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Hardwood biochar and manure co-application to a calcareous soil

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ABSTRACT

Biochar may affect the mineralization rate of labile organic C sources such as manures via microbial community shifts, and subsequently affect nutrient release. In order to ascertain the positive or negative priming effect of biochar on manure, dairy manure (2% by wt.) and a hardwood-based, fast pyrolysis biochar were applied (0%, 1%, 2%, and 10% by wt.) to a calcareous soil. Destructive sampling occurred at 1, 2, 3, 4, 6 and 12 months to monitor for changes in soil chemistry, water content, microbial respiration, bacterial populations, and microbial community structure. Overall results showed that increasing biochar application rate improved the soil water content, which may be beneficial in limited irrigation or rainfall areas. Biochar application increased soil organic C content and plant-available Fe and Mn, while a synergistic biochar–manure effect increased plant-available Zn. Compared to the other rates, the 10% biochar application lowered concentrations of NO_3-N; effects appeared masked at lower biochar rates due to manure application. Over time, soil NO_3-N increased likely due to manure N mineralization, yet soil NO_3-N in the 10% biochar rate remained lower as compared to other treatments. In the presence of manure, only the 10% biochar application caused subtle microbial community structure shifts by increasing the relative amounts of two fatty acids associated with Gram-negative bacteria and decreasing Gram-positive bacterial fatty acids, each by ~1%. Our previous findings with biochar alone suggested an overall negative priming effect with increasing biochar application rates, yet when co-applied with manure the negative priming effect was eliminated.

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1. Introduction

Biochar is a pyrolysis product that may be utilized as a soil amendment. Research has focused a great deal of attention on biochar application in highly weathered soil 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, the use of biochar in semi-arid and arid agricultural soils (which comprise over 2 billion hectares worldwide (Brady and Weil, 1999)) is a relatively new, not extensively studied concept.

Van Zwieten et al. (2010) applied 10 Mg ha^{-1} of biochar to an Aridisol, noting no change in soil fertility status. Yet others (Laird et al., 2010a, 2010b; Brewer et al., 2012; Ippolito et al., 2012a, 2012b) have applied biochar (up to 40 Mg ha^{-1}) to Aridisols and Mollisols, and observed increases in soil extractable P, K, Mg, Fe, and Ca, and decreases in NO_3-N leaching. Increases in soil extractable nutrient concentrations may have been due simply to biochar-borne elemental addition. However, the reduction in NO_3-N leaching may have been due to nutrient retention in biochar micropores (Kameyama et al., 2012), adsorption by biochar (Laird et al., 2010b), biochar-induced microbial immobilization (Streubel et al., 2011; Sarkhot et al., 2012), or greater abundance of microorganisms able to fix or denitrify N (Ducey et al., 2013).

Specific biochar effects on microbiological activity in arid and semi-arid soils have been mostly documented in laboratory incubation studies. Increases in CO_2 evolution have been observed in Mollisols receiving between 20 and 45 Mg biochar ha^{-1} (Rogovska et al., 2011; Streubel et al., 2011; Smith et al., 2010) and in Aridisols receiving 45 Mg ha^{-1} (Smith et al., 2010). Increases in CO_2 evolution have been attributed to biochar initially containing easily degradable C compounds, and to a reduction in soil bulk density and subsequently an improvement in microorganism habitat (Rogovska et al., 2011; Smith et al., 2010). Ippolito et al. (2014) applied up to 200 Mg ha^{-1} of biochar to an Aridisol, also noting increases in CO_2 production over a 12 month

Abbreviations: DTPA, diethylenetriaminepentaacetic acid; EL, ester linked; FAME, fatty acid methyl ester; MRPP, multi response permutation procedure; NMS, nonmetric multidimensional scaling; PLFA, phospholipid fatty acid analysis.

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incubation study. Furthermore, the authors studied microbial community composition, noting a shift toward more bacteria and less fungi with increasing biochar application. This shift was attributed to physiological stress favoring bacteria over fungi, biochar supplying labile C which favored fast growing bacteria over fungi, or to biochar resulting in increased soil water retention and improving the microclimatic conditions favorable for bacteria over fungi. The above information suggests that biochars alone may not greatly improve nutrient retention in arid and semi-arid soils, and at excessive rates may cause shifts in microbial community composition. However, applying biochars at a proper application rate, with nutrient-rich materials such as manures, may provide some benefit in terms of improvements in soil fertility without compromising the microbial community composition.

Biochar co-application with manure has not been extensively studied, despite that manure is already commonly applied to agriculture soils (6.5 million hectares in the US; USDA, 2006) where there is interest in also applying biochar. Thus, a need exists to identify the effects of biochar and manure soil co-applications. To this end, organic C sources such as manures, when added to soils, often leads to a positive priming effect due to increased microbial activity (Sorensen, 1974) associated with supplied energy sources and nutrient release. Furthermore, it is plausible that various rates of biochar can cause either a positive or negative priming effect of added labile organic C sources (e.g., Keith et al., 2011; Liang et al., 2010; Hamer et al., 2004). Based on our previous observations where biochar was applied alone (Ippolito et al., 2014), we hypothesized that relatively low biochar application rates (e.g., 1% and 2% by wt.) would cause no effect, while an excessive biochar application (e.g., 10% by wt.) would cause a negative priming effect even in the presence of manure. Thus, a 12 month laboratory incubation study was conducted with the objective to assess the effect of biochar–manure co-application on soil water content, nutrient concentrations, microbial respiration, bacterial abundance, and microbial community structure in relation to priming effect.

