

Ethylene: potential key for biochar amendment impacts

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Abstract Significant increases in root density, crop growth and productivity have been observed following soil additions of biochar, which is a solid product from the pyrolysis of biomass. In addition, alterations in the soil microbial dynamics have been observed following biochar amendments, with decreased carbon dioxide (CO₂) respiration, suppression of methane (CH₄) oxidation and reduction of nitrous oxide (N₂O) production. However, there has not been a full elucidation of the mechanisms behind these effects. Here we show data on ethylene production that was observed from biochar and biochar-amended soil. Ethylene is an important plant hormone as well as an inhibitor for soil microbial processes. Our current

hypothesis is that the ethylene is biochar derived, with a majority of biochars exhibiting ethylene production even without soil or microbial inoculums. There was increased ethylene production from non-sterile compared to sterile soil (215%), indicating a role of soil microbes in the observed ethylene production. Production varied with different biomass sources and production conditions. These observations provide a tantalizing insight into a potential mechanism behind the biochar effects observed, particularly in light of the important role ethylene plays in plant and microbial processes.

Keywords Biochar · Black carbon · Charcoal · Greenhouse gas

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Introduction

A potential abatement strategy to increasing levels of carbon dioxide (CO₂) in the atmosphere is to sequester atmospheric CO₂ in a stable form. One proposed approach is the use of pyrolysis to convert vegetative biomass into biochar (charcoal, black carbon) that could then be incorporated in soil, thus sequestering atmospheric carbon into a slower cycling pool (Lehmann 2007). Previous research has demonstrated significant plant and soil benefits resulting from biochar amendments, including drastic increases in yield, plant growth, fine root development, and overall increases in soil fertility (Chan et al.

2007; Lehmann and Joseph 2009; Marris 2006; Renner 2007). Laboratory incubations of biochar amended soil have routinely shown a decrease in N₂O production (Spokas and Reicosky 2009; Spokas et al. 2009; Van Zwieten et al. 2009; Yanai et al. 2007) as well as decreases in soil respiration of soil organic matter (Lehmann and Joseph 2009; Spokas et al. 2009) with corresponding reductions in N₂O emissions being observed in biochar treated field plots (Rondon et al. 2005; Rondon et al. 2006). In addition, biochar has also been shown to increase sorption of agrochemicals and thereby reduce leaching potential (Cao et al. 2009; Spokas et al. 2009; Yang and Sheng 2003). Conversely, occasional negative plant and soil effects have also been observed in field and greenhouse studies, including reduced plant growth (Lehmann and Joseph 2009), reduced methane oxidation activity (Spokas and Reicosky 2009; Spokas et al. 2009) and increased soil respiration of soil organic matter (Spokas and Reicosky 2009; Wardle et al. 2008). Potential hypotheses explaining these effects have focused mainly on abiotic interactions (e.g. pH changes, bulk density decreases, alterations in nutrient availability, water retention increases, biochar structure, soil structure alterations), as well as promotion of colonization by arbuscular mycorrhizal fungi (Lehmann and Joseph 2009; Novak et al. 2009; Warnock et al. 2007; Zackrisson et al. 1996). During laboratory incubations of soils with biochar additions, we have observed an additional potential factor in both these positive and negative effects: ethylene production.

Ethylene (C₂H₄), which originates from both natural and anthropogenic sources, has been known for some time to be an important phytohormone acting at very low levels (~10 nL L⁻¹) (Abeles et al. 1992; Arshad and Frankenberger 1991; Arshad and Frankenberger 2002; Frankenberger and Arshad 1995). Ethylene has been implicated in a variety of pleiotropic plant effects such as release of seed dormancy, leaf and flower senescence, fine root hair growth, fruit ripening, increased post-seedling stem growth, increased seed germination, and increased yield in some agricultural crops (Abeles et al. 1992; Frankenberger and Arshad 1995; Arshad and Frankenberger 2002; Ortega-Martinez et al. 2007). However, negative impacts from ethylene have also been observed, such as reduced crop yields, reduced stem growth, increases in root diameter, and stimulated flowering (Abeles et al. 1992;

