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Contrasting effects of biochar versus manure on soil microbial communities and enzyme activities in an Aridisol

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HIGHLIGHTS

- Biochar, manure, and biochar + manure effects were studied in the field.
- Microbial communities and enzyme activities were affected by manure but not biochar.
- Mycorrhizal root colonization was negatively affected by manure but not biochar.

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ABSTRACT

Biochar can increase microbial activity, alter microbial community structure, and increase soil fertility in arid and semi-arid soils, but at relatively high rates that may be impractical for large-scale field studies. This contrasts with organic amendments such as manure, which can be abundant and inexpensive if locally available, and thus can be applied to fields at greater rates than biochar. In a field study comparing biochar and manure, a fast pyrolysis hardwood biochar (22.4 Mg ha^{-1}), dairy manure (42 Mg ha^{-1} dry wt), a combination of biochar and manure at the aforementioned rates, or no amendment (control) was applied to an Aridisol ($n = 3$) in fall 2008. Plots were annually cropped to corn (*Zea maize* L.). Surface soils (0–30 cm) were sampled directly under corn plants in late June 2009 and early August 2012, and assayed for microbial community fatty acid methyl ester (FAME) profiles and six extracellular enzyme activities involved in soil C, N, and P cycling. Arbuscular mycorrhizal (AM) fungal colonization was assayed in corn roots in 2012. Biochar had no effect on microbial biomass, community structure, extracellular enzyme activities, or AM fungi root colonization of corn. In the short-term, manure amendment increased microbial biomass, altered microbial community structure, and significantly reduced the relative concentration of the AM fungal biomass in soil. Manure also reduced the percent root colonization of corn by AM fungi in the longer-term. Thus, biochar and manure had contrasting short-term effects on soil microbial communities, perhaps because of the relatively low application rate of biochar.

1. Introduction

Biochar is a form of black carbon (C) created by thermal degradation of organic material (e.g., wood, manure, leaves, etc.) in a low or zero oxygen environment (pyrolysis). It is distinguished from charcoal and similar materials by its use as a soil amendment (Lehmann and Joseph, 2009). Biochar C is recalcitrant in nature (Spokas, 2010) and its reactive surfaces are capable of sorbing and exchanging nutrients and native organic matter (Liang et al., 2006); therefore, there is a great interest in utilizing biochar as a soil amendment to sequester C and improve soil fertility in agricultural soils.

Biochar's ability to enhance soil fertility has been demonstrated in tropical soils, where long-term biochar inputs have helped create highly fertile soil known as Terra Preta, or Amazonian Dark Earth (Sombroek, 1966; Glaser et al., 2001). Furthermore, Amazonian Terra Preta soils have greater microbial biomass, and

Abbreviations: AM fungi, arbuscular mycorrhizal fungi; FAME, fatty acid methyl ester; LSD, least significant difference; MRBP, multi-response blocked permutation; PCA, principal components analysis.

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in some cases, greater bacterial diversity than the surrounding area (Kim et al., 2007). Biochar has also been found to positively affect the abundance of arbuscular mycorrhizal (AM) and ectomycorrhizal fungi in soil (Ishii and Kadoya 1994; Warnock et al., 2007), as well as the percent root colonization of host plants (Solaiman et al., 2010). Thies and Rillig (2009) hypothesized that biochar could have a positive effect on the soil microbial communities by providing a habitat where bacteria and fungi could escape from predators as well as find substrates to meet many of their diverse C, energy, and mineral nutrient needs. This may also lead to a change in soil microbial community composition and diversity.

Laboratory incubation studies that involve biochar amendment to arid and semi-arid soil provided support for a positive effect of biochar on microbial activity. For example, biochar amendment increased soil CO_2 evolution when biochar was added at rates of 20 Mg ha^{-1} to a Mollisol (Rogovska et al., 2011), 40 Mg ha^{-1} to three Mollisols (Streubel et al., 2011), and 45 Mg ha^{-1} to a Mollisol and an Aridisol (Smith et al., 2010). These observations were attributed to an increase in the quantity of easily degradable C sources present in the biochar (Smith et al., 2010), and an improved microbial habitat due to reductions in soil bulk density and improved gas exchange in biochar-amended soil (Rogovska et al., 2011). Ippolito et al. (2014) conducted a 12-month incubation study in which biochar was applied to an Aridisol at rates of 0, 20, 40, or 200 Mg ha^{-1}. The authors also observed increased and sustained CO_2 production in all biochar-amended treatments over the 12-month period. However, the 40 and 200 Mg ha^{-1} biochar rates altered the relative proportion of bacterial and fungal fatty acids, and shifted the microbial community toward greater amounts of bacteria and fewer fungi.

