derived from respiration or nitrification. In soils with low  $O_2$  concentrations, denitrification, codenitrification or dissimilatory nitrate reduction of ammonia to nitrate can be important, whereas in soils with high  $O_2$  concentrations nitrification is an important process. The mixed impact of biochar on GHG emissions may be related to the impact of temperature on the rate that C and N compounds are processed through nitrification and respiration. For example, if the soil temperature is low, respiration may not be high enough to produce anaerobic conditions. Under these conditions, biochar, by enhancing nitrification, can increase  $N_2O$  emission (Prommer et al., 2014). Under higher temperatures, biochar may reduce  $N_2O$  emission by reducing respiration (Chintala et al., 2014b). The objective of this experiment was to determine the impact of corn stover biochar on  $CO_2$  and  $N_2O$  emissions in field experiments with different water and temperature cycles.

## MATERIALS AND METHODS

This experiment was conducted at the Aurora Experimental Farm in Brookings, SD (44°18'20.57" N, 96°40'14.04" W) in the middle of May (from 17th–23rd) and middle of August (from 14th–20th), 2014. The May and August treatments were selected to represent two different temperature regimes. Treatments were applied to a site previously cropped to corn and soybean [*Glycine max* (L.) Merr.] and the field was chisel plowed and disked to control weeds and prepare a seedbed on 12 May. The soil type was a Brandt silty clay loam (fine-silty, mixed, superactive, frigid Calcic Hapludoll), with clay, silt, and sand percentage of 28, 65, and 7%, respectively. The soil initial SOC for the surface 15 cm was 36 Mg ha<sup>-1</sup>, and the C mineralization kinetics were previously discussed in Clay et al. (2015). A more complete description of the study site is available in Clay et al. (1995, 1996, 2015). The water contents at field capacity and the wilting point were 0.315 and 0.177 kg kg<sup>-1</sup>, respectively, and soil bulk density (0–15 cm soil depth) at the beginning of the study was 1.2 g cm<sup>-3</sup>. Plants were excluded from the study area.

### Experimental Design

In the May study, a randomized complete block experiment was used to determine the impact of two N rates (0 and 224 kg Ca(NO<sub>3</sub>)<sub>2</sub>-N ha<sup>-1</sup>), two biochar rates (biochar and no-biochar) and two water-filled porosity (low and high) treatments on  $N_2O$ -N and  $CO_2$ -C emissions. Each treatment was replicated three times. In the August study, the number of N treatments were increased from two to three [0, 224 kg Ca(NO<sub>3</sub>)<sub>2</sub>-N ha<sup>-1</sup>, and

224 kg (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>-N ha<sup>-1</sup>] and each treatment was replicated three times. The two N treatments [(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and Ca(NO<sub>3</sub>)<sub>2</sub>] were used to calculate different components of the N cycle. For example, nitrification rate calculations were based on changes in ammonia concentration in the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> treatment.

The N treatments were prepared by mixing the reagent grade chemical compounds with 100 mL of water, which were subsequently sprinkled onto the bare soil surface. The water or water + N treatments were sprinkled onto all treatments including the no-N and no-biochar controls. For these treatments, the fertilizer were mixed with type 1 water (EC < 0.1 dS m<sup>-1</sup>).

The fertilizer and biochar treatments were applied to PVC rings with a 25 cm diam. and a 15 cm height. Each ring was an individual plot and plants were excluded from the rings. The rings were pushed 5-cm deep into the soil. For the biochar treatments, 1000 kg of corn stover biochar ha<sup>-1</sup> were mixed 7.5-cm deep into the soil. This rate was approximately 10 g of biochar for each kg of soil. The area outside of the ring was not treated with biochar or fertilizer.

