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The effects of walnut shell and wood feedstock biochar amendments on greenhouse gas emissions from a fertile soil

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ABSTRACT

Land application of biochar, as a strategy to enhance soil fertility and reduce greenhouse gas (GHG) emissions is receiving widespread interest. Short-term soil incubations (29 days) were used to investigate the effects of agriculturally relevant biochar applications from two contrasting feedstocks and temperatures on CO_2 and N_2O emissions from a fertile agricultural soil amended with different types of fertilizer (organic and synthetic). In addition, the effects of biochar on the denitrification process were examined using an acetylene based method to ascertain N_2O and N_2 emissions during denitrification. Complementary incubation experiments without soil (biochar and biochar with compost) examined the impact on natural or amended organic matter (compost) and biochar stability and surface chemistry were also investigated. Batch incubations (25 °C) of biochar (softwood pyrolyzed at 410 °C [WF_{410}] and 510 °C [WF_{510}] and walnut shell pyrolyzed at 900 °C [WA_{900}]) amended soils were performed to determine emissions of CO_2 and N_2O due to complete (absence of acetylene [C_2H_2]) and incomplete denitrification (presence of C_2H_2). Similarly, GHG emissions from the complementary incubations were also measured. Concurrent biochar surface compositional changes were investigated with attenuated total reflectance (ATR) Fourier transform infrared (FTIR) spectroscopy. Biochar effects on CO_2 emissions were not significantly different from controls. WA_{900} biochar (high pH) affects N cycling resulting in significantly higher emissions of N_2O under conditions of complete denitrification and of N_2 under conditions examining incomplete denitrification. WF_{410} (highest H/C ratio and lowest surface area) treatments with compost resulted in higher GHGs emissions which is attributed to a priming effect of the compost organic matter (COM). In addition, WF_{410} was most susceptible to degradation, evident from infrared spectroscopic analysis of the biochars. Although these results suggest that not all biochars provide substantial benefits as a soil amendment, the data do demonstrate potential for development of biochars with beneficial impacts on GHG emission mitigation and enhancement of soil C stocks.

1. Introduction

One potential strategy to enhance sequestration of C from plant litter and animal wastes is through production of biochar. Biochar is the product of the pyrolysis of biomass made with the intention of using it as a soil amendment, carbon storage, or filtration of percolating soil water (Lehmann and Joseph, 2009). The product is highly aromatic and has increased C stability relative to original feedstock materials. The use of biochar as a soil amendment has received increased attention since the discovery of the Terra Preta de Indio soils in the Amazon. Although not fully explained, these soils are believed to have received historical applications of anthropogenic black carbon or charcoal and today have higher organic C and improved soil fertility (Glaser et al., 2000, 2001; Lehmann and Joseph, 2009; Sombroek et al., 2003). Additionally, some research suggests that biochar application to soil may help increase N-retention and decrease N_2O emissions, while retaining native C, improving soil fertility, and increasing water retention in soil (Lehmann et al., 2006; Major et al., 2009; Rondon et al., 2007; Singh et al., 2010; Sohi et al., 2010). For these reasons, biochar is often proposed as a strategy to be used in agriculture to reduce GHG emissions and mitigate climate change (Woolf et al., 2010).

While reduced GHG emissions have been observed upon addition of biochar to soil (Case et al., 2012; Singh et al., 2010; Yanai et al., 2007), variable results regarding C and N cycling have also been noted and attributed to biochar and soil physical/chemical properties (Novak and Reicosky, 2009; Novak et al., 2010). Novak et al. (2010) showed increased CO_2 release after 25 and 67 days of incubation (pecan shell biochar with dried switchgrass in loamy sand). Another study investigating 16 biochars with three fertile soils (100 day incubation), also reported increased CO_2 and N_2O emissions in some of the treatments (Novak and Reicosky, 2009). The authors indicated

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Abbreviations: GHG, greenhouse gas; WFPS, water filled pore space; COM, compost organic matter; OM, organic matter; HSD, Honest Significant Difference; ANOVA, Analysis of variance; DOC, Dissolved organic carbon; ATR–FTIR, Attenuated Fourier Transformed Infrared spectroscopy.

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that these results highlight the fact that GHG emissions from biochar amended soils are strongly dependent on the biochar feedstock, pyrolysis method, and soil properties. The diversity of biochar source material, pyrolysis methods, soils, and agricultural systems lends complexity to determining the appropriate circumstances for biochar amendments.

To date no studies have attempted to investigate the effect of biochar on the complete and incomplete denitrification. Biochar has potential to enhance net denitrification because of its effect on several soil properties considered drivers of denitrification namely: water filled pore space (WFPS); inorganic N concentrations; labile C; pH; and oxygen content. Biochar has been shown to increase soil water holding capacity (Karhu et al., 2011; Major et al., 2009); increase soil cation exchange capacity and nutrient retention (Liang et al., 2006), and raise soil pH (Glaser et al., 2002; Novak and Reicosky, 2009), all of which directly or indirectly affect denitrification (Sahrawat and Keeney, 1986).

CO_2 and N_2O emissions from denitrification may occur during the priming of native organic matter following biochar amendment, here defined as changes in the mineralization rate of soil OM (Zimmerman et al., 2011). Both increased (Novak et al., 2010; Wardle et al., 2008) and decreased (Kuzyakov et al., 2009; Spokas and Reicosky, 2009) rates of OM decomposition in the presence of biochar have been observed. Low temperature biochar (made at <450 °C) has been shown to prime OM mineralization and in turn undergo concurrent degradation (Luo et al., 2011; Zimmerman et al., 2011), however, no study has investigated the concurrent surface compositional changes in the biochar.

The aims of this study are to determine 1) how biochar soil amendments (at agriculturally relevant rates of N fertilization) to fertile soils affect C and N cycling; 2) how biochar additions affect the ratio of N_2O and N_2 emissions during denitrification; 3) how these biochar affect the decomposition of compost organic matter (COM); and 4) how the incubations impact the structural stability of biochar and alter their composition of surface functional groups. Due to the fact that denitrification is often considered the predominant process responsible for N_2O emissions in agricultural systems (Opdyke et al., 2009; Senbayram et al., 2009), particular emphasis has been given to this process.

2. Materials and methods

2.1. Soil and biochar

Soil was collected from the Ap horizon in a walnut orchard (Winters, CA). The soil series is Yolo (fine-silty, mixed, nonacid, thermic Typic Xerorthent) and contains approximately 7% sand, 62% silt and 31% clay (silt loam). The compost used was a composite sample from the composting facility at the Agricultural Sustainability Institute Student Farm in Davis, CA. Subsamples were collected for moisture content determination by oven drying at 105 °C and the remainder of the soil and compost were air dried and passed through a 2 mm sieve. The untreated soil was analyzed for total C and N with a C/N Analyzer (ECS 4010 Costech Analyzer), pH and moisture content (Table 1).

