



Designer, acidic biochar influences calcareous soil characteristics



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ARTICLE INFO

Article history:

Received 6 December 2014

Received in revised form 19 May 2015

Accepted 27 May 2015

Available online 12 June 2015

Keywords:

Biochar

Soil water

Micronutrients

Microbial enumeration

Nitrate–nitrogen

ABSTRACT

In a proof-of-concept study, an acidic (pH 5.8) biochar was created using a low pyrolysis temperature (350 °C) and steam activation (800 °C) to potentially improve the soil physicochemical status of an eroded calcareous soil. Biochar was added at 0%, 1%, 2%, and 10% (by wt.) and soils were destructively sampled at 1, 2, 3, 4, 5, and 6 month intervals. Soil was analyzed for gravimetric water content, pH, NO₃-N, plant-available Fe, Zn, Mn, Cu, and P, organic C, CO₂ respiration, and microbial enumeration via extractable DNA and 16S rRNA gene copies. Gravimetric soil water content increased with biochar application regardless of rate, as compared to the control. Soil pH decreased between 0.2 and 0.4 units, while plant-available Zn, Mn, and P increased with increasing biochar application rate. Micronutrient availability decreased over time likely due to insoluble mineral species precipitation. Increasing biochar application raised the soil organic C content and remained elevated over time. Increasing biochar application rate also increased respired CO₂, yet the CO₂ released decreased over time. Soil NO₃-N concentrations significantly decreased with increasing biochar application rate likely due to microbial immobilization or denitrification. Depending on application rate, biochar produced a 1.4 to 2.1-fold increase in soil DNA extracted and 1.4- to 2.4-fold increase in 16S rRNA gene abundance over control soils, suggesting microbial stimulation and a subsequent burst of activity upon biochar addition. Our results showed that there is promise in designing a biochar to improve the quality and water relations of eroded calcareous soils.

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1. Introduction

Biochar is the carbonaceous solid byproduct from thermochemical conversion of carbon-based organic materials that commonly contain elevated cellulose, hemicelluloses, or lignin contents (Spokas et al., 2012). The thermochemical conversion process is known as pyrolysis and occurs when carbon-containing substances are introduced to elevated temperatures in the absence of oxygen at varying residence times, yielding biochar. Pyrolysis temperature may be varied to design a biochar with specific end-product characteristics (Novak et al., 2014).

In general, increasing pyrolysis temperature tends to increase biochar total nutrient content, specific surface area, and pH. Increasing pyrolysis temperature increases loss of easily decomposable substances (Munoz et al., 2003; Kloss et al., 2012), volatile compounds (Cantrell et al., 2012), and elements such as O, H, N, S and thus concentrates other nutrients (e.g., C, Ca, Mg, K) (Antal and Grønli, 2003; Kim et al., 2012; Kinney et al., 2012). However,

pyrolysis conducted at specific temperatures can favor the accumulation of certain nutrients in biochar. For example, total N content tends to be maximized between 300 and 400 °C due to the presence of heterocyclic N-containing compounds (Cantrell et al., 2012), while total P content decreases above 760 °C due to volatilization (Knicker, 2007).

Increasing pyrolysis temperature removes acidic functional groups and causes biochar to become more basic (Novak et al., 2009b; Li et al., 2002; Ahmad et al., 2012; Cantrell et al., 2012). In three biochars studied, Enders et al. (2012) showed that as pyrolysis temperature increased from 300 to 600 °C, biochar pH increased. In addition, greater pyrolysis temperatures promote minerals such as KOH, NaOH, MgCO₃, and CaCO₃ to separate from the solid organic matrix, resulting in elevated pH values (Cao and Harris, 2010; Knicker, 2007). Although this would create a biochar well suited for acidic soil conditions because the biochar may act as a liming source, elevated pyrolysis temperatures would not create biochars conducive for use under arid soil conditions. Arid soils may, however, benefit from biochars with lower pH. Thus, an opportunity exists to develop biochars for the ~1 billion ha (Agrostats, 2009) of soils in arid and semi-arid climatic regimes globally.

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The inherent variability of biochars when used as a soil amendment suggests that the production of biochars can be designed for specific situations (Ippolito et al., 2012a). As an example, Novak et al. (2014) showed that nutrient enriched and nutrient poor feedstocks could be co-blended and pyrolyzed to produce a more nutrient-balanced biochar. In theory, this co-blended biochar can be used on soils without excessively adding plant-available nutrients. However, as outlined by Novak et al. (2014), the designer biochar concept is still in its infancy and requires further evaluation of designer biochar performance in other agricultural soils containing diverse fertility or physical characteristics.

