Unweathered Wood Biochar Impact on Nitrous Oxide Emissions from a Bovine-Urine-Amended Pasture Soil

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Low-temperature pyrolysis of biomass produces a product known as *biochar*. The incorporation of this material into the soil has been advocated as a C sequestration method. Biochar also has the potential to influence the soil N cycle by altering nitrification rates and by adsorbing NH_4^+ or NH_3 . Biochar can be incorporated into the soil during renovation of intensively managed pasture soils. These managed pastures are a significant source of N_2O , a greenhouse gas, produced in ruminant urine patches. We hypothesized that biochar effects on the N cycle could reduce the soil inorganic-N pool available for N_2O -producing mechanisms. A laboratory study was performed to examine the effect of biochar incorporation into soil (20 Mg ha^{-1}) on N_2O -N and NH_3 -N fluxes, and inorganic-N transformations, following the application of bovine urine (760 kg N ha^{-1}). Treatments included controls (soil only and soil plus biochar), and two urine treatments (soil plus urine and soil plus biochar plus urine). Fluxes of N_2O from the biochar plus urine treatment were generally higher than from urine alone during the first 30 d, but after 50 d there was no significant difference ($P = 0.11$) in terms of cumulative N_2O -N emitted as a percentage of the urine N applied during the 53-d period; however, NH_3 -N fluxes were enhanced by approximately 3% of the N applied in the biochar plus urine treatment compared with the urine-only treatment after 17 d. Soil inorganic-N pools differed between treatments, with higher NH_4^+ concentrations in the presence of biochar, indicative of lower rates of nitrification. The inorganic-N pool available for N_2O -producing mechanisms was not reduced, however, by adding biochar.

Abbreviations: MBC, microbial biomass carbon; VOC, volatile organic compound; WSC, water-soluble carbon.

Radiative forcing of the climate system is dominated by long-lived greenhouse gases (Forster et al., 2007). Carbon dioxide concentrations in the atmosphere increased at a rate of $1.9 \mu\text{L L}^{-1} \text{ yr}^{-1}$ for the decade 1995 to 2005, and reached $379 \mu\text{L L}^{-1}$ in 2005 (Forster et al., 2007). Currently the concentration is $386 \mu\text{L L}^{-1}$ (Tans, 2009). Mitigation options to reduce global CO_2 concentrations are required. One strategy that has been advocated is based on the low-temperature pyrolysis of biomass, a process that produces a byproduct known as biochar (Lehmann, 2007). When biochar production occurs from biomass, it represents a net withdrawal of CO_2 from the atmosphere (Lehmann, 2007). While biochar can be decomposed microbially (Czimczik and Masiello, 2007), it is very stable in the environment, based on the current evidence (Lehmann et al., 2009), with residues from forest fires often found to be $>10,000$ yr old (Preston and Schmidt, 2006). Thus the incorporation of biochar into the soil is considered a C sink (Lehmann, 2007). An example of this biochar stability is found in the Terra Preta do Índio (true Indian Black Earth) in the Amazon Basin where past burning and mulching practices have led to black earth soils containing elevated quantities of organic C, with levels up to 70 times those of surrounding silt-clay soils (Sandor et al., 2006), and biochar dated at about 800 yr BCE (Lehmann, 2007). Other recent work, however, has shown the incorporation of fire-derived charcoals to stimulate the loss of native soil C in boreal forests during a 10-yr period (Wardle et al., 2008).

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In addition to the potential for C sequestration there are other benefits to incorporating biochar into soils. For instance, the addition of biochar to soils has been shown to also enhance biological activity, with the classic example being the Terra Preta do Índio. More recently, biochar incorporation into soils has been shown to suppress soil microbial populations contributing to plant disease (Nerome et al., 2005); increase the soil cation exchange capacity and increase nutrient retention and availability in highly weathered soils (Glaser et al., 2002; Liang et al., 2006); enhance N_2 fixation in soybean [*Glycine max* (L.) Merr.] crops when applied to highly weathered and acid soils at rates of 30 to 90 g kg⁻¹ soil, possibly as a result of soil liming or conditioning effects (Rondon et al., 2007); enhance nitrification rates in *Pinus ponderosa* P. Lawson & C. Lawson forest soil due to its effect on soil concentrations of secondary plant compounds (DeLuca et al., 2006); and improve plant fertilizer N use efficiency (Chan et al., 2007).