Two commercially available biochars, namely low temperature (410 °C) wood feedstock (WF_{410}); high temperature (510 °C) wood feedstock (WF_{510}), and a third, high temperature (900 °C) walnut shell (WA_{900}) biochar, were obtained from suppliers (see supporting information of Mukome et al., 2013). The wood biochars were made from a feedstock mixture of primarily Douglas fir (*Pseudotsuga menziesii*) and additional White fir (*Abies concolor*) by slow pyrolysis with 25 min residence time and 50 psi of steam at the end of the process. The walnut shell (*Juglans californica*) biochar was made using a Biomax 50 downdraft gasifier. Details regarding biochar characterization are provided in Mukome et al. (2013). Briefly, samples were sieved to pass through a 2 mm mesh and analyzed for pH (1:2 w/v in water), and surface area analysis (BET N_2 sorption, Quantachrome Autosorb-1). Surface area was determined on ball ground samples and after 16 h outgassing at 120 °C. The ash content was determined by dry oxidation of the biochar at a temperature of 575 ± 25 °C (ASTME1755-95, 1995). The total surface basicity of the biochars was determined by the conventional back titration method (Jindarom et al., 2007). For this, about 0.20 g of biochar was soaked in 25 mL of 0.025 M HCl solution in a centrifuge tube and agitated for 48 h at room temperature. The suspension was centrifuged and the filtered supernatant titrated with 0.025 M NaOH solution to determine the remaining HCl in solution.

Table 1
Properties of soil (Yolo silt loam) and compost used in incubation experiments.

	Soil	Compost
pH_w (1:2)	7.8	9.1
Moisture (wt.%)	3.3	4.6
C (wt.%)	1.94	7.4
N (wt.%)	0.18	0.79
C/N	10.8	9.4

2.2. Incubations

Biochar (0.5 g) was mixed into 50 g of soil for a 1% mixture (w/w), which equates to a field-application rate of approximately 12 metric ton ha^{-1} assuming a 10 cm incorporation depth, as the soil bulk density was 1.2 g/cm^3. Treatments consisted of soil + biochar + compost. Compost was augmented to the different biochar treatments in order to achieve total application rate of 100 mg N kg^{-1} soil or 120 kg N ha^{-1}. A comparative treatment of soil with inorganic fertilizer (Surea) and controls of soil only (S only), and soil with compost (SC) were also setup. N application rates for the urea and compost treatments were 100 mg N kg^{-1} soil or 120 kg N ha^{-1}. Breakdowns of the components of each treatment are shown in the supplementary data, Table S1.

Short term CO_2 and N_2O evolution were determined by placing the soils in 1 L gas tight jars and incubating at 25 °C in the dark for 29 days. The jars were placed in a randomized block design with an initial moisture content of 90% WFPS and allowed to dry down and maintained at a moisture content of 55 to 60% WFPS. Headspace gas samples (20 mL) were withdrawn from the enclosed headspace using gas tight syringes, with two way stopcocks, and immediately injected into pre-evacuated 12 mL gas exetainer tubes (Labco, Buckinghamshire, UK). From the same samples, N_2O and CO_2 were measured via gas chromatography (Shimadzu GC 2014) equipped with an electron capture detector (ECD) for N_2O and a flame ionization detector (FID) for CO_2 detection. The difference in syringe and exetainer volumes ensured the exetainers were over pressured thus minimizing external air diffusion. The incubations were performed in triplicate with daily samplings for the first week and then on days 7, 10, 14, 18, 21, 24 and 29.

The acetylene inhibition method, used to determine the emissions of N_2O to N_2 gases, were set up with 10% v/v of C_2H_2 added after removing an equivalent amount of air from the headspace. C_2H_2 was generated by reacting CaC_2 with distilled water prior to use. After each sampling, the jars were vented to ensure no residual gas was retained. Headspace samples of ambient air similarly capped were used to correct sample gas concentration. For all the incubations, extractable DOC (dissolved organic carbon), NH_4–N (ammonium), NO_3–N (nitrate) and pH were measured before and after incubation. Soil (4 g) was extracted with 40 mL of 0.5 M K_2SO_4 (Jones and Willett, 2006) by shaking for 1 h on a reciprocating shaker, filtering using Whatman no. 42 paper, and then analyzing the filtrates within 48 h. DOC concentrations were determined with a Shimadzu TOC–TN analyzer (Shimadzu Corp., Kyoto, Japan) and NH_4–N (Forster, 1995) and NO_3–N (Doane and Horwath, 2003) concentrations were determined colorimetrically via UV–Vis (Genesys 10S UV–Vis, Thermo Scientific) at a wavelength of 540 nm (NH_4–N) and 650 nm (NO_3–N).

To investigate the effect of biochar on non-biochar C pools (represented by compost and compost extract), incubations of biochar with compost (C), similar to that used before, and compost extract (Cext) only (no soil) were conducted (supplementary information, Table S1). The compost extract was obtained by shaking organic compost (335 g) in Barnstead Nanopure water (1 L) for 24 h, centrifuging at 5600 RCF for 20 min, and filtering through a 0.8 μm cellulose filter. An aliquot (100 mL) of the extract was further concentrated by evaporation under compressed air, without heating, to a final volume of 10 mL. For these incubations, biochar (0.5 g), with appropriate amendments, was placed in 1 L gas tight jars and incubated at 25 °C in the dark for 29 days. The jars were placed in a randomized block design and after wetting the biochar, gas collection and analysis was performed as before.

After the incubation period, subsamples of the biochar were manually isolated from the jars, air dried, and analyzed for changes in surface functionality via attenuated total reflectance (ATR) Fourier transform infrared (FTIR) on a Thermo Nicolet 6700 spectrophotometer with a diamond single bounce ATR accessory (GladiATR, PIKE Technologies, Madison, WI) and DTGS detector at ambient temperature (23 ± 1 °C). All spectra were collected in triplicate using 4 cm^{-1} resolution and 1.2 kHz scanning speed for a total of 128 co-added scans.

