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Switchgrass Biochar Effects on Plant Biomass and Microbial Dynamics in Two Soils from Different Regions

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ABSTRACT

Biochar amendments to soils may alter soil function and fertility in various ways, including through induced changes in the microbial community. We assessed microbial activity and community composition of two distinct clayey soil types, an Aridisol from Colorado (CO) in the U.S. Central Great Plains, and an Alfisol from Virginia (VA) in the southeastern USA following the application of switchgrass *(Panicum virgatum*) biochar. The switchgrass biochar was applied at four levels, 0%, 2.5%, 5%, and 10%, approximately equivalent to biochar additions of 0, 25, 50, and 100 t ha^{-1}, respectively, to the soils grown with wheat (*Triticum aestivum*) in an eight-week growth chamber experiment. We measured wheat shoot biomass and nitrogen (N) content and soil nutrient availability and N mineralization rates, and characterized the microbial fatty acid methyl ester (FAME) profiles of the soils. Net N mineralization rates decreased in both soils in proportion to an increase in biochar levels, but the effect was more marked in the VA soil, where net N mineralization decreased from −2.1 to −38.4 mg kg^{-1}. The 10% biochar addition increased soil pH, electrical conductivity, Mehlich- and bicarbonate-extractable phosphorus (P), and extractable potassium (K) in both soil types. The wheat shoot biomass decreased from 17.7 to 9.1 g with incremental additions of biochar in the CO soil, but no difference was noted in plants grown in the VA soil. The FAME recovery assay indicated that the switchgrass biochar addition could introduce artifacts in analysis, so the results needed to be interpreted with caution. Non-corrected total FAME concentrations indicated a decline by 45% and 34% with 10% biochar addition in the CO and VA soils, respectively, though these differences became nonsignificant when the extraction efficiency correction factor was applied. A significant decline in the fungi:bacteria ratio was still evident upon correction in the CO soil with biochar. Switchgrass biochar had the potential to cause short-term negative impacts on plant biomass and alter soil microbial community structure unless measures were taken to add supplemental N and labile carbon (C).

Key Words: correction factor, extraction efficiency, fatty acid methyl ester profile, nitrogen mineralization, soil microbial community, soil nutrient availability, wheat

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INTRODUCTION

Soil microbial abundance, activity, and composition are crucial to soil quality because of multiple soil functions related to nutrient cycling and availability, carbon sequestration, and the structure and stability of a soil through the binding of stable aggregates. The soil microbial biomass and activity may be enhanced through the incorporation of organic material into soil, which has been demonstrated with the addition of composted materials and by leaving plant residues on-site in both agricultural and forestry ecosystems (*e.g.*, Paustian *et al.*, 2000; Johnson and Curtis, 2001). More recently, soils amended with biochar, the solid product after pyrolyzing waste biomass, has been reported to result in changes in microbial activity and population dynamics in different ways depending upon both soil and biochar properties (Gaskin *et al.*, 2009; Anderson *et al.*, 2011; Bailey *et al.*, 2011; Lehmann *et al.*, 2011; Ducey *et al.*, 2013; Ameloot *et al.*, 2013; Rutigliano *et al.*, 2014).

The mechanisms for biochar effects on microbial communities could vary. An indirect effect could be due to biochar influence on nutrient cycling and water retention in soils, which could increase resources available for microbial uptake. Biochar influence on nutri-

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ents and water may also provide increased soil fertility and crop yield, reduced greenhouse gas emissions, and improved down-stream water quality, while providing an ecologically sound way to dispose of waste materials (*e.g.*, Glaser *et al.*, 2002; Lehmann *et al.*, 2006; Lehmann and Rondon 2006; Laird, 2008). An increase in soil fertility may also be explained by increased cation exchange capacity of soils and liming effect due to ash content in biochar, as well as possible changes in soil microbial activity and cycling of nutrients (Warnock *et al.*, 2007; Steinbeiss *et al.*, 2009; Lehmann *et al.*, 2011).

Biochar can be produced from a large number of feedstock materials including agricultural, forest, and municipal wastes and under conditions that vary in temperature and burning duration (Rutherford *et al.*, 2012). This results in a product that can vary widely in physical and chemical properties such as carbon (C) and nitrogen (N) contents, surface area, ash content, acid-neutralizing capacity, and surface functional groups, which would result in markedly differing reactions when applied to soils (Joseph *et al.*, 2010; Uchimiya *et al.*, 2011). While an increasing body of literature exists on the soil chemical and physical responses to biochar addition, far less is known regarding the effects of biochar addition on microbial activity, abundance, and diversity.

In addition to shifts in microbial communities due to changes in soil characteristics with biochar, microbial responses vary depending upon biochar characteristics, especially surface characteristics and bioavailable compounds present in the biochar, and the pH changes induced upon treatment (Thies and Rillig, 2009). For example, Zimmerman *et al.* (2011) documented an increase in C mineralization in soil/biochar mixtures when the biochar was made from grasses at relatively low temperatures (250–400 °C) and a decrease in C mineralization when biochar was made from hard woods at higher temperatures (525–600 °C). Steinbeiss *et al.* (2009) showed that fungi were more able to utilize biochar created from yeast, while Gram-negative bacteria better-utilized biochar created from glucose, though glucose biochar additions resulted in an overall decrease in microbial biomass. Additionally, Anderson *et al.* (2011) also documented variable results of biochar addition, depending on family of bacteria, where pine-derived biochar induced a positive effect on *Bradyrhizobiaceae*, *Hyphomicrobiaceae*, *Streptosporangineae*, and *Thermomonosporaceae*, but a negative effect on *Streptomycetaceae* and *Micromonosporaceae*.

We require a more complete understanding of the effects of different biochar types on microbial activity and subsequent nutrient cycling and plant responses in diverse soil ecosystems, especially in agricultural settings, if biochar is to be used as a soil amendment. Studies with different biochars differ widely by reporting they are a theoretically non-degrading substrate inaccessible to microbial degradation (Thies and Rillig, 2009; Jha *et al.*, 2010), a potential additional labile C source itself (Liang *et al.*, 2006; Cross and Sohi, 2011) and/or a source of phytotoxic materials such as ethylene, a known nitrification inhibitor (Spokas *et al.*, 2010), as well as harmful salts such as Na or Cl (Lehmann *et al.*, 2011).

Biochar may preferentially sorb essential enzymes or nutrients, especially N, imposing detrimental C and N limitations on plant and microbial growth (Bailey *et al.*, 2011). It has been demonstrated that biochar can sorb substrates during enzyme activity assays (Swaine *et al.*, 2013), and we hypothesize that similar artifacts can occur during the extraction of soil fatty acids for microbial community analysis. Most of the documented biochar-induced soil fertility improvements have occurred in studies on highly oxidized weathered soils (Ultisols and Oxisols), while relatively few studies have been performed in arid alkaline soils (Aridisols), though these soils are agriculturally important in USA (Ippolito *et al.*, 2012).

Our first objective was to investigate the effect of switchgrass-derived biochar additions on the microbial activity and community structure of an Alfisol from the southeastern USA and an Aridisol from the U.S. Central Great Plains. We hypothesize that biochar amendment will affect soil microbial community structure due to the associated changes in soil chemistry and surface effects as well as the dilution effect that the amendment higher rates will have. We also assessed the biochar amendment effects on soil nutrient availability and subsequent biomass and nutrient status of wheat (*Triticum aestivum* L.) grown in these soils. Because studies on the response of microbial communities to biochar applications are still limited by methodology and the sorption of lysed cells and their contents by biochar (*e.g.*, Lehmann *et al.*, 2011), our second objective was to compare the trends observed with and without an extraction efficiency correction factor for the use of fatty acids methyl ester analysis to evaluate shifts in the microbial community structure due to biochar applications in these two soil types.

MATERIALS AND METHODS

Soil sampling sites and soil collection

The agricultural soils used in this study were colle-

cted in spring 2012 from Morgan County, Colorado (40°16′35.25″ N, 103°36′44.69″ W) and from Roanoke County, Virginia, USA (37°23′13.24″ N, 80°6′3.74″ W). Selected soil properties are presented in Table I. The soil from Colorado (CO), an Aridisol (Soil Taxonomy), belongs to the Heldt series (fine, smectitic, mesic Ustertic Haplocambid) (Soil Survey Staff, USDA Natural Resources Conservation Service, 2013) and was collected from an agricultural field planted in corn. The soil from Virginia (VA), an Alfisol (Soil Taxonomy), belongs to the Chilhowie series (very fine, mixed semiactive, mesic Typic Hapludalf) (Soil Survey Staff, USDA Natural Resources Conservation Service, 2013) and was collected from a fallow field last planted in soybean two years prior to collection. We chose these two soils because while they are both under agricultural production and have similar clay content, they are from widely different geographic areas, with different organic matter and mineralogical composition (Table I). This enables us to investigate the effects of biochar in soils developed in different environments. Soil samples (0–10 cm) were collected from 20 random locations at each site and composited into one bulk sample per site. Samples collected from VA were shipped overnight to the laboratory in CO for processing. Upon receiving, both soils were allowed to air-dry for 2–4 d prior to sieving through 2-mm mesh prior to use in the study.

