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ENVIRONMENTAL BENEFITS OF BIOCHAR

Effects of Biochar Addition on Greenhouse Gas Emissions and Microbial Responses in a Short-Term Laboratory Experiment

Gayoung Yoo* and Hojeong Kang

Biochar application to soil has drawn much attention as a strategy to sequester atmospheric carbon in soil ecosystems. The applicability of this strategy as a climate change mitigation option is limited by our understanding of the mechanisms responsible for the observed changes in greenhouse gas emissions from soils, microbial responses, and soil fertility changes. We conducted an 8-wk laboratory incubation using soils from PASTURE (silt loam) and RICE PADDY (silt loam) sites with and without two types of biochar (biochar from swine manure [CHAR-M] and from barley stover [CHAR-B]). Responses to addition of the different biochars varied with the soil source. Addition of CHAR-B did not change CO_2 and CH_4 evolution from the PASTURE or the RICE PADDY soils, but there was a decrease in N_2O emissions from the PASTURE soil. The effects of CHAR-M addition on greenhouse gas emissions were different for the soils. The most substantial change was an increase in N_2O emissions from the RICE PADDY soil. This result was attributed to a combination of abundant denitrifiers in this soil and increased net nitrogen mineralization. Soil phosphatase and N-acetylglucosaminidase activity in the CHAR-B–treated soils was enhanced compared with the controls for both soils. Fungal biomass was higher in the CHAR-B–treated RICE PADDY soil. From our results, we suggest CHAR-B to be an appropriate amendment for the PASTURE and RICE PADDY soils because it provides increased nitrogen availability and microbial activity with no net increase in greenhouse gas emissions. Application of CHAR-M to RICE PADDY soils could result in excess nitrogen availability, which may increase N_2O emissions and possible NO_3 leaching problems. Thus, this study confirms that the ability of environmentally sound biochar additions to sequester carbon in soils depends on the characteristics of the receiving soil as well as the nature of the biochar.

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*Corresponding author (gayoo@khu.ac.kr).

5585 Guilford Rd., Madison, WI 53711 USA

Charcoal formation from biomass has been reported to be a promising strategy to convert easily decomposable biomass into a recalcitrant soil organic matter (Goldberg, 1985; Lehmann et al., 2006; Lehmann et al., 2010). Lehmann et al. (2006) estimated a potential global C sequestration of 0.16 Gt yr^{-1} using current forestry and agricultural wastes for biochar production. Although the importance of soil biochar applications as a long-lasting form of C storage to mitigate climate change has captured public attention recently (Goldberg, 1985), the agricultural importance of biochar dates back much earlier. For example, in Japan, charcoal use in agriculture goes back to at least 1697, when "ash manures" were applied to soils to increase crop yields (Ogawa and Okimori, 2010). The potential benefits of biochar are linked to two purposes: climate change mitigation and improving soil quality and fertility. The C storage purpose emphasizes the long-term stability of biochar in soil (Spokas, 2010; Zimmerman et al., 2011), while the benefit of enhanced soil quality emphasizes the changes in soil nutrients and soil physical and chemical conditions (e.g., pH, cation exchange capacity [CEC], and soil water-holding capacity [WHC]) (Novak et al., 2009; Laird et al., 2010a; Laird et al., 2010b).

The influence of biochar applications on net soil greenhouse gas (GHG) emissions varies with the type of biochar (Van Zwieten et al., 2009a; Spokas and Reicosky, 2009; Lehmann et al., 2010) and soil (Shneour, 1966; Spokas and Reicosky, 2009). Yanai et al. (2007) reported reduced emissions of N_2O in a laboratory chamber when the soil's water-filled pore space (WFPS) was 73%. However, when the soil was rewetted to 83% WFPS, N_2O emissions increased. The authors attributed changes in N_2O emissions to an interaction between pH and aeration of the soil. Because charcoal addition increased the soil's pH, N_2O-producing microbial activity may have been enhanced. Rondon et al. (2006) also observed reduced N_2O evolution in unfertile tropical soils after 8 and 20 t ha^{-1} biochar additions. This reduction in N_2O emissions was explained by increases in soil pH, CEC, and K availability. Singh et al. (2010), on the other hand, reported an initial elevation in N_2O

G. Yoo, Center for Environmental Studies, Dep. of Environmental Science and Engineering, College of Engineering, Kyung Hee Univ., 1 Seochon-dong, Giheung-gu, Yongin-si, Gyeonggi-do, 446-701, Republic of Korea; H. Kang, School of Civil and Environmental Engineering, Yonsei Univ., Shinchon-dong, Seodaemoon-gu, Seoul, Republic of Korea. Assigned to Associate Editor David Laird.

Abbreviations: CHAR-B, biochar from barley stover; CHAR-M, biochar from swine manure; CEC, cation exchange capacity; GHG, greenhouse gas; PCR, polymerase chain reaction; POM, particulate organic matter; WFPS, water-filled pore space.

emissions from soil after the addition of biochar made from poultry manure. The increase was related to the high labile nitrogen (N) content of the biochar itself. Methane emissions are also influenced by adding biochar. Rondon et al. (2005) noted a suppression of CH_4 emissions by adding biochar made from *Calliandra callothyrsus* to soils in grass and soybean stands. Furthermore, Karhu et al. (2011) reported that biochar additions increased CH_4 uptake due to better aeration in biochar-amended soils. However, there have also been observed decreases in net CH_4 oxidation potential of soils after biochar additions (Spokas et al., 2009; Spokas and Reicosky, 2009). The inconsistent effects of biochar additions on soil N_2O and CH_4 emissions are attributed to many factors, such as soil and biochar types, application rates, moisture regime, nutrient availability, and potentially other unknown causes.

Many studies have reported changes in microbial responses due to the addition of biochar. In some research, biochar was reported to stimulate the activity of a variety of agriculturally important soil microorganisms that affect the microbiological activity of soils (Ogawa et al., 1983; Pietikainen et al., 2000). Increased soil surface area resulting from adding biochar could provide additional habitat for microbial colonization by providing areas where they are protected from predator grazing (Saito and Marumoto, 2002; Warnock et al., 2007). Furthermore, the increased WHC of biochar may result in an overall improvement in soil moisture conditions stimulating microbial activity (Pietikainen et al., 2000). Biochars could also serve as a substrate for soil biota. Although many researchers regard biochar as a relatively recalcitrant substance that is altered little by biochemical processes (Nichols et al., 2000; Ascough et al., 2010), biochar surface properties are not strictly inert. Residual pyrolysis compounds, such as bio-oil, may condense on biochar surfaces during production and provide substrates for microbial growth and metabolism (Ogawa, 1994; Steiner et al., 2008) or may inhibit pathogens (Graber et al., 2010) and microbial activity (Clough et al., 2010; Spokas et al., 2010). Pietikainen et al. (2000) investigated changes in phospholipid fatty acid profiles and reported significant differences in the microbial community structure between biochar-amended and control soils. Substrate utilization patterns were also significantly influenced by biochar addition to soils (Pietikainen et al., 2000).

We hypothesized that the nutrient status of biochar is one of the most important factors influencing a soil's initial gas emission in response to biochar addition. Once labile portions of biochar are metabolized and removed, biochars provide little or no energy or C for soil microbes. Accordingly, we assumed that biochar influences the soil microbial community during the initial period of time in the soil by the physical and chemical changes in the soil environment (Cheng et al., 2008).

In this study, we conducted a short-term laboratory incubation of soils with two different types of biochar to investigate changes in soil microbial response and GHG production. Biochar was prepared using barley stover and swine manure, which are typical byproducts of agriculture in Korea. These biochars have unusually high N contents, which may be beneficial in terms of soil fertility and harmful in terms of GHG emissions and nutrient leaching. In this sense, this experiment is necessary to provide a scientific basis for the application of N-rich biochars to soils as a management strategy. To use biochar as a long-term C storage medium, the potential emission or absorption of GHGs needs to be evaluated. On the other hand, to use biochar as a soil amendment to improve soil quality, the potential nutrient benefits and drawbacks must be carefully identified.