Microbial communities and their enzymes are the primary regulators of many soil processes, including nutrient cycling, and changes to microbial community structure and enzyme activity might indicate potential long-term effects of biochar on soil nutrient cycling processes. In the studies noted above, biochar amended to soil at relatively high rates affected microbial activity (20 Mg ha^{-1} or more) and microbial community structure (40 Mg ha^{-1} or more). Such rates have also been proven to affect the availability of plant nutrients in soil, perhaps because microbial communities were affected. For example, Ippolito et al. (2012b) observed a decrease in P and NO_3-N leaching from two Aridisols when biochar was applied at approximately 40 Mg ha^{-1}. Other studies with Aridisols and Mollisols have shown increases in plant-available soil nutrients with biochar applications of up to 40 Mg ha^{-1} (Brewer et al., 2012; Ippolito et al., 2012a; Laird et al., 2010a,b). In contrast, Van Zwieten et al. (2010) noted no change in extractable soil nutrients after 10 Mg ha^{-1} biochar was applied to an Aridisol. However, biochar application rates of 40 Mg ha^{-1} or more may be impractical for field studies due to biochar's cost and limited availability, in contrast to organic amendments such as manure which can be abundant and inexpensive if locally available, and thus can be applied at greater rates than biochar in the field.

In a short-term field experiment, Lentz and Ippolito (2012) studied biochar effects on the chemical properties of the Portneuf soil series up to two years following application of either 22.4 Mg ha^{-1} biochar, 42 Mg ha^{-1} manure, or both. The authors observed no change in extractable soil nutrients with biochar application, whereas manure significantly affected soil fertility. No data were collected on the response of soil microbial communities or enzymes, however, and it is plausible that the soil fertility results could be due to differential responses of microbial communities and their enzyme activities to biochar and manure. Therefore, the objective of this study was to compare the effects of biochar, manure, and co-application of biochar and manure on soil microbial community biomass and structure, AM fungi, and enzyme activities in the field trials of Lentz and Ippolito (2012), as a means to explain the contrasting fertility results. Microbial responses were quantified from fresh soil samples collected in 2012, four years after amendment application, and cryopreserved samples from the first growing season in 2009, so that longer- and shorter-term effects of biochar and manure could be assessed.

2. Materials and methods

2.1. Study site, soil, and amendments

A long-term field study was established in fall 2008 near Kimberly, Idaho (42°31′N, 114°22′W, elevation of 1190 m) to quantify the effects of a single biochar or manure application on crop productivity and soil fertility. In 2012, the study's objectives were expanded to include soil biological properties and root colonization by AM fungi. The soil was a Portneuf silt loam (coarse-silty, mixed superactive, mesic Durinodic Xeric Haplocalcids), pH 7.6, containing 20% clay, 56% silt, 24% sand, 1.2% organic carbon, and having an 8.8% calcium carbonate equivalency. For 33 years prior to this study, the site was cropped to an alfalfa–corn–bean–grain rotation, and no manure had been applied since 1986. Additional details of the study site are described in Lentz and Ippolito (2012).

Manure and biochar chemical characteristics are presented in Table 1. Dairy cattle (*Bos* species) solid manure was obtained from unconfined piles from a local dairy. The material contained little or no straw bedding and comprised 55.3% solids at time of application. The biochar material was provided by Dynamotive Energy Systems (West Lorne, Ontario, Canada) and was marketed under the name CQuest. It was derived from oak and hickory hardwood sawdust and created by fast pyrolysis at 500 °C. The biochar had an ash content of 14% as determined by the ASTM methods for wood charcoal (600 °C). The biochar had an oxygen:carbon ratio of 0.22, a surface area of 0.75 m^2 g^{-1}, and a pH of 6.8. Additional details regarding the manure and biochar treatments are provided in Lentz and Ippolito (2012).

2.2. Experimental design

The experimental design was a randomized complete block design with three replicates and four treatments (control, biochar, manure, and biochar plus manure). Plots were 4.6 m wide and 5.2 m long and included eight planted rows. Each plot was separated by a 1.5 m-wide border. Due to limited biochar availability, it was not possible to enlarge the plots or add additional blocks. Treatments were applied once in November 2008. Details of the field operations are provided in Lentz and Ippolito (2012), but in

Table 1
Selected chemical properties of biochar and manure applied to the experimental plots in November 2008. Adapted from Lentz and Ippolito (2012).

Property	Units	Biochar	Manure
pH		6.8	8.8
EC	dS m^{-1}	0.7	13.4
Ash	%	14	N/A[†]
Total C	%	66.2	26.4
Total N	%	0.32	2.15
Organic N	%	0.32	2.12
NO_3-N	mg kg^{-1}	1.5	80.6
NH_4-N	mg kg^{-1}	1.2	220
K	mg kg^{-1}	3400	13,500
Ca	mg kg^{-1}	3700	22,000
Mg	mg kg^{-1}	1500	8230
Na	mg kg^{-1}	200	3750
P	mg kg^{-1}	300	4080

[†] N/A = Not Applicable.

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brief, the field was prepared by growing spring barley (*Hordeum vulgare* L.) in 2008 and moldboard plowing to a 20-cm depth after barley harvest. Solid manure was hand-applied to the soil surface on November 21, 2008, at a rate of 42 Mg ha^{-1} (dry wt). Three days later, biochar was hand-applied to appropriate plots at a rate of 22.4 Mg ha^{-1} (dry wt) and immediately after, all plots were rototilled to a depth of 15 cm. The field was roller harrowed on April 21, 2009, and Round-Up ready silage corn (*Zea mays* L.) (Monsanto, St. Louis, MO) was planted annually in May and harvested in October during the 2009–2012 study. Corn was managed with standard conventional methods, and detailed information regarding fertilization and irrigation is provided in Lentz and Ippolito (2012).