The current tropospheric N_2O concentration is significantly higher than the preindustrial concentration of 0.27 $\mu\text{L L}^{-1}$ and is continuing to increase (0.26% yr⁻¹), reaching 0.32 $\mu\text{L L}^{-1}$ in 2005 (Forster et al., 2007). Nitrous oxide is both a long-lived greenhouse gas with a global warming potential 298 times that of CO_2 over a 100-yr time horizon (Forster et al., 2007), and the dominant source of ozone-depleting nitrogen oxides in the stratosphere (Crutzen, 1970). The foremost source of anthropogenic N_2O is agriculture, and mitigation options are required. Incorporation of biochar into the soil has been reported to reduce N_2O emissions by 50 and 80% under soybean and grass regimes, respectively, as a result of better aeration and possibly better stabilization of soil C (Rondon et al., 2005). The effects of charcoal addition on N_2O emissions during the rewetting of soil were studied by Yanai et al. (2007), who found that charcoal either stimulated or suppressed N_2O emissions depending on the initial soil moisture content. In their experiment, there was no direct evidence to link charcoal addition with N_2O suppression and it was assumed charcoal addition led to water absorption and improved soil aeration, thus reducing denitrification at 73% water-filled pore space (WFPS), while at 83% WFPS enhanced N_2O fluxes were thought to result from insignificant improvement in soil aeration and stimulation of N_2O -producing activity.

The use of biochar as a mechanism for sequestering CO_2 in soils was inspired by the properties of Amazonian soils (Lehmann, 2007). Any environmental benefits or negative effects of sequestering C as biochar are poorly understood and quantified, however, particularly in relation to its impact on N dynamics (Lehmann et al., 2006). Biochars have been shown, however, to adsorb NH_3 (Asada et al., 2006) and dissolved NH_4^+ (Lehmann et al., 2002).

Nitrous oxide is a soil-derived greenhouse gas resulting from biological processes such as nitrification and denitrification and as such is influenced by the inorganic-N supply. In grazed pastures, urine patches are the dominant source of N_2O due to the intense rate of N application that surpasses the pasture's ability to utilize the deposited urinary N (Haynes and Williams, 1993). In intensively grazed pasture systems, biochar could potentially be incorporated into the soils during pasture cultivation, a practice

performed to renovate pastures, and thus sequester C. Given the previous work to date, which shows biochar influencing the N cycle in soils, it is possible that biochar has the ability to mitigate N_2O emissions arising from ruminant urine patches, especially because N compounds such as NH_4^+ and NH_3 are produced in the urine patch and uptake of these N forms by biochar may reduce the soil N pool(s) available for N_2O -production mechanisms. There is a lack of information, however, with regard to N transformation processes when ruminant urinary N is excreted onto soils containing biochar. The objective of this study was to assess the impact of incorporating biochar into a pasture soil on N_2O emissions arising from the application of bovine urine.

MATERIALS AND METHODS

A laboratory experiment was set up to determine the effect of biochar incorporation on the fluxes of N_2O following the application of bovine urine to a pasture soil. The study comprised four treatments replicated in a randomized block design, with four replicates of each treatment. The treatments consisted of a control (deionized water instead of urine), urine only, biochar only, and biochar plus urine as described below.

A Wakanui silt loam pasture soil, defined under the New Zealand soil classification system as a mottled, immature pallic soil (Hewitt, 1998), was collected (0–10-cm depth) from the Lincoln University Dairy Farm, Canterbury Plains (43°38.48 S, 172°26.39 E). The soil was then sieved (4 mm) with any aggregates >4 mm, stones, and vegetation (foliage, roots, and surface organic matter) discarded. Biochar, manufactured from *Pinus radiata* D. Don at a temperature of 600°C, was freshly made and unweathered and was crushed and sieved to pass through a 5-mm mesh.

A qualitative analysis of volatile organic compounds (VOCs) in the biochar samples was determined using an automated headspace solid-phase micro-extraction (SPME) in conjunction with gas chromatography–mass spectrometry (GC-MS). Biochar samples were placed into 20-mL SPME vials and quickly capped. A CTC Combi-Pal auto sampler (CTC Analytics AG, Zwingen, Switzerland) incubated the vials at 40°C for 40 min while the enclosed headspace of the vial was exposed to a 2-cm-long DVB/CAR/PDMS combination SPME fiber (Supelco, Bellefonte, PA), which was preconditioned for 10 min at 250°C under a He atmosphere before use. Desorption of the headspace volatiles occurred when the fiber was inserted into the heated injection port (250°C for 5 min) of a Shimadzu GC-MS-QP2010 GC-MS equipped with two gas chromatograph columns in series, namely an Rtx-Wax 30-m by 0.25-mm i.d. by 0.5- μm film thickness (polyethylene glycol, Restek, Bellefonte, PA) and an Rxi-1ms 15-m by 0.25-mm i.d. by 0.50- μm (100% dimethyl polysiloxane, Restek). Helium was used as the carrier gas with the GC-MS set to a constant linear velocity of 32.3 mL s⁻¹. The injector was operated in splitless mode for 5 min, then switched to a 20.5:1 split ratio. The column oven was held at 40°C for 5 min (during desorption of the SPME fiber), then heated to 250°C at 4°C min⁻¹ and held at this temperature for 15 min. The total run time was 72.5 min. The interface and mass spectrometry source temperatures were set at 250 and 200°C, respectively. The mass spectrometer was operated in electron impact mode at an ionization energy of 70 eV and a mass range of 33 to 403 m/z . The data acquisition software used was GCMSolutions (version 5.0, Shimadzu) in full scan mode. Volatile organic compounds were identified by matching

mass spectra with the spectra of reference compounds in the National Institute of Standards and Technology EPA/NIH Mass Spectral Library database. The SPME results are noted in Table 1.