The biochar was produced from corn stover collected in the fall of 2012. The baled biomass was pelletized to size of 6 by 1500 mm. Biochar was produced in a two stage continuous process in which the reactor temperature increased from 150 to 850°C over a 4 h and 4 min period (Biochar Solutions, Inc, Carbondale, CO). The specific surface area, pH, electrical conductivity (EC), cation exchange capacity (CEC), total C, and N of corn stover biochar were 1.76 ha kg<sup>-1</sup>, 10.0, 0.80 dS m<sup>-1</sup>, 24 cmol<sub>c</sub> kg<sup>-1</sup>, 480 g kg<sup>-1</sup>, and 4.1 g kg<sup>-1</sup>, respectively (Chintala et al., 2014b). The C/N ratio of the biochar was 117. The soil pH and electrical conductivity of the soil and biochar mixture was measured with a 1:1 soil to water mixture. When added to soil, the biochar increased the soil pH (1:1 water) from 5.77 (±0.03) to 6.20 (±0.29), and in the August experiment, biochar addition did not influence the 1:1 soil to water EC (0.37 ± 0.11 dS m<sup>-1</sup>) value.

For the high WFP treatment, 300 mL of water (0.61 cm) was initially uniformly added across the treatment area to saturate the soil. Thereafter, 100 mL (0.2 cm) of water was sprayed across the treatment area three times daily. For the low WFP treatment, water was not added during experiments. The WFP for the biochar and non-biochar treatments are shown in Table 1. Soil temperature of the surface 7.5 cm was measured in the plots using a Taylor 3516 Digital Instant Read Thermometer (Taylor Precision Product, Inc. Las Cruces, NM). The average soil temperatures in May at the 7.5-cm depth were 15°C (range of 5.4–21.9) and 14°C (range of 5.6–21.8) in the low and high WFP, respectively, whereas in August, the average soil temperatures were 24°C (range of 19.6–31.8) and 22°C (range of 18.0–26.9) in the low and high WFP, respectively. Apparent nitrification rate for the August NH<sub>4</sub>-N treatment was calculated with the equation,  $\{[(\text{Initial } NH_4 + NH_4\text{-N added}) - \text{remaining } NH_4] / 7 \text{ d}\}$ . This equation does not account for N mineralization.

### Measuring Greenhouse Gases

To trap the  $N_2O$  and  $CO_2$  greenhouse gases, 10 mL gas samples were collected with a syringe at 0 and 20 min after the PVC cylinders were covered and sealed. Each cover had a

Table 1. The water-filled porosities (WFP) for the three soil depths as influenced by the experiment date (May or August).

Treatment	May: Soil depth, cm			August: Soil depth, cm		
	0–7.5	7.5–15	15–30	0–7.5	7.5–15	15–30
	% WFP					
High WFP						
Biochar	68.0	64.0	61.0	67.5	62.5	62.5
No-biochar	67.0	65.0	62.0	67.0	61.7	61.7
<i>P</i>	0.86	0.48	0.13	0.53	0.65	0.24
Low WFP						
Biochar	45.0	57.0	61.0	42.5	57.0	60.0
No-biochar	45.0	56.0	58.0	42.3	56.3	60.0
<i>P</i>	0.47	0.42	0.52	0.64	0.54	0.57

septum through which a 10 mL gas sample was collected. The gas was injected into a 10 cm vial filled with He. Each vial had a prefitted septa.

Gas samples were taken three times per day (0700, 1300, and 1900 h) for seven consecutive days. The gas samples were analyzed for N<sub>2</sub>O and CO<sub>2</sub>-C by GC-2014 Gas Chromatograph (Shimadzu Co., Kyoto, Japan) immediately after sampling (Chintala et al., 2014a). The gas chromatograph was calibrated with traceable gas standard (Scotty Analyzed Gases, Air Liquide America Specialty Gases LLC, Plumsteadville, PA). When gas samples were measured, soil moisture and temperatures were concurrently measured for the surface 7.5 cm using a Theta Probe Type ML2x (Delta-Y Devices Ltd, Cambridge, England) and a thermocouple placed at the 7.5-cm soil depth.

### Soil Analysis

In the May and August experiments at time zero, soil samples were collected adjacent to the rings from 2 depths (0–15 and 15–30 cm), and at completion of the experiment, samples were collected from the rings. Bulk density was determined and soil samples were analyzed for soil moisture, air dried, ground, and analyzed for ammonium and nitrate concentrations using a 10:1 1 M KCl solution to soil ratio (Kim et al., 2008). Ammonium and nitrate concentrations were used to determine net inorganic N accumulations, relative amount of nitrate in the surface soil, and assess the relative impact of biochar on nitrification. Gravimetric values were converted to volumetric values using the measured soil bulk densities.