TABLE I

Selected properties of the Colorado (CO) and Virginia (VA) soils used in this study

Item	Soil	
	CO	VA
Soil classification	Aridisol	Alfisol
Texture	Clay	Clay
Sand (%)	28.3	34.8
Silt (%)	29.1	20.3
Clay (%)	42.6	44.9
CEC[a)] (cmol kg^{-1})	26.9	21.7
Organic matter (g kg^{-1})	15	69
Mineralogy (g kg^{-1})		
Feldspar	158	2
Calcite	20	0
Goethite	0	14.5
Kaolinite (1:1)	27 (crystalline)	27 (poorly crystalline)
Smectite (2:1)	215	151
Illite (2:1)	106	181
Muscovite (2:1)	79	91

[a)] Cation exchange capacity.

Mineralogy of the two soils was determined *via* X-ray diffraction (XRD) and quantified using the RockJock software program (Eberl, 2003). The CO soil is distinguished from the VA soil in that it contains a relatively large quantity of feldspar and calcite (Table I). The clays are also much more different than the abundance numbers suggest. The VA soil has an intimately intermixed illite/smectite clay (with abundant thin illite crystals), whereas the CO soil has much more discrete smectite that swells to 17 Å with glycol, and a very thick well-defined illite that closely resembles a real muscovite. Halloysite in these fits usually means more poorly crystalline kaolinite, so the two soils have similar kaolinite abundances, but the VA soil has poorer crystallinity relative to the CO soil. The VA soil also has a much greater organic matter content of 69 g kg^{-1} relative to 15 g kg^{-1} in the CO soil.

Biochar

Biochar derived from switchgrass (*Panicum virgatum*) (Biochar Solutions, Inc. Pueblo, USA; exact preparation methods proprietary) was produced using a beta base unit in a two-stage slow-pyrolysis continuous process. In stage one, the feedstock was carbonized in a controlled aerobic environment at a temperature between 500–700 °C for less than one minute. In the second stage, material was held in a hot gas anaerobic environment for up to 14 min between 300–550 °C. The switchgrass biochar produced was ground and sieved through 2-mm mesh and its properties are presented in Table II.

TABLE II

Some properties of biochar made from switchgrass used in this study

Item	Value
pH (H_2O)	9.33
Ash (g kg^{-1})	238.1
C (g kg^{-1})	717.4
N (g kg^{-1})	9.3
H (g kg^{-1})	12.9
O (g kg^{-1})	21.1
Volatile matter (g kg^{-1})	66.0
Fixed C (g kg^{-1})	695.9
C:N ratio	77.14
CEC[a)] (cmol kg^{-1})	53.9
Surface area (m^2 g^{-1})	67.10
Total pore volume (mL g^{-1})	0.95

[a)] Cation exchange capacity.

Growth chamber experiment

Soil and biochar mixtures were achieved by thoroughly hand-mixing soil and biochar materials in ratios to form a total unit volume of 5 L packed to a density of 1.0 g cm^{-3} dry weight soil to represent biochar levels of 0%, 2.5%, 5%, and 10%, approximately equivalent to 0, 25, 50, and 100 t ha^{-1}, respectively, as-

suming a 10-cm incorporation depth. Soil and biochar mixtures were weighed and placed in 6-L planting pots. The VA soil and biochar mixtures at four different biochar levels were replicated 3 times ($n = 4$), totaling 12 subtreatments. The CO soil and biochar mixtures at four different biochar levels were replicated four times ($n = 4$), totaling 16 subtreatments, given that more CO soil was available. The pots with the CO and VA soils were treated as separate experiments, and the replicates were distributed within randomized complete blocks according to the biochar level.

Each pot was seeded with 10 seeds of *Triticum aestivum* and one week after germination the plants were thinned to two plants per pot. Nitrogen and phosphorus (P) fertilizers were then added to each pot as liquid KNO_3 and granule superphosphate, equivalent to 168 kg ha^{-1} of N and 67.2 kg ha^{-1} of P, respectively. Plants were allowed to grow for 8 weeks following germination in a growth chamber at the USDA-ARS Central Great Plains Research Center. At this point the plants had not reached the heading stage. The 8-week period was chosen to allow for enough time to observe effects on the soil microbiology due to biochar addition, but before the plants became pot bound. Plants were grown at 22 °C with a 13-h day (irradiance level corresponding to a fluorescence maximum of 400 μmol) and relative humidity was held constant at 60%. Deionized water was added weekly to each pot according to measured pot weight loss over time to maintain relatively constant soil moisture over time.

Plant and soil analyses

At harvest, shoots were clipped at the soil surface, dried at 50 °C, and weighed. Shoot material was ground using a Wiley mill and analyzed for C and N contents using a Leco CHN analyzer (Leco Corporation, St. Joseph, USA). The C:N ratios of the wheat shoots in the VA soil were very low, at approximately 10. Because of this, we tested for mineral N accumulation in the shoots of the VA plants. For this, we extracted 0.1 g of dried ground shoots with 2 mol L^{-1} KCl and analyzed the extracts using a Lachat Quickchem analyzer (Lachat Instruments, Loveland, USA). The average NO_3+NH_4 content of the VA shoots was 13.9 mg N g^{-1}, with 98.4% of it as NO_3^-. In contrast, the CO shoots had 0.24 mg g^{-1} mineral N. Because of the apparent NO_3^- accumulation in the VA plants, we subtracted the mineral N content form the total N content to determine the shoot N and shoot C:N ratios reported in this study.

Soil N mineralization rates (nitrification, ammonification, and total mineralizable N) were determined by extracting N with 2 mol L^{-1} KCl at the onset and conclusion of an 18-d incubation period at 25 °C. Nitrate (NO_3-N) and ammonium (NH_4-N) contents were determined on an auto-analyzer (Olsen Agricultural Laboratory, Inc., McCook, USA). Soil Ca and Mg were extracted using 1 mol L^{-1} NH_4OAc at pH 7.0 and soil P was extracted using Mehlich-III or bicarbonate (0.5 mol L^{-1} $NaHCO_3$) at pH 8.5. Calcium and Mg were analyzed using atomic adsorption/emission and P was analyzed on an auto-analyzer by the acid molybdate method (Olsen Agricultural Laboratory, Inc., McCook, USA). All results are expressed on an oven-dry weight basis.

Moist soil samples from the pots were stored frozen prior to analysis for fatty acid methyl ester (FAME) using the ester-linked (EL)-FAME procedure of Schutter and Dick (2000). This method involves the following 4 steps: 1) saponification and methylation of EL fatty acids by incubation of 3 g of soil in 15 mL of 0.2 mol L^{-1} KOH in methanol at 37 °C for 1 h, with the samples being vortexed every 10 min and addition of 3 mL of 1.0 mol L^{-1} acetic acid to neutralize the pH of the mixture at the end of incubation; 2) partition of FAMEs into an organic phase by adding 10 mL of hexane followed by centrifugation at 480 × g for 10 min; 3) transferring the hexane layer to a clean glass test tube that the hexane can be evaporated under a stream of N_2; and 4) redissolution of FAMEs by adding 100 μL of 1:1 methyl tert-butyl ether and hexane containing methyl nonadecanoate (19:0) as an internal standard (0.5 mg mL^{-1}). Samples were vortexed and transferred to a 250-μL glass insert in a 2-mL gas chromatography (GC) vial.

Analysis for FAMEs was conducted using an Agilent 6890 N gas chromatograph with a 25 m × 0.32 mm × 0.25 μm (5% phenyl)-methylpolysiloxane Agilent HP-5 fused silica capillary column (Agilent, Santa Clara, USA) and flame ionization detector (Hewlett Packard, Palo Alto, USA) with ultra-high purity hydrogen as the carrier gas. The temperature program ramped from 170 °C to 270 °C at 5 °C min^{-1} and then ramped to 300 °C for 2 min to clear the column. Peak identification and area calculation was performed using the TSBA6 aerobe program (Microbial ID, Inc., Newark, USA). The FAMEs are described by the number of C atoms, a colon, the number of double bonds, and then the position of the first double bond from the methyl (ω) end of the molecule. Other notations are used for methyl (Me), cis (c) and trans (t) isomers, and iso (i) and anteiso (a) branched FAMEs. Selected FAMEs were used as microbial markers according to previous research (Zelles, 1999), and included Gram-

positive bacteria (i15:0, a15:0, i17:0, a17:0), Gram-negative bacteria (cy17:0, cy19:0), and actinomycetes (10Me16:0, 10Me17:0, 10Me18:0). Fungal markers included saprophytic fungi (18:1ω9c, 18:2ω6c) and arbuscular mycorrhizal fungi (AMF) (16:1ω5c). Absolute amounts of FAMEs (nmol g^{-1} soil) were calculated according to Zelles (1999) using the 19:0 internal standard. Bacterial sums were calculated using the Gram-positive, Gram-negative, and actinomycete markers listed above; fungal sums were calculated using both saprophytic and AMF fungal markers listed above, and the fungi:bacteria ratio was calculated by dividing the fungal sum by the bacterial sum.