Arshad and Frankenberger 1991; Yang and Hoffman 1984). Ethylene also impacts the soil, with observed reductions in microbial ammonium nitrification (Porter 1992) and soil methanotrophic activity (Jäkel et al. 2004), and it has been postulated to affect spore germination of fungi (Abeles et al. 1992; Arshad and Frankenberger 2002). Observations of ethylene soil gas concentrations have ranged from 0.5 to 18 μL L⁻¹ (Arshad and Frankenberger 2002; Burford 1975; Campbell and Moreau 1979; Ioannou et al. 1977; Sheard and Leyshon 1976; Smith and Russell 1969). In the soil, microorganisms are the major sources and sinks of ethylene, with a proposed model of ethylene being produced under both aerobic and anaerobic conditions, while ethylene oxidation occurs only under aerobic conditions (Zechmeister-Boltenstern and Smith 1998). Small alterations in the soil ethylene balance may affect both soil microbial and plant growth (Abeles et al. 1992). Increased soil ethylene production has been observed following other soil organic amendments (Arshad and Frankenberger 1990) and in water logged soils (Sheard and Leyshon 1976; Frankenberger and Arshad 1995). However, this is the first study to document the formation of ethylene from biochar and soil-biochar additions. We hypothesize that this ethylene could be an additional potential mechanism for the soil and plant responses observed from biochar amendments.

Materials and methods

To evaluate ethylene production potential we examined 12 different biochars, as well as a steam-activated charcoal, through sealed aerobic laboratory incubations (Table 1). Production temperatures and proximal and ultimate analyses on the biochars are presented in Table 1. Proximal and ultimate analyses were conducted by Hazen Research, Inc (Golden, CO) and surface area analyses were completed by Pacific Surface Science, Inc (Ventura, CA).¹

Soil for the laboratory studies was collected at the University of Minnesota's Research and Outreach Station in Rosemount, MN (44°45' N, 93°04' W). Soil

¹ Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable.

Table 1 Properties of biochars evaluated

Label	Parent Biomass	Supplier ^a	Pyrolysis Temperature (°C)	Surface Area (m ² /g)	% C	% O	% N	% VM	% Ash	% Fixed C
Charcoal	Activated coconut charcoal (steam activated; water rinsed)	Willinger Brothers	450	976.2	83	<0.1	0.4	1.7	15.3	82.5
Biochars										
BC-1	Hardwood sawdust (CQuest™)	Dynamotive	500 (fast)	10.4	66.5	13.1	0.3	28.8	15.4	55.4
BC-2	Macadamia nut	Biochar Brokers	N/A	6.9	93.2	1.7	0.7	16.8	1.9	81.2
BC-3	Hardwood chip	Best Energies	550 (slow)	66.3	71.1	20.6	0.1	34.8	4.8	60.5
BC-4	Dried distillers grain	ISTC	350 (slow)	0.28	69	6.6	7.5	44.7	11.3	43.1
BC-5	Dried distillers grain	ISTC	400 (slow)	0.28	69.4	5.9	7.4	37.6	11.9	49.4
BC-6	Corn cobs	ISTC	350 (slow)	<0.10	78.9	12.9	0.7	33.2	2.9	63.9
BC-7	Corn cobs	ISTC	400 (slow)	<0.10	82.6	8.8	0.6	24.8	3.8	71.4
BC-8	Wood waste (mixed)	ISTC	400 (slow)	3.5	79.9	11.9	0.8	26.8	3.7	69.5
BC-9	Wood waste (mixed)	ISTC	450 (slow)	26.8	80.8	11.4	0.8	23.7	3.7	72.6
BC-10	Wood pellets	Chip Energy	500 (slow)	1.8	73.4	18.8	0.2	12.4	6.4	81.3
BC-11	Wood waste (mixed)	Chip Energy	400–500 (updraft gasifier)	33.5	31.5	<0.1	0.3	20.4	67.0	12.5
BC-12	Peanut Hulls	EPRIDA	481 (slow)	1.0	59.0	2.7	12.0	39.8	15.0	45.2

a - Names are necessary to report factually on available data; however, the USDA neither guarantees nor warrants the standard of the product, and the use of the name by USDA implies no approval of the product to the exclusion of others that may also be suitable. Abbreviations: *fast* less than 2 second resident time; *slow* greater than 2 s; *C* Carbon; *N* Nitrogen; *O* Oxygen; *VM* Volatile Matter; *ISTC* Illinois Sustainable Technology Center; *EPRIDA* Earth people research innovation development acknowledgement; *NA* designates data not available

at the site is a Waukegan silt loam (fine-silty over skeletal mixed, super active, mesic Typic Hapludoll) containing approximately 22% sand, 55% silt, and 23% clay with a pH (1:1 H₂O) of 6.3–6.6, 2.6% organic carbon and a slope < 2%. This site was farmed in a conventionally tilled (moldboard plow) corn (*Zea mays L.*) and soybean [*Glycine max (L.) Merr.*] rotation for the last 8+ years. The soil was sampled following corn harvest. Surface soil (0–5 cm) was collected, sieved to <2 mm and homogenized for the incubation study. The following sets of incubations (3 replicates) were established for each of the twelve biochar types and the activated charcoal (Table 2):