The amount of inorganic N contained in the surface 15 cm prior to the May study was 33.4 kg of inorganic-N ha<sup>-1</sup>. After the experiment was completed, the low and high WFP no-fertilizer control treatments contained 42.1 and 33.0 kg inorganic-N ha<sup>-1</sup> in the surface 15 cm, respectively. The amount of inorganic N in the surface 15 cm prior to the August study was 46.4 kg inorganic-N ha<sup>-1</sup>, and after the experiment was completed, the low and high WFP no-fertilizer control treatments contained 68.4 and 70.4 kg inorganic-N ha<sup>-1</sup>, respectively.

The Fourier transformation of the air temperatures, CO<sub>2</sub>-C, and N<sub>2</sub>O-N emissions were used to determine the phase angle and amplitudes of the soil moisture, temperature, CO<sub>2</sub>-C, and N<sub>2</sub>O (amplitude) diurnal cycles. In this transformation, the data was fit to the equation,

$$y_c(t) = A_c \left[ \cos \left( \frac{2\pi ct}{T} - \phi_c \right) \right]$$

where  $T$  is the interval,  $y_c(t)$  is the gas concentration at time  $t$ ,  $A_c$  is amplitude of the cosine curve,  $\phi_c$  is phase angle of the cosine curve, and  $c$  is the frequency of the wave cycles (Carr, 1995). The amplitude represents the height of diurnal cycle peak, whereas the phase angle or shift represents the offset of peak in the cosine wave. The peak time of diurnal cycle was determined by converting the phase angle to 24 h basis. In this experiment,  $T$  is 1 (a day in 24 h period) and  $c$  is 1 (a complete cycle). The statistical analysis was conducted in PROC GLM in SAS (SAS Institute, 2008). The Fisher's LSD at the 0.1 level of significance were used to separate means. May and August experiments were analyzed separately.

The influence of biochar on dissolved organic C was evaluated using surface soil from the study site where moist soil was mixed with two biochar rates (none and 1%) and incubated for 7 d at 20°C. The gravimetric soil moisture was 25% and the soil was placed into covered containers. The containers had a radius of 8 cm and a height of 10 cm and volume of 2 L. The soil depth was 7 cm and each treatment was replicated four times. At the completion of the experiment, 20 g of soil was mixed with 20 mL of water, centrifuged, and analyzed for dissolved organic C (Rice et al., 2012).

## RESULTS AND DISCUSSION

### Nitrous Oxide and Carbon Dioxide Emissions

During the May experiment, the CO<sub>2</sub>-C, N<sub>2</sub>O-N emissions, and soil temperature followed diurnal cycles (Fig. 1) and had similar diurnal cycle phase shifts (Table 2). Biochar did not influence the phase angles for either GHG. The diurnal CO<sub>2</sub> and N<sub>2</sub>O emissions patterns were attributed to microbial respiration that increased with soil temperature, and N<sub>2</sub>O and CO<sub>2</sub> solubility that decreased with increasing temperature. For example, increasing the temperature from 15 to 35°C reduces N<sub>2</sub>O and CO<sub>2</sub> solubility in water 42 and 43%, respectively. Smith et al. (1998) and Clay et al. (1990) had similar results and reported that GHG emissions increased with soil temperature. However, there were distinct differences between the N<sub>2</sub>O-N and CO<sub>2</sub>-C emissions pattern. These differences were associated with the time delay between the start of the experiment and the strong diurnal N<sub>2</sub>O emission cycles that were observed 2 to 4 d later (Fig. 1). This delay was not observed for CO<sub>2</sub>-C emissions.

