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Hardwood Biochar Influences Calcareous Soil Physicochemical and Microbiological Status

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The effects of biochar application to calcareous soils are not well documented. In a laboratory incubation study, a hardwood-based, fast pyrolysis biochar was applied (0, 1, 2, and 10% by weight) to a calcareous soil. Changes in soil chemistry, water content, microbial respiration, and microbial community structure were monitored over a 12-mo period. Increasing the biochar application rate increased the water-holding capacity of the soil–biochar blend, a trait that could be beneficial under water-limited situations. Biochar application also caused an increase in plant-available Fe and Mn, soil C content, soil respiration rates, and bacterial populations and a decrease in soil NO_3–N concentration. Biochar rates of 2 and 10% altered the relative proportions of bacterial and fungal fatty acids and shifted the microbial community toward greater relative amounts of bacteria and fewer fungi. The ratio of fatty acid 19:0 cy to its precursor, 18:1ω7c, was higher in the 10% biochar rate soil than in all other soils, potentially indicating an environmental stress response. The 10% application rate of this particular biochar was extreme, causing the greatest change in microbial community structure, a physiological response to stress in Gram-negative bacteria, and a drastic reduction in soil NO_3–N (85–97% reduction compared with the control), all of which were sustained over time.

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Biochar, a product of pyrolysis, is generally considered biomass-derived char intended specifically for soil application (Sohi et al., 2010). The practice of using charred biomass as a soil amendment was documented as early as 100 to 150 yr ago (Allen, 1847; Lefroy, 1883; Hall, 1910). More recently, research has focused a great deal of attention on the use of biochar in highly weathered systems (e.g., Lehmann et al., 2003; Glaser et al., 2004; Novak et al., 2009; Hass et al., 2012; Major et al., 2012; Schomberg et al., 2012). However, biochar use for improving soils in less weathered temperate and arid systems is a relatively new concept. Soils under arid and semiarid climates are used extensively for grazing and for dryland and irrigated agriculture and encompass approximately 1 billion ha globally (Agrostats, 2009). Thus, biochar use in these settings may dramatically affect system dynamics, especially regarding soil nutrient relations, microbial activity, and water retention.

Nutrient responses have been observed for various biochar–arid soil combinations. When biochar was mixed into an Australian Aridisol at 10 Mg biochar ha^{-1}, no change in extractable soil nutrients was observed (Van Zwieten et al., 2010). However, when a Mollisol was amended with an equivalent of 12 Mg ha^{-1} biochar, soil-extractable P, as well as K and Fe, increased as compared with unamended soil (Brewer et al., 2012). Laird et al. (2010a) amended a Mollisol with an equivalent of up to 20 Mg biochar ha^{-1}, noting an increase in soil-extractable P, K, Mg, and Ca. Ippolito et al. (2012a) added approximately 40 Mg biochar ha^{-1} to two Aridisols and observed a decrease in P leaching, suggesting that P retention was a function of surface functional groups, the presence of Fe and Al oxides, and precipitation with Ca and Mg.

Biochar application to temperate and aridic soils can also affect the soil NO_3–N status. In a column study, Laird et al. (2010b) incubated a Mollisol containing up to 20 Mg biochar ha^{-1}. After 45 wk of weekly leaching, the 20 Mg ha^{-1} biochar treatment lost 26% more NO_3–N than control columns. The authors attributed the increased NO_3–N loss to enhanced organic N mineralization stimulated by the high biochar application rate (Laird et al., 2010b). In contrast, Streubel et al. (2011) showed a decrease in N mineralization in several soils amended

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Abbreviations: EC, electrical conductivity; FAME, fatty acid methyl ester; MRPP, multiresponse permutation procedure.

with various types of biochars (up to 39 Mg ha^{-1}). Kameyama et al. (2012) studied NO_3–N retention by calcareous Japanese soils amended with biochar. The authors showed that biochar NO_3–N sorption was related to base functional groups present and that increased retention of NO_3–N in biochar micropores decreased NO_3 leaching. Ippolito et al. (2012b) studied biochar application to two Aridisols, showing that NO_3–N leaching decreased with biochar addition to both soils at a rate equivalent to approximately 40 Mg ha^{-1}. Biochar-borne C likely stimulated microbial growth and thus increased N immobilization (Ippolito et al., 2012b).

The effects of biochar on soil microorganisms have received much less attention than changes in soil chemical properties (but see Lehmann et al. [2011] and Pietikäinen et al. [2000]). Nowhere is this more evident than in less weathered temperate and arid systems. Sarkhot et al. (2012) amended an Alfisol with ~22 Mg ha^{-1} biochar and observed a 67% reduction in cumulative CO_2 flux as compared with control soils. Kuzyakov et al. (2009) incubated ~54 Mg biochar ha^{-1} in an Alfisol for 3.2 yr and noted that total CO_2 efflux did not change markedly. In contrast, Smith et al. (2010) applied up to 44.8 Mg ha^{-1} biochar to a Mollisol and an Aridisol and determined soil respiration response over 49 d. Both soils showed an initial (within the first week) increase in CO_2 production with increasing biochar rates, and the authors confirmed that biochar was the C source contributing to the flux. Streubel et al. (2011) added up to 39 Mg ha^{-1} of various biochars to three Mollisols and measured C mineralization over a 30-wk period. Similar to the findings of Smith et al. (2010), the authors observed greater C mineralization during the initial study stages. This was partly explained by the biochars containing labile C. However, the authors noted that biochar application rate had no significant effect on the total C mineralized. Rogovska et al. (2011) added an equivalent of up to 20 Mg ha^{-1} biochar to a Mollisol and measured cumulative CO_2 release over 268 d. Increasing biochar rate increased CO_2 emissions at each date measured. The authors attributed this to a decrease in soil bulk density that enhanced gas exchange or to biochar's inner porosity adsorbing organic compounds, enhancing microorganism habitat and thus accelerating decomposition. Luo et al. (2011) added two biochars to an Alfisol at an equivalent rate of 50 Mg ha^{-1} and measured cumulative CO_2 evolution over 180 d. Both biochars increased CO_2 evolved, yet evolution was maximal within the first 3 d. In contrast to the findings of Smith et al. (2010), the authors demonstrated that some biochars caused a priming effect on native soil organic C in the first several days of the experiment.