The objectives of this study were to identify the effects of biochar additions to soils on (i) soil CO_2, CH_4, and N_2O emissions and soil enzyme activities and microbial communities and (ii) to relate changes in soil GHG emissions with the biogeochemical properties of soils, especially N dynamics.

Materials and Methods

Soil Sampling and Analysis of Basic Physicochemical Properties

Soils for the incubation studies were collected on 22 Apr. 2010 from the surface (0–10 cm depth) of a pasture (PASTURE) and an adjacent rice paddy (RICE PADDY) in Chunan si, Chungcheongnam-Do, Korea. According to the USDA Soil Classification system, both soils are Utisols. Three soil cores were collected from different locations in the PASTURE and RICE PADDY sites and composited. In the laboratory, soil samples were passed through a 2-mm sieve and air-dried for 2 wk. Soil texture was determined using the hydrometer method, pH was determined with a glass electrode using a 1:1 (w/v) soil to deionized water ratio, and NO_3^- and NH_4^+ concentrations were determined using the salicylate microplate method (Sims et al., 1995). To quantify labile soil organic carbon (C), particulate organic matter (POM) was separated from the bulk soil by dispersing 20-g soil samples with 50 mL of sodium hexametaphosphate solution (50 g L^{-1} H_2O). The suspensions were shaken at high speed on a reciprocal shaker for 1 h and passed through a 53-μm sieve (Wander et al., 1998). Samples were ground to analyze total and labile C and N contents by combustion analysis using a Carlo Erba NS 1500 C/N analyzer (Carlo Erba, Milan, Italy).

Biochar Preparation

Two different biochars were used in this study. Biochar prepared from barley stover (CHAR-B) was first air dried and ground to pass through 2-mm sieve. Barely stover (160 g) was added to a 5-L reactor, and 10% of sodium hydroxide (>98.0%) and potassium hydroxide (>85%) were mixed with 2 L of distilled water. The headspace of the reactor was circulated with N gas to remove oxygen, and then the reactor was heated to 320°C at 144 bar. After 30 min of reaction, the reactor was cooled using an air compressor for 15 h to reduce the temperature of the liquefaction products to room temperature. The liquefaction products were filtrated to remove the liquid phase, and the filtrates were dried at 70°C for 2 d.

Biochar from swine manure (CHAR-M) was synthesized using pyrolysis of swine manure. Using a centrifuge at 3000 rpm, the liquid contained in the waste was separated, and the sludge was obtained using a membrane filter press. The sludge was dehydrated in two steps, first using a jacket dryer at 300 to 400°C and then using a moving dryer at 500 to 600°C. Finally, using a closed screw-type reactor, the sludge was carbonized at 600 to 800°C. The procedures used to produce CHAR-B and

CHAR-M are patented by MSBEEE Inc. Korea. The pH of each biochar was determined in a 1:5 ratio of air-dried material to deionized water (w/v). The particle size distribution of CHAR-B was determined by a particle size analyzer (Mastersizer-S; Malvern Instruments, Herrenberg, Germany). The CHAR-M biochar included larger particle sizes and therefore could not be analyzed by the same machine. Therefore, we used a sieve shaker (ATL-7077; Retsch Co., Newtown, PA) for particle size determination of the CHAR-M. The surface areas of biochars were measured by the BET method (Monosorb MS-22; Quantachrome Corp., Boynton Beach, FL) (Lim, 2004).

Incubation

To investigate the effect of biochar additions on GHG emissions and soil microbial activity, soil microcosms were constructed using 0.30-L glass jars with a septum. Each jar received 50 g of 105°C dry-weight–equivalent soil. Treatments consisted of the addition of CHAR-B or CHAR-M. Biochar control (CON), which is soil without biochar, was also used and compared with the treatments. All the treatments and controls were performed with six replications. The application rate of biochar was 2% by weight, which was in the lower level of additions reported by Kolb et al. (2009) and Yanai et al. (2007) and equivalent to 20 ton ha^{-1} if calculated based on 10 cm incorporation depth in the field. After charcoal addition, the soil water was adjusted at 70% WHC and incubated in the dark at 30°C for 8 wk. During the incubation, the containers were sealed except when the lids of the containers were opened every 3 d to reaerate the microcosms. The headspace air was recirculated with ambient air using a fish tank bubbler.

Measurements

Gas samples in the headspace were collected at 4, 7, 17, 24, and 36 d after the initiation of incubation. Concentrations of CO_2, CH_4, and N_2O were measured by a gas chromatography (CP3800; Varian, Cary, NC). Details about trace gas measurements can be found in Jang et al. (2011) and Park and Kang (2010). To check the degree of aeration in the incubation jars, a separate set of soil samples was incubated, and the oxygen concentration in the jar headspace was measured daily for 1 wk with the jars sealed air-tight. Gas emission rates were calculated as the change in gas concentration relative to the initial gas concentration divided by the time the container was closed. After the 8-wk incubation, destructive soil sampling was conducted. We measured available N contents (NO_3^- and NH_4^+) and soil pH using fresh soil samples. Sample pH was determined with a glass electrode using 1:1 (w/v) soil:deionized water ratio. The NO_3^- and NH_4^+concentrations were determined using the salicylate microplate method (Sims et al., 1995). Net N mineralization was measured by the difference between initial and final contents of NO_3^- and NH_4^+ in the soils. The fumigation-extraction method was used to measure microbial C and N contents (Vance et al., 1987; Solaiman, 2007) for the fresh soil samples. The extraction factors used were 0.45 for C and 0.54 for N.

Extracellular hydrolysis by enzymes has been reported as a critical step in inorganic matter decomposition and nutrient cycling in soil ecosystems. Furthermore, enzyme activities are associated with trace gas emissions from wetland ecosystems (Kang et al., 1998). The activities of four enzymes (β-glucosidase, phosphatase, N-acetylglucosaminidase, and arylsulphatase) were determined using methylumbelliferyl compounds as model substrates (Kang and Freeman, 1999). These enzymes play a key role in the decomposition of cellulose, organic phosphorus, chitin, and organic sulfur, respectively. These enzyme activities have been assessed in relation to biogeochemical processes in soil ecosystems, including mineralization rates of organic matter, trace gas fluxes, changes in hydrochemistry, and general activity of microbes.

To estimate the bacterial, fungal, and archaeal biomass, we used quantitative real-time polymerase chain reaction (PCR). We performed quantitative PCR using an I-CyclerTM (version 3.0a; Bio-Rad, Hercules, CA) and SYBR Green (Bio-Rad) as a detection system. Each 20-μL sample contained the specific primer set for each group. Primers used were 341F (5′-CCTACGGGAGGCAGCAG-3′)-797R (5′-GGACTACCAGGGTCTAATCCTGTT-3′) for bacteria (Lane, 1991; Nadkarni et al., 2002), ITS1F (5′-CTTGGTCATTTAGAGGAAGTAA-3′)-ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primer pair for fungi (Gardes and Bruns, 1993; White et al., 1990), and Arch349F (5′-GYGCASCAGKCGMGAAW-3′)-Arch806R (5′- GGACTACVSGGGTATCTAAT-3′) for archaea (Takai and Horikoshi, 2000). The amplification followed a three-step PCR for the bacterial 16S rRNA gene and the fungal ITS region using 40 cycles with denaturation at 94°C for 25 s, primer annealing at 50°C (for bacteria and fungi) or 52°C (for archaea) for 25 s, and extension at 72°C for 25 s. Two independent real-time PCR assays were performed on each soil DNA extract. Standard curves were created using a 10-fold dilution series of plasmids containing the bacterial 16S rRNA gene, fungal ITS region, and archaeal 16S rRNA gene from environmental samples for bacterial, fungal, and archaeal communities, respectively.

Statistical Analyses

Analysis of variance was performed using the MIXED procedure of SAS 9.1 (SAS Institute, 2001) on soil CO_2, CH_4, and N_2O emission rates; β-glucosidase, phosphatase, N-acetylglucosaminidase, and arylsulphatase activity; microbial biomass C and N; and abundances of bacteria, archae, and fungi. Soil (PASTURE and RICE PADDY), treatment (CON, CHAR-B, and CHAR-M), and date were the fixed variables. Least square means were used to test for significant differences among soils and treatments at the 1, 5, and 10% probability levels.