2.3. Soil sampling

Soils were sampled in late June 2009 and again in early August 2012 (the R1/silking stage). In 2009, four cores (0–30 cm deep) were collected from each plot and composited into one bag. In 2012, four cores (0–30 cm deep) were collected, two on each side of the corn row near the center of each plot (to eliminate potential edge effects), and composited. The different sampling schemes reflect the study's initial objectives (corn yield and soil fertility) and the biological objectives added in 2012. Sampling in 2012 was targeted near corn plants in order to collect roots along with soil. Samples were stored on ice and transported in ice chests to the laboratory for analysis. Subsamples of soil from 2009 were either air-dried and analyzed for soil chemical properties (see below) or cryopreserved at −80 °C for three years prior to microbial community and enzyme analyses. Soils from 2012 were sorted by hand to remove roots, and roots were stored at 4 °C for staining of AM fungi. Soil from 2012 was then divided and either stored at −20 °C for microbial community and enzyme analyses or air-dried and stored at room temperature for chemical analyses.

2.4. Soil chemical analyses

Soil pH was determined using a 1:1 soil:deionized water extract (Thomas, 1996). Total C and N were determined by dry combustion (Nelson and Sommers, 1996; Thermo-Finnigan FlashEA1112; CE Elantech Inc., Lakewood, NJ). Inorganic C analysis was determined using a modified pressure-calcimeter method (Sherrod et al., 2002). Total organic C was determined by difference between total and inorganic C. A 2 mol L^{-1} KCl extract method was used to determine NO_3-N and NH_4-N content (Mulvaney, 1996). Extractable P was determined by ammonium bicarbonate diethylenetriaminepentaacetic acid (AB-DTPA) extraction (Soltanpour and Schwab, 1977).

2.5. Soil enzymes

Potential soil enzyme activities were analyzed according to the standard florescence enzyme protocols described in Steinweg et al. (2013) and Bell et al. (2013). The six enzymes assayed were three C-cycling enzymes (β-D-cellobiosidase, β-glucosidase, and β-xylosidase), 1 C/N cycling enzyme (N-acetyl-β-glucosaminidase), 1 N cycling enzyme (leucine aminopeptidase), and 1 P cycling enzyme (phosphatase). All assays included appropriate blanks, where soil suspensions were incubated in the absence of enzyme substrate. Standard curves were prepared for each replicate plot soil sample by incubating soil suspensions in the presence of increasing concentration of 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (MUC) standard. Incubations were conducted at 25 °C for 2.5 h. Fluorescence measurements of the plates were read on a Tecan Infinite® M200 microplate (Tecan, Mannedorf, Switzerland) at 365 nm excitation and 450 nm emission wavelengths.

2.6. Fatty acid methyl ester (FAME) extractions

Fatty acids were extracted from soil samples using the ester-linked fatty acid methyl ester (FAME) method (Schutter and Dick, 2000). With this method, cells within a soil sample are lysed and ester-linked fatty acids are released and extracted directly from neutral, glyco and phospholipids (Schutter and Dick, 2000). An advantage of this method over the phospholipid fatty acid (PLFA) method is that the FAME method extracts biomarker fatty acid 16:1ω5c from the neutral and glycolipids of AM fungi, and not just the PLFA fraction. Because certain bacteria contain 16:1ω5c in their PLFAs, the PLFA method is not able to distinguish between AM fungal and bacterial biomass (Zelles, 1997). In contrast, the FAME method extracts the biomarker from neutral and glycol lipids associated with AM fungi's extrametrical hyphae, and thus provides a more accurate representation of AM fungal biomass in soil (Olsson 1999; Grigera et al., 2007; Moeskops et al., 2012). In brief, 3 g of soil was extracted with 0.2 M methanolic KOH during a 37 °C, 1-h incubation with periodic mixing followed by pH neutralization with 1.0 M acetic acid. Hexane was then added to divide the FAMEs into an organic phase, followed by centrifugation (480*g* for 10 min). The hexane layer was transferred to a clean tube and each tube was placed under a gentle stream of N_2 to evaporate the hexane. Finally, each sample was redissolved in hexane and transferred to a gas chromatograph (GC) vial and 20 μg of internal standards (13:0 and 19:0) were added before the hexane solvent was completely evaporated. Samples were then sent to the University of Delaware, where FAMEs were dissolved in 1:1 hexane:methyl-tert-butyl ether and analyzed on a HP 6890 Series II gas chromatograph (Hewlett–Packard, Palo Alto, Calif.) equipped with a 25 m × 0.2 m fused silica capillary column (5% diphenyl–95% dimethylpolysiloxane) and a flame ionization detector. FAMEs were identified and their relative peak areas determined by the MIS Aerobe method of the MIDI system (Microbial ID, Newark, DE). Biomarker FAMEs were assigned to the following groups: i14:0, i15:0, a15:0, i16:0, i17:0, a17:0 for Gram-positive bacteria; 16:1ω7c, 16:1ω9c, 17:0 cyclo, 19:0 cyclo for Gram-negative bacteria; 18:1ω9c and 18:2ω6c for fungi, and 16:1ω5c for AM fungi (Schutter and Dick, 2000; Moeskops et al., 2012).