The addition of C to soils in the form of biochar may also be important for pedological processes such as soil structure development, leading to increasing soil water storage (Ippolito et al., 2012b). However, changes in the soil water status of less weathered systems have not been fully documented. Novak et al. (2012) showed that adding approximately 45 Mg ha^{-1} of biochar to two Aridisols increased the soil water content between 3 and 7% as compared with control soils. Chan et al. (2007) showed that adding 50 or 100 Mg ha^{-1} of biochar to an Alfisol caused a significant increase in water held at field capacity. Streubel et al. (2011) calculated water-holding capacity for five different western U.S. soils amended with four different biochars applied at 0, 9.8, 19.5, and 39 Mg ha^{-1}. Biochar application led to increases in half of the biochar–soil treatments tested, whereas the other half showed no change in water-holding capacity.

The above research focused mainly on short-term responses to biochar land application in less weathered systems. Longer-term research focusing on nutrient dynamics is needed to quantify the legacy of biochar applications and to assess their true value in aridic conditions (Spokas et al., 2012). Thus, Lentz and Ippolito (2012) began a long-term study in 2008 to identify the effect of fast pyrolysis hardwood biochar application (22.4 Mg ha^{-1}) to corn silage (*Zea mays* L.) grown in a southern Idaho Aridisol. Differences in yield were not evident within 1 yr after application; however, a 36% yield decrease occurred in year 2. Yield suppression was due to reduced N or S availability or uptake, potentially caused by biochar aging (Lentz and Ippolito, 2012). Aging may be necessary for bacteria to inhabit biochar pores, and once occupied the biochar pores limit bacterial biomass nutrient availability (Lehmann et al., 2011). Unfortunately, microbial-mediated nutrient alterations and changes in soil microbial community structure were not documented; this is a research gap that needs to be addressed (Ippolito et al., 2012a). Thus, the objective of the current research was to use laboratory incubation studies to support the in-field soil chemistry findings of Lentz and Ippolito (2012) and to understand changes in the soil microbial respiration, bacterial populations, and overall community structure as affected by biochar application.

Materials and Methods

Biochar Characteristics

A hardwood biochar supplied by Dynamotive Energy Systems Inc. was manufactured from oak (*Quercus* spp.) and hickory (*Carya* spp.) hardwood sawdust using fast (i.e., flash) pyrolysis at 500°C in a fluidized-bed kiln with a 5-s residence time. The biochar ash content was determined by Hazen Laboratory (Hazen Research, Inc.) using a modified ASTM method (D1762–84) for wood charcoal (600°C). Biochar total C and N were determined by dry combustion (Nelson and Sommers, 1996) using a CN analyzer (Thermo-Finnigan FlashEA1112; CE Elantech Inc.). Biochar pH and electrical conductivity (EC) were determined on a saturated paste extract (Thomas, 1996; Rhoades, 1996). Biochar NO_3–N and NH_4–N content were determined using a 2 mol L^{-1} KCl extract (Mulvaney, 1996), and organic N content was determined as the difference between total and inorganic N. Biochar total metal concentrations were determined by using an $HClO_4$–HNO_3–HF-HCl digestion (Soltanpour et al., 1996) followed by elemental analysis using inductively coupled plasma–atomic emission spectrometry.

Soil Characteristics

The top 30 cm of soil was obtained from the edge of a field site 1.7 km southwest of Kimberly, Idaho (42°31′ N, 114°22′ W; mean elevation of 1190 m; annual precipitation of 251 mm). The soil was classified as Portneuf (coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcid) and is extensive in southern Idaho, occupying approximately 117,000 ha (USDA-NRCS, 2013). The soil was air dried, passed through a 2-mm sieve, and analyzed for pH (Thomas, 1996) and EC (Rhoades, 1996) using a 1:1 soil:deionized water extract, total elements, and NH_4–N and NO_3–N as previously described. The soil was then

pulverized and analyzed for inorganic C analysis using a modified pressure-calcimeter method (Sherrod et al., 2002) and for total C and N as mentioned above. Soil organic C was determined as the difference between total and inorganic C. Biochar and soil chemical characteristic data are presented in Table 1.

Soil-Biochar Incubation

The effect of hardwood biochar on the Portneuf soil was investigated during a 12-mo incubation study. Biochar was thoroughly mixed into soil at 0, 1, 2, and 10% (~0, 20, 40, and 200 Mg ha^{-1}; dry wt./wt.). The 10% biochar application rate was used to identify potential upper level benefits or detriments to the soil. Soil and biochar mixtures (300 g total) were placed in 8 cm × 8 cm × 8 cm plastic pots lined with a plastic liner to prevent leaching. The pots were placed in a growth chamber (22°C and 30% humidity) and watered twice weekly with reverse osmosis water to 80% of field capacity to account for water loss due to evaporation. Pots were destructively sampled at 1, 2, 3, 4, 6, and 12 mo with four replicates per treatment used for each time step.