Results and Discussion

Soil and Biochar Characterization and Oxygen Level in the Headspace

Physical and chemical characteristics of the soil samples are summarized in Table 1. Soil texture was silt loam for the PASTURE and RICE PADDY soils. Total and particulate organic C contents were two times greater in the PASTURE soil than in the RICE PADDY soil. Available N (NH_4^+ and NO_3^-) contents in both soils were not different, whereas POM

Table 1. Basic physicochemical characteristics of the soils from the PASTURE and RICE PADDY sites.

	Sand (>50 μm)	Silt (2–50 μm)	Clay (<2 μm)	pH	C/N	Total C	Total N	POM C	POM N	NH_4^+	NO_3^-
	%					mg kg^{-1} soil					
Pasture	15 (0.12)†	60 (0.23)	25 (0.22)	7.85 (0.02)	13.7 (0.15)	11.0 (0.19)	0.8 (0.09)	6.4 (0.01)	0.5 (0.01)	1.4 (0.09)	1.9 (0.14)
Rice paddy	9 (0.15)	64 (0.35)	27 (0.17)	7.83 (0.02)	9.0 (0.21)	5.2 (0.17)	0.6 (0.06)	3.1 (0.01)	0.2 (0.02)	1.3 (0.12)	1.6 (0.16)

† The numbers in parentheses are SEM (n = 3).

N content was higher in the PASTURE soil than in the RICE PADDY soil.

The physicochemical properties of CHAR-B and CHAR-M are summarized in Table 2. Total C content in CHAR-M was higher than in CHAR-B, whereas N content in CHAR-M was lower than in CHAR-B. The C/N ratio of CHAR-B was 5.8, and that of CHAR-M was 12.8. The very low C/N ratio of CHAR-B indicates that during the production of our chars, C might have been combusted rather than pyrolyzed. For metal contents, Ca and Mg were higher in CHAR-B than in CHAR-M, whereas Al, Fe, K, and Na were higher in CHAR-M than in CHAR-B.

The oxygen level in the headspace of the microcosms is reported in Table 3. Because we opened the lids of the containers every 3 d, the oxygen level after 72 h is our primary concern. The O_2 concentration drop was the biggest within the first 24 h, which corresponds to the highest CO_2 production rates in the same period. The O_2 concentrations in the headspace did not differ among treatments and did not drop below 15% within the first 72 h. This indicates that our incubation experiments were conducted under aerobic conditions. However, this may not ensure that all the soil pores were aerobic. Linn and Doran (1984) reported that when the soil WFPS was >60%, aerobic microbial activity decreased. Because our water adjustment was 70% WHC, we consider that our incubation environment was overall aerobic with some anaerobic pores. This consideration is important for the discussion of soil CH_4 and N_2O production.

Soil Carbon Dioxide and Methane Production

There was a significant interaction between treatment, soil, and date on CO_2 emissions (Table 4). For the RICE PADDY soil, cumulative CO_2 emissions were significantly lower for the soil amended with CHAR-M than for the soils with CON and CHAR-B treatments (Fig. 1). For the PASTURE soil, CO_2 emissions from the soil amended with CHAR-M was higher than the other treatments only in the initial stage of the incubation. After 20 d of incubation, no treatment effect on CO_2 evolution was found. The CO_2 evolution pattern from the PASTURE soil was similar to that observed by Smith et al. (2010) in their laboratory study. They also found an increase in CO_2 emissions from soils amended with biochar made from switchgrass (*Panicum virgatum*); this increase lasted only for the first few days. Their results support the assertion that biochar is not completely inert but has some labile C that is readily available to soil microorganisms for use as an energy source for a short time. Kolb et al. (2009) also found a positive effect of biochar additions on basal respiration for four soils with different textures. Surprisingly, addition of CHAR-M to the RICE PADDY soil did not stimulate CO_2 evolution; indeed the amount of CO_2 evolved was smaller than that emitted from the CHAR-B–amended or CON soils. This difference shows the importance of biochar and soil properties in determining the effects of biochar amendments on CO_2 emissions. We speculated that soil in the PASTURE site was N limited (there was a higher C/N ratio in PASTURE soil than the RICE PADDY soil); hence, when CHAR-M, which has high available N

Table 2. Physico-chemical properties of the biochars used for this study. There was no replicate for metal content measurement.

	Particle size			pH	Total C	Total N	Surface area	Metal content					
	>56 μm	10–56 μm	<10 μm					Al	Ca	Fe	Mg	K	Na
	%			mg kg^{-1} soil				mg kg^{-1} soil					
CHAR-B†	93.8 (3.98)‡	–6.2 (1.44)		6.68 (0.05)	309 (21.55)	53 (6.52)	40.60 (3.72)	825	12,610	1,935	9,883	1,260	1,089
CHAR-M§	24.0 (2.39)	59.0 (6.38)	17.0 (3.39)	7.21 (0.07)	474 (18.68)	37 (5.98)	75.63 (6.19)	6,535	4,006	6,187	1,334	5,834	18,500

† Biochar from barley stover.

‡ The numbers in parentheses are SEM (n = 3).

§ Biochar from swine manure.

Table 3. Oxygen level in the headspace of the incubation containers (n = 3).

Soil	Treatment†	Time (h)							
		0	24	50	72	96	126	144	168
		%							
Pasture	CON	18.00	16.47	16.00	15.93	16.05	16.25	15.82	15.57
	CHAR-M	18.00	15.54	15.43	15.80	15.96	16.02	15.77	15.67
	CHAR-B	18.00	15.48	15.80	15.66	15.96	15.97	15.63	15.74
Rice paddy	CON	18.00	15.65	15.70	15.59	16.05	15.85	15.66	16.06
	CHAR-M	18.00	15.57	15.50	15.66	15.60	15.21	16.06	15.93
	CHAR-B	18.00	14.74	15.66	15.37	15.76	16.03	16.05	15.44

† CHAR-B, biochar from barley stover; CHAR-M, biochar from swine manure; CON, control (soil without biochar).

Table 4. Analysis of variance results examining the effects of the biochar from swine manure and biochar from barley stover additions to the PASTURE and RICE PADDY soils on CO_2, CH_4, and N_2O emissions, enzyme activities, microbial biomass, and microbial communities.

Source	Soil	Treatment	Soil × treatment	Date	Soil × date	Treatment × date	Soil × treatment × date
			Pr > F				
CO_2	<0.0001***	0.0009***	0.3949	<0.0001***	<0.0001***	0.1018	0.0357**
CH_4	<0.0001***	0.0274**	0.8567	<0.0001***	<0.0001***	0.4276	0.9889
N_2O	0.0671*	<0.0001***	0.0040***	<0.0001***	0.2142	0.0005***	0.0076***

	Enzyme activity				Microbial biomass			Microbial community		
	Phosphatase	Sulfatase	β-Glucosidase	N-acetyl glucosaminidase	C	N	C/N	Bacteria	Archae	Fungi
Soil	0.5820	0.3510	0.0075***	0.2931	0.4309	0.4308	0.2196	0.0032***	0.0004***	0.0003***
Treatment	0.0286**	0.3455	0.3731	0.0955*	0.1635	0.0012***	0.0530	0.7509	0.7906	0.0020***
Soil × treatment	0.4036	0.1499	0.0653*	0.5959	0.3418	0.3585	0.2662	0.7525	0.7897	0.0020***

*,**,*** Significant at the 0.1, 0.05, 0.01 probability levels, respectively.

(Table 5), was added CO_2 production was stimulated, whereas addition of CHAR-M to the RICE PADDY soil decreased CO_2 emissions.