For the control and urine-only treatments, the sieved soil (118 g) was packed to a depth of 6 cm into polyvinyl chloride cores (7 cm high by 5 cm i.d.). In the biochar-only and biochar-plus-urine treatments, the biochar was uniformly mixed with the sieved soil at a biochar rate equivalent to 20 Mg ha⁻¹ at a ratio of 3.9 g biochar/91 g soil. This resulted in different masses of soil in each of these treatments but it is entirely realistic to expect that, following biochar incorporation into soil, any urine applied to the soil would come into contact with a reduced mass of soil, assuming the same volume of a soil-plus-biochar matrix is wetted by the urine. Thus, as noted below, gaseous fluxes are expressed on an area basis and not per gram of soil. The biochar rate of 20 Mg ha⁻¹ was arbitrarily chosen after referral to other studies, where rates have ranged from 10 to 100 Mg ha⁻¹ (Chan et al., 2007, 2008), and what might be considered a feasible rate to incorporate into a pasture soil in practice. To prevent soil loss, a fine nylon mesh (<0.5 mm) was attached to the base of the soil cores before packing. Sixteen soil cores were used for N₂O gas measurements (see below), while a further 112 soil cores (4 treatments × 4 replicates × 7 destructive sampling events) were also set up for destructive soil analyses over time (see below). The soil cores were incubated at 18°C for 7 d before the application of bovine urine. The layout of the soil cores was randomized. The chemical characteristics of the soil and biochar are shown in Table 1.

Fresh urine was collected from Friesian dairy cows (*Bos Taurus*) that had been fed barley (*Hordeum vulgare* L.)–molasses pellets. The urine was collected and immediately stored at 4°C until its application (within 18 h). The collected urine contained 10 g N L⁻¹. Urine treatments received 0.015 L of urine at an N application rate equivalent to 760 kg N ha⁻¹, which is typical of a urinary-N deposition event under grazed dairy pastures (Haynes and Williams, 1993).

Following urine application, each soil core was covered with Parafilm that had been pierced (2 mm) to allow gas diffusion between the soil core headspace and the atmosphere, but which limited rapid soil drying. The soil cores were then incubated (18°C) until sampling as described below. Moisture levels were maintained by spraying deionized water onto the soil cores twice a week, with the amount of water determined by regularly monitoring the soil core weights.

Nitrous oxide fluxes were sampled by placing the intact soil cores into Mason jars (internal volume of 0.058 L) that had been previously flushed with compressed air. The jars were then sealed with screw-top lids pre-fitted with septa pierced with 3.8-cm-long 16-gauge hypodermic needles (part no. 305198, Precision-Glide, Becton-Dickinson, Franklin Lakes, NJ). Each needle was topped with a three-way stop-cock (no. 2C6201, Baxter Healthcare Corp., Waukegan, IL) to which a 20-mL glass syringe was attached when gas sampling. Before gas sampling, the syringe was flushed twice with ambient air and then flushed twice with headspace air, after which a gas sample was removed. The headspace gas (10 mL) was injected into pre-evacuated 6 mL Exetainer vials (Labco Ltd., High Wycombe, UK). This allowed the vials to be overpressurized, thus eliminating the possibility of external air diffusing into the vials. Gas sample vials were reduced to ambient pressure immediately before analysis using a double-ended needle. The gas samples were analyzed on an automated SRI 8610 gas chromatograph (GC)

Table 1. Chemical properties of the soil and biochar used in this study.

	Soil	Biochar
Cation exchange capacity, cmol kg ⁻¹	20.0	0.8
Phosphate retention, %†	18.0‡	3.4§
K, cmol _c kg ⁻¹	0.4	1.8
Ca, cmol _c kg ⁻¹	9.8	0.7
Mg, cmol _c kg ⁻¹	2.1	0.2
Na, cmol _c kg ⁻¹	0.2	0.3
pH in water	4.9 (1:2.5)¶	8.7 (1:10)
N, g kg ⁻¹	4.0	2.0
C, g kg ⁻¹	40.0	706.0
C/N ratio	10.0	353.0
Volatile organic compounds detected	not determined	acetaldehyde, α-pinene, β-pinene, trans-pinocarveol

†According to Blakemore et al. (1987), where the substrate is shaken for 16 h at a buffered pH of 4.65 using a standard solution containing 1000 mg L⁻¹ of P.