To that end, we expanded the findings of Ippolito et al. (2012b) who suggested that a low pyrolysis temperature, low pH biochar could improve environmental quality by reducing nutrient losses in calcareous soils. Here, in a proof-of-concept study we designed a low pH, steam activated biochar for potentially improving the soil physicochemical status of an eroded calcareous soil in south-central Idaho. Topsoil in many locations of the area have been eroded due to ~100 years of flood irrigation, leaving the calcareous subsoil (pH 7.8–8.2; USDA-NRCS, 2001) exposed. Subsoil organic C content has been measured as about half of the soil surface (0.45% vs 0.94% organic C; Robbins et al., 2000) in this area of south-central Idaho. Lower organic matter content in eroded soils has been shown to significantly reduce available soil water content as compared to non-eroded soils (e.g., Frye et al., 1982). Thus, our hypotheses were that increasing application rates of an acidic pH biochar to an eroded calcareous soil will (1) improve the soil water status by reducing evaporative losses, (2) lower soil pH and (3) increase plant nutrient availability. In theory, an acidic pH designed biochar could neutralize excess soil OH⁻ groups and thus increase micronutrient concentration by increasing the dissolution of micronutrient carbonate mineral phases.

2. Materials and methods

2.1. Initial soil analysis, biochar analysis, and experimental setup

The experimental setup and design has been described elsewhere (Ducey et al., 2013). Briefly, subsoil was obtained near Kimberly, Idaho (42° 31' 07.50" N, 114° 22' 33.50" W), was classified from the Portneuf series (coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcid), and was part of an eroded soil experiment whereby the topsoil (0–30 cm) was removed (Robbins et al., 1997, 2000; Lentz et al., 2011). The top 30 cm of exposed subsoil was collected, air-dried, and passed through a 2-mm sieve. Initial soil analysis included pH (Thomas, 1996) and EC (Rhoades, 1996) using a 1:1 soil:deionized water extract, NO₃-N using a 2 M KCl extract (Mulvaney, 1996), and total C and N by dry combustion (Nelson and Sommers, 1996). Soil inorganic C was determined using a modified pressure–calimeter method (Sherrod et al., 2002) and organic C was determined by difference between total and inorganic C. The amount of CaCO₃ present in the soil was determined by converting inorganic C to CaCO₃. Soil total elemental concentrations were determined by HClO₄–HNO₃–HF–HCl digestion (Soltanpour et al., 1996) followed by analysis using inductively coupled plasma atomic emission spectrometry.

Biochar feedstock was full maturity switchgrass (*Panicum virgatum*), dried at 40 °C, and then hammer milled to pass a 6-mm sieve. Switchgrass was pyrolyzed at 350 °C under N₂ gas, then steam activated at 800 °C and allowed to cool down to room temperature; for more detailed information regarding biochar production see Ducey et al. (2013). Lower pyrolysis temperatures (e.g., 350 °C) helps retain acidic functional groups and lower ash contents, causing biochar to be more acidic (Ahmad et al., 2012; Cantrell et al., 2012; Enders et al., 2012; Novak et al., 2009b). Steam activation

can remove tar-like compounds with a subsequent increase in surface area (e.g., Borchard et al., 2012), and thus may lead to an increase in nutrient retention. Biochar surface area was determined by N₂ adsorption isotherms using the Brunauer, Emmett, and Teller (BET) equation (Brunauer et al., 1938), pH was determined in deionized water (1% w v⁻¹; Novak et al., 2009b), EC via a saturated paste extract (Rhoades, 1996), and total C and N, NO₃-N, and total elements as previously described. Soil and biochar physicochemical characteristics are presented in Table 1.

Switchgrass biochar was thoroughly mixed into soil (300 g), by hand, at 0%, 1%, 2%, or 10% (by wt.), and a completely randomized experimental design with four replicates was utilized; six sets of four replicates of each treatment were created for destructive sampling, described below. Although likely not conducive for use in production agricultural settings, the 10% biochar application rate was utilized in order to identify upper level benefits or detriments to the soil. Soil and biochar mixtures were placed in 8 cm × 8 cm × 8 cm plastic pots lined with a plastic liner to inhibit leaching. All pots were then placed in a constant temperature growth chamber (22 °C, 30% humidity) and watered twice weekly with reverse osmosis-treated water to bring all pots to 80% of field capacity (by wt.). Field capacity was determined prior to the experiment by using four of the plastic pots lined with cheesecloth and filled with 300 g soil only (no biochar), saturated, and allowed to freely drain for 48 h. Soil bulk density was also determined after mixing biochar into soils by filling a 100 mL graduated cylinder with the soil mixture, tapping the cylinder gently on a countertop, and topping off the cylinder with additional soil mixture. A soil–biochar-mixture weight per unit volume was then determined.