The RICE PADDY site receives regular N fertilization additions. Hence, we speculate that the microbial community in the RICE PADDY soil was adapted to high N conditions. In this context, the addition of char may have suppressed the metabolic activity of the soil microbial community. Spokas et al. (2009) conducted an experiment to separate CO_2 emission of biochar from CO_2 emissions of soils to verify a stimulating effect of biochar additions on soil respiration. They concluded, after subtracting the amount of CO_2 evolved from the biochar treatment from the CO_2 evolved from the soil+biochar treatments, that the biochar amendments had suppressed CO_2 production compared with the control soil. The exact cause of this reduction was not discussed. The observation of Spokas et al. (2009) was partially mirrored in our results for the RICE PADDY soil, supporting the hypothesis that biochar, in addition to sequestrating C, might aid in stabilizing soil organic C by reducing the rate of mineralization (Amonette et al., 2003; Zimmerman et al., 2011).

Net production of CH_4 was decreased by the addition of CHAR-M to both soils (Fig. 2). Although our incubation environment had enough oxygen to ensure general aerobic condition, the incubated soils may contain some anaerobic pores where a methanogenic community could produce CH_4. Our results on CH_4 emissions can be interpreted in three ways: (i) Production of CH_4 was inhibited, (ii) CH_4 uptake was stimulated, or (iii) evolved CH_4 was adsorbed by CHAR-M. Our results on CH_4 emissions are consistent with those of Rondon et al. (2005) and Karhu et al. (2011), who observed decreased CH_4 emissions from soils treated with biochar relative to controls. They speculated that the decreased CH_4 emissions were due to an increase in soil aeration caused by the biochar addition, which caused a reduction in soil bulk density. Our results favored the first mechanism because CO_2 production was also decreased by the addition of CHAR-M. However, it is not clear whether addition of CHAR-M inhibited methanogenic microbes or stimulated methanotrophic microbes. Identification and quantification of methanogenic and methanotrophic microbes would greatly improve our understanding of the mechanisms. The third mechanism may explain the different responses between CHAR-M and CHAR-B. Although CHAR-M had a higher surface area (75.63 g m^{-2}) than CHAR-B (40.60 g m^{-2}), informal experimental results showed that adsorption of evolved CH_4, measured using a fixed bed adsorption method (Belmabkhout et al., 2009), was not different between CHAR-B–treated and CHAR-M–treated soils (data not shown). Further experiments should be conducted to investigate the gas adsorption capacity of biochar materials.

Soil Nitrous Oxide Emission and Nitrogen Availability

Temporal changes in soil N_2O emissions differed by treatment and soil (Table 4). For the PASTURE soil, N_2O emissions decreased by the addition of CHAR-B, whereas for the RICE PADDY soil, N_2O emissions were enhanced by the addition of CHAR-M (Fig. 3). Nitrous oxide emissions

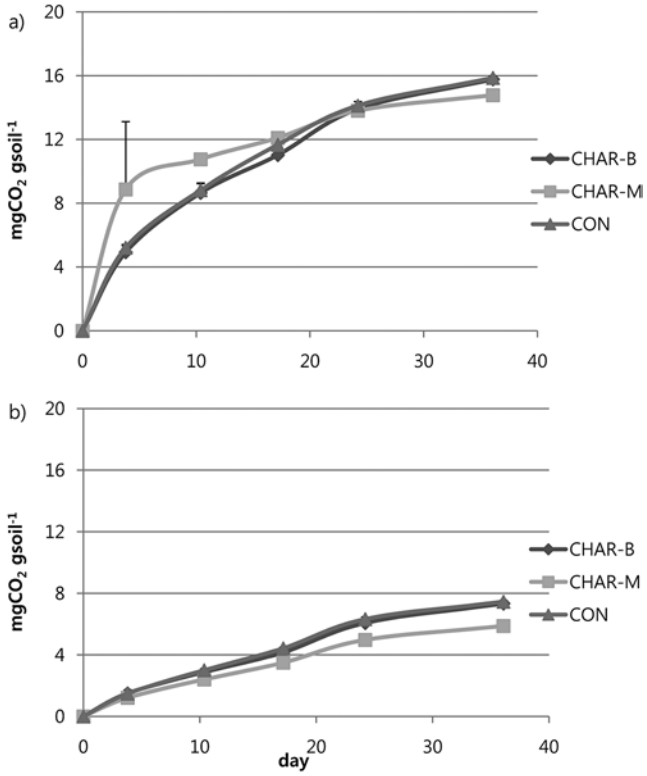

Fig. 1. Accumulative CO_2 evolution during incubation of the (a) PASTURE and (b) RICE PADDY soils amended with biochar from barley stover (CHAR-B) and biochar from swine manure (CHAR-M). The bars are 1 SEM (n = 6). Some of error bars are too small to be discernible.

Table 5. Changes in concentrations of NO_3^- and NH_4^+ in soils before and after incubation and net mineralization of nitrogen (n = 6).

Soil	Treatment†	Before incubation		After incubation		Net mineralization
		NO_3^-	NH_4^+	NO_3^-	NH_4^+	$\Delta(NH_4^+ + NO_3^-)$
		mg g soil^{-1}				
Pasture	CON	1.89	1.41	10.64	4.28	11.60a‡
	CHAR-M	8.90	1.30	25.03	3.87	18.70b
	CHAR-B	2.16	1.27	12.70	4.17	13.40a
Rice paddy	CON	1.60	1.30	5.88	3.82	6.80a
	CHAR-M	16.40	1.43	28.85	3.86	14.90b
	CHAR-B	1.93	1.43	6.74	4.04	7.40a

† CHAR-B, biochar from barley stover; CHAR-M, biochar from swine manure; CON, control (soil without biochar).

‡ Values in the same column followed by the same letter are not significantly different at $P < 0.05$.

generally originate from denitrification or nitrification processes (Pathak, 1999). We adjusted the water content of soil samples to 70% WHC. Although this is an aerobic condition, there may have been some saturated pores in the incubated soils. Therefore, we cannot exclude denitrification as a cause of N_2O evolution. A significant increase in N_2O evolution from the RICE PADDY soil amended with CHAR-M could be attributed to a higher availability of N, which was consistent with Singh et al. (2010). Nitrogen mineralization was enhanced with the CHAR-M treatment for both soils (Table 3). This increase is probably related to the fact that the C/N ratios of the CHAR-M and CHAR-B were relatively low compared with biochars used in other studies (Spokas and Reicosky, 2009; Chan et al., 2008; Smith et al., 2010). Accordingly, biochar addition stimulated N mineralization like other organic amendments to soils, which suggests a need for caution when applying high-N biochars to soils.

For the RICE PADDY soil, net N mineralization expressed as $\Delta(NO_3^- + NH_4^+)$ was enhanced by 119% by the addition of CHAR-M compared with the CON soil, whereas net N mineralization for the PASTURE soil was enhanced by 61% compared with the CON soil. It has been widely reported that soils in rice paddies have a large variety of denitrifiers (Hussain and Pan, 2011). We propose that a combination of abundant preexisting denitrifiers in our RICE PADDY soil and a high level of N availability could cause a rapid increase in N_2O emissions through denitrification. On the other hand, for the PASTURE soil, there was no net increase in N_2O emissions by the addition of CHAR-M despite the high level of N availability. This difference is probably due to the lower level of denitrifiers in the microbial community of the PASTURE soil.

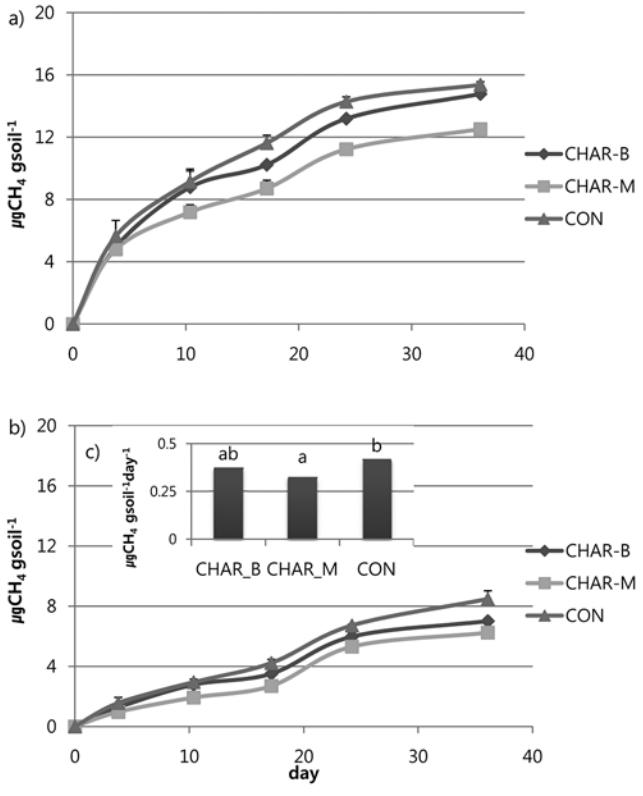

Fig. 2. Accumulative CH_4 evolution during incubation of the (a) PASTURE and (b) RICE PADDY soils amended with biochar from barley stover (CHAR-B) and biochar from swine manure (CHAR-M). (c) Averaged CH_4 evolution from the two soils by treatments. The bars are 1 SEM (n = 6). Some of error bars are too small to be discernible.