On the sampling day, a soil subsample was obtained and stored in a −80°C freezer for microbial analysis. All soils were analyzed for NO_3–N and NH_4–N as previously described and for available Fe, Zn, Mn, and Cu using diethylenetriaminepentaacetic acid extraction (Lindsay and Norvell, 1978). Substrate-induced soil respiration rates, based on CO_2 production, were determined by a method similar to that described by Dungan et al. (2003). Briefly, 50 g of moist soil, 0.5 g of glucose, 0.01 g of K_2HPO_4, and 0.075 g of NH_4Cl were thoroughly mixed and placed in a 100-mL air-tight mason jar. A vial containing 5 mL of 1 mol L^{-1} NaOH was placed inside each jar, and the jars were sealed. After 24 h the vials were removed, excess $BaCl_2$ was added to the NaOH, phenolphthalein indicator was added, and the NaOH was titrated to a clear endpoint with 1 mol L^{-1} HCl. Duplicate measurements were made on all soils. Soil water content on the day of sampling was determined to convert the above soils data to a dry weight basis. Soils were then air dried, passed through a 2-mm sieve, and analyzed for pH and EC, and a subsample was pulverized and analyzed for organic C as previously described. Soils from months 1, 6, and 12 were analyzed for gravimetric water content by using a pressure plate extractor at matric potentials of 0, −10, −33, −100, and −300 kPa (Dane and Hopmans, 2002; Reynolds and Topp, 2008). Analysis was performed in a constant temperature room to minimize temperature effects on soil water composition (Bachmann et al., 2002).

Table 1. Chemical properties and total elemental analysis of the hardwood biochar and Portneuf soil.

Property	Units	Biochar	Portneuf soil
pH		6.8	8.2
EC	dS m^{-1}	0.7	0.3
Ash	%	14	ND†
Total C	%	66.2	3.53
Inorganic C	%	ND	2.33
Organic C	%	ND	1.20
Total N	%	0.32	0.08
Organic N	%	0.32	0.08
NO_3–N	mg kg^{-1}	1.5	18.1
NH_4–N	mg kg^{-1}	1.2	0.57
K	mg kg^{-1}	3,400	2,590
Ca	mg kg^{-1}	3,700	74,500
Mg	mg kg^{-1}	1,500	13,100
Na	mg kg^{-1}	200	280
P	mg kg^{-1}	300	330
Al	mg kg^{-1}	300	720
Fe	mg kg^{-1}	1400	700
Zn	mg kg^{-1}	14.1	27.7
Mn	mg kg^{-1}	118	218
Cu	mg kg^{-1}	16.8	4.83
Ni	mg kg^{-1}	4.9	6.6
Mo	mg kg^{-1}	<0.05	<0.01
Cd	mg kg^{-1}	<0.05	0.12
Pb	mg kg^{-1}	2.0	6.4
B	mg kg^{-1}	12.3	14.7

† Not determined.

Quantitative Polymerase Chain Reaction Analysis

Bacterial community DNA was extracted from 0.5 g (dry weight) of previously frozen soil using a UltraClean Soil DNA Isolation Kit (MO BIO laboratories, Inc.) as recommended by the manufacturer. Afterward, 5 μL of DNA was used in a 30-μL quantitative PCR mixture to estimate the concentration of 16S gene copy numbers in each sample as described by Nadkarni et al. (2002). The quantitative PCR was performed with a Bio-Rad multicolor iQ5 real-time PCR detection system. Purified DNA from *Escherichia coli* (ATCC 11775) was used as a standard to calculate gene copy numbers.

Fatty Acid Methyl Ester Analysis

Frozen soils from incubation periods of 2, 4, 6, and 12 mo were analyzed for microbial community structure according to fatty acid methyl ester (FAME) profiles. Before analysis, replicate 4 from each biochar rate within the 2-mo incubation treatment had been used for other analyses; therefore, only three replicate soils were analyzed from the 2-mo incubation period, whereas four replicates were analyzed for the 6- and 12-mo incubation periods. Microbial FAMEs were extracted following the ester-linked FAME procedure described by Schutter and Dick (2000), where 3 g soil were extracted with 0.2 mol L^{-1} KOH during a 37°C, hour-long incubation with periodic mixing followed by the addition of 1.0 mol L^{-1} acetic acid to neutralize the pH of the tube contents. Soil FAMEs were partitioned into an organic phase by the addition of hexane, which was removed from the aqueous phase after centrifugation at 480 *g* for 10 min. After the addition of an internal standard (20 μg of 19:0), samples were analyzed by gas chromatography analysis (Agilent 6890 gas chromatograph, Agilent Technologies, Inc.) by the University of Delaware. The capillary column was an Ultra 2 Agilent #1909 1B-102 crosslinked 5% phenyl methyl silicone that was 25 m long with an internal diameter of 0.2 mm and a film thickness of 0.33 μm. Flame ionization detection was achieved at 250°C using a carrier gas of hydrogen at a flow rate of 0.8 mL min^{-1}. Samples were run using the Microbial ID Eukary method and peak naming table. Biomarkers of specific functional groups were assigned according to Frostegård and Bååth (1996) and Schutter and Dick (2000). Bacterial biomarkers were the sum of i15:0, a15:0, 15:0, i160, 16:1ω9c, 16:1ω7c, i17:0, a17:0, 17:0 cy, 17:0, and 19:0 cy. The FAMEs 18:2ω6c and 18:1ω9c were used as the indicators of saprophytic fungi.