Determination of an extraction efficiency factor for FAME analysis

Because biochars may be strong sorbents (depending on biochar characteristics and age) that could interfere in the efficiency of a standard extraction method (Thies and Rillig, 2009; Durenkamp *et al.*, 2010; Liang *et al.*, 2010; Gomez *et al.*, 2014), we assessed the extraction efficiency of our FAME method to determine if any resulting change in FAMEs shown may be an artifact of the methodology used. 100 L of 19:0 FAME standard (150 nmol) was added to each soil sample. Three additional biochar-only samples with and without the standard added were extracted and analyzed as described above for the FAME method on the GC along with two samples of the standard only. The extraction efficiency was calculated as the ratio of the sample peak area to the standard peak area. The amount of FAMEs initially extracted was then recalculated using this extraction efficiency as a correction factor. We present both initial and corrected values for each individual FAME marker, assuming that each marker has the same extraction efficiency as the standard 19:0 used in the assay.

Statistical analyses

Soil N mineralization, shoot biomass, and soil characteristic changes attributed to biochar application were analyzed using one-way analysis of variance (ANOVA) for each soil individually, followed by Tukey's honest significant difference (HSD) test ($P = 0.05$) to compare means. All statistical analyses were performed using SAS JMP version 7.0 (SAS Institute Inc., Cary, USA). Exploratory principal component analysis (PCA) was performed on the FAME data correlation matrix using the Vegan software package in R (version 2.0-2) and PCA plots were generated to compare the effects of biochar treatment levels and soil types on FAME markers.

RESULTS

Shoot biomass and N content

Shoot biomass of *T. aestivum* grown in the VA soil exhibited no significant ($P = 0.2908$) differences with biochar additions, but was significantly ($P < 0.0001$) decreased in plants grown in the CO soil with increasing biochar addition (Fig. 1a). Plants grown in the CO soil with 2.5%, 5%, and 10% biochar exhibited a 19%, 28%, and 49% smaller biomass, respectively, than plants grown in the CO soil without biochar.

In the control without biochar, the VA plants contained 22.4 g kg^{-1} organic N, whereas the CO plants contained approximately 9.3 g kg^{-1} organic N (Fig. 1b). No significant ($P > 0.05$) biochar effects were no-

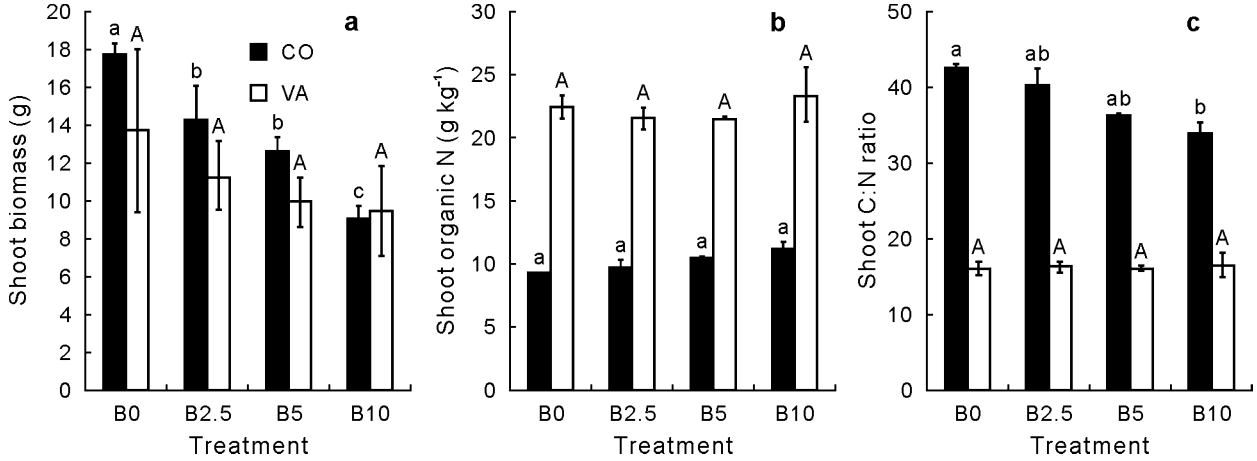

Fig. 1 Shoot biomass (a), organic N content (b), and C:N ratio (c) of wheat grown in the CO and VA soils with switchgrass biochar treatments at different levels. Values are means of 4 (CO soil) or 3 (VA soil) replicates and errors bars represent standard errors of the means. Bars with the same lowercase letter(s) within the CO soil and the uppercase letter(s) within the VA soil are not significantly different at $P > 0.05$. See Table I for details of the CO and VA soils. B0, B2.5, B5, and B10 are the treatments of 0% (control), 2.5%, 5%, and 10% biochar levels, approximately equivalent to biochar additions of 0, 25, 50, and 100 t ha^{-1}, respectively.

ted in shoot organic N concentrations in either CO or VA soils, though plants grown in the CO soil exhibited a significant ($P = 0.01$) decline in shoot C:N ratio with biochar addition (Fig. 1c). The C:N ratio declined from 41.6 in plants grown in the CO soil without biochar to 33.3 in the CO soil with 10% biochar. No significant ($P > 0.05$) differences in shoot C:N ratio occurred in plants grown in the VA soil at any biochar level.

Selected soil properties

The pH values in the CO soil increased significantly ($P = 0.0003$) from 8.10 to 8.33 with the highest biochar level (10%) compared to the non-amended control, and pH in the VA soil also increased significantly ($P < 0.0001$) from 6.1 to 7.2 (Table III). Exchangeable P from both extraction methods assessed (Mehlich-III and bicarbonate) increased in both CO and VA soils with biochar addition. For example, exchangeable P from the Mehlich-III extraction in the CO soil increased from 121 to 172 mg kg^{-1} soil (nearly 43%) with 10% biochar. In the VA soil, exchangeable P from the Mehlich-III extraction increased from 78 to 104 mg kg^{-1} soil (33%) with 10% biochar. Exchangeable K increased in both soils with increasing biochar addition, and exchangeable Ca decreased in the CO soil with biochar, but not in the VA soil. No significant ($P = 0.052$) changes were noted in exchangeable Mg concentration, though Mg also tended to decline with increasing biochar addition in the CO soil. Significant changes to CEC occurred only at the 10% biochar level in the VA soil (data not shown), which declined from 21.73 cmol kg^{-1} with 0% biochar to 15.63 cmol kg^{-1} with 10% biochar.

Initial extractable soil NH_4^+ content was similar between the two soils without biochar (3.53 and 3.46 mg kg^{-1} in the CO and VA soils, respectively), though extractable NO_3^- was significantly higher in the VA soil (0.93 and 14.45 mg kg^{-1} in the CO and VA soils, respectively). Total net N mineralization over the 18-d incubation decreased in both soils with biochar addition, becoming more negative ($P = 0.0007$ for CO and $P = 0.0246$ for VA) with increasing biochar addition (Fig. 2). Net ammonification did not change with biochar addition in either soil ($P > 0.05$ for both soils), and net nitrification was the main component of total net N mineralization in these soils and significantly declined with increasing biochar addition in both soils. In the CO soil, total net N mineralization decreased from −0.9 mg N kg^{-1} in the 0% biochar control to −8.7 mg N kg^{-1} with 10% biochar. With 10% biochar addition to the VA soil, total net N mineralization decreased from 8.0 mg N kg^{-1} in the control to −28.7 mg N kg^{-1}.

Microbial community structure according to FAMEs

Total FAMEs (nmol g^{-1} soil) extracted from both

TABLE III

Selected properties of the CO and VA soils[a)] following treatments of switchgrass biochar at different levels

Soil	Soil property	Treatment[b)]			
		B0 (control)	B2.5	B5	B10
CO	pH (H_2O)	8.1±0.06[c)]a[d)]	8.0±0.03a	8.2±0.03b	8.3±0.03b
	Loss-on-ignition (LOI) organic matter (g kg^{-1})	25±0.3a	27±0.3ab	28±0.1b	31±0.8c
	Electrical conductivity (S cm^{-1})	1.27±0.02a	1.31±0.02a	1.32±0.01ab	1.41±0.04b
	Mehlich-III-extractable P (mg kg^{-1})	121.0±1.0a	142.5±2.96b	155.8±4.78b	172.3±4.71c
	Bicarbonate-extractable P (mg kg^{-1})	24.3±2.33a	33.0±1.91a	45.3±1.03b	53.8±3.42b
	Exchangeable Ca (mg kg^{-1})	3 713±95.3c	3 400±114.1bc	2 780±113.6a	2 822±204.2ab
	Exchangeable Mg (mg kg^{-1})	734.7±26.9a	725.8±29.8a	632.3±29.4a	595.3±48.4a
	Exchangeable K (mg kg^{-1})	599.3±27.2a	834.5±43.7ab	995.8±59.3b	1 499±83.0c
VA	pH (H_2O)	6.1±0.03a	6.5±0.03b	6.8±0.88c	7.2±0.03d
	LOI organic matter (g kg^{-1})	106±1.2b	100±0.1a	101±0.1a	101±0.1a
	Electrical conductivity (S cm^{-1})	0.79±0.08a	0.95±0.05a	1.02±0.03a	1.30±0.03b
	Mehlich-III-extractable P (mg kg^{-1})	78.0±3.61a	69.3±1.20a	79.7±3.48a	104.0±4.04b
	Bicarbonate-extractable P (mg kg^{-1})	61.7±0.88a	57.3±1.20a	70.7±0.67b	78.7±1.33b
	Exchangeable Ca (mg kg^{-1})	2 936±104.0a	3 163±254.6a	2 826±389.4a	2 273±89.7a
	Exchangeable Mg (mg kg^{-1})	239.3±9.3a	257.7±9.6a	246.7±9.5a	254.0±10.5a
	Exchangeable K (mg kg^{-1})	235.3±11.0a	326.0±7.6b	481.0±27.3c	749.7±14.2d

[a)] See Table I for details of the CO and VA soils.
[b)] B0, B2.5, B5, and B10 are the treatments of 0% (control), 2.5%, 5%, and 10% biochar levels, approximately equivalent to biochar additions of 0, 25, 50, and 100 t ha^{-1}, respectively.
[c)] Means±standard errors ($n = 4$ for the CO soil and $n = 3$ for the VA soil).
[d)] Means followed by the same letter(s) in a row are not significantly different at $P > 0.05$.