‡ 5 g/25 mL.

§0.5 g/25 mL.

¶ Mass of soil or biochar/volume of water.

interfaced to a Gilson 222XL liquid autosampler fitted with a double concentric injection needle that allowed rapid purging of the gas sample. The GC configuration was similar to that first used by Mosier and Mack (1980) and included a 6-m-long analytical column preceded by a 1-m-long pre-column, both 3-mm o.d. stainless steel tubes packed with Haysep Q. A 10-port gas-sampling valve was automated on the GC to send the O₂-free N₂ carrier gas (40 mL min⁻¹) through the columns in series (in inject mode) or to backflush the pre-column. At the posterior end of the analytical column, a four-port gas-sampling valve was synchronized to send the gas stream to the ⁶³Ni electron capture detector at 320°C. Samples for N₂O were taken at 0.5, 1, or 2 h after the jars were sealed and N₂O standards were used to create a standard curve. Soil core N₂O gas flux samples were taken 20 times during a 53-d period (Days 1, 2, 3, 5, 7, 9, 11, 13, 15, 17, 21, 25, 29, 31, 33, 37, 41, 45, 49, and 53).

Ammonia volatilization was also measured at the same time as the N₂O sampling periods by placing a piece of Whatman no. 42 filter paper impregnated with 20 µL of 14.6 mol L⁻¹ orthophosphoric acid in the headspace of the Mason jar. These acid traps were removed after 1 h and extracted with 10 mL of deionized water, with the extract analyzed for NH₄-N as described below. The hourly NH₃ fluxes were then integrated to yield the total NH₃-N emission during the 17 d following urine application. After this time, NH₃ emissions were not significantly different from the controls.

Destructive soil analyses were performed on Days 1, 3, 6, 10, 20, 35, and 55. Bulk density determinations were performed by dividing the oven-dry mass of soil (± biochar) by the volume occupied by the soil in the cores. Gravimetric water content determinations were made after drying the soil (± biochar) at 105°C for 24 h. Analyses conducted included surface soil pH, inorganic-N concentrations, water-soluble C (WSC), and microbial biomass C (MBC). Soil surface pH was determined after applying one drop of deionized water to the soil surface, whereupon the pH was measured with a flat-surface pH probe (Broadley-James, Irvine, CA). The top 2 cm of the soil core was then removed and mixed well in a small plastic bag for approximately 10 s. Soil subsamples were then taken for analyses requiring field-moist soil, and the remainder was air dried at 20°C.

Fig. 1. (a) Nitrous oxide flux (log-transformed) vs. time (error bars are $\pm$ standard error of the mean, $n = 4$); (b) cumulative N_2O -N flux after 53 d (error bars are $\pm$ standard error of the mean, $n = 4$).

Inorganic-N concentrations were determined by extracting 2 g of moist soil with 20 mL of 2 mol L^{-1} KCl for 60 min, filtering the extracts (Whatman no. 42), and performing the analyses on an Alpchem FS3000 twin-channel flow injection analyzer for NO_3^- -N, NO_2^- -N, and NH_4^+ -N with appropriate standards. The WSC concentrations were determined by extracting 2 g of moist soil with 20 mL of deionized water (Ghani et al., 2003), with the extracts filtered (Whatman no. 42) and analyzed for total organic C (TOC) on a Shimadzu TOC analyzer. The amount of MBC was assessed using the chloroform-fumigation technique (Vance et al., 1987). In brief, duplicate samples of moist soil (5 g) were used, with one sample fumigated immediately with purified chloroform for 18 to 24 h while the unfumigated sample was extracted with 0.5 mol L^{-1} K_2SO_4 (1:4 soil/extractant) for 30 min on an end-over-end shaker before filtering (Whatman no. 42) and analyzing for

TOC as described above. The fumigated sample was then extracted and also analyzed as above. For each soil sample, the MBC was calculated by subtracting the TOC values for the unfumigated treatment from values for the fumigated treatment and multiplying by a constant value of 0.45 (Jenkinson et al., 2004), which accounts for the efficiency of the soil microbial biomass extraction.

Statistical analyses were performed using Minitab (Minitab Inc., 2000) with one-way analysis of variance used to compare treatment effects at any given time. Soil pH values were converted to H^+ ion concentrations before analysis, with mean and confidence interval values converted back to pH values for graphical presentation. Nitrous oxide flux data were log-transformed ($\ln + 1$) before statistical analysis.