Statistical Analyses

Statistical analysis was performed on all soil data (except soil water and FAME analysis) using a split with time design in the Proc GLM model (SAS software version 9.2; SAS Institute, 2008) at $\alpha = 0.05$. Soil water statistical analysis was performed within individual months 1, 6, or 12 (i.e., not split by time). A Fisher's protected LSD (Steel and Torrie, 1980) was calculated when significant differences were observed within treatments or between time intervals. Multivariate analyses of microbial community FAMEs were conducted with the PC-ORD statistical package (version 6, MjM Software). Concentrations of FAMEs were converted to relative mol% and screened for outliers. One sample from the 4-mo incubation set (replicate 3 of the 1% biochar rate treatment) was identified as an outlier and removed from subsequent analyses. Principal components analysis was applied to analyze community FAME profiles in two-dimensional space. Multiresponse permutation procedure (MRPP) tests were conducted in PC-ORD to determine if predefined groups of microbial communities (based on biochar rate, incubation time, or biochar rate within an incubation time) were significantly different from each other ($\alpha = 0.05$). The MRPP 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 a value of 1 means that 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).

Results and Discussion

The effect of biochar application rate was, in most instances, dependent on time. Biochar typically caused an increase in the soil constituent of concern, and the effect decreased over time; the opposite was observed for soil NO_3–N. Thus, interaction effects are presented, but the discussion focuses on main effects only.

Soil Water

Increasing fast pyrolysis biochar application rate increased the water-holding capacity of the soil blends as determined from saturation to −300 kPa, and the response was consistent during months 1, 6, and 12 (Table 2). For example, at field capacity (−33 kPa) and after 12 mo of incubation, the respective 1, 2, and 10% biochar–soil blends contained 1, 4, and 19% greater gravimetric soil water as compared with the 0% biochar rate. This was similar to results reported by Novak et al. (2012), who applied 2% switchgrass biochar (by weight) to two Aridisols and noted a 3 to 7% increase in soil water content as compared with soils not receiving biochar even after 127 d. Streubel et al. (2011) also noted increases in water-holding capacity of several western U.S. silt loam soils after biochar addition as low as 0.4% by weight. The increase in soil water content in the current study was likely associated with the porosity of biochar (Bruun et al., 2012). The increase in soil water content via biochar application could be of value to arid region crop producers where rainfall quantities are low and reliance on irrigation is high (Novak et al., 2012).

Soil Chemical Characteristics

Increasing biochar application rate, averaged over time, caused a slight but significant increase in soil pH and a decrease in soil EC (Supplemental Fig. S1 and S2). The increase in pH is unexplainable because the initial soil and biochar pH values were 8.2 and 6.8, respectively. The decrease in EC may have been due to salt sorption by biochar because the capacity of charcoal to sorb salts has been long known (Bartell and Miller, 1923). Results by Thomas et al. (2013) further substantiate the potential for salt sorption by biochar. Change in pH over time, averaged over biochar rate, decreased soil pH by a maximum of 0.3 pH units (pH values for month 1 versus month 6 were 8.34 and 8.00, respectively). The soil buffering capacity prevented major changes in soil pH, even at the highest biochar application rate. The EC increased slightly over time, but by month 12 the EC values were statistically equal to the initial values. Lentz and Ippolito (2012) also showed no difference in EC between

Table 2. Biochar-amended (0, 1, 2, or 10% by wt) Portneuf soil mean (*n* = 4) percent gravimetric soil water content at 0, −10, −33, −100, and −300 kPa for soils incubated for 1, 6, or 12 months.

Month	Biochar application rate	Matric potential				
		0 kPa	−10 kPa	−33 kPa	−100 kPa	−300 kPa
		Gravimetric water content				
		%				
1	0	52.8 (2.1)†c‡	29.2 (0.4)c	21.4 (0.2)b	15.2 (0.1)b	11.5 (0.1)a
	1	54.5 (0.6)c	30.2 (0.2)bc	21.4 (0.1)b	15.1 (0.1)b	11.6 (0.1)a
	2	58.5 (0.5)b	30.4 (0.1)b	21.7 (0.1)b	15.4 (0.2)b	11.4 (0.0)a
	10	67.2 (0.4)a	36.0 (0.4)a	24.4 (0.2)a	16.9 (0.3)a	11.6 (0.1)a
6	0	60.2 (0.8)c	36.6 (0.4)c	28.6 (0.3)b	21.1 (0.2)b	17.5 (0.2)b
	1	61.7 (0.6)bc	38.0 (0.2)b	28.7 (0.2)b	21.2 (0.2)b	17.5 (0.3)b
	2	63.3 (0.4)b	38.5 (0.3)b	29.3 (0.2)b	21.6 (0.2)b	17.6 (0.2)ab
	10	74.0 (0.9)a	43.8 (0.6)a	32.2 (0.5)a	23.4 (0.3)a	18.3 (0.2)a
12	0	54.2 (0.6)b	37.0 (0.1)d	29.4 (0.3)c	21.3 (0.1)b	18.2 (0.8)b
	1	56.4 (0.8)b	38.6 (0.3)c	29.6 (0.2)bc	21.8 (0.2)b	17.9 (0.2)b
	2	58.4 (0.8)b	40.1 (0.2)b	30.6 (0.3)b	22.5 (0.3)ab	18.1 (0.2)b
	10	75.3 (2.9)a	52.2 (0.7)a	35.0 (0.4)a	26.0 (2.6)a	20.0 (0.3)a

† Values inside parentheses indicate 1 SEM.

‡ Within a column and a given month, values followed by the same letters are not significantly different at $\alpha = 0.05$ as determined using a Fisher's protected LSD.

a control and a 22.4 Mg biochar ha^{-1} under field conditions that used the same soil as in the current study.