2.2. Destructive soil sampling

Pots were destructively sampled at 1, 2, 3, 4, 5, and 6 month intervals. At time of sampling, soils were analyzed for pH, and NO₃-N (as previously described), and for available Fe, Zn, Mn, and Cu using a diethylenetriaminepentaacetic acid (DTPA) extraction (Lindsay and Norvell, 1978). Substrate induced CO₂ respiration was determined by thoroughly mixing 50 g of moist soil, 0.5 g of glucose, 0.01 g of K₂HPO₄, and 0.075 g of NH₄Cl in a 100 mL mason jar, as described by Dungan et al. (2003). A vial containing 5 ml of 1 M NaOH was placed inside the jar and the jars were sealed. Following 24 h of incubation at room temperature the vials were removed, excess BaCl₂ was added to the NaOH, phenolphthalein indicator was added, and the NaOH was titrated to a clear endpoint with 1 M HCl; measurements were made in duplicate for all soils.

Table 1

Properties and total elemental analysis of the switchgrass biochar and Portneuf subsoil.

Property	Units	Switchgrass biochar	Portneuf subsoil
Surface area	m ² g ⁻¹	219	ND
pH		5.8	7.6
EC	dS m ⁻¹	0.70	0.77
Ash	%	5.86	ND
Total C	%	88.0	2.98
Inorganic C	%	ND ^a	2.02
Organic C	%	ND	0.96
CaCO ₃	%	ND	16.8
Total N	%	0.68	0.08
Organic N	%	0.68	0.08
NO ₃ -N	mg kg ⁻¹	2.6	18.1
P	mg kg ⁻¹	700	300
Fe	mg kg ⁻¹	100	7000
Zn	mg kg ⁻¹	10.3	28.0
Mn	mg kg ⁻¹	64.6	220
Cu	mg kg ⁻¹	3.4	4.83

^a ND = not determined.

Gravimetric soil water content, on day of sampling, was determined to convert the above soils data to a dry weight basis, and to identify potential changes in soil water status and losses due to evaporation associated with biochar treatment. After the above sampling occurred, soils were then 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, and a subsample was pulverized and analyzed for total, inorganic, and organic C as previously mentioned. Soils were also analyzed for Olsen (NaHCO₃)-extractable P (Kuo, 1996).

2.3. Soil DNA extraction

For DNA extraction, two pairs of the four replicate samples from each biochar treatment and month were composited, resulting in 48 samples (two samples per treatment and month). A total of 0.5 g of soil from each composite sample was used for microbial DNA extraction using a PowerLyzer PowerSoil DNA Isolation kit (MO BIO Laboratories, Inc., Carlsbad, CA), according to manufacturers specifications. Measurement of DNA concentration and purity were determined by 260/280 nm and 260/230 nm measurements respectively, using a Nanodrop spectrophotometer (Nanodrop Technologies, Wilmington, DE).

Quantitative Real-Time polymerase chain reaction assays (qPCR) were used to measure 16S rRNA gene copy numbers. Assays were carried out using SYBR GreenER qPCR SuperMix (Invitrogen, Carlsbad, CA) in a total reaction volume of 25 μ L. Final reaction concentrations of reagents consisted of 1X SYBR GreenER qPCR SuperMix, 200 nM each of the primers 515F (5'-TGCCAGCAGCCGCGGTAA-3') and 927R (5'-CTTGTGCGGGCCCCCGTCAATTC-3'), and 1 μ L of a 1:100 dilution of DNA template. The qPCR reaction conditions were conducted as follows: (i) an initial denaturation at 95 °C for 5 min; (ii) 50 cycles of denaturation at 95 °C for 30 s, annealing at 55 °C for 30 s, and elongation at 72 °C for 30 s; (iii) melting curve analysis to confirm amplification product specificity. Fluorescent measurements were taken during the annealing phase of each cycle. All qPCR assays included negative controls without template, as well as reactions containing between 10¹ and 10⁹ DNA copies to generate standard curves and calculate amplification efficiencies according to the equation: $E = 10^{[1/\text{slope}]}$ (Pfaffl, 2001). DNA standards consisted of linearized plasmids carrying the appropriate target gene (Hou et al., 2010), which were sequenced to confirm their identity and primer binding site. Each assay was performed in triplicate, with triplicate measurements for each sample.

2.4. Statistics

An analysis of variance was performed on all soil data using Proc GLM model, SAS software version 9.2 (SAS Institute, 2008). The completely randomized design factorial model included

treatment, month, and their interaction as dependent variables. We tested our hypotheses using an $\alpha = 0.05$, and mean separation was calculated using a Fisher's Protected Least Significant Difference (LSD; Steel and Torrie, 1980) when significance was observed within treatments or between time intervals.