2. Materials and methods

2.1. Biochar, manure, and soil characteristics

A hardwood biochar (<0.5-mm particle size), made from oak and hickory sawdust, supplied by Dynamotive Energy Systems Inc. (Vancouver, British Columbia, Canada) was manufactured using fast 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, Golden, CO) using a modified ASTM method D1762-84 for wood charcoal (600 °C), total C and N were determined by dry combustion (Nelson and Sommers, 1996; Thermo-Finnigan FlashEA1112; CE Elantech Inc., Lakewood, NJ), and specific surface area was determined from isotherms fitted to the Brunauer, Emmett, and Teller (BET) equation (Brunauer et al., 1938). Biochar pH and EC were determined on a saturated paste extract (Thomas, 1996; Rhoades, 1996), NO_3-N and NH_4-N content using a 2 M KCl extract (Mulvaney, 1996), and organic N content as difference between total and inorganic N. Biochar total metal concentrations were determined by $HClO_4$–HNO_3–HF–HCl digestion (Soltanpour et al., 1996) followed by elemental analysis using a PerkinElmer inductively coupled plasma-optical emission spectrometer Optima 8300 (PerkinElmer; Waltham, MA).

Dairy cattle solid manure was collected from a pen at a local open-lot dairy, and contained 55.3% solids. Total C and N, total elemental concentrations, NO_3-N and NH_4-N, and pH and EC were determined as previously described.

Soil (0–30 cm) was obtained from the edge of a field located 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, classified as Portneuf (coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcid; USDA-NRCS, 2013), was air-dried, passed through a 2-mm sieve, and then 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 sieved soil was also pulverized and analyzed for inorganic C using a modified pressure-calcimeter method (Sherrod et al., 2002) and total C and N as mentioned above. Soil organic C was determined by difference between total and inorganic C. Biochar, manure, and soil characteristic data are presented in Table 1.

2.2. Soil–biochar incubation

The effect of hardwood biochar and manure application to the Portneuf soil was investigated during a 12 month incubation study. Biochar was applied and thoroughly mixed by hand into soil at 0, 1, 2, and 10% (~0, 20, 40, and 200 Mg ha^{-1}; wt:wt). The 10% application rate was chosen to help identify upper level soil detriments by biochar application. Manure was then added to all soils at a rate of 2% (by wt.). Soil–biochar–manure mixtures (300 g total) were placed in 8 cm × 8 cm × 8 cm plastic pots using 4 replicates per treatment. Pots were lined with plastic liners to prevent leaching, placed in a growth chamber (22 °C; 30% humidity) and watered twice weekly with reverse osmosis water to 80% of field capacity. Field capacity was determined for each biochar–manure soil mixture prior to the experiment by lining four pots with cheesecloth, filling the pots with 300 g soil, saturating with reverse osmosis water, and allowing to freely drain over a 48 h period. Within the growth chamber, pots were separated by month and then randomized within each month. Pots were destructively sampled at 1, 2, 3, 4, 6, and 12 month intervals.

During monthly destructive sampling, a soil subsample was removed, placed in a ziplock storage bag, and stored in a −80 °C freezer for microbial analysis. The remainder of soils were analyzed for NO_3-N and NH_4-N as previously described, as well as

Table 1
Properties and total elemental analysis of the hardwood biochar, manure, and Portnuef soil.

Property	Units	Biochar	Manure	Portnuef soil
Surface area	$m^2\ g^{-1}$	0.75	ND	ND
pH		6.8	8.8	8.2
EC	$dS\ m^{-1}$	0.7	13.4	0.3
Ash	%	14	ND	ND
Total C	%	66.2	26.4	3.53
Inorganic C	%	ND[a]	ND	2.33
Organic C	%	ND	ND	1.20
Total N	%	0.32	2.15	0.08
Organic N	%	0.32	2.12	0.08
NO_3-N	$mg\ kg^{-1}$	1.5	80.6	18.1
NH_4-N	$mg\ kg^{-1}$	1.2	220	0.57
K	$mg\ kg^{-1}$	3400	13,500	2590
Ca	$mg\ kg^{-1}$	3700	22,000	74,500
Mg	$mg\ kg^{-1}$	1500	8230	13,100
Na	$mg\ kg^{-1}$	200	3750	280
P	$mg\ kg^{-1}$	300	4080	330
Al	$mg\ kg^{-1}$	300	3520	720
Fe	$mg\ kg^{-1}$	1400	4480	700
Zn	$mg\ kg^{-1}$	14.1	167	27.7
Mn	$mg\ kg^{-1}$	118	169	218
Cu	$mg\ kg^{-1}$	16.8	76.5	4.83
Ni	$mg\ kg^{-1}$	4.9	3.4	6.6
Mo	$mg\ kg^{-1}$	<0.05	0.49	<0.01
Cd	$mg\ kg^{-1}$	<0.05	0.34	0.12
Pb	$mg\ kg^{-1}$	2.0	1.9	6.4
B	$mg\ kg^{-1}$	12.3	27.3	14.7

[a] ND = not determined.