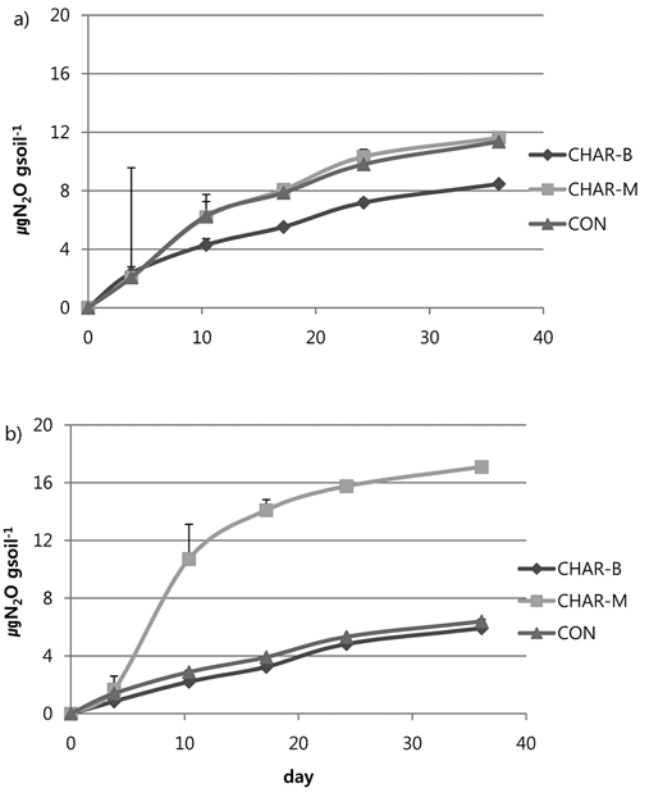

Fig. 3. Accumulative N_2O evolution during incubation of the (a) PASTURE and (b) RICE PADDY soils amended with biochar from barley stover (CHAR-B) and biochar from swine manure (CHAR-M). The bars are 1 SEM (n = 6). Some of error bars are too small to be discernible.

The observed decrease in N_2O emissions when CHAR-B was added to the PASTURE soil is consistent with the studies of Yanai et al. (2007) and Rondon et al. (2005). These authors explained the decreased emissions by changes in the soil chemical environment induced by biochar addition, especially increases in pH and CEC. In our experiment, there was no significant change in soil pH among the CON, CHAR-M, and CHAR-B soils, which were 7.57, 7.47, and 7.50 for the PASTURE soil and 7.51, 7.48, and 7.53 for the RICE PADDY soil, respectively. Hence, pH change cannot explain the decrease in N_2O emissions that occurred when CHAR-B was added to the PASTURE soil. There must have been some other factors to suppress denitrification or nitrification in the CHAR-B–treated soils, especially in soil from the PASTURE site. This result is in agreement with our finding of decreased fungal biomass in the CHAR-B–treated soil from the PASTURE site (Fig. 6). The CHAR-B might contain inhibiting materials for certain microbial groups, in this case denitrifiers and nitrifiers. Clough et al. (2010) suggested that compounds from biochar could potentially be toxic to microbes. Again, identification of specific microbes might enhance our level of understanding of the mechanisms behind N_2O emissions.

Soil Enzyme Activity and Microbial Community

Soil enzyme activities were influenced by biochar additions to soils, but the change pattern was not consistent with soil or treatment (Table 4). Phosphatase activity was significantly increased in both soils by adding CHAR-B but not by adding CHAR-M (Fig. 4). N-acetylglucosaminidase activity was higher for the CHAR-B treatment than for the CHAR-M treatment. The lower N-acetylglucosaminidase activity for the CHAR-M treatment may be related to the high content of available N in the CHAR-M–treated soils (Table 3). The CHAR-B treatment had lower β-glucosidase activity than the control only in the PASTURE soil. Fansler et al. (2009) also observed a significant decrease (75%) in β-glucosidase activity measured in biochar-amended soils when compared with control soils. They asserted that the significant reduction of β-glucosidase activity might be related to the fact that biochar chemically altered or blocked binding sites for the substrate. Recently, Bailey et al. (2011) reported that β-glucosidase is inhibited by the presence of biochar, whereas an opposite response was observed for N-acetylglucosaminidase. Considering those factors, our results suggest that increased enzyme activities were induced by microbial proliferation during the incubation, but lower activities might be partly explainable by higher nutrient availability (N-acetylglucosaminidase by CHAR-M) or by chemical blocking of substrates by biochar (β-glucosidase in the PASTURE soils). The negative correlation between β-glucosidase activity and CO_2 emission ($r = -0.4955$; $p < 0.05$) suggests that evolved CO_2 might have been adsorbed by biochars. Further sorption experiments are needed to determine the underlying mechanisms.

Microbial biomass N and C/N ratios were greatly enhanced and decreased, respectively, in both soils by the addition of CHAR-M (Fig. 5). Increased microbial biomass N suggests microbial immobilization of N. There was no significant change in microbial biomass C due to the addition of CHAR-M or CHAR-B. This finding is consistent with Van Zwieten et al. (2009b), who also observed no change in soil microbial biomass C after addition of poultry litter biochars in an incubation study. On the other hand, decreased microbial biomass C due to biochar addition was reported by Dempster et al. (2010). The significant decrease in microbial C/N ratio in the CHAR-M–treated soil suggested a possible shift in microbial community structure.

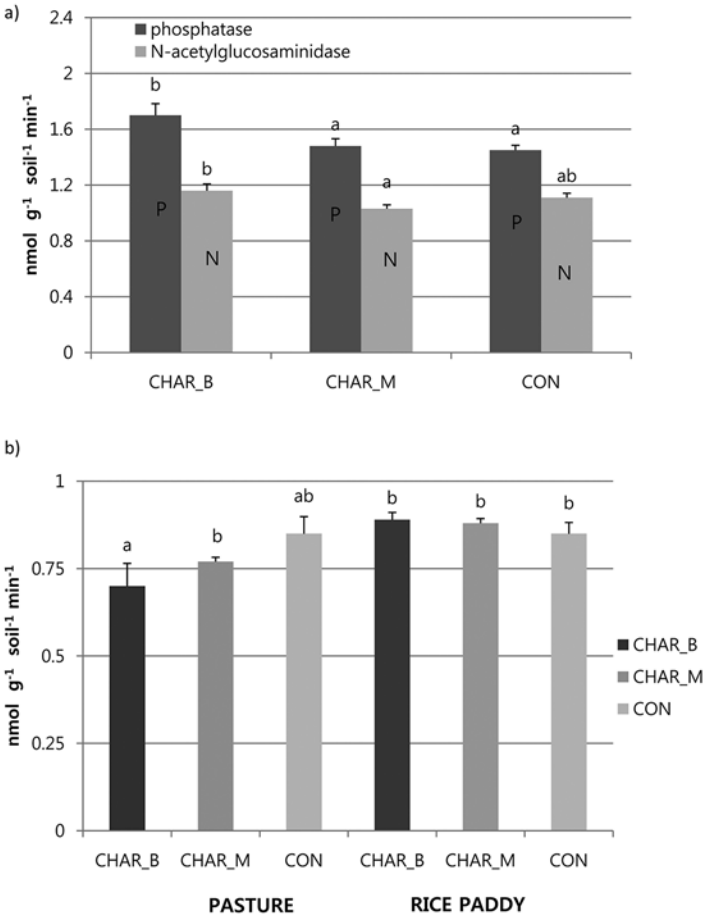

Fig. 4. Changes in soil enzyme activities influenced by biochar from barley stover (CHAR-B) and biochar from swine manure (CHAR-M) addition to soils. Averaged values of (a) phosphatase activity and N-acetylglucosaminidase activity and (b) β-glucosidase activity for the PASTURE and RICE PADDY soils. Bars with different letters are significantly different at a 10% probability level (*n* = 6).