2.3. Gas flux calculations

Gas flux was calculated by converting the gas concentrations into mg L^{-1} according to Eq. (1):

$$f = \frac{\left(\frac{C_t[V_h + (V_w \alpha)]}{CF_n}\right) MP}{RTWt} \tag{1}$$

where f is the gas flux (μg gas g^{-1} soil day^{-1}); C_t (μL gas L^{-1}) is the gas concentration in the gas phase at time t; V_h (mL) is the volume of the headspace; V_w (mL) is the volume of water in the soil during the incubation; α (mL gas mL^{-1} water) is the Bunsen absorption coefficient = 0.759 for CO_2 and 0.544 for N_2O at 25 °C; CF_n is the sampling correction factor (1 for the first sample); where M is the atomic weight of C or N (g mol^{-1}); P is the standard atmospheric pressure (101.325 kPa); R is the universal gas constant (8.31451 L kPa mol^{-1} K^{-1}); T is temperature in Kelvin (298.15°K); W is the oven dry mass of soil (g); and t is the time between the first and second gas sample collection. Emissions were corrected for background gas by subtracting concentrations measured in controls of ambient air. For the calculations, the N_2O in the headspace volume was assumed to be equilibrium with the N_2O in the soil solution.

Emissions of CO_2 and N_2O were calculated as arithmetic means of the triplicate samples. Data was analyzed using analysis of variance (ANOVA) to test for significant differences between the treatments. If a difference existed, the Tukey's Honest Significant Difference (HSD) test was used to determine which pair-wise treatments were significantly different at the $P < 0.05$ level.

3. Results

3.1. Soil and biochar characterization

The soil and compost used in the study both had a $pH > 7$ and the C/N ratios were 10.8 and 9.4 respectively (Table 1). Differences in pH, ash content, surface area and H/C ratios are apparent for the biochar made from wood feedstock compared to the walnut shells (Table 2). The WA_{900} biochar has the highest ash content, and surface area. WA_{900} biochar is also strongly basic with a basicity value (11.7 meq/g) an order of magnitude greater than the two wood biochars (0.8 and 0.43 meq/g). The difference in pyrolysis temperature results in differences in the two wood feedstock biochars, particularly in the C/N ratio (320 and 233), H/C ratio (0.3 and 0.76), and surface area (156 and 2.8 m^2 g^{-1}) for WF_{510} and WF_{410}, respectively. Comprehensive data on biochar characterization is provided in Mukome et al. (2013).

3.2. Soil–biochar incubations

Upon initial addition of amendments, only the pH of the soils with the walnut shell biochar ($WA_{900}C$) treatments increased significantly from 7.8 to 8.6 (Table 3). At the end of the incubations, the soil pH changed for only the Surea treatment (in the absence of C_2H_2), 7.9 to 7.4 and the $SWF_{510}C$ treatment (only in the presence of C_2H_2), 7.8 to 8.2.

In the absence of C_2H_2, the greatest emission of CO_2 occurred on the first day of incubation with values ranging from 39 to 91 mg CO_2–C kg^{-1} soil, the only day when there were significant differences in the treatments (Fig. 1). There was no statistically significant difference in the cumulative CO_2 emissions between the six treatments and the greatest cumulative emission of CO_2 arose from the soil/compost/WF_{410} treatment (636 mg CO_2–C kg^{-1} soil). In the presence of C_2H_2, the cumulative CO_2 emission increased between 8 and 48%, with the greatest increase in the $SWA_{900}C$ treatment and the lowest in the soil only and SC treatments (supplementary information, Fig. S1); however, there was no statistical difference in the daily and cumulative treatments.

N_2O emissions from the $SWA_{900}C$ treatment in the C_2H_2 free incubations were significantly different from most of the treatments through 7 days and particularly on Days 2 and 3 (Fig. 2a). By Day 10, the treatment significantly different from all the other treatments was Surea (Fig. 2a table). The largest emissions occurred on the first day (ranging from 162 to 411 μg N_2O–N kg^{-1} soil for the Surea and $SWA_{900}C$ treatment, respectively) with substantial emissions continuing for another four days. As expected the cumulative N_2O emissions in the presence of C_2H_2 increased significantly for all the treatments when compared to emissions in the absence of C_2H_2 (Fig. 2b), but Day 1 was the only time period which showed significant differences between treatments.

The increase in N_2O emission for all the treatments in the presence of C_2H_2 indicates that the C_2H_2 inhibition method successfully inhibited N_2O reductase activity (Fig. 2b). Differences in the N_2O emissions in Fig. 2a and b were used to calculate N_2 emissions and the results used to determine N_2O/N_2 ratios (Fig. 3).

Analysis of the soil chemical data (Table 3) for all incubations also showed only significant differences between $SWA_{900}C$ and all other treatments after incubation (pH and NH_4–N). Differences in the DOC data were apparent from Day 0 with similar DOC levels for the Surea treatment and the WF treatments; and the SC treatment showing a greater concentration of DOC while the $SWA_{900}C$ treatment showed a substantial decrease (Table 3). After 29 days of incubation (without C_2H_2), the DOC increased for all treatments except the SC and Surea treatments, with the greatest increase observed in the soil only treatment (Table 3). Under the same conditions, NH_4–N increased for only the soil alone treatment; was almost unchanged for

Table 2
Physical and chemical characteristics of WA_{900} (walnut shell biochar), WF_{510} (wood feedstock biochar) and WF_{410} (wood feedstock biochar) used in the incubations.

Biochar	Processing method	pHw (1:2)	Ash (wt.%)	C (wt.%)	N (wt.%)	C/N ratio	CEC (cmolc/kg)	Basicity (meq/g)	H:C ratio	BET surface area (m^2/g)
Walnut shell (900 °C)	Gasification	9.7	46.6	55.3	0.47	118	33.4	11.7	0.22	227
Wood stock* (510 °C)	Fast pyrolysis	7.3	3.1	83.9	0.36	233	13.2	0.8	0.3	156
Wood stock* (410 °C)	Fast pyrolysis	7.1	2.7	65.7	0.21	313	10.7	0.43	0.76	2.82

* Feedstock: Douglas fir (mostly) and White fir.