RESULTS

Nitrogen Gas Fluxes

From Days 1 to 7, the control soil had higher N_2O fluxes than the biochar control treatment, but after Day 9 the trend was for the N_2O fluxes from the biochar control to be significantly higher ($P < 0.01$) than the control treatment (Fig. 1a). The cumulative losses of N_2O -N from the control and biochar control treatments did not differ ($P = 0.11$) during the 53-d period and were 0.98 (0.19) and 1.71 (0.33) g N_2O -N m^{-2} respectively (SEM in parentheses).

It was 9 d after treatment applications before the urine-treated soils had N_2O fluxes significantly higher than those of the non-urine-treated soils. After this time, the urine-treated soils generally had significantly higher N_2O fluxes than the non-urine-treated soils until the end of the study (Fig. 1a). During the first 3 d, there were no differences in N_2O fluxes between the urine-treated soils. After this time, the biochar-plus-urine treatment had higher N_2O fluxes than in the urine treatment ($P < 0.01$), through until Day 30 with the exception of Day 15. The N_2O fluxes in the urine treatment peaked on Day 33 and slowly decreased thereafter (Fig. 1a). The biochar-plus-urine treatment N_2O flux peaked on Day 21 and then declined and did not differ from the urine treatment between Days 30 to 41, but after this time they were lower than in the urine treatment (Fig. 1a). As a percentage of urinary N applied, the cumulative N_2O -N flux in the biochar-plus-urine treatment (28.6% [5.9]) did not differ statistically from the urine treatment (16.8% [2.7]) (SEM are in brackets) (Fig. 1b).

Mean NH_3 -N fluxes peaked earlier and were higher ($P < 0.01$) in the biochar-plus-urine (1927 $\text{mg m}^{-2} \text{d}^{-1}$) treatment than in the urine-only treatment (1143 $\text{mg m}^{-2} \text{d}^{-1}$) on Day 1. The NH_3 -N emissions in the urine-only treatment peaked on Day 2 (1459 $\text{mg m}^{-2} \text{d}^{-1}$). The NH_3 -N fluxes were significantly lower ($P < 0.05$) in the urine-only treatment than in the biochar-plus-urine

Fig. 2. Soil surface pH vs. time (error bars are $\pm$ standard error of the mean, $n = 4$).

treatment for the first 3 d, and after this time the $\text{NH}_3\text{-N}$ fluxes did not differ between the urine treatments. Cumulative $\text{NH}_3\text{-N}$ fluxes reflected the differences in the peak flux rates between urine-treated soils, and after 3 d the cumulative fluxes in the urine-only treatment were significantly lower ($P < 0.01$) than in the biochar-plus-urine treatment and were 4.8 (0.2), and 7.1 (0.3)%, respectively, as a percentage of N applied (SEM in parentheses). After 17 d, the cumulative $\text{NH}_3\text{-N}$ fluxes as a percentage of N applied differed ($P < 0.05$), with higher cumulative fluxes in the biochar-plus-urine amended soil with $\text{NH}_3\text{-N}$ losses by volatilization equal to 16.7 (1.9) and 20.2 (1.6)% of N applied in the urine-only and biochar-plus-urine treatments, respectively (standard deviations in parentheses).

Soil pH

During the 55 d of the experiment, the mean soil surface pH ranged from 4.16 to 8.67. At Day 1, the treatments that received urine had mean soil surface pH values > 8.5 , which only began to decrease on Day 6, finally reaching mean values of < 4.4 by Day 55 (Fig. 2). Throughout the study, in the two treatments that received urine, there were generally no statistically significant differences in the soil surface pH values. Urine-treated soils had higher soil pH values than either the control or biochar control treatments during the first 20 d ($P < 0.01$) and lower ($P < 0.01$) pH values on Day 55 (Fig. 2). In the urine-treated soils, there was a significant correlation of surface soil pH with soil $\text{NH}_4\text{-N}$ concentrations ($P < 0.001$, $r = 0.95$). The soil surface pH of the biochar control treatment was only statistically higher than the control soil on Day 3 ($P < 0.05$) when values were 6.6 and 6.1, respectively.

Inorganic Nitrogen Concentrations

Maximum soil inorganic-N concentrations in the control and the biochar control treatments were 18, 0.3, and 93 mg kg^{-1} soil for $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$, respectively, with no statistically significant differences in the concentrations of any form of inorganic N between these two treatments throughout the study (Fig. 3). In the two treatments that received urine, the soil $\text{NH}_4\text{-N}$, $\text{NO}_2\text{-N}$, and $\text{NO}_3\text{-N}$ concentrations were greater ($P < 0.01$), on

Fig. 3. Soil (a) $\text{NH}_4\text{-N}$, (b) $\text{NO}_2\text{-N}$, and (c) $\text{NO}_3\text{-N}$ concentrations vs. time (error bars are $\pm$ standard error of the mean, $n = 4$).