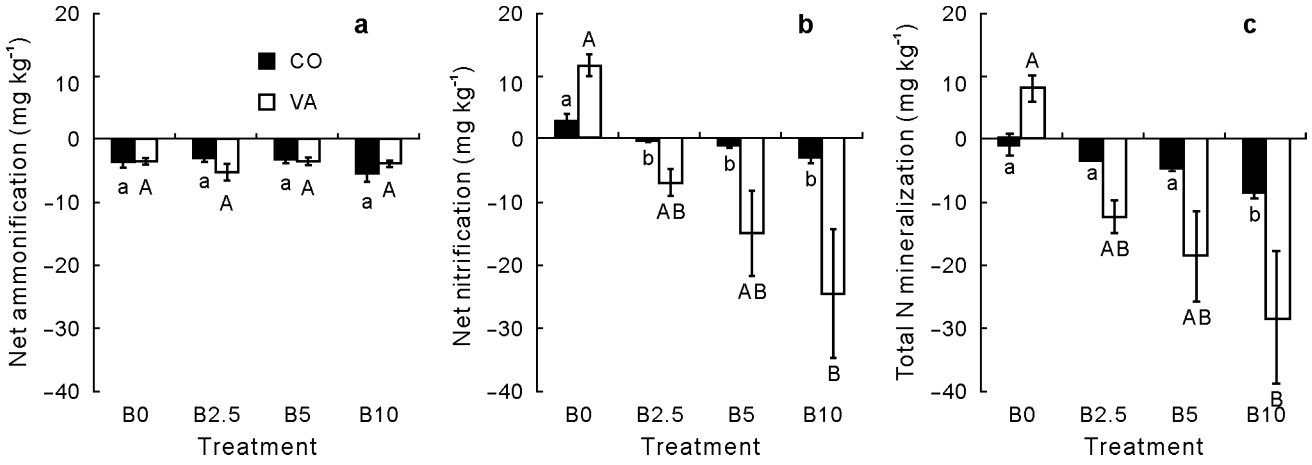

Fig. 2 Soil N mineralization as measured by net ammonification (a), net nitrification (b), and total net mineralization (c) in an 18-d incubation of the CO and VA soils with switchgrass biochar treatments at different levels. Values are means of 4 (CO soil) or 3 (VA soil) replicates and errors bars represent standard errors of the means. Bars with the same lowercase letter(s) within the CO soil and the same uppercase letter(s) within the VA soil are not significantly different at $P > 0.05$. See Table I for details of the CO and VA soils. B0, B2.5, B5, and B10 are the treatments of 0% (control), 2.5%, 5%, and 10% biochar levels, approximately equivalent to biochar additions of 0, 25, 50, and 100 t ha^{-1}, respectively.

soils decreased significantly ($P < 0.0001$ and $P = 0.0072$ in the CO and VA soils, respectively) with biochar addition (Fig. 3), with a more pronounced effect in the CO soil than the VA soil using values before any corrections were applied. In the CO soil, significant declines in total FAMEs were found at the 2.5% and 10% biochar levels, but no difference occurred at the 5% biochar level. In the VA soil, significant declines in total FAMEs occurred only at the 10% biochar level (Fig. 3). A shift in the relative fungal and bacterial community composition also occurred in the CO soil with 10% biochar, as noted by a significant ($P = 0.0442$) decrease in the fungi:bacteria ratio, though no such shift ($P > 0.05$) occurred in the VA soil upon biochar addition at any level. Overall, a significant decrease of each extractable FAME marker occurred in both soils with 10% biochar, with the noted exceptions of the fungal markers 16:1ω5c in CO soil and 18:2ω6c in the VA soil, both of which showed no difference from those of the respective control and at any other biochar addition level (data not shown).

The FAME markers responded differently to biochar addition at the lower levels (2.5% and 5%), depending on soil type. Generally, extractable FAMEs that are associated with fungi tended to decrease with biochar levels in the CO soil and those associated with bacteria tended to decrease with biochar addition in the VA soil (Table IV). With 2.5% biochar addition in the CO soil, there were significantly lower concentrations of the actinomycete marker 10Me17:0 and fungal markers 18:1ω8:1 and 16:1ω6:1, though with 5% biochar, decreases in the FAMEs 10Me17:0 and 18:1ω8:1 were noted. However, in the VA soil, 2.5% biochar addition yielded no changes in any of the measured markers relative to the control, though a 5% biochar addition led to a decrease in all extractable bacteria markers (Gram-positive, Gram-negative, and actinomycete), with the exception of the actinomycete marker 10Me17:0, where no change relative to the control was noted. The largest relative decrease of any measured marker occurred with the fungal marker 18:2ω8:2 in both the CO and VA soils with 10% biochar addition. The 18:2ω8:2 showed a 60% or 42.9% decrease by the addition of 10% biochar relative to the control in the VA soil or the CO soil, respectively.

Extraction efficiency of FAMEs and evaluation of shifts in microbial community structure

The extraction efficiency of an added external fatty acid (19:0) was reduced by incremental additions of biochar compared to the control (Table V) and the extraction efficiency of pure biochar was 0.84. The extraction efficiency was higher in the unamended CO soil, but experienced a higher degree of decline with biochar addition than the VA soil. The extraction efficiency at the 10% biochar level ranged from 0.50 to 0.55 regardless of the soil type. When the original extracted FAME values were corrected for these extraction efficiency values, the effects attributed to biochar addition became negligible in all but one FAME marker measured (18:2ω8:2 in the CO soil at the 10% biochar level) (Table IV).

Principal components analysis using all indicator FAME markers together (actual concentration, nmol