The effects of biochar application and time since application on diethylenetriaminepentaacetic acid–extractable Fe, Zn, Mn, and Cu are shown in Fig. 1. Increasing biochar application rate caused an increase in available soil Fe and Mn but had no effect on soil Zn or Cu. In alkaline soils, Zn and Cu form strong associations with Fe and Al (hydr)oxides and thus may limit their availability. Iron and Mn observations were potentially due to these biochar elements being in readily available forms and their concentrations being an order of magnitude larger than biochar Zn or Cu concentrations. In addition, soil Fe and Mn are typically complexed by soil organic matter in neutral and alkaline soils. The addition of the fast pyrolysis biochar may have also supplied labile organic C or primed decomposition of natural soil organic matter, leading to an increase in Fe and Mn in the soil solution. Lentz and Ippolito (2012) observed an increase in Mn availability when biochar was applied to the same soil under field conditions and considered this a potential benefit. Over time, available Fe and Mn concentrations decreased while available Zn and Cu increased (Fig. 1). The decreases in available Fe and Mn were likely due to mineral forms changing from more to less available over time. The slight increases in Zn and Cu concentrations may have been due to the slight decrease in soil pH over time. Nonetheless, the increases in Zn and Cu content did not raise their concentrations to above those considered marginal for certain crops (1 mg kg^{-1} available Zn or Cu) (Davis and Westfall, 2009; Davis et al., 2009; Espinoza et al., 2006).

Biochar is mostly C (66.2%) (Table 1); thus, increasing biochar application caused an increase in soil organic C content (Fig. 2a). After 12 mo of incubation, soil organic C content for the 1, 2, and 10% biochar rates were 48, 216, and 686% greater than the control, respectively. Results were similar to those observed by other researchers (e.g., Rogovska et al., 2011; Bolan et al., 2012). Soil organic C content decreased over time suggesting that either a portion of the biochar-added C was labile and available for microorganisms or that the biochar application stimulated degradation of native soil organic C (Wardle et al., 2008; Hamer et al., 2004).

Biochar Induced Effects on Nitrogen and Carbon Dynamics

Biochar additions stimulated microbial activity in these soils, increased substrate-induced respiration and bacterial populations, and altered microbial community structure. Soil NO_3–N concentrations significantly decreased with increasing biochar application rate potentially due to microbial immobilization and perhaps reduced nitrification activity over time (Fig. 2b). Our biochar contained low NO_3–N concentrations (Table 1), a common feature of biochars in general (Belyaeva and Haynes, 2012) and likely attributable to gaseous N loss during pyrolysis (Amonette and Joseph, 2009). Furthermore, the biochar C/N ratio was ~ 207/1, which was

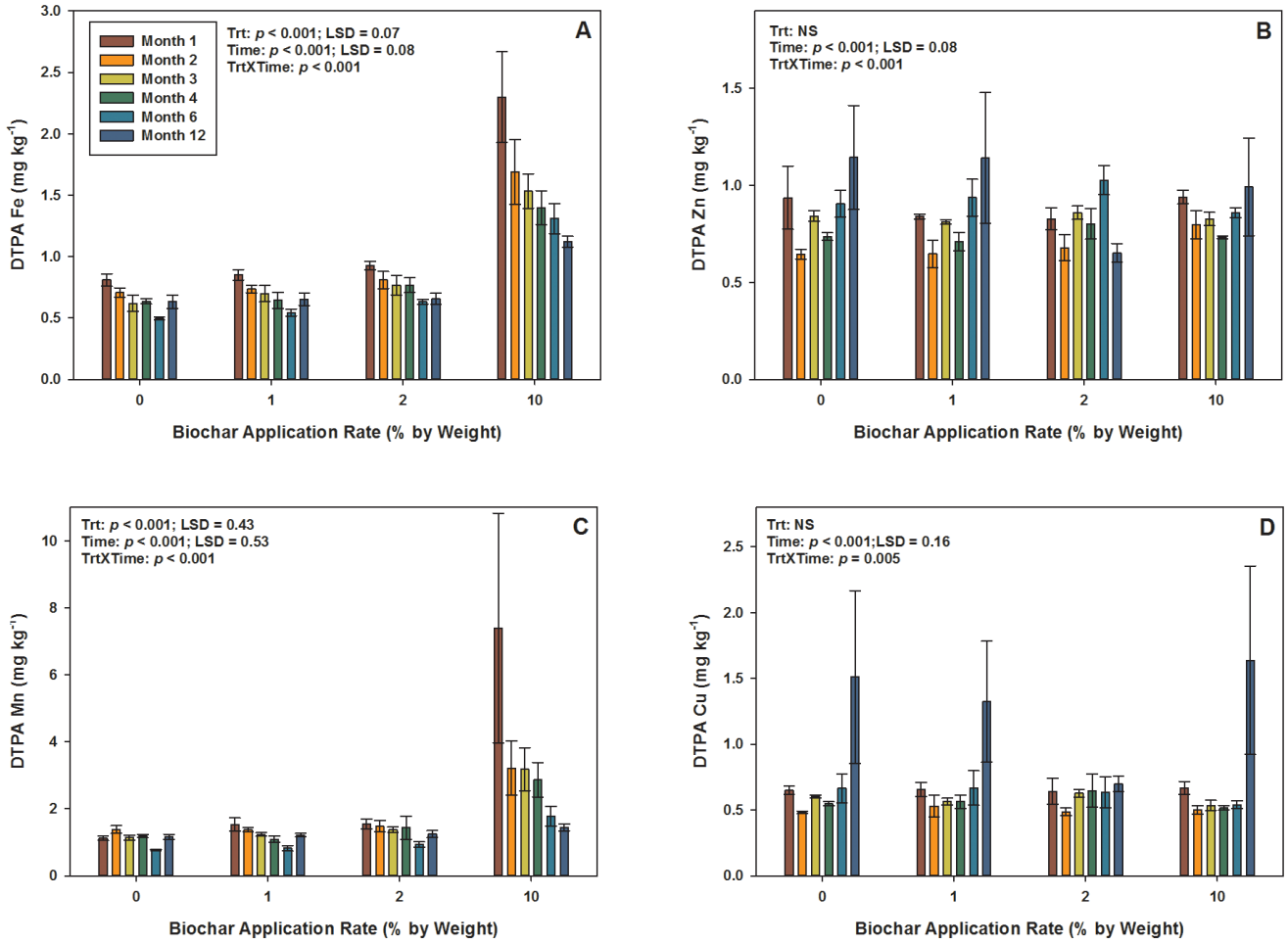

Fig. 1. The effect of increasing biochar application rate and time since application on diethylenetriaminepentaacetic acid (DTPA)-extractable soil iron (a), zinc (b), manganese (c), and copper (d). Error bars represent 1 SEM (n = 4). Trt, treatment.