all sampling occasions than in either the control or biochar control treatments. The maximum mean $\text{NH}_4\text{-N}$ concentrations on Day 1 in the urine-treated soils were 1643 and 1602 $\text{mg NH}_4\text{-N kg}^{-1}$ soil for the urine and biochar-plus-urine treatments, respectively (Fig. 3a), with no significant difference between the urine-treated soils. The soil $\text{NH}_4\text{-N}$ concentrations in these treatments then declined with time, with the minima occurring on Day 55 with respective concentrations of 73 and 111 $\text{mg NH}_4\text{-N kg}^{-1}$ soil. The biochar-plus-urine treatment had higher soil $\text{NH}_4\text{-N}$ concentrations than the urine-only treatment on Days 10 and 20 ($P < 0.01$) (Fig. 3a). The rates of decrease in the soil $\text{NH}_4\text{-N}$ concentrations between Days 10 to 20 were 52 and 72 $\text{mg NH}_4\text{-N kg}^{-1}$ soil d^{-1} for the urine and urine-plus-biochar treatments, respectively.

Maximum mean $\text{NO}_2\text{-N}$ soil concentrations in the two urine-treated soils of 9 and 98 $\text{mg NO}_2\text{-N kg}^{-1}$ soil occurred on Days 10 and 20 for the urine and biochar-plus-urine treatments, respectively (Fig. 3b). Soil $\text{NO}_2\text{-N}$ concentrations declined fol-

Soil $\text{NO}_3\text{-N}$ concentrations did not differ between the control and biochar control soils. Peak soil $\text{NO}_3\text{-N}$ concentrations in the urine treatments occurred between Days 20 and 35 (range of $427\text{--}530\text{ mg NO}_3\text{-N kg}^{-1}$) before concentrations decreased (Fig. 3c). In the urine-treated soils, statistical differences ($P < 0.01$) in soil $\text{NO}_3\text{-N}$ concentrations occurred only on Day 55 when the biochar-plus-urine treatment had higher concentrations than the urine-only treatment, with values of 397 and $155\text{ mg NO}_3\text{-N kg}^{-1}$, respectively.

Soil bulk densities in the biochar-amended soil treatments (range 0.78–0.83 Mg m⁻³) were significantly lower ($P < 0.01$) than in the urine-only treatment ($P < 0.01$), where bulk densities ranged from 0.97 to 0.98 Mg m⁻³ soil. There were no significant trends or differences between treatments in terms of soil moisture content, with the saturation of the biochar-plus-urine, urine-only, biochar control, and control treatment soils averaging 74 (3), 68 (3), 92 (3), and 86 (7)%, respectively (SEM in parentheses) during the experiment.

Figure 2 is a line graph showing the degradation of water-soluble carbon (WSC) over 55 days for four treatments: Control, Biochar control, Urine, and Biochar+urine. The y-axis represents WSC in mg kg^{-1} , ranging from 0 to 1600. The x-axis represents time in days, from 0 to 55. The Control and Biochar control treatments show a rapid decline in WSC, reaching near baseline levels by day 35. The Urine treatment shows a slower decline, reaching near baseline levels by day 55. The Biochar+urine treatment shows the slowest decline, maintaining high WSC levels throughout the 55-day period.

Days	Control (mg kg^{-1})	Biochar control (mg kg^{-1})	Urine (mg kg^{-1})	Biochar+urine (mg kg^{-1})
0	120	100	120	120
2	180	180	120	120
4	120	120	120	120
6	120	120	120	120
8	120	120	120	120
10	120	120	120	120
20	120	120	120	120
35	120	120	120	120
55	120	120	120	120

There was a trend for the average soil MBC concentrations to be higher under the biochar control treatment (range 297–830 mg kg⁻¹ soil) than the control (range 148–671 mg kg⁻¹ soil) but this was only statistically significant on Days 6 and 20 ($P < 0.05$). Microbial biomass C in these two treatments increased with time, peaking on Day 35. There were no significant differences in MBC concentrations between the urine-treated soils. The MBC declined rapidly after urine application and then gradually increased, with peak MBC values occurring on Days 35 (Fig. 5).