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for diethylenetriaminepentaacetic acid (DTPA; i.e. available) extractable Fe, Zn, Mn, and Cu (Loeppert and Inskeep, 1996). Substrate-induced soil respiration rates were determined by thoroughly mixing 50 g of moist soil with 0.5 g of glucose, 0.01 g of K_2HPO_4, and 0.075 g of NH_4Cl in 100 mL air tight mason jars. A vial containing 5 mL of 1 M NaOH was placed inside each jar to capture respired CO_2, and the jars were sealed. After 24 h the vials were removed, excess $BaCl_2$ was added to the NaOH, several drops of phenolphthalein indicator were added, and the NaOH was titrated to a clear endpoint with 1 M HCl (Dungan et al., 2003). Duplicate measurements were made on all soils. Soil water content was determined on all samples to convert the above soils data to a dry weight basis. Soils were then air-dried, passed through a 2-mm sieve, and a subsample was pulverized and analyzed for total, inorganic, and organic C as previously mentioned. Soils from months 1, 6, and 12 were also 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) in a constant temperature room (22 °C). Soils were packed to a bulk density of 1.00 g cm^{-3} into 4.7-cm diameter × 1.8-cm tall metal rings. To keep the soil within the rings, a nylon sheet (6 μm mesh opening) was secured to the bottom of each ring with a rubber band. Soils within the rings were saturated in a plastic tub with a water level of approximately 1.5 cm for about two days. Soils were then transferred to a pressure pot, matric potentials were established over time (from 0 to −300 kPa), and after water ceased to be emitted from the side of the pressure pot at each potential, the samples were removed and weighed for soil moisture content.

Soils preserved in the −80 °C freezer were analyzed for bacterial counts ($n = 3$) using 0.5 g of soil (dry wt.) and an UltraClean Soil DNA Isolation Kit (MO BIO laboratories, Inc., Carlsbad, CA) following manufacturer's instructions. After extraction, 5 μL of DNA was used in a 30 μL quantitative PCR (qPCR) reaction mixture with universal primers and probe (100 nM each) to estimate the concentration of 16S rDNA copy numbers in each sample as described by Nadkarni et al. (2002). The qPCR was performed with a Bio-Rad (Hercules, CA) multicolor iQ5 real-time PCR detection system. Purified DNA from *Escherichia coli* (ATCC 11775) was used as a standard to calculate gene copy numbers.

Frozen soils from the control and 10% biochar rate treatments, and from incubation periods of 2, 6, and 12 months, were analyzed for microbial community structure according to fatty acid methyl ester (FAME) profiles. Prior to analysis, replicate 4 from each biochar rate within the 2-month incubation treatment had been sacrificed for other analyses; therefore only three replicate soils were analyzed from the 2-month incubation period, whereas four replicates were analyzed for the 6- and 12-month incubation periods. Microbial FAMEs were extracted following the ester-linked (EL) FAME procedure described by Schutter and Dick (2000), where 3 g soil were extracted with 0.2 M KOH during a 37 °C, 1 h-long incubation with periodic mixing, followed by addition of 1 M acetic acid to neutralize the pH of the tube contents. Soil FAMEs were partitioned into an organic phase by addition of hexane, which was removed from the aqueous phase after centrifugation at 480g for 10 min. After the addition of an internal standard (20 μg of 19:0), samples were analyzed by gas chromatography (Agilent 6890 gas chromatograph, Agilent Technologies, Inc., Palo Alto, CA) at the University of Delaware (Newark, DE) using the Microbial ID (Newark, DE) 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, i16:0, 16:1ω9c, 16:1ω7c, i17:0, a17:0, 17:0 cy, 17:0, and 19:0 cy. FAMEs 18:2ω6c and 18:1ω9c were used as the indicators of saprophytic fungi.

2.3. Statistical analyses

The experimental design was a split plot with time design, with statistical analysis performed using the Proc GLM model, SAS software version 9.2 (SAS Institute, 2008). Soil water content statistical analysis was performed only within individual months 1, 6, or 12. We utilized an $\alpha = 0.05$, and calculated a Fisher's Protected Least Significant Difference (LSD; Steel and Torrie, 1980) when significance was 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, Gleneden Beach, OR). Due to the non-normal distribution of the FAME data set, relative % molar concentrations of FAMEs were analyzed by non-metric multidimensional scaling (NMS) with the SØrensen distance measure; NMS is a non-parametric, multivariate technique that ordinates each community in two- or three-dimensional space so that community similarities and dissimilarities can be visualized. Between-community distances are preserved as well as possible in the reduced dimensional space, compared to the actual between-community distances in multivariate space where the number of dimensions is equivalent to the number of dependent variables, or FAMEs in this case (Borg and Groenen, 2005). Multi response permutation procedure (MRPP) tests were conducted 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 ($\alpha = 0.05$).

3. Results and discussion

3.1. Interpretation of statistical analysis

Biochar, co-applied with manure, 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 (Figs. 1 and 2). In the presence of manure, the effect of biochar on soil properties was, in most instances, modified by time (i.e., significant effects of biochar and time). Significant treatment x time interactions occurred for most variables; however, the impact of the interaction on soil variables was not as strong as the impact of either biochar application rate or time. For example, changes in a soil variable over time may have been slightly delayed at the greatest biochar treatment, as compared to the lower biochar treatments, by only about one month (e.g. Fe; Fig. 1A). Yet the final, year-long effect of time on a soil variable was consistent across all biochar treatments. Meaning, we did not observe long-lasting deviations in temporal trends based on biochar application rate. Thus, interaction effects are presented in the results, but the discussion mostly focuses on the main effects of treatment and time.