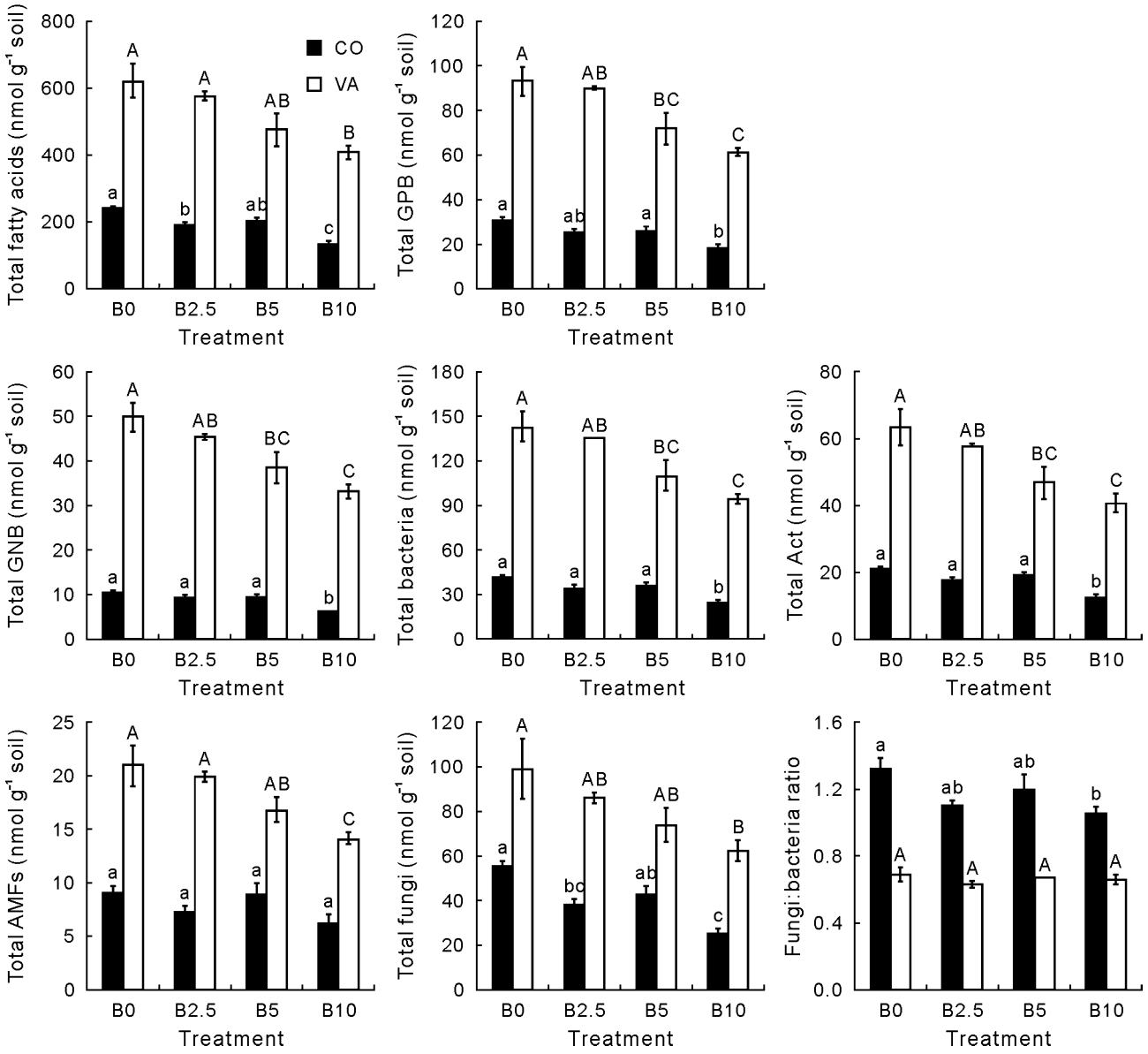

Fig. 3 Changes in microbial community structure as indicated by initially extracted (no extraction efficiency corrections have been applied) fatty acid methyl ester (FAME) markers in the CO and VA soils with switchgrass biochar treatments at different levels. GPB = Gram-positive bacteria; GNB = Gram-negative bacteria; Act = actinomycete; AMF = arbuscular mycorrhizal fungi. Values are means of 4 (CO soil) or 3 (VA soil) replicates and errors bars represent standard errors of the means. Bars with the same lowercase letter(s) within the CO soil and the same uppercase letter(s) within the VA soil are not significantly different at $P > 0.05$. See Table I for details of the CO and VA soils. B0, B2.5, B5, and B10 are the treatments of 0% (control), 2.5%, 5%, and 10% biochar levels, approximately equivalent to biochar additions of 0, 25, 50, and 100 t ha^{-1}, respectively.

g^{-1} soil) identified distinct microbial community structures in the control without biochar and the soils with biochar added, especially at the 10% biochar level when no correction was applied (Fig. 4a, c). For the CO soil, there was a greater separation of the 10% biochar level from the control, and no significant differences between the 2.5% and 5% biochar levels. For the VA soil, there was a greater separation for these four biochar levels than in the CO soil. When the correction factor was applied, the PCA plots continue to indicate a shift in the microbial community structures for both soils. For the CO soil, the lower biochar levels (0%, 2.5%, and 5%) clustered together and there was only a distinct separation at the 10% biochar level (Fig. 4b). For the VA soil, only the 2.5% biochar level clustered with the control and the other biochar levels (5% and 10%) continued to show a separation (Fig. 4d).

TABLE IV

Microbial biomass and individual fatty acid methyl ester (FAME) markers for microbial groups in the CO and VA soils[a)] with switchgrass biochar treatments at different levels

Soil	Microbial community	Treatment[b)]			
		B0 (control)	B2.5	B5	B10
		nmol g^{-1} soil			
CO	Total FAMEs	237.58[c)] (263.98)[d)]	188.35* (272.18)	200.43 (287.97)	131.83* (239.25)
	Gram-positive bacteria				
	i15:0	11.50 (12.77)	9.55 (13.81)	10.20 (14.65)	7.28* (13.21)
	a15:0	8.53 (9.48)	6.67 (9.64)	6.94 (9.97)	4.83* (8.77)
	i17:0	4.89 (5.43)	4.39 (6.34)	4.29 (6.17)	2.78* (5.04)
	a17:0	5.58 (6.20)	4.60 (6.64)	4.60 (6.61)	3.11* (5.66)
	Gram-negative bacteria				
	cy17:0	3.51 (3.90)	2.95 (4.27)	3.15 (4.53)	2.18* (3.96)
	cy19:0	7.09 (7.88)	6.40 (9.25)	6.42 (9.23)	4.09* (7.43)
	Actinomycetes				
	10Me16:0	14.28 (15.87)	12.20 (17.63)	13.35 (19.18)	9.22* (15.09)
	10Me17:0	2.31 (2.56)	1.84* (2.66)	1.84* (2.65)	1.17* (2.12)
	10Me18:0	4.42 (4.92)	3.71 (5.36)	3.69 (5.30)	2.40* (4.35)
	Fungi				
	16:1ω6:1	9.04 (10.04)	7.19 (10.40)	8.92 (12.82)	6.13 (11.13)
	18:1ω8:1	27.31 (30.34)	18.53* (26.78)	20.04* (28.79)	12.08* (21.93)
	18:2ω8:2	17.82 (19.80)	12.09* (17.48)	13.41 (19.27)	7.13* (12.93*)
	Fungal sum	54.16 (60.18)	37.82* (54.65)	42.37 (60.88)	25.34* (45.99)
	Bacterial sum	41.11 (45.67)	34.56 (49.94)	35.60 (51.15)	24.28* (44.06)
	Fungi:bacteria ratio	1.32 (1.32)	1.09 (1.09)	1.20 (1.20)	1.05* (1.05*)
VA	Total FAMEs	621.07 (817.19)	576.43 (798.38)	478.07 (903.71)	408.47* (816.93)
	Gram-positive bacteria				
	i15:0	35.02 (46.08)	34.62 (47.96)	27.10* (51.22)	23.86* (47.72)
	a15:0	28.79 (37.88)	29.03 (40.20)	22.50* (42.54)	19.62* (39.25)
	i17:0	14.30 (18.81)	13.24 (18.34)	10.82* (20.46)	8.66* (17.32)
	a17:0	14.81 (19.48)	13.44 (18.62)	11.27* (21.30)	9.20* (18.40)
	Gram-negative bacteria				
	cy17:0	9.10 (11.98)	8.39 (11.62)	6.93* (13.10)	5.76* (11.52)
	cy19:0	39.23 (51.62)	35.71 (49.47)	30.40* (57.47)	26.24* (52.47)
	Actinomycetes				
	10Me16:0	38.18 (50.23)	36.33 (50.31)	28.47* (53.82)	25.19* (50.38)
	10Me17:0	7.09 (9.32)	6.20 (8.58)	5.33 (10.07)	4.36* (8.72)
	10Me18:0	18.03 (23.72)	15.31 (21.21)	13.08* (24.73)	11.24* (22.49)
	Fungi				
	16:1ω6:1	20.89 (27.49)	19.86 (27.51)	16.72 (31.62)	14.12* (28.24)
	18:1ω8:1	57.77 (76.02)	50.68 (70.20)	44.14 (83.44)	37.24* (74.48)
	18:2ω8:2	18.99 (24.99)	15.16 (20.99)	12.62 (23.85)	10.84 (21.69)
	Fungal sum	98.76 (129.95)	85.70 (118.70)	73.48 (138.91)	62.20* (124.41)
	Bacterial sum	142.71 (187.77)	135.70 (187.95)	110.15* (208.21)	94.49* (188.98)
	Fungi:bacteria ratio	0.69 (0.69)	0.63 (0.63)	0.67 (0.67)	0.66 (0.66)

*Significant with respect to the control at $P < 0.05$.
[a)] See Table I for details of the CO and VA soils.
[b)] B0, B2.5, B5, and B10 are the treatments of 0% (control), 2.5%, 5%, and 10% biochar levels, approximately equivalent to biochar additions of 0, 25, 50, and 100 t ha^{-1}, respectively.
[c)] Measured mean ($n = 4$ for the CO soil and $n = 3$ for the VA soil).
[d)] Values in parentheses are a corrected mean calculated using the extraction efficiency correction factor determined for each soil and biochar mixture in this study.

DISCUSSION

Soil chemical characteristics and plant growth as affected biochar levels

With additions of switchgrass biochar at different levels, we documented gradual increases in soil pH and exchangeable P content, and decreased N net mineralization in both soil types. The soil chemical properties investigated in this study generally underwent the same changes in response to biochar addition in both soils, though the scale of change differed between the two soils. For example, the increase in pH was much

TABLE V

Measured extraction efficiency from standard fatty acid methyl ester (FAME) 19:0 for the CO and VA soils[a)] with switchgrass biochar treatments at different levels

Soil	Treatment[b)]	Extraction efficiency[c)]
CO	B0 (control)	0.900
	B2.5	0.692
	B5	0.696
	B10	0.551
VA	B0 (control)	0.760
	B2.5	0.722
	B5	0.529
	B10	0.500
Biochar		0.844

[a)] See Table I for details of the CO and VA soils.
[b)] B0, B2.5, B5, and B10 are the treatments of 0% (control), 2.5%, 5%, and 10% biochar levels, approximately equivalent to biochar additions of 0, 25, 50, and 100 t ha^{-1}, respectively.
[c)] Calculated from known spiked samples and the 19:0 standard as the ratio of the sample peak area to standard peak area.

greater in the VA soil relative to the CO soil, given its lower initial pH (6.17) and the pH of the added biochar of 9.33. Additionally, the scale of decline in total N mineralization and immobilization of N was greater in the VA soil, presumably attributable to its larger initial N and organic matter contents relative to the CO soil.