1. 0.5 g biochar + no soil + no water (biochar alone dry)
2. 0.5 g biochar + no soil + 1 mL water (biochar alone wet)
3. 5 g soil + 0.74 mL water (soil control field capacity)

4. 5 g soil + 5 mL water (soil control saturated)
5. 0.5 g biochar + 5 g soil + 0.74 mL water (field capacity)
6. 0.5 g biochar + 5 g soil + 5.0 mL water (saturated)
7. 5.0 mL water (control)

The next set of incubations (3 replicates) was established for the oxygen dependency and soil sterilization effects [1 h; steam @ 125°C; dry heat sterilization for 4 h (125°C)] (Table 3). These incubations were established solely with the macadamia nut biochar:

1. 0.5 g macadamia nut biochar + no soil + 0.74 mL water (0 mL L⁻¹ oxygen)
2. 0.5 g macadamia nut biochar + 5 g sterilized soil + 0.74 mL water (0 mL L⁻¹ oxygen)
3. 0.5 g macadamia nut biochar + 5 g soil + 0.74 mL water (0 mL L⁻¹ oxygen)
4. 0.5 g macadamia nut biochar + no soil + 0.74 mL water (200 mL L⁻¹ oxygen)

Table 2 Net ethylene production rates from laboratory incubations

	Ethylene Production (ng C ₂ H ₄ 0.5 g _{soil} ⁻¹ d ⁻¹)											
Soil Control (field capacity)	<0.5											
Soil Control (1:1 slurry)	0.6 (0.3)											
Charcoal	BC-1	BC-2	BC-3	BC-4	BC-5	BC-6	BC-7	BC-8	BC-9	BC-10	BC-11	BC-12
Ethylene Production (ng C ₂ H ₄ 0.5 g _{char} ⁻¹ d ⁻¹)	<0.5	9.5 (3.5)	<0.5	2.4 (0.9)	1.7 (0.8)	<0.5	<0.5	<0.5	<0.5	3.0 (1.0)	<0.5	0.9 (0.4)
Dry Biochar (air-dry)	<0.5	2.5 (0.1)	27.2 (8.8)	0.5 (0.4)	19.6 (0.7)	12.9 (1.0)	5.0 (0.6)	0.5 (0.6)	<0.5	3.2 (1.2)	<0.5	2.7 (1.2)
Wet Biochar (1:2 slurry)	Biochar Corrected Ethylene Production (ng C ₂ H ₄ g _{soil} ⁻¹ d ⁻¹)											
Soil + Biochar (field capacity)	<0.5	14.7 (1.7)	<0.5	6.9 (0.8)	4.0 (0.2)	0.4 (0.1)	<0.5	<0.5	<0.5	2.8 (1.2)	<0.5	1.5 (0.4)
Soil + Biochar (1:1 slurry)	<0.5	6.6 (0.2)	18.7 (8.9)	0.9 (0.4)	6.6 (0.4)	4.0 (0.2)	6.5 (0.5)	1.5 (0.5)	<0.5	4.5 (2.1)	<0.5	6.2 (1.8)

Average of triplicate incubations of 10% (w/w) biochar additions at two different moisture conditions (field capacity and 1:1 saturated slurry; details in text) are given along with the standard deviation of the observations in parentheses

- 0.5 g macadamia nut biochar + 5 g sterilized soil + 0.74 mL water (200 mL L⁻¹ oxygen)
- 0.5 g macadamia nut biochar + 5 g soil + 0.74 mL water (200 mL L⁻¹ oxygen)
- 0.5 g macadamia nut biochar + no soil + 0.74 mL water (400 mL L⁻¹ oxygen)
- 0.5 g macadamia nut biochar + 5 g sterilized soil + 0.74 mL water (400 mL L⁻¹ oxygen)
- 0.5 g macadamia nut biochar + 5 g soil + 0.74 mL water (400 mL L⁻¹ oxygen)

There were also corresponding controls of sterile and non-sterile soil at each of the oxygen concentrations (Table 3).