Inorganic-N concentrations were obviously higher in the urine-treated soils as a result of the urinary N applied. Since there were no differences in soil $\text{NH}_4\text{-N}$ concentrations between the urine-treated soils on Day 1, and given that the highest soil $\text{NH}_4\text{-N}$ concentrations occurred on Day 1, it is reasonable to assume that the higher soil $\text{NH}_4\text{-N}$ concentrations that were still present on Days 10 and 20 in the biochar-plus-urine treatment were the result of reduced depletion of the $\text{NH}_4\text{-N}$ pool due to treatment effects on the nitrification, immobilization, and volatilization processes. The greater loss of $\text{NH}_3\text{-N}$ in the biochar-plus-urine treatment may have slowed the rate of $\text{NH}_4\text{-N}$ depletion at Day 20 by inhibiting nitrifiers (Villaverde et al., 1997). Following pyrolysis of biomass and the formation of char, microbially toxic compounds (e.g., polyaromatic hydrocarbons) may reside on or in the char (Kim et al., 2003) and such compounds, or VOCs, can have bactericidal properties (Ward et al., 1997). The VOC analysis performed was only qualitative and did not determine the relative quantities of VOCs in the biochar. The VOCs present in the biochar have been previously found in *Pinus* species (α - and β -pinene, pinocarveol [Kurose et al., 2007; Simpson and McQuilkin, 1976]) or are products of the biochar manufacturing process (acetaldehyde). Inhibition of *Nitrosomonas* has been reported for α -pinene (Ward et al., 1997). It has also been noted (De Luca et al., 2006), however, that biochar can stimu-

late nitrification by removing inhibitory phenolic compounds in *Pinus* forest soils. Acetaldehyde was unlikely to be inhibiting nitrification because it has been reported as a product of substrate utilization by ammonia monooxygenase (McCarty, 1999). The lack of any difference between the urine-only and biochar-plus-urine treatments with respect to NO_3 formation at Day 55 indicates that any effect that biochar had on slowing the rate of NH_4 oxidation (i.e., between Days 10 to 20) was short term in nature.

The 2 mol L^{-1} KCl method for extracting soil $\text{NH}_4\text{-N}$ does not differentiate between $\text{NH}_4\text{-N}$ in soil solution and $\text{NH}_4\text{-N}$ on cation exchange sites. Biomass-derived biochar in the Terra Preta do Índio has been shown to enhance the charge density (potential cation exchange capacity [CEC] per unit surface area) compared with adjacent soils lacking biochar. Those soils, however, were 600 to 8700 yr old and the enhanced CEC was a consequence of biochar particles becoming oxidized and covered with adsorbed organic matter. This is in stark contrast to the biochar in the current experiment, which was freshly made and unweathered and had a low CEC. This agrees with the work of others (Lehmann, 2007) where the CEC of fresh biochar was reported to be low, with only aged biochar possessing high CEC. Thus the relatively slower rate of $\text{NH}_4\text{-N}$ depletion seen in the biochar-plus-urine treatment cannot be attributed to an enhanced pool of $\text{NH}_4\text{-N}$ on CEC sites. Absorption of $\text{NH}_4\text{-N}$ into the biochar material may also have occurred, and this may have provided a reservoir from which $\text{NH}_4\text{-N}$ could subsequently diffuse back into the soil solution, thus protecting the $\text{NH}_4\text{-N}$ pool.

Previous work on highly weathered soils has shown biochar to have beneficial effects on the soil microbial biomass (Steiner et al., 2008). In the absence of urine, the soil microbial biomass increased in the current study, although the reasons for this are not clear. In the presence of urine, however, there was a tendency for the MBC to be lower in the biochar-plus-urine treatment, although it was not statistically significant. The lower microbial biomass in the presence of urine may have been due to stress from osmotic or soil pH changes resulting from the urine application.

The elevated levels of WSC occurred in the urine treatments as a result of the high soil pH conditions following urine-urea hydrolysis, which were sufficiently high to solubilize soil organic matter. The fact that there were higher levels of WSC in the biochar-plus-urine treatment may have been due to a lack of microbial utilization of the WSC. While it was not statistically significant, this may have been due to the tendency for the microbial pool to be lower in the biochar-plus-urine treatment, as noted above.

Following the hydrolysis of the urea in the bovine urine, the formation of NH_3 in the soil can be considerable when the pH is high (>8.0). Asada et al. (2006) demonstrated the chemical adsorption of NH_3 , in aqueous solutions, onto biochar derived from bamboo. Thus NH_3 formed following urea hydrolysis could certainly have been absorbed by the biochar in the current study. If NH_3 was absorbed, it may have been transformed to $\text{NH}_4\text{-N}$ following the subsequent decline in soil pH (<8.0) and this could have contributed to the elevated $\text{NH}_4\text{-N}$ pools seen in the biochar-plus-urine treatment.

Soil pH results provided further evidence for reduced $\text{NH}_4\text{-N}$ depletion rates at Day 20, via nitrification, in the biochar-plus-urine treatment. The rapid increase in soil pH following urine application is a consequence of urea hydrolysis. The subsequent initial decrease in soil pH following urine application is a result of NH_3 volatilization (Sherlock and Goh, 1984) and this was accentuated by the net release of H^+ ions during the nitrification process (Wrage et al., 2001). Thus the slower the nitrification rate, the slower the decline in soil pH, which explains the strong correlation observed between soil pH and the soil $\text{NH}_4\text{-N}$ concentrations. The biochar-plus-urine treatment had both elevated soil pH and NH_4^+ concentrations at Day 20, further indicating that nitrification rates had been lower or delayed at this time. It is also worth noting that the liming effect of the biochar on the soil used here was insignificant compared with the changes in pH caused by urine application, and that biochar had a very limited liming effect, with a significant difference in soil pH due to biochar addition only significant on Day 3 compared with the control. The liming effect of biochar has been noted in other studies, but again, these positive liming results have been achieved after biochar addition to highly weathered soils (Chan et al., 2007).