Previous studies have reported an increase in CEC, contrary to the no change in the CO soil and the decrease of CEC at 10% biochar noted in the VA soil. For example, in a loamy Mollisol collected from Iowa, USA, Laird *et al.* (2010) reported a 20% increase in CEC from 17.1 to 20.8 cmol kg^{-1} with the addition of biochar at 20 g kg^{-1} soil. Changes in CEC have also been shown to be a function of biochar age, as changes in surface oxidation over time increase the number of negatively charged sites as biochar ages (Cheng *et al.*, 2008; Zimmerman *et al.*, 2011). The CEC of a given

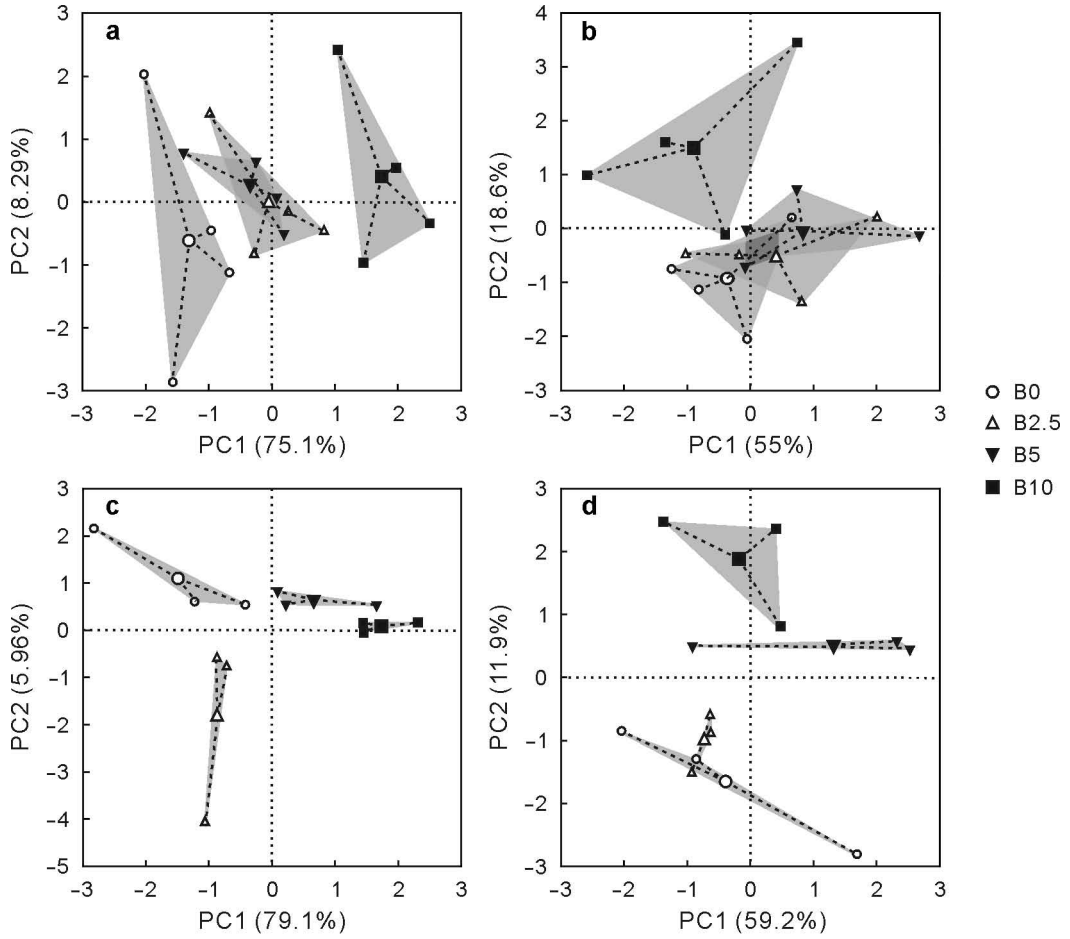

Fig. 4 Principal component (PC) analysis using all indicator fatty acid methyl ester (FAME) markers together indicating changes in the microbial community structure of the CO soil (a and b) and VA soil (c and d) with switchgrass biochar treatments at different levels: initially extracted FAMEs (actual concentration, nmol g^{-1} soil) with no extraction efficiency corrections applied (a and c) and the corrected FAMEs using the extraction efficiency correction factor determined in this study (b and d). See Table I for details of the CO and VA soils. B0, B2.5, B5, and B10 are the treatments of 0% (control), 2.5%, 5%, and 10% biochar levels, approximately equivalent to biochar additions of 0, 25, 50, and 100 t ha^{-1}, respectively.

biochar/soil mixture would thus likely increase over a longer time and results of the biochar addition on the CEC measurement would be a function of the time since application. It is feasible therefore that if given a longer incubation period this biochar treatment may result in an increase in CEC. Our result of decreasing CEC is especially surprising given the high initial CEC of the biochar (53.9 cmol kg^{-1}). This result needs to be verified by repeated studies to elucidate possible explanations. The increase in exchangeable P noted here has also been documented in other studies. For example, when corn-derived biochar was added to an agricultural soil at 20 g kg^{-1} soil with additional N fertilizer, Mehlich-III-extractable P increased by 5.4 mg kg^{-1} soil above the control soil (Nelson *et al.*, 2011).

The decline in total net N mineralization documented in both soils could also be attributed to a decline in microbial activity due to the presence of phytotoxic materials such as ethylene, a known nitrification inhibitor (Spokas *et al.*, 2010), as well as harmful salts such as Na or Cl (Lehmann *et al.*, 2011). Our results do indicate a slight increase in extractable salts with biochar in both soils (Table III). Other possible explanations for a decline in net N mineralization include increasing rates of microbial immobilization and/or denitrification rates with increasing biochar levels. Another study investigating N cycling in biochar-treated soils indicated no change in net N nitrification at 30 and 60 t ha^{-1} biochar, and an increase in nitrification at 30 t ha^{-1} after 3 months (Castaldi *et al.*, 2011), while Güereña *et al.* (2013) documented a 3-fold increase of labeled N^{15} in the soil microbial biomass. Alternatively, some biochar may preferentially sorb essential enzymes or nutrients, especially N, potentially imposing detrimental C and N limitations on plant and microbial growth (Anderson *et al.*, 2011; Zimmerman *et al.*, 2011; Schomberg *et al.*, 2012). Adsorption and immobilization of mineral N to biochar surfaces may also be likely in this study as evidenced by the negative total N mineralization, with concomitant declines (the CO soil) or no change (the VA soil) in plant shoot C:N.

A nearly 50% decrease in shoot biomass occurred in plants grown in the CO soil with 10% biochar, along with a significantly lower shoot C:N ratio in these plants relative to plants grown without biochar (Fig. 2d). No effect attributable to biochar addition on plant growth occurred in the VA soil. This negative effect on plant yield in the CO soil does not appear to be entirely associated with N availability given that the shoot N concentrations showed no change attributable to biochar addition and an incrementally lower C:N ratio was noted in plants grown with increasing biochar levels. The liming effect on the already alkaline CO soil might have caused nutrient solubility and fertility issues for the wheat plants. Other studies have also demonstrated a decline in plant yield with biochar addition and in a review of biochar effects on plant yield by Spokas *et al.* (2012), approximately 50% of the 46 reviewed studies reported an increase in plant biomass, 30% reported no change, and 20% of the studies reported a negative effect on plant biomass. Most of the studies that reported an increase in plant biomass occurred in soils that were severely degraded, highly weathered, or nutrient-poor, with large potential for improvement with amendments (Spokas *et al.*, 2012). Many explanations have been posed for negative impacts on plant yield, and may be associated with imposed nutrient limitations by the sorption of base cations such as ammonium (Yao *et al.*, 2012) or other nutrients, or possibly the addition of harmful volatile organic compounds with biochar.

Soil microbial community structure as affected by biochar levels

As reviewed by Lehmann *et al.* (2011), several factors may be linked with a decline in microbial activity and/or change in community structure attributable to biochar addition to soil. These include 1) changes in soil chemical and physical properties, such as pH and particle size distribution, 2) addition of potentially toxic compounds from biochar such as salts or heavy metals, 3) a decrease in symbiotic fungi as a result of increased nutrient and water availability to plants, and 4) the direct sorption of C compounds (or other essential nutrients) to biochar surfaces, decreasing the availability of C substrate for microbes. We suggest two additional candidate explanations that deserve discussion, including 5) a simple dilution caused by an addition of an inert substance to the soil and/or 6) an interference in the analytical efficiency due to strong sorption of microbial byproducts to biochar surfaces.

The negative effect of biochar addition to these soils on microbial activity and nutrient availability does not appear to be (solely) a function of simple dilution. This is evidenced by the nearly 44% decline in total mineralizable N in the VA soil with just a 10% biochar addition (Fig. 1). The magnitude of this decline suggests that additional reactions are occurring in the soil/biochar mixtures beyond simple dilution by an inert substance.