The last set of incubations (3 replicates) examined the impact of ethylene headspace additions on the net greenhouse gas production potentials, without biochar additions:

- 5 g non-sterile soil + 0.74 mL water
- 5 g non-sterilized soil + 0.74 mL water + 1 μL L⁻¹ ethylene
- 5 g non-sterilized soil + 0.74 mL water + 5 μL L⁻¹ ethylene
- 5 g non-sterilized soil + 0.74 mL water + 25 μL L⁻¹ ethylene
- 5 g non-sterilized soil + 0.74 mL water + 50 μL L⁻¹ ethylene
- 5 g non-sterilized soil + 0.74 mL water + 275 μL L⁻¹ ethylene

These incubations were initially run for 10 days prior to the ethylene injection to allow equilibration and document uniformity, and then 30 days following the ethylene injection. In addition, these incubations were extracted at day 30 and analyzed for nitrate and ammonium. Soils were extracted with 30 mL of 2 M KCl for 1 h. After settling for 24 h, extracts were filtered (no. 42; Whatman, Maidstone, UK) and stored (-20°C) until analysis. Filtrate samples were analyzed for ammonium and nitrate using a flow-through injection analyzer (Lachat, Milwaukee, WI).

All soil incubations were conducted in sterilized 125 mL serum vials (Wheaton Glass, Millville, NJ) and sealed with red butyl rubber septa (Grace, Deerfield, IL). To prepare the various oxygen concentrations (Table 3), after sealing the incubations the headspace was purged with either ultra high purity nitrogen or 400 mL L⁻¹ oxygen in nitrogen (Minnesota Oxygen Supply; Minneapolis, MN) using a

Table 3 Effect of oxygen concentration and soil sterilization on ethylene production from macadamia nut biochar (BC-2)

Oxygen Concentration	Ethylene Production (ng C ₂ H ₄ d ⁻¹)				
	Soil Controls				
	Biochar only (no soil)	Non-sterile soil	Sterilized Soil	Biochar + sterilized soil	Biochar + non-sterile soil
0 mL L ⁻¹	36 (7)	<0.5	12 (7)	41 (11)	57 (32)
200 mL L ⁻¹	24 (7)	<0.5	16 (9)	55 (12)	120 (9)
400 mL L ⁻¹	30 (9)	<0.5	22 (5)	63 (13)	180 (19)

Average of triplicate incubations of 10% (w/w) biochar additions and corresponding controls at field capacity and with standard deviation of the observations in parentheses

double needle arrangement with the flush gas flow of 10 L min⁻¹ for 2 min. The 200 mL L⁻¹ oxygen treatment was ambient lab air and was not flushed. For the incubations in Table 1, headspace was not modified.

Periodic gas samples were withdrawn from the incubations for analysis on both a gas chromatographic (GC)-flame ionization detector (FID) and a GC-mass spectrometer (GC-MS) system to quantify ethylene production over a maximum 15 to 30-day incubation period. To sample the incubations, initially 5 mL of air (known composition) was injected into the sealed incubation vials. The syringe was flushed three times to allow for adequate mixing of the serum bottle headspace. Then 5 mL of headspace was pulled back into the syringe and injected into a 10 mL headspace vial, previously flushed with helium, for later analysis. The GC-FID system was interfaced using a headspace sampler (Agilent, Foster City, CA, model 7,694) with a 1.0 mL sample loop to a Hayesep-N column (3.2 mm×1.8 m; Grace; Deerfield, IL; 32 mL min⁻¹ He flow rate). For the GC-MS system, the system described in Spokas and Reicosky (2009) was used, with the exception that the ethylene was quantified from the RT-QSPLOT (0.32 mm×30 m, Restek, Bellefonte, PA; 2 mL min⁻¹ He flow rate) at 5.07 min (m/z=28 was used for quantification and detection of ethylene). It should be noted that acetylene and ethylene are overlapping peaks on the RT-QSPLOT with the oven program. However, ethylene was successfully separated from the acetylene peak using m/z=28. Overall quantification limit was 200 nL C₂H₄ L⁻¹ on the GC-FID and 400 nL C₂H₄ L⁻¹ on the GC-MS. The GC-MS was used as a confirmation tool and the quantification of the ethylene was performed on the GC-FID system.

Ethylene peaks were confirmed with a 96.9% spectral quality match (after background spectra subtraction; 62% prior to background subtraction) to the NIST library (Perkin Elmer). Both GC systems were calibrated using multiple traceable ethylene standards (Scott Specialty Gases; Troy, MI and Minnesota Oxygen Supply; Minneapolis, MN).