The N<sub>2</sub>O-N emissions were generally higher in the high WFP (WFP = 67%) than the low WFP (WFP = 45%) treatments. The impact of WFP on N<sub>2</sub>O-N emissions is consistent with Linn and Doran (1984) and it supports the

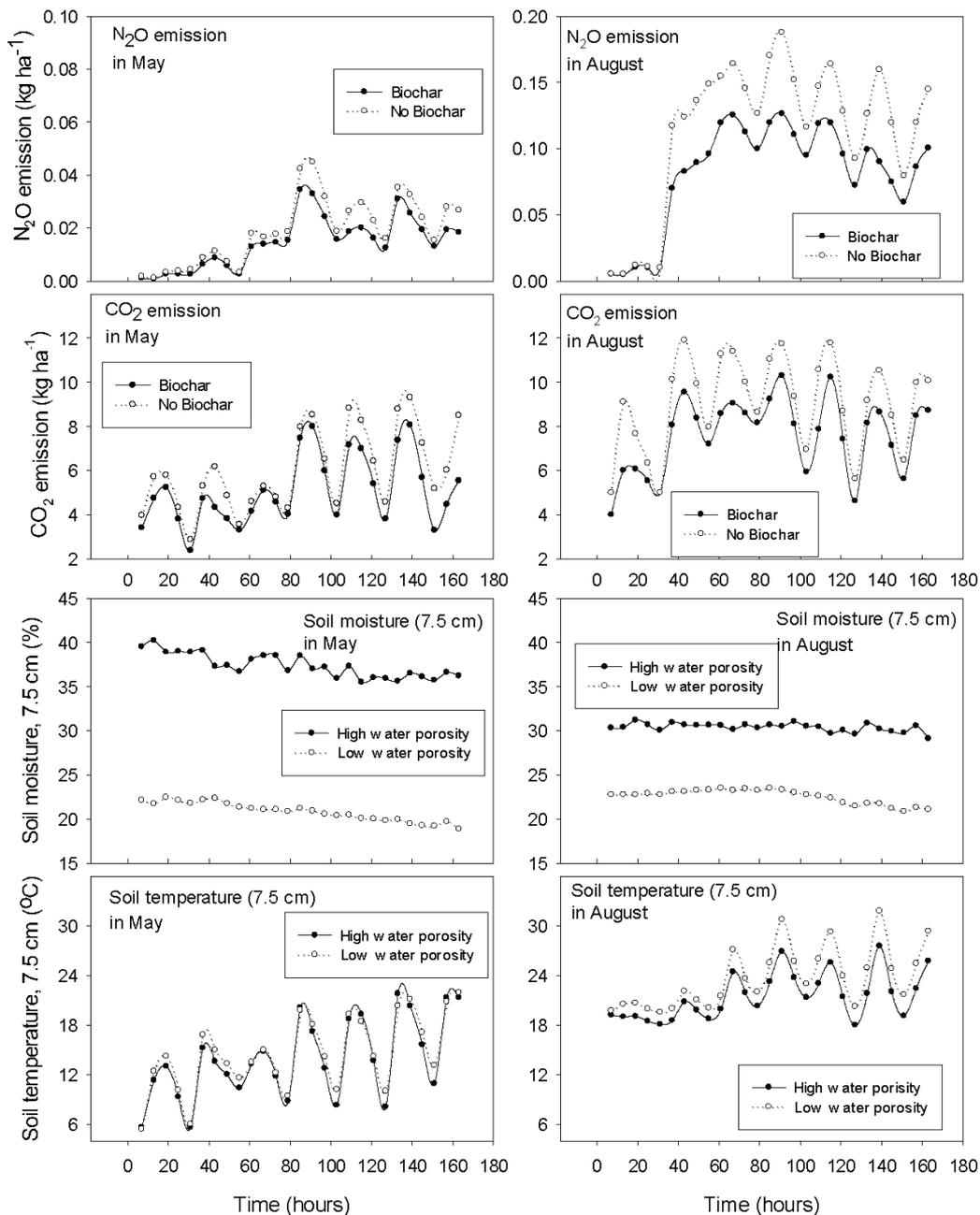


Fig. 1. The  $N_2O$ -N and  $CO_2$ -C emission, soil moisture (0–7.5 cm), and temperatures (7.5 cm) in the biochar and non-biochar experiments conducted in May and August. All treatments were fertilized with  $224 \text{ kg Ca(NO}_3)_2\text{-N ha}^{-1}$ .