2.7. AM fungal root colonization

Arbuscular mycorrhizal fungal colonization of corn roots were quantified in 2012 using the magnified gridline intersect method detailed in McGonigle et al. (1990). Fine, fibrous roots were hand-picked from soil samples and washed in water to remove all particulates. Root staining followed the method outlined by the International Culture Collection of (Vesicular) Arbuscular Mycorrhizal Fungi (INVAM) (http://invam.wvu.edu/methods/mycorrhizae/staining-roots). Roots were placed in rectangular plastic cassettes with 0.9 mm holes, and cleared in hot 10% KOH to remove cytoplasmic contents from cells. To minimize agitation, the KOH was heated in a large beaker over a Bunsen burner until boiling, the burner was turned off, and cassettes were immediately added for a 10-min soaking period. Afterwards, the roots were washed five times in water and then immersed in 2% HCl for 20 min. Next, roots were stained with trypan blue, rinsed five separate times with DI water and stored at 4 °C. Roots were mounted on glass slides and for each sample, 100 intersects were examined under a microscope at 400× magnification for AM fungal hyphae, arbuscules, and vesicles.

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2.8. Statistical analyses

Univariate data were analyzed by one-way analysis of variance tests for a randomized complete block design, using PROC GLM in SAS (ver. 9.3, SAS Institute, Cary, North Carolina), using plot replicates as statistical replicates ($n = 3$). Mean effects were separated using a Fisher's Protected Least Significant Difference (LSD) at the $\alpha = 0.05$ level. Contrast statements were included for β-glucosidase activity and total FAME concentration in 2012, to allow for specific treatment group comparisons. For microbial community analysis, FAME data were converted from nmol g^{-1} soil to relative percent basis. Data were then analyzed by principal components analysis (PCA) and multi-response blocked permutation (MRBP) tests with the PC-ORD statistical package (MjM software, Gleneden Beach, OR, 1999). The MRBP test generates a *P*-value and two additional test statistics (A and T). Values of A vary between 0 and 1 and describe the within-group variability. Values between 0 and 0.03 indicate a high level of heterogeneity within a group, whereas 1 means all members of the group are identical. Values of T describe the degree of difference between groups; T becomes more negative as the difference in community structure between groups becomes greater (McCune and Grace, 2002). Pearson's correlation coefficients were calculated using PROC CORR in SAS to identify FAME and enzyme variables significantly correlate with community distributions along PC's 1 of 2 of the PCA.

3. Results

3.1. Soil chemical properties

Treatment effects on soil chemical properties in June 2009 are shown in Table 2. Manure and biochar + manure treatments increased total N 1.2- and 1.4-fold, respectively, compared to the control, while adding biochar alone did not change total soil N. The biochar + manure treatment contained the greatest quantity of organic C (1.86%) as compared to all other treatments. When applied individually, biochar or manure increased organic C 1.6-fold, or 1.5-fold, respectively, over control. Relative to the control, biochar + manure increased extractable P 6-fold, while manure alone produced a 4-fold increase. Manure and biochar + manure treatments more than doubled soil NO_3-N concentrations. Adding biochar alone had no influence on soil extractable P, NO_3-N, or NH_4-N as compared to the control. In 2012, nearly four years after treatment applications, soil chemical properties were unaffected by biochar, manure, or biochar + manure (Table 2).

Table 2
Mean (±1 SE) soil (0–30 cm depth) total N, organic C, pH, ammonium bicarbonate diethylenetriaminepentaacetic acid (AB-DTPA) extractable P, NH_4-N, and NO_3-N under corn in June 2009 and August 2012, after a November 2008 application of 22 Mg ha^{-1} biochar, 42 Mg ha^{-1} manure, or biochar plus manure to experimental research plots (n = 3).

Treatment	Total N	Organic C	pH	AB-DTPA Ext.P	NH_4-N	NO_3-N
	%	%	mg kg^{-1}			
June 2009						
Biochar	0.09b[†]	1.21b	7.59	0.37b	1.38	16.2b
	(0.001)	(0.05)	(0.09)	(0.07)	(0.05)	(2.18)
Manure	0.11a	1.14bc	7.49	1.67a	2.48	48.1a
	(0.009)	(0.13)	(0.03)	(0.67)	(0.74)	(10.9)
Biochar + manure	0.13a	1.86a	7.60	2.37a	2.47	49.9a
	(0.015)	(0.25)	(0.08)	(0.73)	(0.27)	(3.36)
Control	0.09b	0.77c	7.60	0.40b	1.37	16.3b
	(0.003)	(0.06)	(0.12)	(0.23)	(0.08)	(0.55)
LSD	0.02	0.38	ns[‡]	1.19	ns	17.6
Pr > F	0.0078	0.0023	0.65	0.015	0.11	0.0043
August 2012						
Biochar	0.12	0.77	7.73	8.33	1.40	5.56
	(0.003)	(0.03)	(0.03)	(1.20)	(0.20)	(0.33)
Manure	0.13	0.81	7.77	9.67	1.70	4.50
	(0.003)	(0.04)	(0.03)	(1.77)	(0.15)	(0.25)
Biochar + manure	0.12	0.81	7.77	10.7	2.10	4.50
	(0.003)	(0.03)	(0.03)	(2.33)	(0.06)	(0.50)
Control	0.13	0.78(0.01)	7.80	8.50	1.40	3.40
	(0.001)		(0.06)	(0.76)	(0.35)	(0.84)
LSD	ns	ns	ns	ns	ns	ns
Pr > F	0.93	0.13	0.65	0.38	0.23	0.19

[†] Within columns by year, means followed by different letters are significantly different at $\alpha = 0.05$.
[‡] ns = not significant.