In our experiment, there were no treatment effects on bacterial and archaeal gene copy number, whereas a significant effect on fungal gene copy number was observed (Fig. 6). For the PASTURE soil, the total biomass of fungi was smaller with CHAR-B addition than with CHAR-M addition or CON, whereas for the RICE PADDY soil, there was a larger fungal biomass with the CHAR-B than with the CHAR-M and CON treatments. Our results from the RICE PADDY soil are consistent with those of Warnock et al. (2007) and Steinbeiss et al. (2009), who reported positive effects of biochar additions on mycorrhizal abundance and fungi. Four possible mechanisms linking biochar additions to the stimulation of fungi were suggested by Warnock et al. (2007): (i) the change in soil nutrient availability by biochar addition,(ii) the indirect effect of biochar through effects on other soil microbes, (iii) the change in

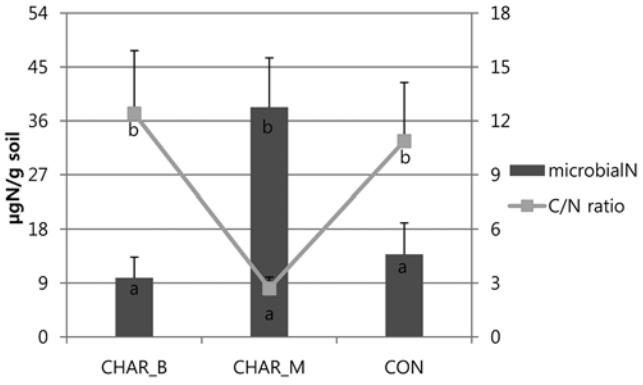

Fig. 5. Influence of biochar from barley stover (CHAR-B) and biochar from swine manure (CHAR-M) additions to soils on microbial biomass N and C/N ratio. Bars and lines with different letters are significantly different at a 10% probability level (*n* = 6).

plant-fungus signaling processes, and (iv) biochar's provision of refugia from hyphal grazers. Our results on N availability do not to support the first mechanism because the magnitude of change in N availability was greater for the CHAR-M than for the CHAR-B treatments (Table 3). If the first hypothesis were correct, a significant change in fungi biomass with the CHAR-M treatment should have been observed. Additions of biochar to soil generally change nutrient status by altering soil pH, CEC, WHC, etc. (Lucas and Davis, 1961; Lehmann et al., 2003; Topoliantz et al., 2005). The changes in soil nutrient status other than N would influence the microbial community, and these changes might have been reflected in our findings of fungal biomass. The second mechanism seems to be a more probable explanation for our results on changes in fungi biomass. Our biochar might contain certain materials toxic or beneficial to the growth of certain groups of microbes, and these effects would be manifested differently in the context of soil environments. Biochar can stimulate the growth of certain bacteria, such as the *Paenibacillus* species, which is a growth enhancer of fungi mycelium (Hildebrandt et al., 2002). In our experiment, materials beneficial for fungal growth contained in CHAR-B might be effective in the environment of the RICE PADDY soil, whereas material toxic to fungi contained in the CHAR-B might be effective in the environment of the PASTURE soil. Because we did not grow plants in the incubated soils, we could not make any evaluation of the third mechanism emphasizing the interactions between plants and fungi. The fourth proposed mechanism is about a physical process in which biochar provides places to colonize fungal hyphae. This mechanism is a probable explanation of the enhanced fungal biomass in the CHAR-B–amended RICE PADDY soil. However, we could not clarify the inconsistent effects of CHAR-B on the PASTURE and RICE PADDY soils with this mechanism. Taxa-specific information in each treated soil would be required to acquire a mechanistic understanding. For example, Khodadad et al. (2011) have reported that biochar additions can induce opposite responses in CO_2 emissions and gene copy number of 16S rRNA depending on characteristics of the biochar. More labile biochar could provide new C sources, resulting in higher microbial respiration, whereas refractory biochar provides a suitable habitat for microbial proliferation (Zimmerman et al., 2011; Spokas, 2010). In addition

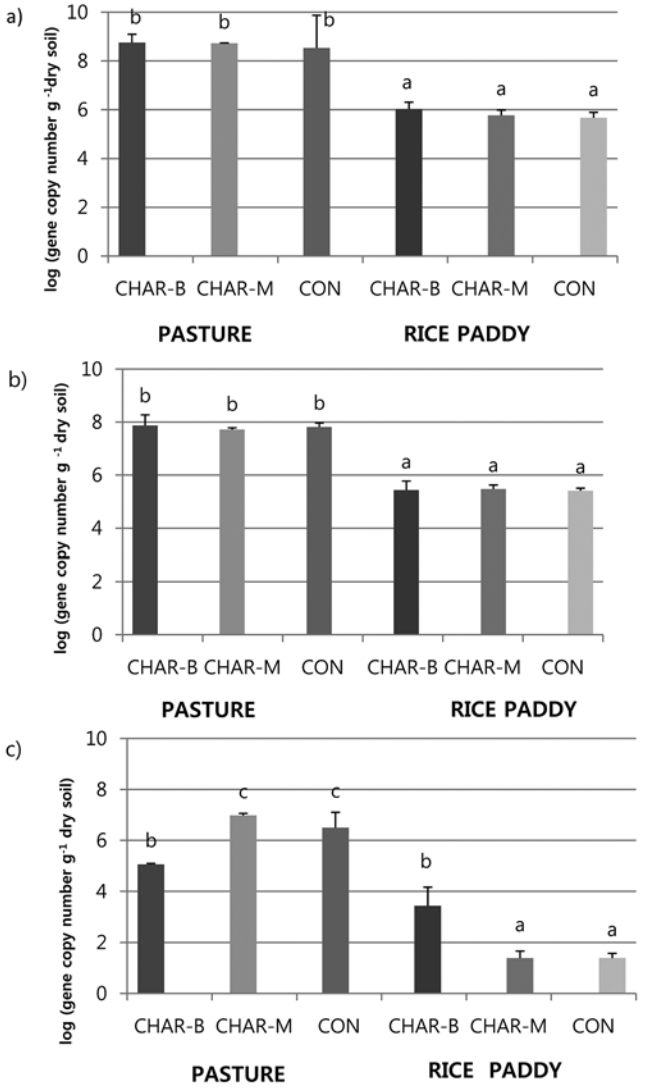

Fig. 6. Changes in gene copy number per gram dry soil of (a) bacteria, (b) archae, and (c) fungal biomass. Bars with different letters are significantly different at a 10% probability level.

to differences in biochar characteristics, ecosystem differences have been shown in other studies to be an important factor in the response of the soil to biochar additions (Shneour, 1966; Spokas and Reicosky, 2009). More specifically, our results suggest that soil type influences the effects of biochar additions on fungal biomass.

Conclusions

Our results confirm that a portion of biochar can stimulate microbial communities, depending on the condition of the soil to which the biochar is added. In the PASTURE soil, there appeared to be a reduction in CO_2 and CH_4 production by the addition of CHAR-M, except for the initial burst of CO_2. In the RICE PADDY soil, the benefit from reduced emissions of CO_2 and CH_4 may be offset by increased N_2O evolution after addition of CHAR-M. Stimulation of N_2O emissions from the RICE PADDY soil was caused by a combination of increased N mineralization and presumably abundant preexisting denitrifiers in the CHAR-M–amended RICE PADDY soil. Considering that there was no significant increase in GHG

emissions caused by the addition of CHAR-B to either soil, we suggest that CHAR-B is a more appropriate amendment material because it exhibited better GHG mitigation potential.