The relatively earlier occurrence of the soil $\text{NO}_2\text{-N}$ peak and the relatively low maximum $\text{NO}_2\text{-N}$ soil concentration in the urine treatment demonstrated that NO_2^- oxidation occurred sooner and with a faster turnover of the $\text{NO}_2\text{-N}$ pool than in the biochar-plus-urine treatment, although the $\text{NO}_2\text{-N}$ concentrations in the biochar-plus-urine treatment were not statistically different from the urine-only treatment, they were an order of magnitude higher and occurred 10 d later. The latter fact supports the theory of slower nitrification rates, but it does not readily explain why the soil $\text{NO}_2\text{-N}$ concentrations were an order of magnitude higher in the biochar-plus-urine treatment. It could have been due to some inhibiting compound contained in the biochar, as noted above. Alternatively, the higher fluxes of NH_3 in the urine-plus-biochar treatment may have been sufficiently high to inhibit NO_2^- oxidizers (Smith et al., 1997; Vadivelu et al., 2007). Biochars have also been reported to be capable of adsorbing anions, both NO_3^- (Mizuta et al., 2004) and phosphate (Beaton et al., 1960; Lehmann et al., 2005), so adsorption of NO_2^- onto the biochar surface may have caused the elevated $\text{NO}_2\text{-N}$ concentrations measured in the biochar-plus-urine treatment.

The elevated N_2O fluxes in the biochar-plus-urine treatment between Days 15 and 29 are intriguing. For N_2O to form via biological mechanisms, it must involve the $\text{NO}_2\text{-N}$ pool (Stevens and Laughlin, 1998). Given that the elevated $\text{NO}_2\text{-N}$ concentrations did occur in the biochar-plus-urine treatment during the period of elevated N_2O fluxes and the fact that there was a slower $\text{NH}_4\text{-N}$ depletion rate, the higher N_2O fluxes may have been a consequence of greater "leakage" from the nitrification process either via a reaction of NO_2^- or compounds in the nitrification pathway that are precursors to this, such as hydroxylamine (Wrage et al., 2001). Our statistical analysis forced us to conclude that the cumulative N_2O emissions from the biochar-amended soil in the presence of urine were no different from

those of the urine-only treatment. There were large standard deviations around the cumulative means, however, and increased replication may have presented us with a different answer. The cumulative losses measured in this experiment were high and due to favorable experimental conditions for N_2O loss in terms of temperature, constant moisture, an abundance of inorganic-N substrate, and no competition from plant uptake or leaching. A field-based experiment, where factors are less favorable and with plant competition present, would produce lower losses of N_2O -N as a percentage of urine N applied. More intricate ^{15}N experiments are required to fully understand the reason(s) why the N_2O fluxes were higher during the period of Days 15 to 29.

Clearly, further studies are required to elucidate the N transformations and fluxes that occur when biochar is incorporated into soil receiving bovine urine if biochar is to be sequestered into pasture soils. It also needs to be noted that different biochar–soil combinations may well provide varying results. Future studies with ^{15}N tracer studies will be highly beneficial in determining the N_2O source mechanisms and fate of applied N, especially with regard to adsorption of N forms onto biochar. Further studies also need to be performed under field conditions where other N loss pathways such as leaching and plant uptake can be studied, in addition to the effect of biochar on N use efficiency in pastures. We would also support the suggestion made for an international biochar standard(s) to allow better comparisons of published literature (Schmidt and Masiello, 2007).

CONCLUSIONS

This laboratory study assessed the impact of incorporating biochar into a pasture soil on N_2O emissions following the application of bovine urine. It was hypothesized that the addition of biochar might mitigate N_2O emissions by reducing the size of the inorganic-N pool available via adsorption of $\text{NH}_4\text{-N}$ or the absorption of NH_3 . The incorporation of biochar failed to mitigate N_2O emissions in this laboratory-based study, however, and in fact stimulated N_2O emissions for a period. Despite this, the cumulative fluxes of N_2O -N after 53 d did not differ between biochar-amended soils receiving urine and soils treated with urine only. Differences occurred in the inorganic-N pool dynamics under urine when the biochar amendment was present, but $\text{NH}_4\text{-N}$ and $\text{NO}_2\text{-N}$ concentrations were elevated and biochar amendment did not reduce the soil inorganic-N pool available for N_2O production mechanisms.

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