3.2. Soil enzyme activities

None of the soil enzyme potential activities were significantly affected by any of the soil amendments in 2009 or 2012, perhaps because of the large variability among treatments (Table 3). Potential activities of β-D-cellobiosidase and β-xylosidase tended to be highest in plots receiving biochar + manure in 2009, but the effect was not statistically significant ($P = 0.12$). In 2012, there was a trend for greater β-glucosidase activity in biochar-amended soil (either with or without manure; $P = 0.13$; Table 3). A contrast test was conducted, but there was no significant difference in β-glucosidase activity between biochar/biochar + manure amendments versus manure/control amendments ($P = 0.75$).

3.3. Microbial community biomass and structure

Microbial biomass in 2009, as estimated by total concentration of FAMEs, was significantly affected by the amendment applied (Fig. 1A). Total FAME concentration was greater in manure (2.3-fold) and biochar + manure treatments (2.6-fold) as compared to the control. Adding biochar alone, however, did not increase microbial biomass. In 2012, no significant difference in total FAME biomass was detected among the treatments (Fig. 1A). A contrast test was conducted with 2012 total FAME biomass data to determine if FAME biomass in biochar-amended soil was significantly different from all other treatments, and it was not ($P = 0.42$).

Populations of microorganisms were differentially affected by treatments in 2009. Following the trend of total microbial biomass, the concentration of FAMEs for Gram-negative bacteria and fungi were elevated in plots receiving manure or biochar + manure in 2009 (Table 4). Fungal FAME concentrations were ~3-fold greater in manure treated plots compared to control plots or plots receiving biochar alone. Overall, the manure treatments increased the ratio of fungi:bacteria, as indicated by the ratio of fungal:bacterial FAMEs (Table 4). When FAMEs were normalized by total concentration and expressed on a relative percent basis, the percent fungal FAME was 7–10% greater, and the percent bacterial FAMEs were 2–3% lower, in manure treated plots than in control or biochar-alone treated plots (Table 4). However, these differences disappeared by 2012, when no significant treatment effects were detected (Table 4).

The relative percent of FAME biomarker for AM fungi (16:1ω5c) was significantly affected by treatments in 2009, with decreased percentages (~1%) of 16:1ω5c in manure and biochar + manure treatments than in soil receiving biochar alone or no amendment (Table 4). In 2012, all soil communities contained similar amount of 16:1ω5c, and there were no significant effects of the soil amendments.

Table 3
Mean (±1 SE) potential soil (0–30 cm depth) enzyme activities under corn in June 2009 and August 2012, after a November 2008 application of 22 Mg ha^{-1} biochar, 42 Mg ha^{-1} manure, or biochar plus manure to experimental research plots (n = 3).

Treatment	β-glucosidase	β-D-cellobiosidase	β-xylosidase	N-acetyl-β-glucosaminidase	Phosphatase	Leucine aminopetidase
	nmol product g^{-1} dry soil h^{-1}					
June 2009						
Biochar	44.0 (11.5)	14.7 (3.9)	7.7 (1.3)	10.8 (1.1)	64.1 (7.0)	202 (13)
Manure	52.9 (3.2)	10.8 (4.0)	9.3 (2.6)	6.3 (2.0)	79.5 (11.2)	199 (44)
Biochar + manure	54.8 (2.8)	21.3 (2.7)	20.2 (6.2)	11.4 (3.5)	72.0 (9.2)	176 (44)
Control	43.8 (10.2)	17.7 (4.2)	12.6 (2.8)	11.6 (1.0)	64.0 (6.7)	180 (17)
LSD	ns[†]	ns	ns	ns	ns	ns
Pr > F	0.64	0.12	0.12	0.13	0.65	0.87
August 2012						
Biochar	112 (13)	51.0 (8.6)	48.6 (13.3)	17.4 (2.0)	174 (10)	223 (21)
Manure	72.3 (23.5)	44.1 (18.3)	51.5 (26.9)	14.2 (1.9)	164 (15)	222 (17)
Biochar + manure	117 (8)	48.5 (6.1)	34.3 (14.5)	19.9 (6.5)	163 (2)	254 (2)
Control	67.5 (21.6)	21.3 (14.3)	11.9 (11.4)	10.1 (3.2)	152 (2)	214 (28)
LSD	ns	ns	ns	ns	ns	ns
Pr > F	0.13	0.37	0.48	0.47	0.52	0.58

[†] ns = not significant.