In general, there was an incremental decline in extractable fatty acids with increasing biochar levels in both soils, with 45% and 34% decline with 10% biochar

addition in the CO and VA soils, respectively. This is in agreement with a similar recovery assay performed by Liang *et al.* (2010), who demonstrated a 21%–41% lower recovery of microbial biomass C (MBC) when soils were fumigated and extracted with K_2SO_4 in Brazilian Anthrosols rich in black carbon. The lower recovery of MBC was found to be due to re-adsorption onto black carbon surfaces and they suggested a correction factor (0.26) to account for the low MBC recovery during the initial extraction method. O'Neill (2006) also demonstrated a lower extraction efficiency of microbial DNA from the same Anthrosols relative to similar soils low in black carbon. In our study, the degree to which individual FAME markers were affected depended on the soil and the biochar application rate, where it appears that FAMEs associated with fungi tended to decrease with biochar addition in the CO soil to a greater extent than in the VA soil and FAMEs associated with bacteria tended to decrease with biochar addition in the VA soil.

When the decreased extraction efficiency of FAME markers due to sorption to biochar surfaces was accounted for and the correction factor was applied to the extracted values, the differences in FAMEs that were initially measured became statistically negligible, thus yielding the result that biochar addition had little effect on the total FAMEs in either CO or VA soil. However, the change in the fungi:bacteria ratio was not affected by the correction factor, and a decline in the fungi:bacteria ratio was still evident in the CO soil with biochar addition, becoming significant at the 10% level, indicating a shift to a bacteria-dominated community. This population shift to favor bacteria was also documented by Jones *et al.* (2012) with biochar field application at 50 t ha^{-1}. Although we tested a correction factor for the soils evaluated, PCA performed on the actual concentration of all FAMEs (nmol g^{-1} soil) showed a similar separation of the microbial communities as was found when a correction factor was applied. These shifts in microbial community structure are likely a result of soil conditions that favor bacteria over fungal species, especially in the CO soil. Such soil conditions that favor bacterial growth include higher soil pH (Bardgett *et al.*, 1996), lower soil C:N ratio (Pennanen *et al.*, 2001), and relatively greater amounts of labile organic C sources (Zak *et al.*, 1996; Buyer *et al.*, 2002; Allison *et al.*, 2005). Ecological consequences of such a shift in community structure may include a decrease in soil C storage as bacteria have a lower C assimilation efficiency than fungi (Holland and Coleman, 1987; Lundquist *et al.*, 1999).

Even using the more conservative, corrected estimates of microbial measurements, the results indicate that the switchgrass biochar had the potential to cause short-term negative impacts on the nutrient availability (Fig. 1) and alter the microbial community structure (Fig. 4) in some soils. As pointed out by Lehman *et al.* (2011), the reasons for changes in microbial abundance may differ for different groups of microorganisms. Three distinct mechanisms have been discussed specifically for AMF increases in biochar-amended soils, which may be applicable to other microbial groups: 1) protection of the extraradical mycelium from grazers by internal pore systems of biochar particles (Warnock *et al.*, 2007; Lehmann *et al.*, 2011); 2) sorption of signaling compounds, detoxification of allelochemicals, changes in soil physical and chemical properties, or indirect effects through alterations of other soil microbial populations (Warnock *et al.*, 2007; Elmer and Pignatello, 2011); and 3) stimulation of spore germination of AMF by biochar produced *via* hydrothermal carbonization, as was found by Rillig *et al.* (2010). However, this trend for AMF could be soil-dependent as others have found its decreases with biochar additions (Gaur and Adholeya, 2000; Birk *et al.*, 2009; Warnock *et al.*, 2010).

It is unknown how the microbial community measured here may respond over time, as changes in the surface properties of the biochar evolve over time to alter the availability of substrate or nutrient compounds in the soil (Cheng *et al.*, 2008; Zimmerman *et al.*, 2011). Thus, future studies should continue for long-term implications and especially at the biochar treatment levels of 10% or greater. A shift in microbial community structure in soils may alter nutrient cycles and affect the turnover of the active pool of soil organic C. For example, Cross and Sohi (2011) suggested that negative priming (lower than expected C mineralization) upon biochar addition could lead to an increased stabilization of native soil organic matter, as more labile C compounds become sorbed onto the biochar surface and are protected from degradation, effectively slowing decomposition and increasing C storage over time. However, this slower turnover of soil organic material may negatively impact crop yield in agricultural soils if nutrient deficits are imposed as C and N mineralization are slowed.

CONCLUSIONS

Switchgrass biochar could have a negative effect on N mineralization during crop growth, which partially explained depressed plant growth on the biochar-amended CO soil. A correction factor was necessary for

the proper interpretation of FAME data. The fatty acid analysis showed that besides the effects on N cycling, the switchgrass biochar could also alter the soil microbial community composition. It is unknown how the microbial community measured here may respond over time, as changes in the surface properties of the biochar evolve over time to alter the availability of substrate or nutrient compounds in the soil. Thus, future studies should continue for long-term implications and especially at different treatment levels in order to determine how different microbial communities across soil types may respond to biochar treatments. Future studies should also include steps to determine extraction efficiency of each method to separate true differences in soil function from artifacts introduced by difficulties in the extraction of biochar material.

REFERENCES

Allison, V. J., Miller, R. M., Jastrow, J. D., Matamala, R. and Zak, D. R. 2005. Changes in soil microbial community structure in a tallgrass prairie chronosequence. *Soil Sci. Soc. Am. J.* **69**: 1412–1421.

Ameloot, N., Graber, E. R., Verheijen, F. G. A. and De Neve, S. 2013. Interactions between biochar stability and soil organisms: review and research needs. *Eur. J. Soil Sci.* **64**: 379–390.

Anderson, C. R., Condron, L. M., Clough, T. J., Fiers, M., Stewart, A., Hill, R. A. and Sherlock, R. R. 2011. Biochar induced soil microbial community change: implications for biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia.* **54**: 309–320.

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

Bardgett, R. D., Hobbs, P. J. and Frostegård, Å. 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. *Biol. Fert. Soils.* **22**: 261–264.

Birk, J. J., Steiner, C., Teixeira, W. C., Zech, W. and Glaser, B. 2009. Microbial response to charcoal amendments and fertilization of a highly weathered tropical soil. *In* Woods, W. I., Teixeira, W. G., Lehmann, J., Steiner, C., WinklerPrins, A. M. G. A. and Rebellato, L. (eds.) Amazonian Dark Earths: Wim Sombroek's Vision. Springer, Amsterdam. pp. 309–324.

Buyer, J. S., Roberts, D. P. and Russek-Cohen, E. 2002. Soil and plant effects on microbial community structure. *Can. J. Microbiol.* **48**: 955–964.

Castaldi, S., Riondino, M., Baronti, S., Esposito, F. R., Marzaioli, R., Rutigliano, F. A., Vaccari, F. P. and Miglietta, F. 2011. Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. *Chemosphere.* **85**: 1464–1471.

Cheng, C. H., Lehmann, J. and Engelhard, M. H. 2008. Natural oxidation of black carbon in soils: changes in molecular form and surface charge along a climosequence. *Geochim. Cosmochim. Acta.* **72**: 1598–1610.

Cross, A. and Sohi, S. P. 2011. The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. *Soil Biol. Biochem.* **43**: 2127–2134.

Ducey, T. F., Ippolito, J. A., Cantrell, K. B., Novak, J. M. and Lentz, R. D. 2013. Addition of activated switchgrass biochar to an aridic subsoil increases microbial nitrogen cycling gene abundances. *Appl. Soil. Ecol.* **65**: 65–72.

Durenkamp, M., Luo, Y. and Brookes, P. C. 2010. Impact of black carbon addition to soil on the determination of soil microbial biomass by fumigation extraction. *Soil Biol. Biochem.* **42**: 2026–2029.

Eberl, D. D. 2003. User's Guide to RockJock—A Program for Determining Quantitative Mineralogy from Powder X-ray Diffraction Data. U.S. Geological Survey Open-File Report 2003-78. U.S. Geological Survey, Reston.

Elmer, W. H. and Pignatello, J. J. 2011. Effect of biochar amendments on mycorrhizal associations and *Fusarium* crown and root rot of asparagus in replant soils. *Plant Dis.* **95**: 960–966.

Gaskin, J. W., Das, K. C., Tassistro, A. S., Sonon, L., Harris, K. and Hawkins, B. 2009. Characterization of char for agricultural use in the soils of the southeastern United States. *In* Woods, W. I., Teixeira, W. G., Lehmann, J., Steiner, C., WinklerPrins, A., Rebellato, L. (eds.) Amazonian Dark Earths: Wim Sombroek's Vision. Springer, Amsterdam. pp. 433–443.

Gaur, A. and Adholeya, A. 2000. Effects of the particle size of soil-less substrates upon AM fungus inoculum production. *Mycorrhiza.* **10**: 43–48.

Glaser, B., Lehmann, J. and Zech, W. 2002. Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal—a review. *Biol. Fert. Soils.* **35**: 219–230.