3.2. Soil water content

Biochar applied at 10% (by wt.) increased gravimetric soil water content from saturation to −300 kPa, and the response was consistent from month 1 to 6 to 12 (Table 2). This was similar to results observed by Ippolito et al. (2014) who applied up to 10% biochar alone. Results were also similar to other studies where lower biochar rates (0.4–2%) were applied alone (Case et al., 2012; Liu et al., 2012; Novak et al., 2012; Streubel et al., 2011). Biochar porosity likely increased the water content, similar to that observed by Sun et al. (2013), Bruun et al. (2012), and Novak et al. (2012). The increase in soil water content due to biochar application alone may be of value to crop producers who experience sporadic rainfall or solely rely upon irrigation (Novak et al., 2012), with benefits

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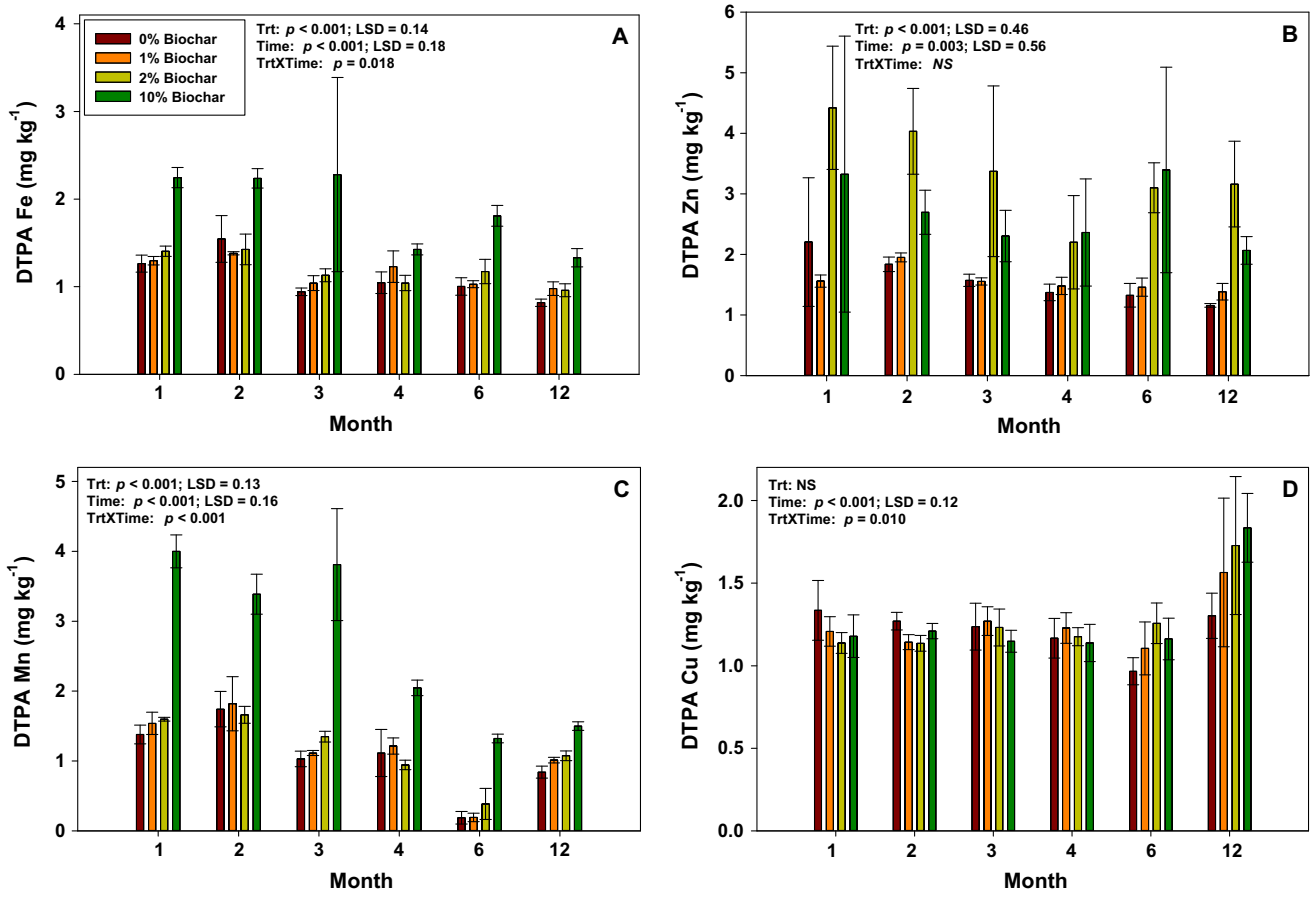

Fig. 1. The effect of increasing biochar application rate (by wt.), applied with a constant rate of manure (2% by wt.), and time since application on diethylenetriaminepentaacetic acid (DTPA) extractable soil (A) iron, (B) zinc, (C) manganese, and (D) copper. Error bars represent one standard error of the mean ($n = 4$). Trt = biochar treatment. Time = time since biochar application. TrtXTime = the interaction between treatment and time.

potentially realized over the long-term (Spokas et al., 2012) as biochars are fairly recalcitrant to degradation.