Table 3
Variations in pH, dissolved organic carbon (DOC), nitrate (NO_3), ammonium (NH_4) concentrations and net N mineralization rates of soil treatments after 29 days of incubation in the absence of C_2H_2. Day 0 values are from analysis of bulk samples.

	pH			DOC (mg/kg)			NO_3–N (mg/kg)			NH_4–N (mg/kg)		
	Day 0	Day 29	Day 29[¥]	Day 0	Day 29	Day 29[¥]	Day 0	Day 29	Day 29[¥]	Day 0	Day 29	Day 29[¥]
Soil	7.8 *a*[*]	7.8 (0.07)*a*	7.7 (0.04)*a*	191*a*	247 (15)*a*	192 (6)*a*	2.9*a*	38 (5)*a*	nd	5.7*a*	8.2 (0.1)*a*	35 (3)*a*
SC	7.8 *a*	7.9 (0.05)*a*	7.9 (0.04)*a*	216*b*	197 (6)*b*	221 (2)*a*	6*b*	50 (7)*a*	nd	5.7*a*	0.6 (0.1)*a*	28(2)*a*
S_{urea}	7.9 *a*	7.4 (0.08)*a*	7.9 (0.02)*a*	197*a*	194 (6)*b*	196 (10)*a*	2.9*a*	135 (16)*b*	nd	6.7*b*	1.2 (0.6)*a*	101 (4)*b*
$SWF_{410}C$	7.8 *a*	7.8 (0.01)*a*	7.9 (0.06)*a*	201*a*	233 (20)*ab*	199 (4)*a*	4.9*b*	43 (5)*a*	nd	5.6*a*	5.5 (0.6)*b*	29 (4)*a*
$SWF_{510}C$	7.8 *a*	7.8 (0.02)*a*	8.2 (0.02)*b*	202*a*	206 (7)*ab*	242 (23)*b*	4.9*b*	51 (2)*a*	nd	5.6*a*	3.8 (0.6)*b*	30 (2)*a*
$SWA_{900}C$	8.6 *b*	8.6 (0.02)*b*	8.5 (0.04)*c*	160*a*	192 (9)*b*	192 (5)*a*	5*b*	63 (11)*a*	nd	5.7*a*	0.6 (0.1)*a*	11 (2)*c*

Values in parenthesis are standard errors of the means.
S = Soil, C = Compost, Cext = Compost water extract.
nd — not detected.

[*] A one-way ANOVA was used to examine for significance of biochar additions at $p<0.05$ and different lowercase letters denote significant differences.

[¥] Data from treatments in the presence of C_2H_2.

the $SWF_{410}C$ treatment; and decreased for all the other treatments (Table 3). The treatments with no C_2H_2 had more NO_3–N than NH_4–N at the end of the incubations while the C_2H_2 dosed treatments, had an increase in NH_4–N but no NO_3–N present.

3.3. Compost–biochar incubations

Treatments with WF_{410} biochar had consistently higher emissions of CO_2 than WF_{510} (Fig. 4; figure of daily emissions analogous to soil incubations in supplementary data, Fig. S3) and was not impacted by C_2H_2. Addition of compost/compost extract to the wood feedstock biochar did not result in an additive effect on CO_2 emissions when compared to the individual components. CO_2 production from $WF_{410}Cext$ (refer to Supporting data, Table S1 for composition) was significantly greater than $WF_{410}C$ while emissions for $WF_{510}C$ and $WF_{510}Cext$ were not different. The $WF_{410}C$ and $WF_{410}Cext$ treatments both had greater emissions of CO_2 than those of compost and compost extract alone but all were less than biochar alone (WF_{410}). For the WF_{510} biochar, combinations of the biochar and compost or compost extract ($WF_{510}C$ and $WF_{510}Cext$) resulted in lower emissions of CO_2 than the compost and compost extract alone. There was a net negative emission of CO_2 from the three WA_{900} treatments when compared to the experimental controls for the entire incubation period evidenced by the negative emissions (Fig. 4). Over the first 15 days, the head space CO_2 was non-detectable in all three treatments.

The greatest emission of N_2O occurred on the second day of the incubations in the $WF_{410}C$ treatment with a emission of 37 µg

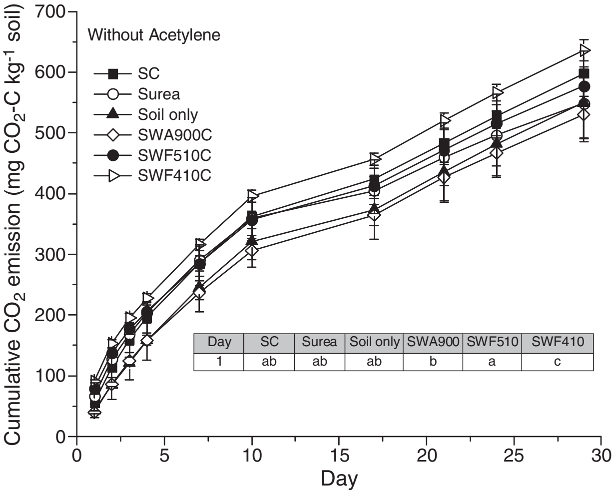

Fig. 1. Cumulative CO_2 emission from incubations of soil only, soil/compost, soil/urea and soil/compost/biochar of the three biochars; WA_{900}, WF_{410} and WF_{510} in the absence of C_2H_2 after 29 days. Means and standard errors of the incubations are shown. Only days with significant differences in emissions are shown in the inset table with treatments with different lowercase letters denoting significant differences at $p<0.05$. S = soil, C = compost, WA_{900} = walnut shell biochar, WF_{410} = low temperature wood feedstock biochar, and WF_{510} = high temperature wood feedstock biochar.

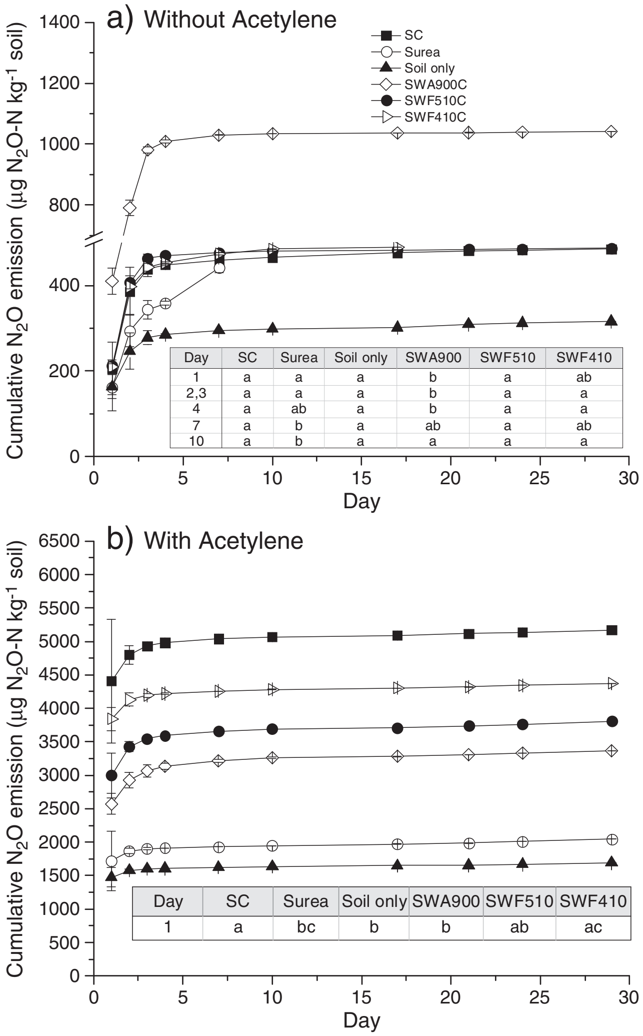

Fig. 2. Cumulative N_2O emission from incubation of soil only, soil/compost, soil/urea and soil/compost/biochar of the three biochars; WA_{900}, WF_{410} and WF_{510} a) in the absence and b) presence of C_2H_2 after 29 days. Means and standard errors of the incubations are shown. Only days with significant differences in emissions are shown in the inset table with treatments with different lowercase letters denoting significant differences at $p<0.05$. S = soil, C = compost, WA_{900} = walnut shell biochar, WF_{410} = low temperature wood feedstock biochar, and WF_{510} = high temperature wood feedstock biochar.