Gomez, J. D., Denef, K., Stewart, C. E., Zheng, J. and Cotrufo, M. F. 2014. Biochar addition rate influences soil microbial abundance and activity in temperate soils. *Eur. J. Soil Sci.* **65**: 28–39.

Güereña, D., Lehmann, J., Hanley, K., Enders, A., Hyland, C. and Riha, S. 2013. Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system. *Plant Soil.* **365**: 239–254.

Holland, E. A. and Coleman, D. C. 1987. Litter placement effects on microbial and organic matter dynamics in an agroecosystem. *Ecology.* **68**: 425–453.

Ippolito, J. A., Novak, J. M., Busscher, W. J., Ahmedna, M., Rehrah, D. and Watts, D. W. 2012. Switchgrass biochar affects two Aridisols. *J. Environ. Qual.* **41**: 1123–1130.

Jha, P., Biswas, A. K., Lakaria, B. L. and Rao, A. S. 2010. Biochar in agriculture—prospects and related implications. *Curr. Sci.* **99**: 1218–1225.

Johnson, D. W. and Curtis, P. S. 2001. Effects of forest management on soil C and N storage: meta-analysis. *Forest Ecol. Manag.* **140**: 227–238.

Jones, D. L., Rousk, J., Edwards-Jones, G., DeLuca, T. H. and Murphy, D. V. 2012. Biochar-mediated changes in soil quality and plant growth in a three year field trial. *Soil Biol. Biochem.* **45**: 113–124.

Joseph, S. D., Camps-Arbestain, M., Lin, Y., Munroe, P., Chia, C. H., Hook, J., Van Zweiten, L., Kimber, S., Cowie, A., Singh, B. P., Lehmann, J., Foidl, N., Smernik, R. J. and Amonette, J. E. 2010. An investigation into the reactions of biochar in soil. *Soil Res.* **48**: 501–515.

Laird, D. A. 2008. The charcoal vision: a win-win-win scenario for simultaneously producing bioenergy, permanently sequestering carbon, while improving soil and water quality. *Agron. J.* **100**: 178–181.

Laird, D. A., Fleming, P., Davis, D. D., Horton, R., Wang, B. and Karlen, D. L. 2010. Impact of biochar amendments on

the quality of a typical Midwestern agricultural soil. *Geoderma*. **158**: 443–449.

Lehmann, J., Gaunt, J. and Rondon, M. 2006. Biochar sequestration in terrestrial ecosystems—a review. *Mitig. Adapt. Strat. Glob. Change*. **11**: 403–427.

Lehmann, J., Rillig, M. C., Thies, J., Masiello, C. A., Hockaday, W. C. and Crowley, D. 2011. Biochar effects on soil biota —A review. *Soil Biol. Biochem*. **43**: 1812–1836.

Lehmann, J. and Rondon, M. 2006. Bio-char soil management on highly weathered soils in the humid tropics. *In* Uphoff, N. (ed.) Biological Approaches to Sustainable Soil Systems. CRC Press, Boca Raton. pp. 517–530.

Liang, B., Lehmann, J., Sohi, S. P., Thies, J. E., O'Neill, B., Trujillo, L., Gaunt, J., Solomon, D., Grossman, J., Neves, E. G. and Luizão, F. J. 2010. Black carbon affects the cycling of non-black carbon in soil. *Org. Geochem*. **41**: 206–213.

Liang, B., Lehmann, J., Solomon, D., Kinyani, J., Grossman, J., O'Neill, B., Skjemstad, J. O., Thies, J., Luizão, F. J., Petersen, J. and Neves, E. G. 2006. Black carbon increases cation exchange capacity in soils. *Soil Sci. Soc. Am. J*. **70**: 1719–1730.

Lundquist, E. J., Scow, K. M., Jackson, L. E., Useugi, S. L. and Johnson, C. R. 1999. Rapid response of soil microbial communities from conventional, low input, and organic farming systems to a wet/dry cycle. *Soil Biol. Biochem*. **31**: 1661–1675.

Nelson, N. O., Agudelo, S. C., Yuan, W. Q. and Gan, J. 2011. Nitrogen and phosphorus availability in biochar-amended soils. *Soil Sci*. **176**: 218–226.

O'Neill, B. E. 2006. Microbial communities in Amazonian dark earth soils analyzed by culture-based and molecular approaches. M.S. Thesis, Cornell University, Ithaca,.

Paustian, K., Six, J., Elliott, E. T. and Hunt, H. W. 2000. Management options for reducing CO_2 emissions from agricultural soils. *Biogeochemistry*. **48**: 147–163.

Pennanen, T., Strömmer, R., Markkola, A. and Fritze, H. 2001. Microbial and plant community structure across a primary succession gradient. *Scand. J. Forest Res*. **16**: 37–43.

Rillig, M. C., Wagner, M., Salem, M., Antunes, P. M., George, C., Ramke, H. G., Titirici, M.-M. and Antonietti, M. 2010. Material derived from hydrothermal carbonization: Effects on plant growth and arbuscular mycorrhiza. *Appl. Soil. Ecol*. **45**: 238–242.

Rutherford, D. W., Wershaw, R. L., Rostad, C. E. and Kelly, C. N. 2012. Effect of formation conditions on biochars: Compositional and structural properties of cellulose, lignin, and pine biochars. *Biomass Bioenerg*. **46**: 693–701.

Rutigliano, F. A., Romano, M., Marzaioli, R., Baglivo, I., Baronti, S., Miglietta, F. and Castaldi, S. 2014. Effect of biochar addition on soil microbial community in a wheat crop. *Eur. J. Soil Biol*. **60**: 9–15.

Schomberg, H. H., Gaskin, J. W., Harris, K., Das, K. C., Novak, J. M., Busscher, W. J., Watts, D. W., Woodroof, R. H., Lima, I. M., Ahmeda, M., Rehrah, D. and Xing, B. 2012. Influence of biochar on nitrogen fractions in a Coastal Plain soil. *J. Environ. Qual*. **41**: 1087–1095.

Schutter, M. E. and Dick, R. P. 2000. Comparison of fatty acid methyl ester (FAME) methods for characterizing microbial communities. *Soil Sci. Soc. Am. J*. **64**: 1659–1668.

Spokas, K. A., Baker, J. M. and Reicosky, D. C. 2010. Ethylene: Potential key for biochar amendment impacts. *Plant Soil*. **333**: 443–452.

Spokas, K. A., Cantrell, K. B., Novak, J. M., Archer, D. W., Ippolito, J. A., Collins, H. P., Boateng, A. A., Lima, I. M., Lamb, M. C., McAloon, A. J., Lentz, R. D. and Nichols, K. A. 2012. Biochar: A synthesis of its agronomic impact beyond carbon sequestration. *J. Environ. Qual*. **41**: 973–989.

Soil Survey Staff, USDA Natural Resources Conservation Service. Web soil survey. Available online at http://websoilsurvey.nrcs.usda.gov/ (verified on February 10, 2013).

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

Swaine, M., Obrike, R., Clark, J. M. and Shaw, L. J. 2013. Biochar alteration of the sorption of substrates and products in soil enzyme assays. *App. Environ. Soil Sci*. **2013**: Article ID 968682, doi:10.1155/2013/968682.

Thies, J. E. and Rillig, M. C. 2009. Characteristics of biochar: biological properties. *In* Lehmann, J. and Joseph, S. (eds.) Biochar for Environmental Management: Science and Technology. Earthscan Publisher, London. pp. 85–106.

Uchimiya, M., Wartelle, L. H., Klasson, K. T., Fortier, C. A. and Lima, I. M. 2011. Influence of pyrolysis temperature on biochar property and function as a heavy metal sorbent in soil. *J. Agr. Food Chem*. **59**: 2501–2510.

Warnock, D. D., Lehmann, J., Kuyper, T. W. and Rillig, M. C. 2007. Mycorrhizal responses to biochar in soil—concepts and mechanisms. *Plant Soil*. **300**: 9–20.

Warnock, D. D., Mummey, D. L., McBride, B., Major, J., Lehmann, J. and Rillig, M. C. 2010. Influences of nonherbaceous biochar on arbuscular mycorrhizal fungal abundances in roots and soils: Results from growth-chamber and field experiments. *Appl. Soil Ecol*. **46**: 450–456.

Yao, Y., Gao, B., Zhang, M., Inyang, M. and Zimmerman, A. R. 2012. Effect of biochar amendment on sorption and leaching of nitrate, ammonium, and phosphate in a sandy soil. *Chemosphere*. **89**: 1467–1471.

Zak, D. R., Ringelberg, D. B., Pregitzer, K. S., Randlett, D. L., White, D. C. and Curtis, P. S. 1996. Soil microbial communities beneath *Populus grandidentata* grown under elevated atmospheric CO_2. *Ecol. Appl*. **6**: 257–262.

Zelles, L. 1999. Fatty acid patterns of phospholipids and lipopolysaccharides in the characterization of microbial communities in soil: a review. *Biol. Fert. Soils*. **29**: 111–129.

Zimmerman, A. R., Gao, B. and Ahn, M. Y. 2011. Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biol. Biochem*. **43**: 1169–1179.