The soil water response to biochar application was less noticeable at lower biochar rates, likely due to a masking effect of the 2% manure application (i.e. ~40 Mg ha^{-1}). In support of this finding, Rasoulzadeh and Yaghoubi (2010) noted an increase in soil water content at field capacity (−33 kPa) over a control when cattle manure was applied at 30 Mg ha^{-1}; a greater soil water content was observed when the application rate was increased to 60 Mg ha^{-1}. Arriaga and Lowery (2003) found a similar soil water retention response following 10 years of cattle manure application at an annual loading rate of ~15 Mg ha^{-1}. However, few other studies have studied changes in soil water content with co-application of biochar and other organic amendments. Liu et al. (2012) studied the effect of biochar (0, 5, 10, and 20 Mg ha^{-1}) co-applied with a mixture of composted green waste, wood, and soil (32.5 Mg ha^{-1}); co-application at 20 Mg biochar ha^{-1} doubled water holding capacity as compared to the control.

In comparing manure alone with biochar + manure treatments (Table 2), we noted that water retention for both increased with time, yet the increase was consistently greater for soils amended with >1% biochar than for manure-only treatments. For example, at a soil matric potential of −10 kPa, water retention for manure-only increased 1.3× between months 1 and 12, compared to a 1.7× increase for the 10% biochar treatment (Table 2). This suggests that the water-associated benefits from both amendments require time to develop, and that the physical changes induced in manure-amended soils with regards to increased water retention differed from those of biochar.

3.3. Soil chemical characteristics

The effect of biochar and manure co-application, and time since co-application, on DTPA extractable Fe, Zn, Mn, and Cu are presented in Fig. 1. The 10% biochar application rate, co-applied with 2% manure, significantly increased DTPA-extractable Fe and Mn over the other three treatments. This was likely due to biochar-borne Fe and Mn being in readily available forms, as shown by Ippolito et al. (2014). Although decreases in soil pH can increase metal extractability, no significant treatment effect on soil pH was observed (data not shown). Lentz and Ippolito (2012) observed an increase in Mn availability when biochar was applied to the same soil under field conditions. The 2% and 10% biochar rates caused an increase in extractable Zn as compared to the control and 1% biochar rate. A synergistic effect between biochar and manure may have caused the increase in extractable soil Zn at the higher (2% and 10%) as compared to the lower (0% and 1%) biochar application rates. The increasing Zn concentration with the 2% and 10% biochar + 2% manure application is important because DTPA-extractable Zn concentrations less than about 1.5 mg kg^{-1} are considered marginal for certain crops such as corn and potatoes (Davis and Westfall, 2009; Davis et al., 2009; Espinoza et al., 2006). Treatment responses were not observed for extractable soil Cu. Over time, available Fe, Zn, and Mn concentrations tended to decrease likely due to mineral forms changing from more to less available, as previously observed when biochar was applied alone (Ippolito et al., 2014). In the current study, available soil Cu content was greater in month 12 as compared to the other months. Although Cu is known to form strong associations

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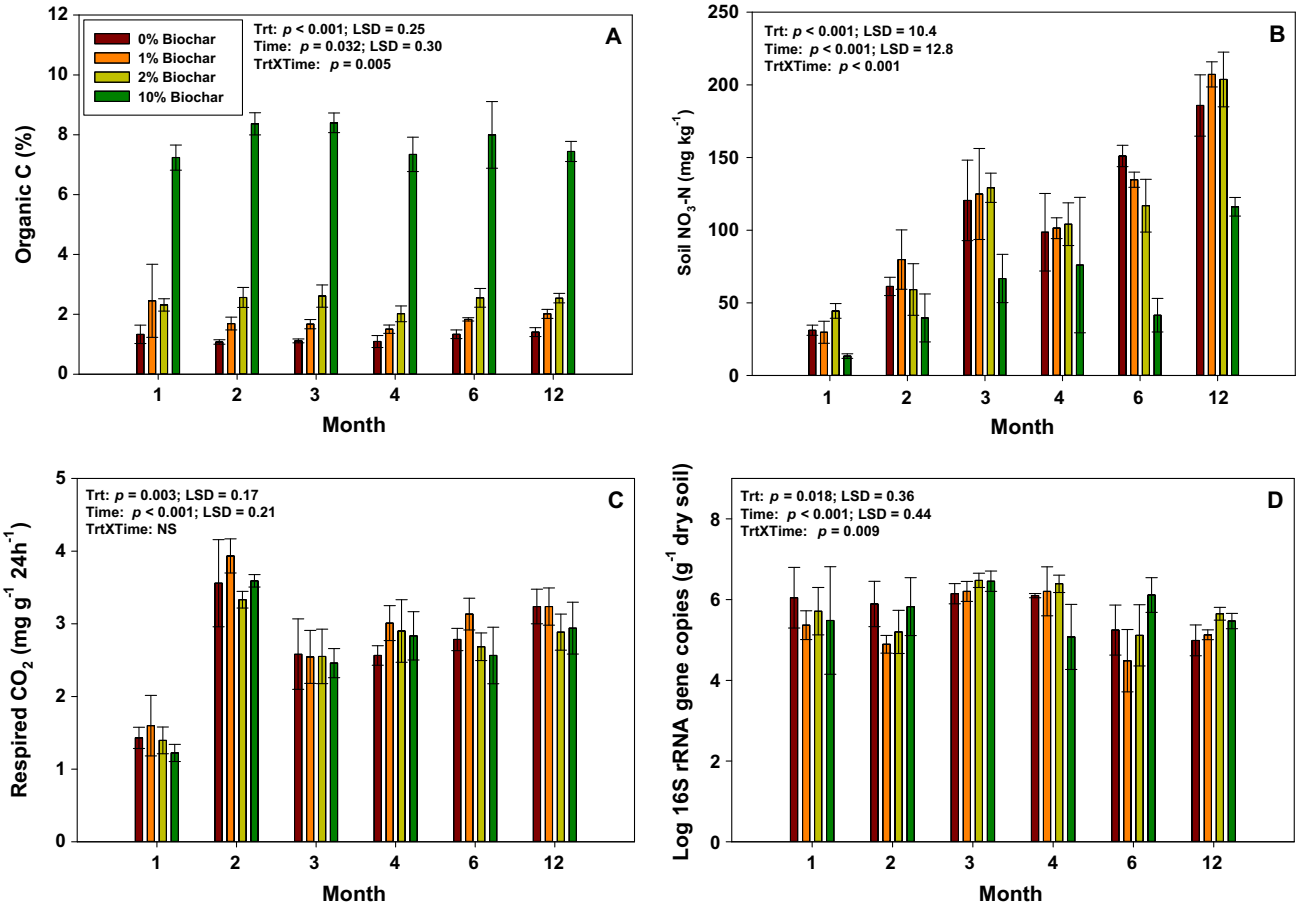