much greater than the assumed approximately 25/1 minimum ratio required to induce an immobilization response (Borchard et al., 2012). Other researchers have suggested that biochar-induced immobilization occurs at biochar application rates comparable to our study (20–22 Mg ha^{-1}) (Sarkhot et al., 2012; Lentz and Ippolito, 2012; Shenbagavalli and Mahimairaja, 2012; Ducey et al., 2013).

Soil NO_3–N increased with time regardless of the amount of biochar applied; however, the magnitude of this increase declined with increasing biochar application from 39.4 mg kg^{-1} for 1% to 5.3 mg kg^{-1} for 10% biochar (Fig. 2b). This suggests that biochar may have inhibited the remineralization and nitrification of microbial tissue–bound N in these soils. Throughout the experiment, soil NH_4–N concentrations were no greater than 1 mg kg^{-1} and in many instances were below detection (data not shown). The linearity of soil NO_3–N changes across the 0, 1, and 2% biochar treatments at each sampling date suggests that the effect was proportional to added biochar mass. A similar response was also observed in a 2-yr field study using the same soil type (Lentz and Ippolito, 2012). Reduction in NO_3–N content with biochar application could be due to several soil processes. For example, Prendergast-Miller et al. (2011) found that soils amended with 60 Mg biochar ha^{-1} contained greater NO_3–N contents in both the biochar particles and rhizosphere as compared with 20 Mg biochar ha^{-1} or control treatments; thus, NO_3–N entrapment could have occurred. Rajkovich et al. (2012) showed that increasing biochar application rates (up to 7%) likely caused N immobilization and a subsequent reduction in foliar N content. Ducey et al. (2013) applied 10% biochar (by wt) to an eroded calcareous soil, noting a greater presence of N fixing and denitrification genes as compared with control soil. It may also be possible that biochar application effectively reduced N-cycle microbial dynamics (e.g., Cayuela et al., 2013).

The pattern of soil CO_2 respiration across time was similar for all treatments during the first 3 mo, with a minima occurring in the first month and maxima occurring in the second or third months (Fig. 2c). After the third month, control and biochar treatments diverged and respiration of the control declined, whereas that of biochar-amended soils, in most cases, remained near their peak levels. Hence, the soil CO_2 respiration of the biochar-amended soils exceeded that of the control. This may have been a function of the fast-pyrolysis biochar used because the feedstock is typically not completely pyrolyzed. This may leave relatively easily degradable C sources intact (e.g., cellulose, lignin) to serve as a C source. Furthermore, the 1% biochar application rate produced greater respired CO_2 on average compared with all other treatments. The fact that biochar increased CO_2 respiration is in disagreement with others (Dempster et al., 2012; Kammann et al., 2011; Spokas et al., 2009; Spokas and Reicosky, 2009). However, Rogovska et al. (2011) added biochar at rates up to 2% (by wt) to soil, noting that all biochar applications increased CO_2 emissions over control soil, similar to the current