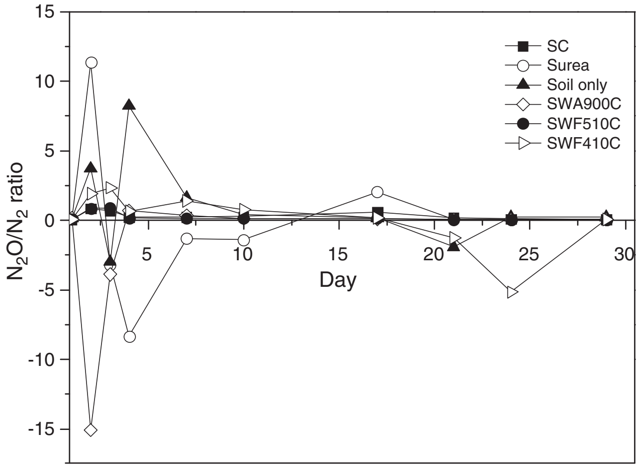

Fig. 3. N_2O/N_2 emission ratios for the soil only, soil/compost, soil/urea and soil/compost/biochar of the three biochars; WA_{900}, WF_{410} and WF_{510} treatments calculated from measurements of N_2O in the headspace of C_2H_2 and non-C_2H_2 amended treatments. Negative values for N_2O/N_2 ratio are due to greater emission of N_2O from non-C_2H_2 than C_2H_2 incubations.

N_2O–N g^{-1} N added in the absence of C_2H_2. The $WF_{410}C$ incubations had a significantly greater cumulative emission of N_2O (47 µg N_2O–N g^{-1} N added) than all the other treatments (Fig. 5a). For the first day, all the treatments had measurable N_2O emissions but subsequent emissions were negative. In the presence of C_2H_2, both wood feedstock biochars stimulated N_2O emissions from the compost, with greater emissions occurring in the WF_{410} treatment. The $WF_{410}C$ treatment had a large increase in N_2O emission from that observed in the absence of C_2H_2 (Fig. 5b).

ATR–FTIR spectra of the biochar before and after incubation for the various treatments reveal alteration of the biochar surface depending on incubation conditions. This modification of biochar was most evident in the spectra of the WF_{410} biochar (Fig. 6) that showed changes in aliphatic (2925 and 2850 cm^{-1}); aromatic carbonyl or carboxyl (1690 cm^{-1}); and polysaccharide (1034 cm^{-1}) peak intensities (assignments of all major peaks are given in the Supplementary data, Table S2). In order to improve our ability to make qualitative and pseudo quantitative comparisons between treatments, IR peak intensity ratios were calculated from the peak intensities of the peaks at 2925 cm^{-1} (ν(C–H) vibrations in CH_3 and CH_2); 2850 cm^{-1} (ν(C–H) vibrations in CH_3; and CH_2) and 1034 cm^{-1} (polysaccharide) relative to the peak at 1690 cm^{-1} (ν(C=O) vibration in aromatic carbonyl/carboxyl C=O stretching) to investigate degradation of the biochar (Table 4). The spectra showed changes consistent with degradation of the biochar under the different treatment conditions. Spectra of the other biochars were collected and showed similar changes so are not included.

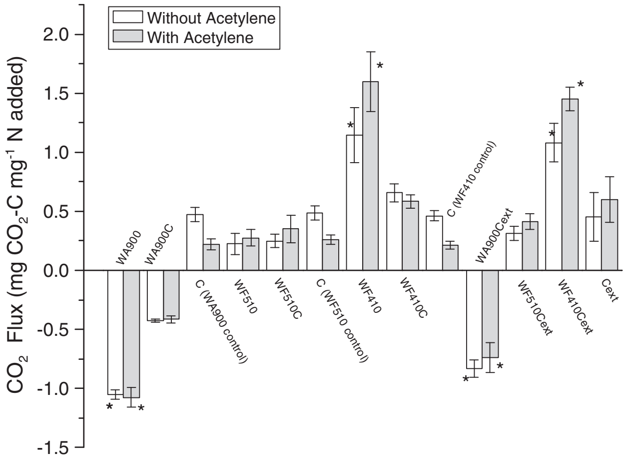

Fig. 4. Cumulative CO_2 emission from incubations of biochar only, biochar/compost and biochar/compost water extract of the three biochars; WA_{900}, WF_{410} and WF_{510} in the absence and presence of C_2H_2 after 29 days. Means and standard errors of the incubations are shown. Due to differing compost additions, the compost only treatments are relative to a biochar (name in parentheses). Asterisks (*) denotes significant differences (p<0.05). C = compost, Cext = compost water extract, WA_{900} = walnut shell biochar, WF_{410} = low temperature wood feedstock biochar, and WF_{510} = high temperature wood feedstock biochar.