Net rates of ethylene production presented in Table 2 were calculated from the linear regression of ethylene concentration versus time during the aerobic portion of the sealed incubation (>100 mL L⁻¹ O₂). This measured rate represented the net soil production and consumption of ethylene during the incubation and was corrected for biochar production by the following formula:

$$\text{net ethylene production (ng C}_2\text{H}_4 \text{ g}_{\text{soil}}^{-1} \text{ d}^{-1}) = \left(\frac{\text{Ethylene}_{\text{soil+biochar}} - \text{Ethylene}_{\text{biochar}}}{(t_d)5 \text{ g}_{\text{soil}}} \right) \quad (1)$$

where Ethylene_{soil + biochar} is the total ethylene production (ng) in the soil + biochar incubation at time t_d, Ethylene_{biochar} is the total ethylene production (ng) in the wet biochar incubation at time t_d, and t_d is the number of days of incubation (Spokas et al. 2009; Spokas and Reicosky 2009). Thereby, the net ethylene is assumed to be corrected for the biochar alone production. The data in Table 3 (BC-2) were not corrected for biochar production and represent net ethylene production per incubation for easier comparison.

Results

Soil without biochar amendments did not produce any detectable ethylene at field capacity and the produc-

tion at saturated conditions (1:1 slurry) was just slightly above the detection limit at $0.6 \pm 0.3 \text{ ng C}_2\text{H}_4 \text{ g}_{\text{soil}}^{-1} \text{ d}^{-1}$ [$0.5 \text{ nL C}_2\text{H}_4 \text{ g}_{\text{soil}}^{-1} \text{ d}^{-1}$]. On the other hand, five biochars, even without any soil or microbial inoculum, produced ethylene in the dried state ranging from 0.9 to $9 \text{ ng C}_2\text{H}_4 \text{ 0.5 g}_{\text{char}}^{-1} \text{ d}^{-1}$ [0.7 to $7.2 \text{ nL C}_2\text{H}_4 \text{ 0.5 g}_{\text{char}}^{-1} \text{ d}^{-1}$] (Table 2). Furthermore, ten of the biochars produced ethylene following water additions ranging from 0.5 to $27.2 \text{ ng C}_2\text{H}_4 \text{ 0.5 g}_{\text{char}}^{-1} \text{ d}^{-1}$ [0.4 to $21.8 \text{ nL C}_2\text{H}_4 \text{ 0.5 g}_{\text{char}}^{-1} \text{ d}^{-1}$] (Table 2). No ethylene production was observed from the activated charcoal, BC-9 (mixed wood waste) and BC-11 (mixed wood waste) with or without moisture additions.

When the biochar was mixed with soil, six out of the twelve biochar-amended soil samples exhibited increased ethylene production compared to the unamended soil at field capacity, ranging from 0.4 to $14.7 \text{ ng C}_2\text{H}_4 \text{ g}_{\text{soil}}^{-1} \text{ d}^{-1}$ [0.3 to $11.8 \text{ nL C}_2\text{H}_4 \text{ g}_{\text{soil}}^{-1} \text{ d}^{-1}$]. Please note that this production has been corrected for the production of ethylene from the wet biochar (Equation 1). The six biochars that did not exhibit ethylene production at field capacity were the biochars made from wood materials (BC-1, 3, 8, 9, and 11) as well as the higher temperature corn cob biochar (BC-7). Slurry biochar + soil + water incubations exhibited ethylene production which was either equal to or increased from the production observed at field capacity (Table 2). Interestingly, the two biochars (BC-9 and BC-11; woodwaste biochars) that had no observable production in saturated conditions were the same two biochars that had no ethylene production in the water + biochar incubations. The activated charcoal had no observable ethylene production when mixed with soil at both water contents (Table 2).

The highest ethylene-producing soil-biochar combination (BC-2; macadamia nut biochar) was also evaluated with different oxygen concentrations and with soil sterilization (Table 3). As seen in the table, rates of ethylene production of the biochar alone were statistically equal $\sim 30 \text{ ng C}_2\text{H}_4 \text{ d}^{-1}$ regardless of headspace oxygen concentrations. Similarly, the sterilized soil + biochar incubations were independent of oxygen concentrations with an average ethylene production observed of $53 \text{ ng C}_2\text{H}_4 \text{ d}^{-1}$. However, the non-sterile soil + biochar showed a statistically significant increase as a function of oxygen concentrations, with the highest rate ($180 \text{ ng C}_2\text{H}_4 \text{ d}^{-1}$) occurring at the 400 mL L^{-1} oxygen concentration.