Fig. 2. The effect of increasing biochar application rate (by wt.), applied with a constant rate of manure (2% by wt.), and time since application on soil (A) organic C, (B) NO_3-N, (C) substrate-induced respired CO_2, and (D) bacterial population. Error bars represent one standard error of the mean ($n = 4$). Trt = biochar treatment. Time = time since biochar application. TrtXTime = the interaction between treatment and time.

Table 2
Biochar + manure (2% by wt.) amended Portneuf soil mean ($n = 4$) percent gravimetric soil water content for soils incubated for either 1, 6, or 12 months. Values inside parenthesis indicate one standard error of the mean. Within a column and a given month, values followed by the same letters are not significantly different at $\alpha = 0.05$, as determined by Fisher's protected LSD.

Month	Biochar application rate (% by wt.)	Matric potential (kPa)				
		0	−10	−33	−100	−300
Gravimetric water content (%)						
1	0	57.5(1.4)c	29.7(0.1)a	21.6(0.2)b	16.2(0.1)b	12.2(0.1)a
	1	60.9(1.2)bc	33.9(4.1)a	21.8(0.2)b	16.0(0.1)bc	12.0(0.1)a
	2	61.7(0.9)b	29.3(0.3)a	21.6(0.0)b	15.9(0.1)c	12.0(0.0)a
	10	70.3(1.4)a	29.0(2.8)a	23.8(0.3)a	16.8(0.1)a	12.3(0.1)a
	P > F	<0.001	0.488	<0.001	<0.001	0.263
	LSD	3.9		0.7	0.3	
6	0	62.5(0.8)c	37.3(0.5)b	28.6(0.1)b	21.6(0.2)b	17.7(0.1)b
	1	63.9(1.0)b	39.2(0.4)b	28.8(0.2)b	21.9(0.1)b	17.8(0.1)b
	2	64.7(1.9)b	39.3(0.6)b	28.8(0.3)b	21.8(0.3)b	17.8(0.3)b
	10	78.6(1.0)a	46.9(1.2)a	32.6(0.4)a	24.6(0.6)a	19.4(0.3)a
	P > F	<0.001	<0.001	<0.001	<0.001	<0.001
	LSD	3.8	2.3	0.8	1.1	0.7
12	0	57.4(1.8)b	38.0(0.4)c	29.4(0.2)c	22.4(0.3)b	18.0(0.2)b
	1	56.1(2.6)b	40.0(0.3)b	29.6(0.2)c	22.0(0.2)b	17.7(0.1)b
	2	59.8(1.5)b	40.2(0.4)b	30.6(0.4)b	22.7(0.2)b	18.0(0.2)b
	10	78.2(1.4)a	50.2(0.3)a	34.6(0.3)a	25.8(0.4)a	19.8(0.3)a
	P > F	<0.001	<0.001	<0.001	<0.001	<0.001
	LSD	5.8	1.1	0.9	0.8	0.7

with organic phases, manure mineralization may have lead to the increased Cu content in month 12.