hypothesis that  $O_2$  flux slowed with increasing WFP. However, because nitrate increased  $N_2O$ -N emissions in both the low and high WFP treatments, it is likely that denitrification/co-denitrification occurred in both WFP treatments (Table 2). In addition: (i) in the low WFP treatments,  $Ca(NO_3)_2$  addition increased  $CO_2$ -C emissions, (ii) inorganic N accumulation (final N – initial N) was less in the unfertilized high WFP ( $12 \text{ kg N ha}^{-1}$ ) than the unfertilized low WFP ( $49 \text{ kg N ha}^{-1}$ ) treatment; (iii) biochar reduced  $CO_2$ -C emissions by 12 and 14% in the low and high WFP treatments, respectively; (iv) the amplitudes of the May  $N_2O$  diurnal cycles were approximately 22% lower in the low WFP than the high WFP treatments when  $Ca(NO_3)_2$  was added; and (v)  $0.21\%$  of the applied  $Ca(NO_3)_2$ -N [ $(N_{2O_{\text{fertilized}}} - N_{2O_{\text{no-fertilizer}}})/N_{\text{applied}}$ ] was emitted as  $N_2O$  over 7 d. Cayuela et al. (2013)

also reported that biochar reduced  $N_2O$  emission, however they attributed the reduction to an enhanced electron shuttle which increased  $N_2$  emissions and an associated reduction in the  $N_2O$  to  $(N_2+N_2O)^{-1}$  ratio. Findings from Harter et al. (2014) support this hypothesis. Our study proposed an alternative hypothesis, where biochar sorbed soluble organic matter which reduced the amount organic substrate available for respiration.

During the August experiment,  $CO_2$ -C,  $N_2O$ -N, soil water, and soil temperature also followed diurnal cycles. In the low water filled porosity treatments (WFP = 42.5%), the  $CO_2$ -C,  $N_2O$ -N, and soil temperature phase shifts were similar and peak values occurred between 1700 and 1900 h, whereas the soil moisture peak occurred 10 h earlier (1600–1800 h). These findings suggest that GHG emissions and soil moisture cycles

**Table 2. Amplitude, peak max time (diurnal cycle phase shift), water-filled porosity (WFP) on soil temperature, accumulated N<sub>2</sub>O and CO<sub>2</sub> gas emission in May and August. The biochar to soil ratio was 1% and the N rates were 0 and 224 kg N ha<sup>-1</sup>.**

WFP	Biochar treatment	N source	Amplitude	Peak max	Total N loss	Amplitude	Peak time	Total C loss	
			kg N <sub>2</sub> O–N (ha×h) <sup>-1</sup>	24 h scale	in 7 d	kg CO <sub>2</sub> –C (ha×h) <sup>-1</sup>	24 h scale	in 7 d	
<b>May</b>									
Low	Biochar	CaNO <sub>3</sub>	0.0023	18	0.24	1.32	18	129	
	No_Bioch	CaNO <sub>3</sub>	0.0029	18	0.31	1.60	19	153	
	No_Bioch	No-N	0.0005	18	0.03	1.40	18	142	
		P	0.0002	0.308	<0.0001	0.430	0.228	0.013	
		LSD (0.1)	0.0003		0.013			9.3	
		Temperature	4.6	17	Avg. = 15°C				
		Soil water	ns	ns	Avg. = 24%				
	High	Biochar	CaNO <sub>3</sub>	0.0047	17	0.39	1.43	18	137
		No_Bioch	CaNO <sub>3</sub>	0.0068	18	0.51	1.75	18	162
		No_Bioch	No-N	0.0005	19	0.04	1.70	17	162
		P	0.0003	0.038	<0.0001	0.444	0.522	0.345	
		LSD (0.1)	0.0009	0.818	0.023				
		Temperature	5.1	17	Avg. = 14°C				
	Soil water	ns	ns	Avg. = 33.3%					
<b>August</b>									
Low	Biochar	CaNO <sub>3</sub>	0.0108	19	1.51	1.33	18	162	
	No_Bioch	CaNO <sub>3</sub>	0.0087	18	1.65	1.44	19	164	
	No_Bioch	No-N	0.0018	18	0.18	1.65	18	209	
	Biochar	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.0082	18	1.59	1.67	18	182	
	No-biochar	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.0098	18	1.73	2.10	18	202	
		P	0.0006	0.195	<0.0001	0.011	0.259	0.045	
		LSD (0.1)	0.0023		0.059	0.303		28.2	
		Temperature	2.5	19	Avg. = 24°C				
		Soil water	0.66	4.83	Avg. = 21.2%				
	High	Biochar	CaNO <sub>3</sub>	0.0159	18	2.21	1.60	18	205
No_Bioch		CaNO <sub>3</sub>	0.0271	19	3.07	2.23	18	245	
No_Bioch		No-N	0.0038	18	0.30	2.17	18	215	
Biochar		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.0128	18	1.84	1.83	18	189	
No-biochar		(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub>	0.0210	19	2.27	2.24	18	217	
		P	0.0002	0.342	<0.0001	0.004	0.355	0.006	
		LSD (0.1)	0.0042		0.141	0.25		18.7	
		Temperature	3.2	19	Avg. = 22°C				
		Soil water	0.96	3.24	Avg. = 35.3				