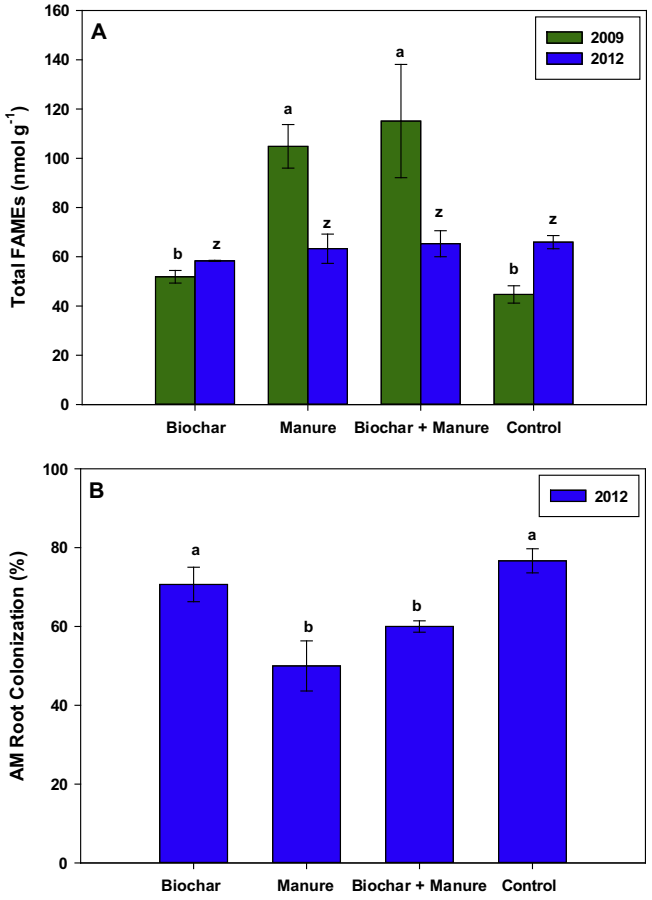

Fig. 1. Concentrations of soil (0–30 cm depth) (A) total FAMEs in 2009 and 2012, and (B) percent AM fungi corn root colonization in August 2012 after a November 2008 application of biochar (22.4 Mg ha^{-1}), manure (42 Mg ha^{-1}), or biochar plus manure to experimental research plots (n = 3). Histogram bars labeled by the same letter, within individual year, are not significantly different (α = 0.05). Error bars represent one standard error of the mean.

In 2012, the percentage of AM fungal colonization in corn roots was analyzed. Data were expressed by summing occurrences of hyphae, arbuscules and vesicles. Manure application decreased mycorrhizal colonization 27% relative to roots from control plots. Root colonization was also lower, at 17%, in biochar + manure application. Biochar did not impact root colonization, with levels that were similar to control (Fig. 1B).

In 2009, PCA revealed shifts in the FAME structure of soil microbial communities in response to soil amendments (Fig. 2A). Communities separated along Principle Component 1 (PC1) according to whether they had received manure (either alone or in combination with biochar) or not. Communities from biochar and control soils grouped along the negative regions of both PC1 and PC2, and clearly separated from manure and biochar + manure plots. According to MRBP tests for a blocked design, marginally significant differences between treatments were found for manure versus biochar (P = 0.062), manure versus control (P = 0.064), and biochar + manure versus biochar (P = 0.073). The distribution of microbial community FAME profiles along PC 1 was significantly correlated with biomarker FAMEs (Table 5). Specifically, the ratio of fungal-to-bacterial FAMEs and the relative percent fungal FAME biomarker were positively and significantly correlated with PC 1 (r = 0.91 and r = 0.94, respectively), meaning that communities to the right of PC 1 (from manure amended plots) were associated with greater concentrations of the fungal FAME biomarker and a higher fungal:bacterial FAME ratio. In contrast, the relative percent of bacteria and AM fungal biomarker 16:1ω5c were negatively correlated with PC 1 (r = −0.78 and r = −0.73, respectively), indicating greater amounts of AM fungi and Gram-negative bacteria in control and biochar-alone treated plots.

In 2012, differences in soil microbial community structures due to treatments were not as evident as was observed in 2009 (Fig. 2B). Furthermore, MRBP analysis showed no significant differences among treatments (P = 0.77). The distribution of microbial community FAME profiles along PC 1 and PC 2 remained correlated to the same FAME variables as in 2009, however, with positive correlations for fungal:bacterial FAME ratio and percent fungal FAME along PC 1, a negative correlation for percent bacterial FAMEs along PC 1, and a positive correlation for percent AM fungal FAME along PC 2 (Table 5).

4. Discussion

The purpose of this study was to determine the short- (~one year) and medium-term (~four years) effects of a biochar amendment on soil microbial communities and enzyme activities, in comparison to a relatively common organic soil amendment (manure). We found that biochar had no significant effects on soil microbial properties and subsequently on nutrient availability. Rather, microbial community biomass and structure were largely affected by manure in the short-term. Both biochar and manure increased soil organic C levels to similar amounts, and even more so when

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Table 4
Mean concentration, ratio or relative percent (±1 SE) of soil biomarker FAMES under corn in June 2009 and August 2012, after a November 2008 application of 22 Mg ha^{-1} biochar, 42 Mg ha^{-1} manure, or biochar plus manure to experimental research plots (n = 3).