Increasing biochar application caused an increase in soil organic C content (Fig. 2A) because biochar is mostly C (66.2%; Table 1). Within each biochar treatment, the organic C content was similar across all sampling periods. After 12 months of incubation, the soil organic C content for the 1%, 2%, and 10% biochar rates were 143%, 181%, and 530% greater than the control; results were similar to

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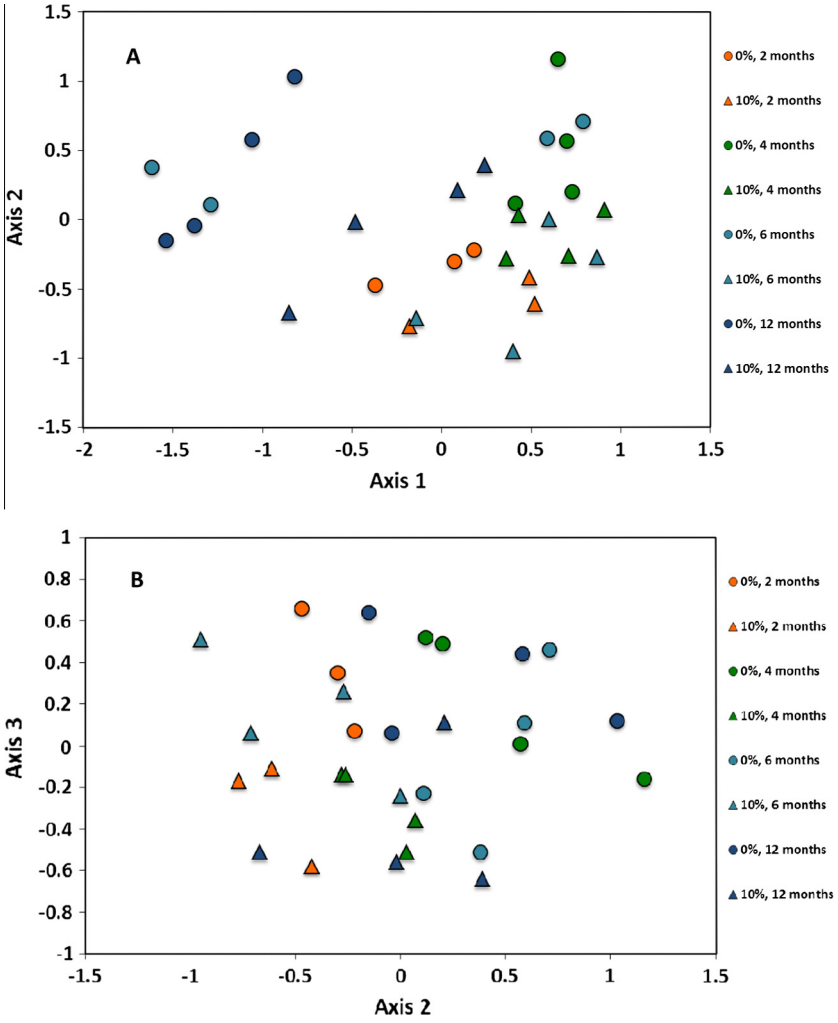

Fig. 3. The effect of increasing biochar application rate (% by wt.), applied with a constant rate of manure (2% by wt.), and time since application on soil microbial community FAME profiles, as determined by non-metric multidimensional scaling (NMS). Community profiles are displayed in multi-dimensional space, with Axis 1 vs. 2 in (A) and Axis 2 vs. 3 in (B).

that observed by other researchers (e.g. Rogovska et al., 2011; Bolan et al., 2012). It has been previously noted that some biochar-added C is available for microorganisms or biochar application can stimulate the degradation of easily degradable compounds present in the natural soil organic C (Hamer et al., 2004;Wardle et al., 2008).

3.4. Soil microbiological responses

In the presence of manure, biochar addition at the 10% rate altered soil microbial N cycling and respiration activities, but did not greatly affect bacterial abundance or microbial community structure. Soil NO_3-N concentrations were significantly lower at the 10% biochar application rates as compared to the other rates (Fig. 2B), at most sampling times, likely due to microbial immobilization and less net mineralization/nitrification. Biochar contained little NO_3-N (Table 1) and the C:N ratio was ~207:1, much greater than the assumed approximately 35:1 minimum ratio when immobilization response is observed (Borchard et al., 2012). However, at the 0%, 1%, and 2% biochar rates, immobilization was less prevalent most likely due to the 2% manure application supplying available N (Table 1).

Over time, soil NO_3-N increased with all biochar rates when applied with a 2% manure rate, likely because of mineralization and nitrification of manure N. Lentz and Ippolito (2012) applied biochar and manure at 22.4 and 42 Mg ha^{-1}, respectively, to the same soil in a field study. The authors noted a decrease in soil NO_3-N within the first year following co-application, followed by a slight increase in NO_3-N likely due to mineralization. In a laboratory incubation study where biochar was applied without manure, Ippolito et al. (2014) showed that the 10% biochar application rate dramatically lowered soil NO_3-N concentrations and prevented NO_3 from accumulating over time, which suggested that excessive applications would not be suitable for crop growth. In the current study the 10% biochar application rate, co-applied with a 2% manure application rate, likely allowed for some net mineralization and nitrification of manure N but limited excessive soil NO_3-N accumulation as compared to the 0%, 1%, and 2% biochar treatments. Thus, mixing biochar with manure, at the appropriate rates during land application, could potentially benefit producers who utilize manure yet observe increasing soil NO_3-N pollution; co-application may lead to more efficient N fertilizer use (Kameyama et al., 2012; Kammann et al., 2011; Chan et al., 2007).