The GHG mitigation potential of biochar should complement the fertilizer function of biochars. In other words, biochars should fulfill both functions with the best possible compromise. Our results showed that both biochars used in this study provided sufficient available N, as evidenced by increases in net mineralization of N. This finding implies that our biochars can play roles as N fertilizers when they are amended to soils. However, an excessive increase in N availability might cause a leaching problem when soils do not have enough N-holding capacity. Future research on leaching is needed to confirm that our biochars have an optimal fertilizer effect, balancing enough N availability with holding (or stabilizing) capacity.

The addition of biochar changed soil enzyme activities and microbial biomass N. These results suggest that increased enzyme activities were induced by microbial proliferation during the incubation, but lowering activities might be partly explainable by higher nutrient availability or chemical blocking of substrates by biochar. Change in microbial communities due to biochar addition was suggested by the observed change in the fungi abundance after adding CHAR-B. To determine whether the change in microbial communities would be beneficial in terms of nutrient retention, C storage, and plant growth, further analyses should be performed by expanding the incubation time and scaling up from a jar to the pot or field scales.

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References

Amonette, J.E., J. Kim, C.K. Russell, A.V. Palumbo, and W.L. Daniels. 2003. Enhancement of soil carbon sequestration by amendment with fly ash. Paper no. 47. *In* Proceedings 2003 Int. Ash Utilization Symp. IAUS, Lexington, KY.

Ascough, P.L., C.J. Sturrock, and M.I. Bird. 2010. Investigation of growth responses in saprophytic fungi to charred biomass. Isot. Environ. Health Stud. 46:64–77. doi:10.1080/10256010903388436

Bailey, V.L., S.J. Fansler, J.L. Smith, and H. Bolton, Jr. 2011. Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. Soil Biol. Biochem. 43:296–301. doi:10.1016/j.soilbio.2010.10.014

Belmabkhout, Y., R. Serna-Guerrero, and A. Sayari. 2009. Adsorption of CO_2 from dry gases on MCM-41 silica at ambient temperature and high pressure.1: Pure CO_2 adsorption. Chem. Eng. Sci. 64:3721–3728. doi:10.1016/j.ces.2009.03.017

Chan, K.Y., L. Van Zwieten, I. Meszaros, A. Downie, and S. Joseph. 2008. Using poultry litter biochars as soil amendments. Aust. J. Soil Res. 46:437–444. doi:10.1071/SR08036

Cheng, C.-H., J. Lehmann, and M.H. Engelhard. 2008. Natural oxidation of black carbon in soils: Changes in molecular form and surface charge along a climosequence. Geochim. Cosmochim. Acta 72:1598–1610. doi:10.1016/j.gca.2008.01.010

Clough, T.J., J.E. Bertram, J.L. Ray, L.M. Condron, M. O'Callaghan, R.R. Sherlock, and N.S. Wells. 2010. Unweathered wood biochar impact on nitrous oxide emissions from a bovine-urine-amended pasture soil. Soil Sci. Soc. Am. J. 74:852–860. doi:10.2136/sssaj2009.0185

Dempster, D.N., D.B. Gleeson, Z.M. Solaiman, D.L. Jones, and D.V. Murphy. 2010. Biochar addition to soil changed microbial community structure and decreased microbial biomass carbon and net inorganic nitrogen mineralized. *In* 19th World Congress of Soil Science, Soil Solutions for a Changing World, Brisbane, Australia.

Fansler, S., H. Bolton, Jr., J. Smith, and V. Bailey. 2009. Biochar reduces apparent soil enzyme activities. *In* 2009 ASA–CSSA–SSSA Int. Annu. Meeting, Pittsburgh, PA. ASA, CSSA, and SSSA, Madison, WI.

Gardes, M., and T.D. Bruns. 1993. ITS primers with enhanced specificity for basidiomycetes-application to the identification of mycorrhizae and rusts. Mol. Ecol. 2:113–118. doi:10.1111/j.1365-294X.1993.tb00005.x

Goldberg, E.D. 1985. Black carbon in the environment: Properties and distribution. John Wiley & Sons, New York.

Graber, E., Y. Meller Harel, M. Kolton, E. Cytryn, A. Silber, D. Rav David, L. Tsechansky, M. Borenshtein, and Y. Elad. 2010. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. Plant Soil 337:481–496. doi:10.1007/s11104-010-0544-6

Hildebrandt, U., K. Janetta, and H. Bothe. 2002. Towards growth of arbuscular mycorrhizal fungi independent of a plant host. Appl. Environ. Microbiol. 68:1919–1924. doi:10.1128/AEM.68.4.1919-1924.2002

Hussain, Q., and G. Pan. 2011. Ammonia oxidizers (amoA) denitrifiers (nirK) dynamics in the rhizophere between hybrid and standard rice cultivars and linkage to N_2O emission. Geophys. Res. Abstr. 13: abstract no. EGU2011-394.

Jang, I., S.-H. Lee, K.-D. Zho, and H. Kang. 2011. Methane concentrations and methanotrophic community structure influence the response of soil methane oxidation to nitrogen content in a temperate forest. Soil Biol. Biochem. 43:620–622. doi:10.1016/j.soilbio.2010.11.032

Kang, H.J., and C. Freeman. 1999. Phosphatase and arylsulfatase activities in wetland soils: Annual variation and controlling factors. Soil Biol. Biochem. 31:449–454. doi:10.1016/S0038-0717(98)00150-3

Kang, H.-J., C. Freeman, and M.A. Lock. 1998. Trace gas emission from a North Wales fen: Role of hydrochemistry and soil enzyme activity. Water Air Soil Pollut. 105:107–116. doi:10.1023/A:1005089122337

Karhu, K., T. Mattila, I. Bergstrom, and K. Regina. 2011. Biochar addition to agricultural soil increased CH_4 uptake and water holding capacity: Results from a short-term pilot field study. Agric. Ecosyst. Environ. 140:309–313. doi:10.1016/j.agee.2010.12.005

Khodadad, C.L.M., A.R. Zimmerman, S.J. Green, S. Hthandi, and J.S. Foster. 2011. Taxa-specific changes in soil microbial composition induced by pyrogenic carbon amendments. Soil Biol. Biochem. 43:385–392. doi:10.1016/j.soilbio.2010.11.005

Kolb, S.E., K.J. Fermanich, and M.E. Dornbush. 2009. Effect of charcoal on microbial biomass and activity in temperate soils. Soil Sci. Soc. Am. J. 73:1173–1181. doi:10.2136/sssaj2008.0232

Lane, D.J. 1991. 16S/23S rRNA sequencing. p. 115–175. *In* E. Stackebrant and M. Goodfellow (ed.) Nucleic acid techniques in bacterial systematics. John Wiley & Sons, New York.

Laird, D.A., P. Fleming, D.D. Davis, R. Horton, B.Q. Wang, and D.L. Karlen. 2010b. Impact of biochar amendments on the quality of a typical midwestern agricultural soil. Geoderma 158:443–449. doi:10.1016/j.geoderma.2010.05.013

Laird, D., P. Fleming, B.Q. Wang, R. Horton, and D. Karlen. 2010a. Biochar impact on nutrient leaching from a Midwestern agricultural soil. Geoderma 158:436–442. doi:10.1016/j.geoderma.2010.05.012

Lehmann, J., D. Guerena, K. Hanley, C. Hyland, A. Enders, and S. Rajkovich. 2010. Environmetal effects of biochars from agricultural wastes: Nutrient leaching, denitrification, and plant growth. *In* ASA–CSSA–SSSA 2010 Int. Annual Meetings, Long Beach, CA. ASA, CSSA, and SSSA, Madison, WI.

Lehmann, J., J. Gaunt, and M. Rondong. 2006. Bio-char sequestration in terrestrial ecosystems: A review. Mitig. Adapt. Strategies Glob. Change 11:403–427.

Lehmann, J., J.P. da Silva, Jr., C. Steiner, and T. Nehls. 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: Fertilizer, manure and charcoal amendments. Plant Soil 249:343–357. doi:10.1023/A:1022833116184

Lim, K. 2004. Principles of adsorption engineering and science. Doo Yang Sa, Seoul, Korea.