Trace amounts of ethylene were produced from the sterilized soil ($\sim 20 \text{ ng C}_2\text{H}_4 \text{ d}^{-1}$) which was also independent of oxygen concentration. There was no observable ethylene production from the non-sterile soil controls.

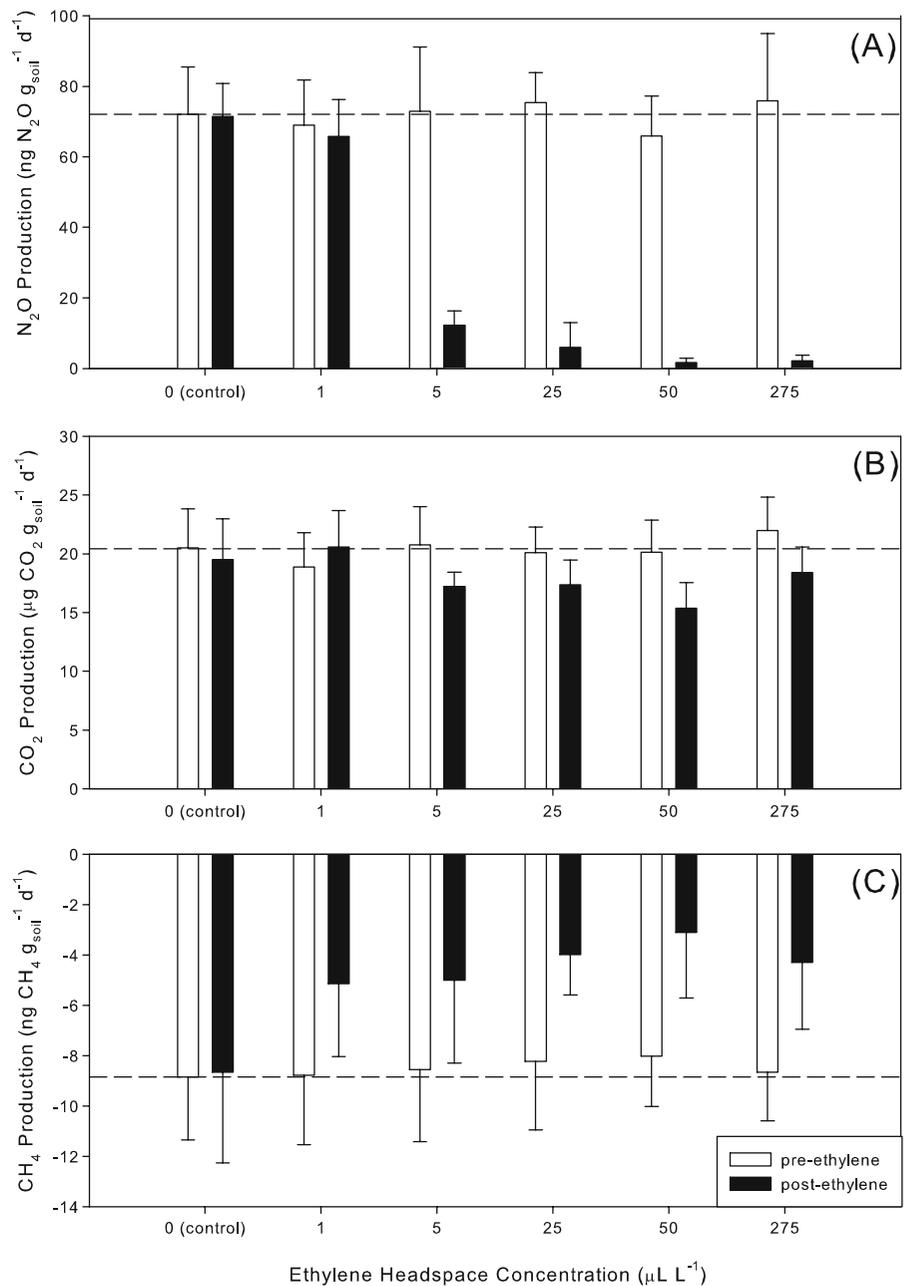
Soil was also incubated in the presence of ethylene to observe the impact on soil greenhouse gas production potentials (Fig. 1). As seen in the figure, there were no statistically significant differences in the three greenhouse gases for the pre-ethylene injection. However, following ethylene injections, the presence of ethylene caused significant reductions in N_2O production and CH_4 oxidation correlated with increasing levels of ethylene (Fig. 1a and c). On the other hand, there was no significant alteration in CO_2 production as a function of ethylene concentrations (Fig. 1b). CO_2 production rates were statistically decreased at some ethylene concentrations, but were not impacted at the highest ethylene concentration evaluated ($275 \text{ } \mu\text{L L}^{-1}$). In addition, ethylene additions caused a decrease in the available nitrate and an increase in the available ammonium as a function of the ethylene headspace concentration at the end of the 30 day incubation (Fig. 2). These inhibitory effects did diminish with time as the ethylene was oxidized, particularly at the lower ethylene levels.