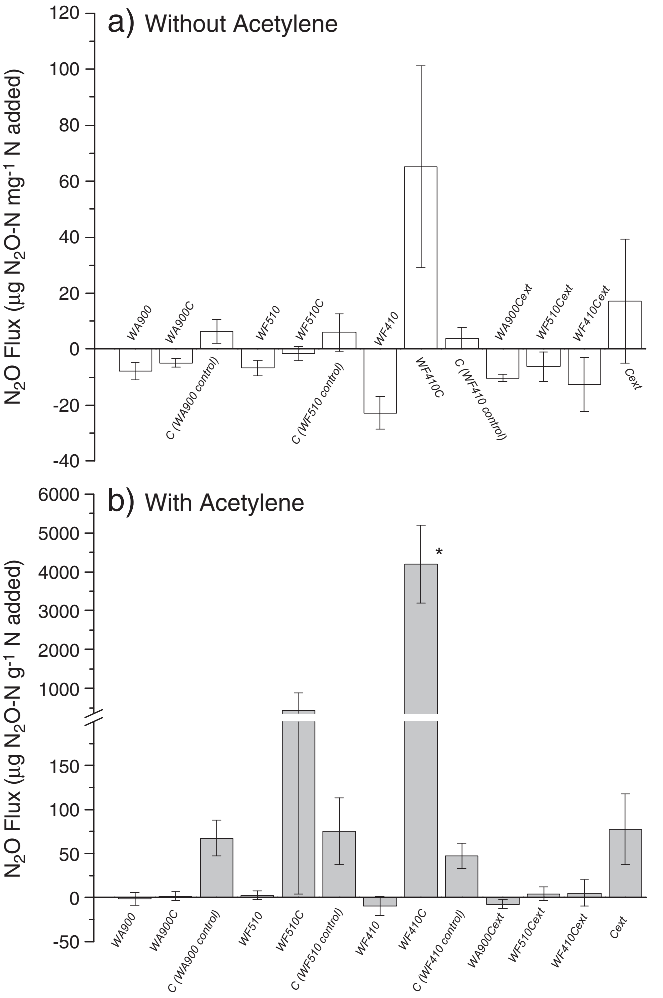

Fig. 5. Cumulative N_2O emission a) in the absence of C_2H_2 and b) in the presence of C_2H_2 of biochar only, biochar/compost and biochar/compost compost water extract of the three biochars; WA_{900}, WF_{410} and WF_{510} after 29 days. Means and standard errors of the incubations are shown. Due to differing compost additions, the compost only treatments are relative to a biochar (name in parentheses). Due to differing compost additions, the compost only treatments are relative to a biochar. Asterisks (*) denotes significant differences (p<0.05). C = compost, Cext = compost water extract, WA_{900} = walnut shell biochar, WF_{410} = low temperature wood feedstock biochar, and WF_{510} = high temperature wood feedstock biochar.

4. Discussion

The three biochars used in this study represent the most popular feedstock (softwood) at a common commercial pyrolysis temperature (410 °C and 510 °C) and a feedstock with very different physiochemical properties compared to wood (walnut shell) that is a by-product of bio-energy production. The walnut shell biochar also presents the probable future scenario for biochar, where local biomass feed stocks will be used as bio-energy sources with the biochar by-product available for soil amendment.

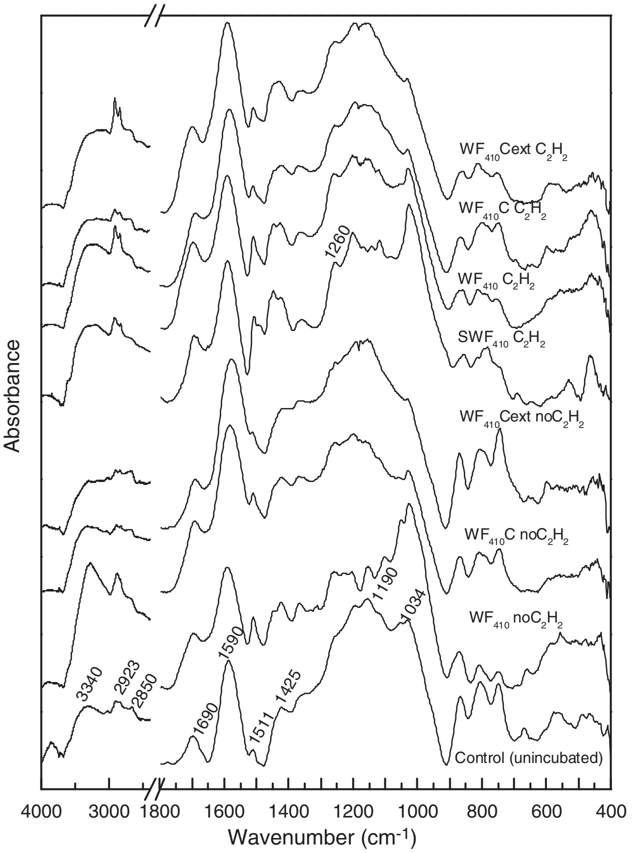

Fig. 6. ATR–FTIR spectra of the low temperature wood feedstock biochar (WF_{410}) biochar treatments in the absence and presence of C_2H_2. C: compost, Cext: compost water extract, and WF410: low temperature wood feedstock biochar.

4.1. Soil–biochar incubations

C and N cycling was evaluated by monitoring CO_2 and N_2O emissions, measuring soluble N concentrations and dissolved organic carbon (DOC). The large emissions of CO_2 and N_2O observed at the beginning of the incubations (Fig. 1 and 2) are likely attributed to increased microbial activity and mineralization resulting from the rewetting of soil and this has been attributed to several processes including increased soil organic carbon turnover from the breakdown of microaggregates and mineralization of substrate from desiccated (due to drying) microbial cells by surviving microbes (Butterly et al., 2010; Garcia-Montiel et al., 2003).

Denitrification was investigated through experiments conducted at high water filled pore space (WFPS) using acetylene (C_2H_2) to inhibit the reduction of N_2O in soils, a well-established method for determination of incomplete denitrification (Berg et al., 1982; Davidson et al., 1986; Hynes and Knowles, 1978; Ryden et al., 1979; Wrage et al., 2004; Yoshinari et al., 1977). The amount of N_2O produced in the presence of C_2H_2 is equivalent to the $N_2O + N_2$ emission, accumulated as N_2O in the headspace of microcosms and represents an estimate of the total N loss by denitrification (Ryden et al., 1979). This method also results in inhibition of nitrification and nitrifier–denitrification processes (Berg et al., 1982; Wrage et al., 2004) potentially resulting in underestimation of denitrification. Several other limitations of this technique have been published and include: the potential for acetylene to act as a C source in conditions where C is limiting (Terry and Duxbury, 1985; Yeomans and Beauchamp, 1982); uneven penetration of the gas into soil microsites (Rudolph et al., 1991); incomplete inhibition at low nitrate concentrations (Knowles, 1990); and incomplete inhibition of N_2O reductase (Qin et al., 2012; Yu et al., 2010). Despite the limitations, this method is validated under conditions where nitrate content is not limiting (Felber et al., 2012; Seitzinger et al., 1993).