Linn, D.M., and J.W. Doran. 1984. Effect of water-filled pore space on carbon dioxide and nitrous oxide production in tilled and no tilled soils. Soil Sci. Soc. Am. J. 48:1267–1272. doi:10.2136/sssaj1984.03615995004800060013x

Lucas, R.E., and J.F. Davis. 1961. Relationships between pH values of organic soils and availabilities of 12 plant nutrients. Soil Sci. 92:177–182. doi:10.1097/00010694-196109000-00005

Nadkarni, M.A., F.E. Martin, N.A. Jacques, and N. Hunter. 2002. Determination of bacterial load by real-time PCR using a broad-range (universal) probe and primers set. Microbiology 148:257–266.

Nichols, G.J., J.A. Cripps, M.E. Collinson, and A.D. Scott. 2000. Experiments

in waterlogging and sedimentology of charcoal: Results and implications. Palaeogeogr. Palaeoclimatol. Palaeoecol. 164:43–56. doi:10.1016/S0031-0182(00)00174-7

Novak, J.M., W.J. Busscher, D.L. Laird, M. Ahmedna, D.W. Watts, and M.A.S. Niandou. 2009. Impact of biochar amendment on fertility of a Southeastern coastal plain soil. Soil Sci. 174:105–112. doi:10.1097/SS.0b013e3181981d9a

Ogawa, M. 1994. Symbiosis of people and nature in the tropics. Farming Japan 28:10–34.

Ogawa, M., and Y. Okimori. 2010. Pioneering works in biochar research, Japan. Austr. J. Soil Res. 48:498–500.

Ogawa, M., Y. Yamabe, and G. Sugiura. 1983. Effects of charcoal on the root nodule formation and VA mycorrhiza formation of soy bean. Abstract no. 578. Presented at The Third International Mycological Congress, 28 Aug.–3 Sept. 1983, Tokyo, Japan.

Park, S.-Y., and H. Kang. 2010. Impact of invasive plant and environmental conditions on denitrification potential in urban riparian ecosystems. Chem. Ecol. 26:353–360. doi:10.1080/02757540.2010.504671

Pathak, H. 1999. Emissions of nitrous oxide from soil. Curr. Sci. (India) 77:359–369.

Pietikainen, J., O. Kiikkila, and H. Fritze. 2000. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos 89:231–242. doi:10.1034/j.1600-0706.2000.890203.x

Rondon, M.A., D. Molina, M. Hurtado, J. Ramirez, J. Lehmann, J. Major, and E. Amezquita. 2006. Enhancing the productivity of crops and grasses while reducing greenhouse gas emissions through bio-char amendments to unfertile tropical soils. *In* 18th World Congress of Soil Science, Philadelphia, PA. 9–15 July 2006. Available at http://www.mendeley.com/research/enhancing-productivity-crops-grasses-while-reducing-greenhouse-gas-emissions-through-biochar-amendments-unfertile-tropical-soils/ (verified 21 Nov. 2011).

Rondon, M.A., J. Ramirez, and J. Lehmann. 2005. Greenhouse gas emissions decrease with charcoal additions to tropical soils. p. 208. *In* Proc. of the Third USDA Sequestration, Soil Carbon Center, Kansas State Univ. USDA, Baltimore, MD.

Saito, M., and T. Marumoto. 2002. Inoculation with arbuscular mycorrhizal fungi: The status quo in Japan and the future prospects. Plant Soil 244:273–279. doi:10.1023/A:1020287900415

SAS Institute. 2001. SAS user's guide. SAS Inst., Cary, NC.

Shneour, E.A. 1966. Oxidation of graphitic carbon in certain soils. Science 151:991–992. doi:10.1126/science.151.3713.991

Sims, G.K., T.R. Ellsworth, and R.L. Mulvaney. 1995. Microscale determination of inorganic nitrogen in water and soil extracts. Commun. Soil Sci. Plant Anal. 26:303–316. doi:10.1080/00103629509369298

Singh, B.P., B.J. Hatton, B. Singh, A. Cowie, and A. Kathuria. 2010. Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. J. Environ. Qual. 39:1224–1235. doi:10.2134/jeq2009.0138

Smith, J.L., H.P. Collins, and V.L. Bailey. 2010. The effect of young biochar on soil respiration. Soil Biol. Biochem. 42:2345–2347. doi:10.1016/j.soilbio.2010.09.013

Solaiman, Z. 2007. Measurement of microbial biomass and activity in soil. Adv. Techniques Soil Microbiol. 11:201–211. doi:10.1007/978-3-540-70865-0_13

Spokas, K.A., and D.C. Reicosky. 2009. Impacts of sixteen different biochars on soil greenhouse gas production. Ann. Environ. Sci. 3:179–193.

Spokas, K.A., W.C. Koskinen, J.M. Baker, and D.C. Reicosky. 2009. Impacts of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. Chemosphere 77:574–581. doi:10.1016/j.chemosphere.2009.06.053

Spokas, K.A. 2010. Review of the stability of biochar in soils: Predictability of O:C molar ratios. Carbon Manage. 1:289–303. doi:10.4155/cmt.10.32

Spokas, K.A., J.M. Baker, and D.C. Reicosky. 2010. Ethylene: Potential key for biochar amendment impacts. Plant Soil 333:443–452. doi:10.1007/s11104-010-0359-5

Steinbeiss, S., G. Gleixner, and M. Antonietti. 2009. Effect of biochar amendment on soil carbon balance and soil microbial activity. Soil Biol. Biochem. 41:1301–1310. doi:10.1016/j.soilbio.2009.03.016

Steiner, C., J.C. Das, M. Garcia, B. Forster, and W. Zech. 2008. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. Pedobiologia 51:359–366. doi:10.1016/j.pedobi.2007.08.002

Takai, K., and K. Horikoshi. 2000. Rapid detection and quantification of members of the archaeal community by quantitative PCR using fluorogenic probes. Appl. Environ. Microbiol. 66:5066–5072.

Topoliantz, S., J.F. Ponge, and S. Ballof. 2005. Manioc peel and charcoal: A potential organic amendment for sustainable soil fertility in the tropics. Biol. Fertil. Soils 41:15–21. doi:10.1007/s00374-004-0804-9

Van Zwieten, L., S. Kimber, S. Morris, K.Y. Chan, A. Downie, J. Rust, S. Joseph, and A. Cowie. 2009a. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant Soil 327:235–246. doi:10.1007/s11104-009-0050-x

Van Zwieten, L., B. Singh, S. Joseph, S. Kimber, A. Cowie, and K.Y. Chan. 2009b. Biochar and emission of non-CO2 greenhouse gases from soil. p. 227–249. *In* J. Lehmann and S. Joseph (ed.) Biochar for environmental management, science and technology. Earthscan, Gateshead, UK.

Vance, E.D., P.C. Brookes, and D.S. Jenkinson. 1987. An extraction method for measuring soil microbial biomass C. Soil Biol. Biochem. 19:703–707. doi:10.1016/0038-0717(87)90052-6

Wander, M.M., B.G. Bidart, and S. Aref. 1998. Tillage impacts on depth distribution of total and particulate organic matter in three Illinois soil. Soil Sci. Soc. Am. J. 62:1704–1711. doi:10.2136/sssaj1998.03615995006200060031x

Warnock, D.D., J. Lehmann, T.W. Kuyper, and M.C. Rillig. 2007. Mycorrhizal responses to biochar in soil: Concepts and mechanisms. Plant Soil 300:9–20. doi:10.1007/s11104-007-9391-5

White, T.J., T.D. Bruns, S. Lee, and J.W. Taylor. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. p. 315–324. *In* M.A. Innis et al. (ed.) PCR protocols: A guide to methods and applications. Academic Press, New York.

Yanai, Y., K. Toyota, and M. Okazaki. 2007. Effects of charcoal addition on N_2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. Soil Sci. Plant Nutr. 53:181–188. doi:10.1111/j.1747-0765.2007.00123.x

Zimmerman, A.R., B. Gao, and M.-Y. Ahn. 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. Soil Biol. Biochem. 10.1016/011.02.005.