were offset from each other, and that gas samples collected at 700 h would underestimate GHG emissions, while samples collected at 1400 h would overestimate emissions. Others have noted that the sampling protocols can impact calculated GHG emissions (Parkin, 2008; Reeves and Wang, 2015).

In the no-biochar low WFP treatments, the percentage of N<sub>2</sub>O–N emissions over 7 d that was attributed to the addition of Ca(NO<sub>3</sub>)<sub>2</sub> or (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> were 0.656 and 0.692%, respectively. In addition, (i) CO<sub>2</sub>–C over 7 d was higher when (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was added than Ca(NO<sub>3</sub>)<sub>2</sub>; (ii) biochar reduced CO<sub>2</sub>–C emissions in the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> treatment; and (iii) biochar reduced N<sub>2</sub>O emissions 22 and 31% in the (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> and Ca(NO<sub>3</sub>)<sub>2</sub> treatments, respectively. Based on these results, nitrification and denitrification/co-denitrification contributed to N<sub>2</sub>O–N emissions in the low and high WFP treatments. It is important to point out that there are differences between our results and those of Archontoulis et al. (2016) and Rabot et al. (2015). Archontoulis et al. (2016) model focused on long-term impacts of biochar, whereas Rabot et al. (2015) evaluated nitrous oxide emissions without considering CO<sub>2</sub> emissions.

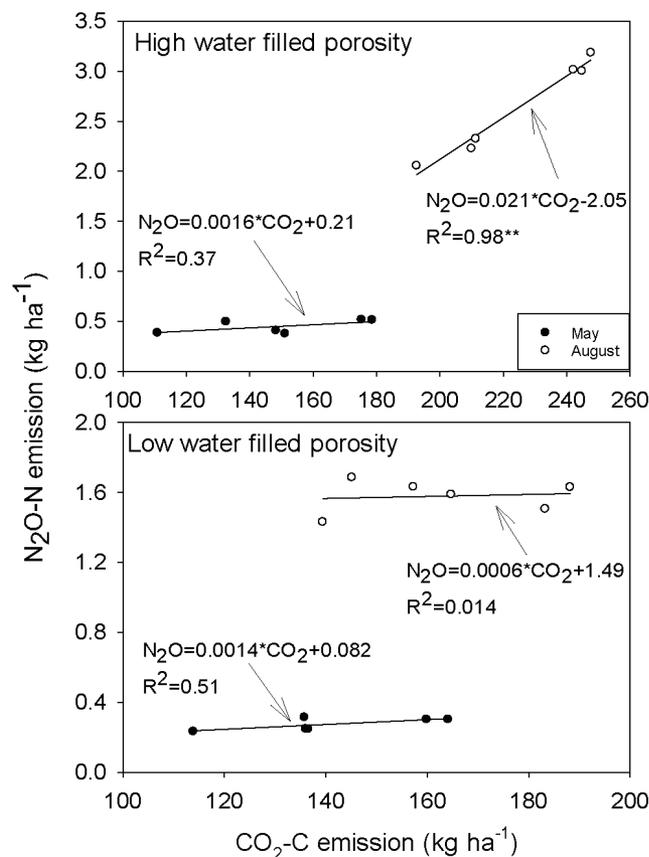
### Soil Inorganic Nitrogen and Nitrification