4.1.1. Soil–biochar: carbon mineralization

The study application rates of these biochars do not significantly affect emissions of CO_2. A similar result was also observed by Novak et al. (2010) on amending a Norfolk Ap horizon soil with pecan shell biochar at application rates of 0, 5, 10 and 20 g kg^{-1}, attributing increased CO_2 emissions to the mineralization of added switchgrass. Several other studies have shown decreased CO_2 emissions with biochar amendment. In a temperate climate study with biochar made from the fast pyrolysis of wood chips (with similar C, N and surface area to WF_{410}), Spokas et al. (2009) found biochar to suppress CO_2 emission from incubations with a Waukegan silt loam from Minnesota assuming the behavior of the biochar alone is the same as in the soil plus char system. Significantly, this suppression was only observed at an application rate of 5 wt.% biochar and greater. Studies such as Yanai et al. (2007) and Spokas and Reicosky (2009) (that have also shown significant reduction of GHG emissions by biochar) have used application rates as high as 60 wt.% biochar, which given the current cost of biochar, are impractical for most farmers.

The only significantly different CO_2 emission observed in the $SWF_{410}C$ (i.e., $SWF_{410} + C$) treatment, Day 1- (Fig. 1), can likely be attributed to biochar mineralization. This result was consistent with a 180 day incubation study of a clay–loam soil (pH 3.7 and 7.6) amended with a grass (*Miscanthus giganteus*) derived biochar (350 and 700 °C) (Luo et al., 2011). Mineralization of the lower temperature biochar was greatest and the maximum emission occurred on the first day. Biochar pyrolyzed at low temperature, as in our study, has a high H/C ratio and this is a good indicator of the susceptibility of a material to rapid degradation by soil microorganisms (Van Zwieten et al., 2009).

The increase in CO_2 emissions of the treatments in the presence of C_2H_2 could indicate utilization of C_2H_2 as C source. However, insignificant differences between the CO_2 emissions in the presence and absence of C_2H_2 for the controls (SC and Soil only) suggest little to minimal occurrence of this phenomenon in this study. In addition, the largest differences in the CO_2 emissions occurred at the beginning of the incubations when the soils had only limited exposure to C_2H_2, thus reducing the importance of this limitation. Insignificant differences in the DOC for most the treatments between the start and completion of the incubation (with and without C_2H_2) is further evidence for negligible conversion of C_2H_2 to a C source (Table 3). WA_{900} (highest surface area and CEC) reduced available DOC at the beginning of the incubation and through the study when compared to the SC control. On-going research in our lab has shown a similar trend

Table 4
Fourier transformed infrared (FTIR) peak intensity ratios of the WF_{410} biochar treatments in the absence and presence of C_2H_2.

Wavenumber ratio	Unincubated control	WF_{410} noC_2H_2	WF_{410}C noC_2H_2	WF_{410}Cext noC_2H_2	WF_{410} C_2H_2	WF_{410}C C_2H_2	WF_{410}Cext C_2H_2	SWF_{410}C C_2H_2
1034/1690[a]	5.93	3.42	1.71	2.70	1.91	1.89	1.65	3.20
2850/1690[b]	2.50	2.03	0.91	1.19	1.13	1.01	1.28	1.19
2923/1690[c]	2.49	1.98	0.88	1.18	1.22	1.05	1.40	1.32

[a] 1034/1690 (polysaccharide/aromatic carbonyl or carboxyl).
[b] 2850/1690 (aliphatic C/aromatic carbonyl or carboxyl).
[c] 2920/1690 cm^{-1} (aliphatic C–H/aromatic carbonyl or carboxyl).

of DOC sorption to the different biochars (as seen in the treatments in the absence of C_2H_2) that correlate well with biochar surface area, as well as the observed increase in DOC in the $SW_{410}C$ treatment from the breakdown of this biochar in solution.

4.1.2. Soil–biochar: nitrogen mineralization

Not all biochars affect denitrification and effects on N mineralization are dependent on the nature of the biochar. In the absence of C_2H_2, the N_2O emissions from the softwood biochar treatments ($SWF_{410}C$ and $SWF_{510}C$) were not significant different to the SC control (Fig. 2a). The high N_2O emission from the WA_{900} biochar treatment (pH 9.7, CEC 33.4 cmol/g and surface area 227 m^2/g), also in the absence of $C_2H_{2,}$ was consistent with the impact of this biochar on the aforementioned drivers of N_2O emission (WFPS, inorganic N concentrations, labile C, pH, and oxygen content) during denitrification resulting in increased N_2O emission (Fig. 2a). This result was also consistent with a study of a clay loam soil amended with cattle feedlot waste biochar at a similar application rate (Scheer et al., 2011). However, several studies have shown biochar to suppress N_2O emissions from amended soils (Spokas and Reicosky, 2009; Yanai et al., 2007). Case et al. (2012) showed N_2O emission suppression from a sandy loam soil amended with biochar (made from a mixture of hardwood trees) and attributed it to increased soil aeration as well as microbial or physical immobilization of nitrate, the substrate for denitrification.

The order of emissions of N_2O from complete denitrification were correlated with the C/N and H/C (proxy for labile carbon) ratios of the biochar (Table 2), with $SWF_{410}C > SWF_{510}C > SWA_{900}C$. Studies have shown the addition of biomass with a C/N ratio above a critical value of 20 results in temporary immobilization of N by microorganisms increasing with increasing C/N ratio (Chan and Xu, 2009) and denitrification is dependent on availability of labile carbon (Sahrawat and Keeney, 1986).

On Day 1, the WA_{900} treatment has the highest N_2O/N_2 ratio consistent with this biochar enhancing N_2O emissions. The change in the magnitude of the ratios on Days 2–4 (Fig. 3) could be due to nitrification inhibition by C_2H_2 (one of the potential limitations of this method). Changes in the N_2O/N_2 ratio are often associated with NO_3^- concentration in soils (Weier et al., 1993). Another explanation for the change in this ratio could be increasing areas of anoxic conditions resulting in a decreased percentage of N_2O evolved until N_2 is the primary gas evolved (Rolston et al., 1978). A similar decrease in N_2O/N_2 emission ratios with increasing pH was observed by Clough et al. (2004) while evaluating soils at a WFPS > field capacity. Our results corroborate the suggestion that the liming effect of biochar at high WFPS encourages denitrification to proceed to dinitrogen (van Zwieten et al., 2010).

Increases in the N_2O/N_2 ratio, as in the urea treatment, have been attributed to rapid nitrification of added NH_4 resulting in increased NO_3^- content which is reduced to N_2O (Vallejo et al., 2006). This mechanism is corroborated by the high NH_4–N determined in this treatment in the absence of C_2H_2 (Table 3).