Biochar increased microbial respiration activity only at the 1% application rate as compared to other rates when averaged over all months (Fig. 2C). It is interesting to note that the 10% biochar rate added 10 times as much biochar-borne C as compared to the 1% rate, yet respiration did not significantly increase over the 1%

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biochar treatment. In general, CO_2 production was least at month 1, rose to a maximum at month 2, and then decreased to an intermediate level through the rest of the incubation period. These findings were similar to that of Rogovska et al. (2011) who added up to 2% biochar to soil and showed increased CO_2 emissions over controls. Others have observed increased (Dempster et al., 2012; Quilliam et al., 2012) and decreased (Spokas et al., 2009) respiration rates associated with increasing biochar application rates.

Averaged over all months, the bacterial abundance decreased with the 1% biochar application as compared to the control or other biochar rates (Fig. 2D). Contrary to our findings, others (Lehmann et al., 2011; Cantrell et al., 2012) have suggested that biochar addition may increase bacterial numbers, possibly due to increases in macro- (e.g., K) or micro-nutrient availability or soil pH. Bacterial concentrations were maximized in months three and four; Ippolito et al. (2014) found a similar response when biochar was applied alone.

In the presence of manure, 10% biochar application caused only subtle shifts in the microbial community structure (Fig. 3A and B). These shifts were mainly attributed to greater percentages of two fatty acids associated with Gram-negative bacteria (17:1ω8c and 17:0 cy) and less percentages of four Gram-positive bacterial fatty acids (i15:0, a15:0 i16:0, and i17:0). Similar to this study, Pietikäinen et al. (2000) found the FAMEs 17:1ω8c and 17:0 cy were enriched within a biochar and in soil humus underlying the biochar, respectively. In the current study, the sum of the two Gram-negative bacterial FAMEs was 1.6% in control soil vs. 2.9% in the 10% biochar rate soil, whereas the sum of the Gram-positive FAMEs were 13.6% in control soil and 12.5% in 10% biochar rate soil. Although small (~1% change for each group of FAMEs), the differences based on biochar rate were statistically significant. Within each incubation period, the separation of microbial community structures based on biochar rate was evident across three dimensions or axes, as shown in Fig. 3A and B. However, the greatest separation of communities occurred along Axis 1 and was due to the effect of long incubation periods on control soil microbial communities (Fig. 3A). Shifts in community FAME profiles during the incubation were due to changes in a few FAMEs. First, the relative proportion of i17:1ω9c, a biomarker for anaerobic sulfate reducers (Vestal and White, 1989), increased over time, from 3.2% at 2 months, 3.7% at 4 months, 4.0% at 6 months, and finally 4.4% at 12 months. The changes between 2 and 4 months, and 4 to 12 months, were statistically significant. In addition, the ratio of FAMEs 19:0 cy-to-18:1ω7c increased significantly over time, from 0.22 at 2 months to 0.37 at 12 months. This ratio is utilized as an indicator of environmental stress, as bacteria synthesize cyclopropane fatty acids (e.g., 19:0 cy) from their monounsaturated precursors (e.g., 18:1ω7c) when starved of labile substrates, or exposed to heat, desiccation, or low pH (Grogan and Cronan, 1997). Thus, it appears that the long incubation period required a physiological adaptation of bacteria, presumably due to exhaustion of labile carbon substrates and oxygen availability.

The FAME data suggest a greater impact of incubation time than biochar co-applied with manure on microbial community structure, which contrasts with results found in a related incubation study where biochar was applied alone (Ippolito et al., 2014). In that study, biochar applied at a 10% rate caused a significant reduction of fungal FAMEs and increased the 19:0 cy-to-18:1ω7c over that of control soil, and these effects were sustained over time. Based on those results, Ippolito et al. (2014) concluded that biochar applied at a 10% rate had excessive negative impacts on a calcareous soil's microbial community. However, when co-applied with 2% manure as in this study, these effects were not observed. The relative proportion of fungal FAMEs was unchanged by biochar (31.3% in control and 30.8% in 10% biochar treatment), as was the 19:0 cy-to-18:1ω7c ratio (0.33 in control and 0.27 in biochar treatment). Thus, manure appeared to moderate the impact of a high application rate of biochar, so that only subtle effects were detected on bacterial populations.

4. Conclusions

Biochar co-application with manure to calcareous soil had several beneficial effects on soil properties. Co-application improved the soil water status, which would be considered beneficial in areas where irrigation or rainfall is limited. When co-applied with manure, a positive synergistic increase in soil-extractable Zn content occurred, which could also be beneficial in Zn-deficient calcareous soils. Previous research suggested that a biochar rate of 10% could be considered excessive and cause significant reductions in soil NO_3-N concentrations and shifts in microbial community structure; a 10% biochar application should be avoided. However, when 10% biochar was applied with 2% manure as in the current study, biochar reduced excess soil NO_3-N accumulation concentrations and thus may benefit producers by leading to more efficient N use. Furthermore, biochar–manure co-application lessened the shifts in microbial community structure, and thus co-application may help reduce significant shifts in ecosystem services associated solely with biochar application. Based on the above conclusions, we accepted our hypothesis that, in the presence of manure, lower biochar application rates (i.e., 1% and 2% by wt.) would not cause a negative priming effect, and we rejected our hypothesis that greater biochar application rates (i.e., 10% by wt.) would cause a negative priming effect. The combination of soil water and nutrient improvements, in conjunction with no major shift in microbial community composition, may actually have beneficial ecosystem services in arid and semi-arid environments.

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