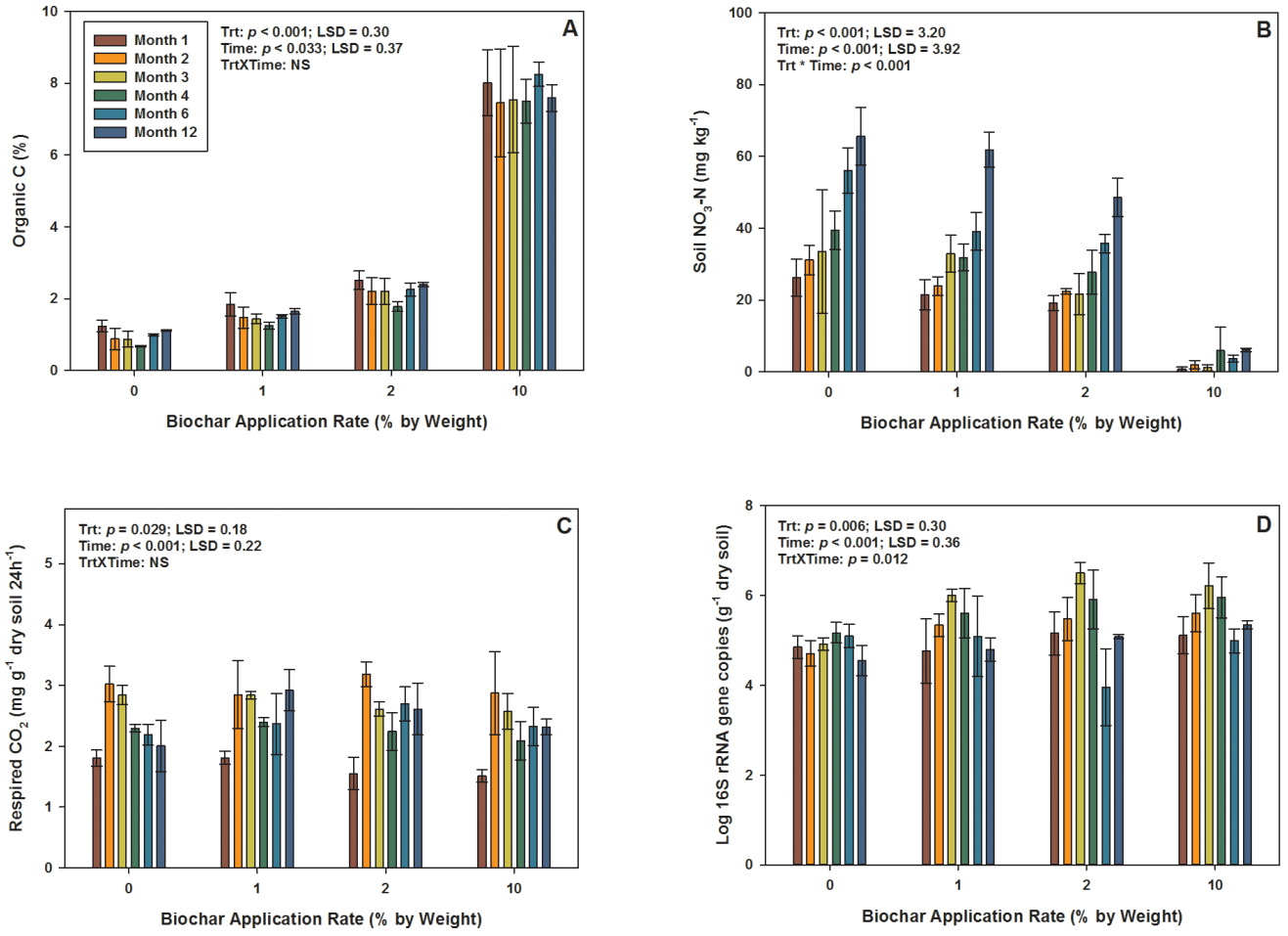

Fig. 2. The effect of increasing biochar application rate and time since application soil organic C (a), NO_3–N (b), substrate-induced respired CO_2 (c), and gene copy numbers of bacterial 16S rRNA (d). Error bars represent 1 SEM (n = 4). Trt, treatment.

study. Furthermore, Dempster et al. (2012) found that a greater biochar application rate (25 Mg ha^{-1}) produced greater CO_2 as compared with a lower biochar rate (5 Mg ha^{-1}). The authors suggested that a negative priming effect occurred at the 5 Mg ha^{-1} rate. Perhaps in the current study our lowest biochar application (~20 Mg ha^{-1}) was not low enough to observe this negative priming effect, or our results may be due to the fact that different biochars and soils elicit different responses.

Microbial Response

The 16S rRNA gene copy data indicate that biochar applications increased the size of soil bacterial populations and altered the pattern of bacterial growth during the year (Fig. 2d). Control populations were constant over time, whereas biochar-treated soils produced peak bacterial populations in months 3 and 4. The peak in biochar bacterial populations and the corresponding peak in respired CO_2 (Fig. 2c) were simultaneous with a period of minimal increases in soil NO_3–N (Fig. 2b), providing evidence that biochar increased immobilization of soil C. However, neither the bacterial population nor respired CO_2 increased dramatically when biochar rate increased from 2 to 10%, which suggests that immobilization was not the primary cause of the corresponding decline in soil NO_3–N between these two treatments.

Lehmann et al. (2011) suggested that biochar addition may increase bacterial populations due to increases in macro- or micronutrient availability or soil pH. In the current study, however, micronutrient availability did not increase drastically and thus was likely not the cause of the increase in bacterial population. Changes in soil pH were also likely not the cause of the bacterial population increase because bacterial populations appear to increase from acidic conditions up to approximately pH 8 (Rousk et al., 2010). Lehmann et al. (2011) also made a compelling case for biochar positively affecting biofilm formation, sorption of inhibitory growth compounds, sorption of microorganisms and improvement of microorganism habitat, and improvement in soil water content. Although most of these hypotheses are plausible, in the current study we only measured changes in soil water content, which were positive. Biochar application can improve the soil water status and may retain moist pore spaces that allow continued bacterial hydration during periods of drying (Lehmann et al., 2011), as likely occurred in between waterings in the current study. Thus, it is plausible that increases in the soil water status, observed at some point in time with all biochar application rates, could have been the direct cause for the increased size of the bacterial population.

Biochar application possibly included some labile C, and this would explain the initial increase in bacterial population. In support of this contention, Smith et al. (2010) also observed greater microbial activity (expressed as C mineralization) during initial study stages (<7 d) likely due to, in part, the presence of biochar-borne labile C. The presence of biochar-borne labile C would also explain the stimulated respiration and N immobilization activities in the early study stages. Upon complete labile C consumption, bacterial populations would decrease as shown in the later months of incubation.

Increasing biochar application rates resulted in greater concentrations of total organic C, water availability, and available Fe and Mn and in a decrease in NO_3–N as compared with the control, which may explain why divergence in microbial community structure increased with increasing application rate (Fig. 3). Regardless of incubation time, microbial communities from 10% biochar rate soils separated from communities exposed to lower biochar application rates along principle components analysis 1 of Fig. 3 and were significantly different from all other biochar rate communities according to the MRPP test (P < 0.0001; A = 0.198; T = −14.7). Microbial community structure in the 2% biochar rate soil was also significantly different from control soil (P = 0.03), although the difference was slight compared with the effect of the 10% biochar rate. Community shifts in response to increasing biochar rates were mainly due to shifts in proportions of fungal (18:2ω6,9c and 18:1ω9c) and some bacterial (i16:0, 18:1ω7c, and 19:0 cy) FAMEs. Fungal biomarkers from the 10% biochar rate comprised 21.0% of the total FAMEs, averaged across the incubation periods, compared with 24.6% of total FAMEs in control soil. Conversely, FAMEs i160, 18:1ω7c, and 19:0 cy increased from 1.4, 9.6, and 0%, respectively, in control soil to 2.1, 14.3, and 2.7% in the 10%