Discussion

Ethylene is known to be produced from the pyrolysis of biomass (Paushkin et al. 1994; Steinburg et al. 1992). However, no observation of ethylene release from the biochar has been noted previously. The net increases observed with the biochar + soil incubations could be due to either increased ethylene production or decreased ethylene oxidation (Table 2).

Rates of ethylene production varied drastically across the different biochars evaluated. This illustrates unknown dependencies on biomass source and conditions of the pyrolysis, since the properties of the resulting biochar vary as a function of the feedstock and conditions of the pyrolysis (Guerro et al. 2005; Sensöz 2003). The largest response, an increase of two orders of magnitude, occurred with BC-2 (macadamia nut biochar) compared to the soil alone. In contrast, soils amended with activated charcoal produced no detectable ethylene, regardless of water content. The lack of ethylene production from the

Fig. 1 Illustration of the impact of various ethylene concentrations in the headspace of aerobic soil incubations on the production rates of (a) N_2O , (b) CO_2 , and (c) CH_4 . Data presented are averages of triplicate incubations with error bars illustrating the standard deviation

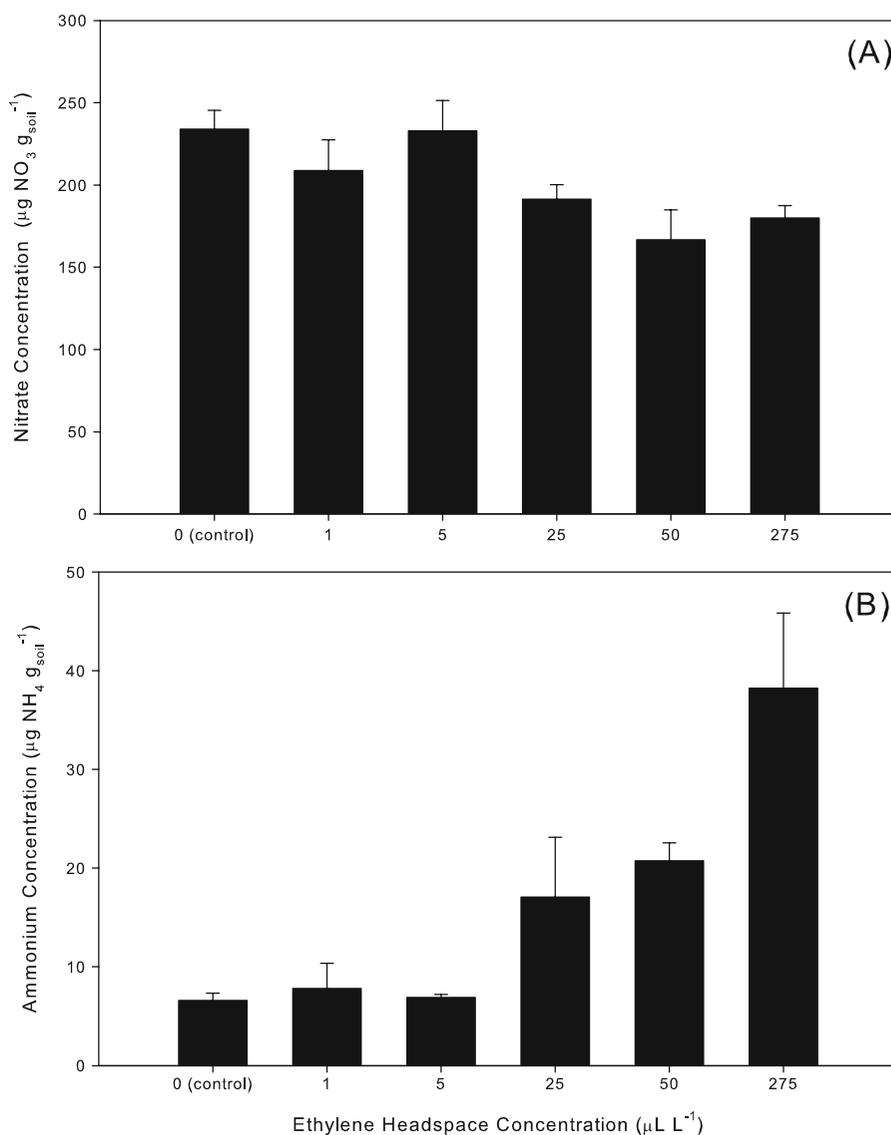


activated charcoal agrees with previous observations of ethylene sorption by activated charcoals (McDermot et al. 1995).