Treatment	Gram+ bacteria	Gram− bacteria	Fungi	AM fungi	Fungi: bacteria	Bacteria	Fungi	AM fungi
	nmol g dry soil^{-1}					%		
June 2009								
Biochar	9.3 (0.6)	6.0bc[‡] (0.5)	12.2b (0.9)	1.73 (0.20)	0.80b (0.04)	29.5a (0.6)	23.5b (0.9)	3.31ab (0.26)
Manure	17.0 (1.1)	10.8ab (2.1)	32.1a (2.8)	2.63 (0.14)	1.10a (0.01)	27.8ab (0.2)	30.7a (0.4)	2.52c (0.12)
Biochar + manure	17.5 (3.8)	11.6a (2.5)	37.2a (7.0)	3.35 (1.73)	1.23a (0.06)	26.6b (0.7)	32.5a (0.7)	2.82bc (0.28)
Control	8.6 (0.7)	4.6c (0.4)	9.9b (0.6)	1.70 (0.03)	0.76b (0.05)	29.5a (0.3)	22.2b (1.3)	3.85a (0.23)
LSD	ns[†]	5.5	14.6	ns	0.17	1.7	3.3	0.70
Pr > F	0.06	0.04	0.008	0.13	0.001	0.01	0.0005	0.01
August 2012								
Biochar	8.7 (0.5)	5.08 (0.12)	13.5 (1.3)	3.77 (0.27)	1.14 (0.16)	20.6 (0.8)	23.2 (2.4)	6.46 (0.44)
Manure	9.7 (1.0)	5.76 (1.00)	13.9 (1.9)	4.36 (0.53)	1.02 (0.02)	21.3 (0.7)	21.7 (1.0)	7.14 (1.35)
Biochar + manure	10.1 (1.2)	5.92 (0.97)	13.7 (1.3)	3.97 (0.37)	0.99 (0.12)	21.6 (1.6)	21.0 (1.2)	6.01 (0.17)
Control	10.0 (0.3)	6.01 (0.35)	14.1 (1.4)	4.18 (0.48)	1.02 (0.14)	21.3 (1.6)	21.2 (1.3)	6.40 (0.92)
LSD	ns	ns	ns	ns	ns	ns	ns	ns
Pr > F	0.60	0.77	0.99	0.80	0.87	0.96	0.81	0.82

[†] ns = not significant.
[‡] Within columns by year, means followed by different letters are significantly different at $\alpha = 0.05$.

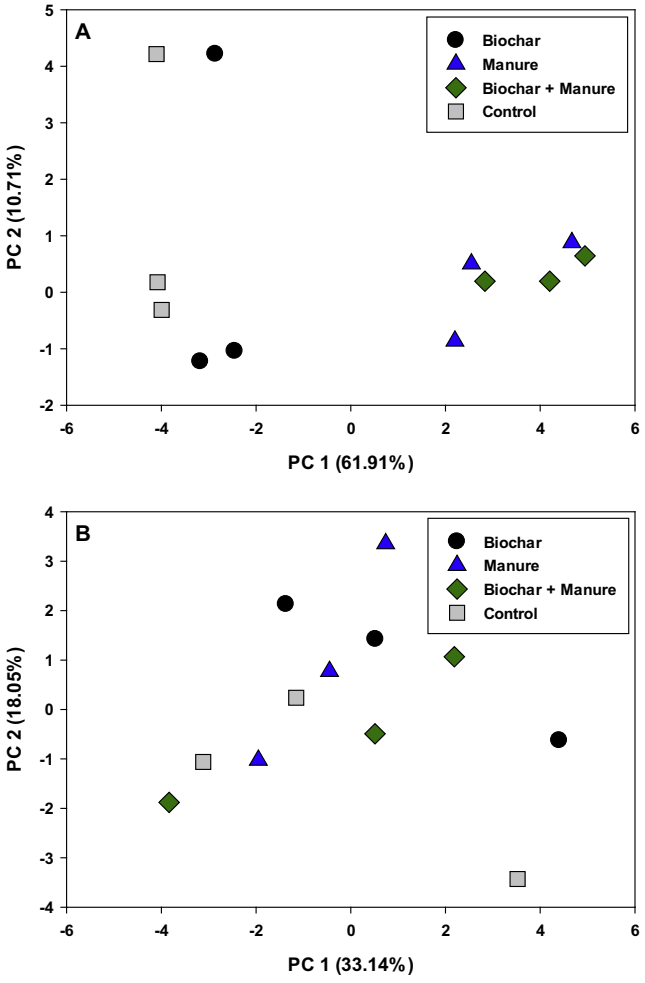

Fig. 2. Principle components analysis (PCA) of soil (0–30 cm depth) microbial community fatty acid methyl esters (FAMEs) under corn in (A) June 2009 and (B) August 2012 after a November 2008 application of biochar (22.4 Mg ha^{-1}), manure (42 Mg ha^{-1}), or biochar plus manure to experimental research plots ($n = 3$).

applied together. Increases in organic C were likely the result of biochar and manure C input since those compounds contain relatively high amounts of organic C. Similar observations were found by Rogovska et al. (2011), Bolan et al. (2012), and Yang et al. (2013). However, the lack of microbial biomass response to biochar indicated that either little of the biochar C was available for microbial degradation or that relatively labile biochar C sources were degraded prior to the 2009 sampling. In addition, biochar did not enhance total N, NH_4-N, NO_3-N, or available P in soil, indicating that the biochar did not contain appreciable quantities of these nutrients (Table 1), or the rate at which it was applied, was not as effective at improving microbial nutrient cycling processes and soil nutrient availability as was manure (Lentz and Ippolito, 2012).