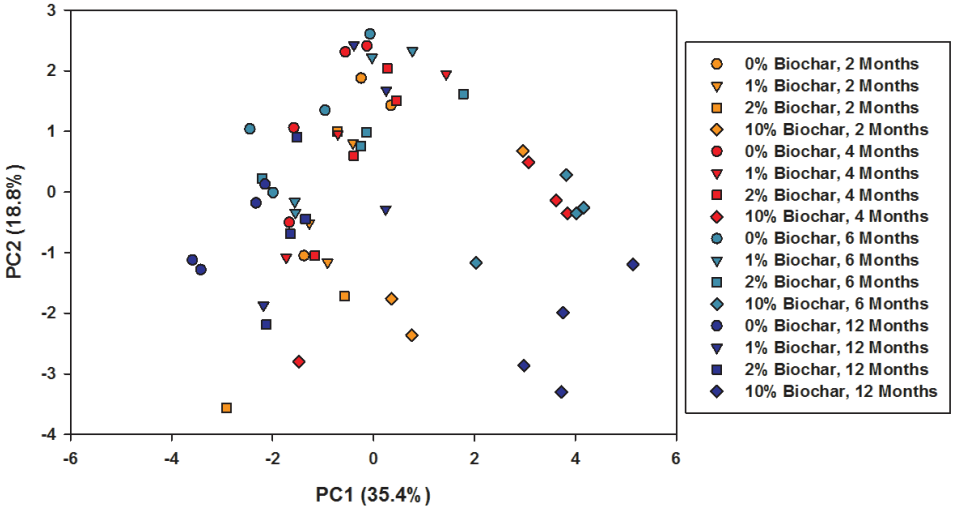

Fig. 3. The effect of increasing biochar application rate (%) and time since application on soil microbial community fatty acid methyl ester profiles as determined by principal components analysis. The percent variance explained by each principal component is shown in parentheses. PC1, principal components analysis 1; PC2, principal components analysis 2.

biochar rate soil. Fatty acid methyl esters 18:1ω7c and 19:0 cy are considered biomarkers for Gram-negative bacteria (Vestal and White, 1989), indicating favorable conditions for growth of these bacteria in response to biochar, particularly at the 10% rate. Alternatively, the ratio of 19:0 cy to its precursor, 18:1ω7c, has been used as an indicator of stress because Gram-negative bacteria synthesize cyclopropane fatty acids (e.g., 19:0 cy) from their monounsaturated precursors (e.g., 18:1ω7c) during periods of starvation, anaerobic conditions, low pH, and high temperatures (Grogan and Cronan, 1997). This ratio ranged from 0 in control soil to 0.03 in the 1 and 2% biochar rate soils and to 0.19 in the 10% biochar rate soil. The difference in values between the 10% biochar rate soil and all other treatments was significant. Similarly, Pietikäinen et al. (2000) detected an increase in 19:0 cy fatty acid in a forest soil humus underlying a layer of charcoal but did not report the effects of charcoal on the ratio of 19:0 cy-to 18:1ω7c. In contrast to our study, they also did not detect a reduction in humus fungal fatty acids in response to charcoal amendment.

In this study, biochar added to a calcareous soil at a rate of 10% altered microbial community structure by reducing soil fungal populations and increasing bacteria, including Gram-negative bacteria. The latter agrees with the above finding that biochar application increased the concentration of bacterial 16S rRNA gene copy numbers in soil. Considering the changes to soil resource availability noted above, biochar may have supplied labile C substrates that favored fast-growing bacteria over fungi. In addition, biochar resulted in greater water retention in soils, which over the course of the study may have limited the negative impacts that soil drying (in between rewettings) could have had on bacteria but not necessarily fungi (Harris, 1981; Schimel et al., 2007). Although biochars have not been studied specifically for their ability to protect bacteria from desiccation, Lehmann et al. (2011) suggested that biochar may retain water-filled pore spaces that allow continued hydration of microbial cells during soil drying. We note that incubation time also affected microbial community structure, regardless of biochar rate, but that the effect of incubation time was minor relative to the effect of biochar rate (Fig. 3). Communities varied significantly only between those incubated for 2 and 12 mo ($P = 0.02$; A = 0.03; T = −2.59).

Biochar studies that have included a microbial component have often measured biochar effects on microbial biomass or the abundance of specific populations (for a review, see Lehmann et al. [2011] and references within). However, few studies (e.g., Jin, 2010) have examined the impact of biochar on the structure or composition of microbial communities in temperate soils. This is unfortunate because shifts in the microbial community may have important functional consequences in terms of decomposition processes, nutrient cycling, and aggregation formation. For example, Dempster et al. (2012) found that in the presence of fertilizer N, biochar applied at 25 Mg ha^{-1} altered the diversity of nitrifying bacteria and reduced nitrification rates in an Australian soil. Ducey et al. (2013) showed that an eroded calcareous soil that received 10% biochar (by wt) contained greater microbial gene abundances associated with N fixation and denitrification. The authors found that gene abundance was correlated with decreasing soil NO_3–N and increasing total N and C content. These studies, in conjunction with the current study, provide evidence that biochar has the potential to improve the soil water and micronutrient status but also to alter soil microbial communities and processes related to C and N cycling. This is especially important if biochar is to be applied globally to the nearly 1 billion ha of soil encompassing semiarid and arid conditions.

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