The rate of ethylene production decreased with increasing biochar production temperature in three parent biomass materials (distillers grain, corn cobs and wood waste) evaluated here (Tables 1 and 2; BC 4–9). These six biochars were produced on the same pyrolysis equipment. This temperature dependency

suggests a possible role of surface-sorbed oils, volatiles or directly sorbed ethylene produced during pyrolysis. However, there was no general relationship observed between ethylene production and volatile material across all of the various biochars. In fact, no overall relationship was observed between any of the composition variables of the biochar and the observed ethylene production. However, typically higher biochar surface areas resulted in lower ethylene produc-

Fig. 2 Illustration of the availability of (a) nitrate and (b) ammonium in soil extracts following the 30 day soil incubation in the presence of various concentrations of ethylene. Data presented are averages of triplicate incubations with error bars illustrating the standard deviation



tion, although not directly correlated (BC-3, 9, and 11; Tables 1 and 2). These factors emphasize the variability in biochar quality as a function of production conditions and biomass feedstock (e.g. Guerro et al. 2005). There is the suggestion from the data that wood materials produced lower amounts of ethylene than other parent materials (Table 2). However, due to the fact that these were different pyrolysis units and production conditions, this conclusion cannot be adequately supported at this time. Furthermore, it is possible that other contaminants were present in the wood waste biochars that could have also affected ethylene production and general micro-

bial activity. We did not confirm this possibility, however.

Ethylene production in the presence of non-sterile soil was 215% higher than ethylene production from sterile soil at ambient oxygen concentrations (Table 3). This observation suggests the involvement of aerobic soil microbes. While ethylene production rates in the sterile soil were lower, rates did not increase significantly with increasing oxygen concentrations. Others have also noted ethylene production from sterilized soil (e.g. Arshad and Frankenberger 2002). We suspect that this is a result of incomplete sterilization, but we cannot rule out abiotic produc-

tion. While the exact mechanism of ethylene formation following biochar additions is not known, the data suggest that biotic processes predominate, with perhaps a small contribution from abiotic pathways.

Ethylene production was observed from biochar and biochar-amended soil, which could be a contributing factor to the observed plant and soil microbial effects. The levels of ethylene production observed here are comparable to other non-biochar studies, where the authors concluded that the levels were high enough to impact soil microbial and plant processes (e.g. Smith and Russell 1969; McCarty and Bremner 1991; Arshad and Frankenberger 2002). The observations seen in the impact of ethylene on the greenhouse gas (GHG) production potentials suggests that ethylene could contribute to GHG reductions that have been previously observed following soil biochar additions (e.g. Spokas and Reicosky 2009; Spokas et al. 2009; Yanai et al. 2007; Van Zwieten et al. 2009). One important note is that in the ethylene injections, ethylene would need to diffuse into the soil matrix in order to contact the soil microbes. However, with the biochar amendments being incorporated in the soil, the source of ethylene is already in the soil matrix. This could contribute to microbial impacts at significantly lower levels than what is observed with the headspace modification experiments.

Ethylene's impact on plant growth has been well established (e.g. Abeles et al. 1992; Arshad and Frankenberger 2002; Frankenberger and Arshad 1995). Therefore, the observed production of ethylene could be a contributing factor to the observations from biochar amended soils, both for microbial and plant processes. The more important implication of this finding is the potential utilization of biochar as a nitrification inhibitor. This would be analogous to the use of calcium carbide (Banerjee and Mosier 1989; Bronson and Mosier 1991; Kashif et al. 2007; Yaseen et al. 2006), which reduces the formation of N_2O and nitrate, particularly in fertilized agricultural soils. However, biochar use as a nitrification inhibitor still requires further investigations into the durations and temporal trends of these observed effects. While we are not suggesting that ethylene is the sole mechanism of biochar impacts, this observed production offers a potential explanation for some of the contrasting effects that have been observed in plant and microbial responses to biochar amendments, particularly for plant growth, microbial activities and fungi colonization.

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