Other researchers have suggested that biochar benefits microbial communities by enhancing the physical and chemical soil characteristics (Lehmann and Joseph 2009; Atkinson et al., 2010; Jindo et al., 2012), providing suitable habitats for microorganisms that protect them from predation (Pietikäinen et al., 2000), supplying labile C substrates for degradation (Thies and Rillig, 2009; Smith et al., 2010), enhancing the availability of macro-nutrients such as N and P (Atkinson et al., 2010; Lammirato et al., 2011), or sorbing compounds that would otherwise inhibit microbial growth (Kasozi et al.,2010). To date, these mechanisms have been poorly studied and are mainly discussed in terms as possible explanations. The results of the current study showed that biochar had no effect on microbial communities compared to control soils. Likewise, Domene et al. (2014) found no effects of biochar on microbial biomass and respiration activity in corn field plots three years after biochar application at rates less than 30 Mg ha^{-1}. In Domene et al.'s (2014) study, microbial biomass did not increase until biochar was added at the highest rate of 30 Mg ha^{-1}. Domene et al. (2014) also observed high variability among

Table 5
Pearson's correlation coefficients (and significance values) between soil FAME variables with community positions along PC 1 in 2009 (Fig. 2A), or with community positions along PC 1 or 2 in 2012 (Fig. 2B) ($n = 12$).

Variable	2009	2012	
	PC 1	PC 1	PC 2
Fungi:bacteria	0.91 ($P < 0.0001$)	0.92 ($P < 0.0001$)	−0.17 ns
% Bacteria	−0.78 ($P = 0.003$)	−0.94 ($P < 0.0001$)	−0.05 ns
% Fungi	0.94 ($P < 0.0001$)	0.74 ($P = 0.006$)	−0.22 ns
% AMF	−0.73 ($P = 0.007$)	−0.23 ns[†]	0.71 ($P = 0.01$)

[†] ns = not significant.

replicate field plots, as in our study, which may have prevented significant biochar effects from being detected. An inconsistent effect of biochar on microbial communities suggests that biochar effects are likely biochar- and soil-specific, related to the rate applied to soil, and/or require greater statistical replication. For example, others have found no effect of biochar on microbial communities when the biochar does not affect the pH of an already neutral or alkaline soil (Meynet et al., 2012), or when biochar does not provide enough labile C substrates (high pyrolysis temperature) or nitrogen (hardwood biochar) to stimulate microorganisms (Bruun et al., 2011; Luo et al., 2011). Biochar in the current study did not affect soil pH (Lentz and Ippolito, 2012) and did not contain appreciable quantities of N (Table 1).

Biochar, with its capacity to sorb a wide range of organic and inorganic molecules, has been shown to inhibit some soil enzymes or their substrates via sorption or by blocking reaction sites (Bailey et al., 2010; Jin, 2010; Lehmann et al., 2011). This did not occur in the present study, as biochar had neutral effects on potential enzymatic activity. We cannot exclude the possibility that any effects from frozen storage may have masked biochar effects on enzyme activities in 2009 soils, however. Some have reported that freezing for longer than one month can cause a decline in enzyme activities (Turner and Romero, 2010), and we did observe lower enzyme activities in 2009 soils than in 2012 soils.

Biochar had neutral effects on soil AM fungal biomass and corn root colonization. Greater differences in microbial biomass, AM fungal biomass and root colonization were observed with the manure treatment. In the short-term, manure amendment increased microbial FAME biomass, and particularly that of the Gram-negative bacterial and fungal components of the microbial community. These positive responses in 2009 were likely due to the recent additions of labile manure C, including incompletely digested plant residue components. Manure amendment would also have added nutrients to soil, including P, which would explain the negative impacts of manure on relative percent AM fungal biomass in 2009 and percent root colonization in 2012. When P and other nutrients are abundant (such as when following manure addition), plants rely less on AM fungi to supply nutrients and root colonization and AM fungal biomass in soil is reduced (Corbin et al., 2003; Covacevich et al., 2006; Gryndler et al., 2006).

In conclusion, this study demonstrated that a 22 Mg ha^{-1} addition of a hardwood-derived, fast-pyrolysis biochar to an Aridisol did not affect microbial community biomass, structure, soil enzyme activities, soil AM fungal biomass, or AM fungal colonization of corn roots. A lack of change in microbial characteristics do, however, align well with the lack of effects on soil nutrient availability measured in this study, and with the field trial findings of Lentz and Ippolito (2012), where little effect on soil chemical characteristics were observed due to biochar application. Biochar land application may be an effective means to sequester C, but at rates practical for field studies (such as the rate employed in this study), biochar may not cause significant shifts in the microbial community status and thus may not cause a change in nutrient cycling activities and nutrient availability. However, if growers wish to apply a carbon-based soil amendment to enhance microbial growth and activity, manure rather than biochar would likely be more effective in the short-term.

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