In May, the percentage of nitrate in the soil was higher in the fertilized than the no-fertilizer treatments at the end of the experiment, and the NH<sub>4</sub>–N concentrations were similar in the biochar and non-biochar treatments (Table 3). In the August experiment when Ca(NO<sub>3</sub>)<sub>2</sub> was added, between 91 and 92% of the remaining inorganic N was in the nitrate form. In the high WFP treatment, N<sub>2</sub>O emissions were primarily attributed to denitrification as opposed to dissimilatory nitrate reduction, because when Ca(NO<sub>3</sub>)<sub>2</sub> was added to the biochar treatment, ammonium concentrations remained unchanged from the beginning (23.8 kg NH<sub>4</sub>–N ha<sup>-1</sup>) to end of the experiment (23.8 kg NH<sub>4</sub>–N ha<sup>-1</sup>).

When (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> was added, the biochar treatments had less remaining ammonium than the non-biochar treatments ( $P = 0.05$ ). These results were attributed to biochar increasing the apparent nitrification rate. Based on changes in the inorganic N concentration in the low WFP (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> treatment, the apparent nitrification rates were 16 kg NH<sub>4</sub>–N (ha × d)<sup>-1</sup> [(8.9 mg (kg × d)<sup>-1</sup>] and 24.8 kg NH<sub>4</sub>–N (ha × d)<sup>-1</sup>

**Table 3.** The amount of relative nitrate and ammonium in the surface 15 cm of soil as impacted by soil water-filled porosity (WFP), N source, and fertilizer application timing.

WFP	Nitrogen	Biochar	May CaNO <sub>3</sub> applied	August	
				CaNO <sub>3</sub> applied	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> applied
				————— NO <sub>3</sub> -N/[(NH <sub>4</sub> +NO <sub>3</sub> )-N] —————	
Low	+N	Biochar	0.94	0.91	0.68
Low	+N	Non-biochar	0.94	0.92	0.62
Low	none	none	0.63	0.64	0.64
High	+N	Biochar	0.93	0.90	0.70
High	+N	Non-biochar	0.93	0.92	0.66
High	none	none	0.61	0.70	0.70
Initial			0.30	0.49	0.49
			<i>P</i>	<0.0001	0.003
			LSD (0.1)	0.02	0.05
				————— kg NH <sub>4</sub> -N m <sup>-2</sup> —————	
Low	+N	Biochar	13.9	22.7	73.9
Low	+N	Non-biochar	14.7	23.6	112.0
Low	none	none	24.6	24.2	24.2
High	+N	Biochar	16.7	22.8	83.3
High	+N	Non-biochar	18.2	22.1	97.3
High	none	none	20.8	21.3	21.3
Initial			23.5	23.8	23.8
			<i>P</i>	0.035	<0.0001
			LSD (0.1)	5.9	13.1



**Fig. 2.** The CO<sub>2</sub>-C and N<sub>2</sub>O-N gas emissions (g m<sup>-2</sup>) over the 7 d of each experiment. The figure shows the May and August experiments and the low (<50% water-filled porosity, WFP) and high WFP (>65% WFP) treatments. All treatments were fertilized with 224 kg Ca(NO<sub>3</sub>)<sub>2</sub>-N ha<sup>-1</sup>.

[(12.1 mg (kg × d)<sup>-1</sup>] in the non-biochar and biochar treatments, respectively. The factors responsible for this increase are poorly understood (Wells and Baggs, 2014), and may be indirectly related to biochar reducing dissolved organic C. It is important to point out that even though biochar appeared to increase nitrification, biochar reduced N<sub>2</sub>O emissions. Biochar enhanced nitrification was previously reported by Prommer et al. (2014).