4.2. Biochar impact on natural organic matter

4.2.1. Compost–biochar: carbon mineralization

In this study, the emissions of GHGs for the incubations of biochar with compost and compost extract correlated with biochar H to C ratios (Figs. 4 and 5, Table 2). Among the wood feedstock biochars, the WF_{510} biochar had a lower H/C ratio (0.30) than the WF_{410} (0.76) and was more resistant to mineralization. Biochars made at higher temperature form more condensed aromatic structures resulting from the loss of more open oxidizable functional groups (Glaser et al., 2002; Liang et al., 2006). The increase in CO_2 emissions of the WF_{410} treatments upon addition of the biochar to compost and compost extract when compared to treatments of compost and compost extract alone (Fig. 4) indicates WF_{410} enhances mineralization or has a priming effect on OM pools. The priming effect of biochar (stimulation and suppression) on more labile OM pools is well documented (Liang et al., 2010; Novak et al., 2010; Zimmerman et al., 2011). Also, as observed in several studies, the high CO_2 emissions from the WF_{410} treatments (Fig. 4) low temperature are not resistant to degradation (Cheng et al., 2006; Kuzyakov et al., 2009) contradicting studies which proposed that biochar should have a limited effect on soil C and N dynamics because it is recalcitrant (Novak et al., 2010).

To confirm the degradation of WF_{410} biochar, a short-term study was initiated to compare wet biochar and dry biochar behavior. The results support the theory that the presence of water leads to elevated CO_2 emissions from biochar (Supplementary data, Fig. S7). Spokas et al. (2009) observed similar emissions from a water blank treatment and suggested biotic/abiotic reactions of water and O_2, as well as mineralization of pyrolysis byproducts sorbed onto the char surface as sources of CO_2.

For the WF_{510} biochar, the composite treatments all had lower GHG emissions than the treatments of the compost and compost extract alone (Fig. 4). This suggests WF_{510} biochar aides in stabilizing the compost and compost extract OM. Suppression of CO_2 has been observed for high temperature ashes and related to their microcrystalline structure and concentration of hydroxyl groups (Fisher et al., 1976). The reduction in CO_2 emissions from WA_{900} incubations is consistent with another study of similar chars having high ash content and high pH (Spokas and Reicosky, 2009). A key phenomenon overlooked by the study is a decrease in headspace CO_2 due to increased solubility of CO_2, into the soil solution, as a function of pH. The increased solubility of CO_2 (g) with increasing pH is a well-known phenomenon and results in a formation of carbonic acid (Jensen, 2003). This perceived suppression of CO_2 due to the biochar amendment would only be a temporary sink as the CO_2 would be re-released in time as the pH of the soil solution increases from carbonic acid formation. This was consistent with our data which showed detectable CO_2 in the headspace after 15 days. Microbial inhibition by WA_{900} may be another potential reason for the reduced levels of CO_2 and to test this, supernatants from mixtures of the biochar and compost as well as compost only were streaked onto agar plates. Visual observation of the plates with the $WA_{900}C$ treatment showed substantially reduced microbial growth and thus the potential of WA_{900} to diminish microbial activity warrants further investigation.

4.2.2. Compost–biochar: nitrogen mineralization

Compared to the observed CO_2 reductions, the effects of the biochar were not as significant for N_2O emissions for the biochar incubations with compost and compost extract. The one exception is the WF_{410} biochar, which appeared to stimulate N mineralization in the compost treatment (Fig. 5a). Interestingly, similarly large emissions of N_2O were not observed in the WF_{410}Cext treatment. We hypothesize that this may be due to the presence of a smaller microbial population in this treatment compared to WF_{410} but this needs requires further investigations aimed at examining microbial activity. The large difference in emissions ($\pm C_2H_2$) for this treatment and WF_{510} show the destabilizing effects of these biochar on compost (Fig. 5b).

4.3. Biochar surface chemistry

Analysis of the FTIR spectra under different conditions also confirms partial degradation of the WF_{410} biochar (Fig. 6), with a decrease in the bands at 2920 and 2850 cm^{-1} (ν(C–H) vibrations in CH_3 and CH_2) an indicator of degradation of the biochar (Smidt and Schwanninger, 2005). The decrease in the ratio of the bands at 2923/1690 cm^{-1} (aliphatic C–H: aromatic carbonyl or carboxyl) in woodchip compost over time has also been used as an indicator for degradation (Smidt et al., 2002) and a similar trend was observed in the biochar bands (Table 4). The peak ratios: 1034/1690 (polysaccharide/aromatic carbonyl or carboxyl), 2850/1690 (aliphatic C/aromatic carbonyl or carboxyl), and 2923/1690 (aliphatic C/aromatic carbonyl or carboxyl) clearly show decreases in ratios from the unincubated (control) biochar to after treatment. The changes indicate a loss of the more labile aliphatic and polysaccharide components of the biochar, and the retention of a more stable aromatic structure (Hsu and Lo, 1999).

The presence of COM ($WF_{410}C$ or $WF_{410}Cext$) resulted in lower ratios than the biochar only (WF_{410}), indicative of increased decomposition, and correlated better with N_2O than CO_2 emissions. The ratios also indicate some stabilization or protection of the biochar surface by interaction with the soil, phenomena attributed to soil minerals (Baldock and Smernik, 2002; Bolan et al., 2012; Glaser et al., 2000).

5. Conclusion

This study shows that amendment of Yolo silt loam soil with biochar at agriculturally relevant application rates does not significantly affect CO_2 emissions (C mineralization) when compared to addition of organic (compost) and inorganic (urea) fertilizers. However, significant enhancement of N mineralization was evident from N_2O emissions for soil incubations with WA_{900}. In the presence of C_2H_2, amendment of the soil with WA_{900} (high pH, CEC and surface area) impacted the initial N_2O/N_2 ratio resulting in increased emission of N_2 relative to N_2O.

Emissions of CO_2 from the interaction of biochar with COM are dependent on the biochar feedstock and pyrolysis temperature. However, the net CO_2 emissions are less for the biochar and compost mixtures (compared to compost alone), suggesting that biochar may stabilize COM and diminish C mineralization. ATR–FTIR spectra of WF_{410}, which is the least aromatic biochar and has the lowest surface area, showed surface degradation of the biochar through decreases in the relative spectral contributions of polysaccharides, carboxyls, aliphatic C. Attributed to its structural lability, incubations with this biochar resulted in the highest emissions of CO_2 and N_2O. This result is consistent with studies that have shown increased emissions of GHGs from soil amended with biochar pyrolyzed at low temperatures, making these types of biochar less amenable to application onto inherently fertile soils. Although data from these short term soil incubations do not show great potential benefits regarding GHG emissions, the incubations of compost and biochar alone suggest that some biochars may serve to enhance C stocks in soils with inherently low NOM content.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found online at http://dx.doi.org/10.1016/j.geoderma.2013.02.004.

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