The impact of biochar on CO<sub>2</sub> emission and associated respiration was attributed to biochar reducing dissolved organic C concentration in the Brandt silty clay loam from 44.5 to 37.5 μg (g soil)<sup>-1</sup> (*P* = 0.035). Decreases in the dissolved organic C, decrease the concentration of substrates available for microbial respiration and requirement to transfer electrons to nitrate. This reduction is attributed to biochar sorbing the dissolved organic C into its structure. Biochar has been reported to sorb other organic compounds. For example, Clay et al. (2016) reported that the biochar used in this experiment reduced the amount of positive and negatively charged herbicides in the soil solution. Prommer et al. (2014) had similar results and reported that biochar increased total organic C and decreased dissolved organic C, whereas Jones et al. (2011) reported that biochar increased simazine (6-chloro-*N,N'*-diethyl-1,3,5-triazine-2,4-diamine) sorption, which reduced its leaching and degradation. Biochar has also been reported to sorb inorganic compounds such as phosphate (Chintala et al., 2014c).

## CONCLUSIONS

Findings from this experiment show that biochar influenced C and N cycling, and that the relationship between N<sub>2</sub>O-N and CO<sub>2</sub>-C emission and soil respiration (CO<sub>2</sub>-C) was dependent on temperature and WFP. Biochar reduced CO<sub>2</sub>-C emissions in the high WFP Ca(NO<sub>3</sub>)<sub>2</sub> treatments in the May and August experiments 15.4 and 16.3 kg ha<sup>-1</sup>, respectively. Associated with this reduction was a 15.7% reduction in dissolved organic C due to

biochar additions. In the May experiment, the average temperature was between 13 and 14°C and N<sub>2</sub>O–N and CO<sub>2</sub>–C emissions were not correlated in either the low or high WFP Ca(NO<sub>3</sub>)<sub>2</sub> treatments. However in the August experiment, the average temperature was between 22 and 24°C and N<sub>2</sub>O–N and CO<sub>2</sub>–C emissions were highly correlated ( $r^2 = 0.98^{**}$ ) in the Ca(NO<sub>3</sub>)<sub>2</sub> high WFP treatment. The strong correlation between N<sub>2</sub>O–N and CO<sub>2</sub>–C emissions suggests that in August, respiration and N<sub>2</sub>O emissions were strongly linked (Fig. 2).

The temperature increase from May to August most likely was responsible for the increasing correlation between N<sub>2</sub>O and CO<sub>2</sub>. In addition, biochar increased the apparent nitrification from 16 to 25 kg NH<sub>4</sub>–N d<sup>-1</sup> in the August low WFP (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>–N ha<sup>-1</sup> treatment. The factor responsible for this increase is unknown, however it might be related to the biochar induced decrease in soluble organic C. In general, N<sub>2</sub>O–N emission increased with WFP and N rate and were reduced by biochar. The impact of biochar on reducing N<sub>2</sub>O–N emission was attributed to biochar reducing mineralizable soluble organic carbon (SOC) followed by a reduction in respiration. At both dates and WFP treatments, multiple mechanisms contributed to N<sub>2</sub>O emissions. Soils with different O<sub>2</sub> flux characteristics or biochars with different abilities to sorb organic compounds may have different results.

This research also showed that soil temperature and N<sub>2</sub>O and CO<sub>2</sub> emissions followed diurnal cycles and had common phase angles, which were not influenced by biochar. The diurnal cycle is attributed to decreasing GHG solubility with increasing temperature and/or that microbial activity mirrored the temperature cycle. The GHG diurnal cycles complicates the collection of representative gas samples. For example, if the samples were collected at 700 h, the GHG would be underestimated and if the samples were collected at 1400 h GHG would be overestimated. Because biochar did not influence the phase angle, a common sampling protocol could be used for both treatments. For this system, a single representative sample should be collected between 0900 and 1100 h. Soils with different temperature diurnal cycle phase shifts would require different sampling protocols. This interpretation is consistent with Parkin and Venterea (2010).

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