3. Results and discussion

3.1. Interpretation of statistical analysis

Biochar typically caused an increase in a given soil constituent, and the effect decreased over time; the opposite was observed for soil pH and NO₃-N. Significant treatment \times time interactions were observed for most variables studied, yet the interaction on soil variables was not as strong as the effect of biochar rate or time since application. Meaning, we did not observe drastic changes in soil response due to biochar rate over the course of the study. Thus, interaction effects are presented, but for ease of discussion the focus is on main effects only.

3.2. Soil water

Gravimetric soil water content tended to increase with biochar application regardless of rate as compared to the control, and the response was consistent over time (Table 2). The data suggests that evaporative losses were reduced (because no leaching or transpirational losses could have occurred) with biochar application regardless of application rate, and thus we accepted our initial hypothesis. Ippolito et al. (2014a,b) observed a similar response to the 1% and 2% biochar observations when hardwood biochar was applied at the same rates to soil collected from the same location. Novak et al. (2012) also found a similar response when 2% switchgrass biochar (by wt.) was incubated in two Aridisols. The authors noted a 3–7% increase in soil water content as compared to soils not receiving biochar, and the response was consistent over 127 days of incubation. Struebel et al. (2011) showed that water holding capacity increased in western US silt loam soils following increasing biochar application rates (0.4%, 0.75%, and 1.5% by wt.). The increase in soil water content in the current and other studies was likely associated with the large surface area and porosity of biochar (Bruun et al., 2012). Low temperature switchgrass biochar surface area was 219 m² g⁻¹ (Table 1); biochar surface areas can range from less than 1 m² g⁻¹ (Bruun et al., 2012) to over 600 m² g⁻¹ (Rajkovich et al., 2012). Surface area tends to increase with increasing pyrolysis temperatures (e.g., Ahmad et al., 2012; Kloss et al., 2012; Chen et al., 2008, 2012). However, the low pH switchgrass biochar created for the current study was pyrolyzed at a relatively low temperature (350 °C), and thus may not have initially resulted in elevated surface area. It should be noted, however, that steam activation, as performed in the current study, can

Table 2
Portneuf subsoil moisture content (%) prior to destructively sampling at each month, and all months combined. Values within parentheses represent one standard error of the mean. Different lowercase letters within a row represent a significant difference at $p = 0.05$, based on mean separation as determined by Fisher's protected least significant difference (LSD).

Month	Biochar application rate (%)				LSD
	0	1	2	10	
Soil moisture content (%)					
1	8.3 (1.0)a	12.7 (0.7)ab	15.3 (1.4)b	11.9 (1.9)ab	4.6
2	8.2 (0.3)a	13.0 (1.0)c	13.9 (0.6)c	10.5 (0.2)b	1.6
3	11.7 (0.2)a	15.2 (0.4)b	14.1 (0.4)b	12.4 (0.5)a	1.3
4 ^A	3.2 (0.2)a	5.2 (0.5)b	5.1 (0.6)b	7.8 (0.8)c	1.7
5	10.8 (0.7)a	12.3 (1.2)ab	14.2 (0.7)b	14.2 (1.3)b	2.4
6	8.1 (0.6)a	11.9 (0.9)b	11.0 (0.6)b	12.3 (0.3)b	1.8
All months combined	8.4 (1.5)a	11.7 (1.7)b	12.3 (1.9)b	11.5 (1.4)b	1.9

^A Month 4 pots were likely not watered at the same time period prior to destructive sampling as the other months.

remove low-volatile tar constituents with a concomitant increase in surface area (e.g., Borchard et al., 2012). In addition, steam activation may have exposed hydrophilic surface functional groups which are theorized to increase the extent of water sorption (Novak et al., 2012). Thus, we expected that steam activation would have created greater biochar surface area and increased hydrophilic group exposure, leading to the increase in gravimetric soil water content in biochar-receiving soils.

It is important to note that although not determined throughout the study, the initial soil bulk density values for the 0%, 1%, 2%, and 10% biochar application rates were 1.03, 0.93, 0.91, and 0.70 g cm⁻³, respectively. Decreases in soil bulk density with increasing organic carbon additions, and the effect on the soil water status, has been reported by Bauer and Black (1992). The authors noted that gravimetric water content changed very little from field capacity to wilting point as soil organic carbon content increased, yet the bulk density decreased with increasing soil organic carbon. Our finding, along with the support of Bauer and Black (1992), suggests that volumetric water content (i.e., gravimetric water content X bulk density) possibly decreased with increasing biochar application rate, and so soils at greater biochar application rates likely could retain greater quantities of water as compared to lower biochar application rates. Further research is needed to evaluate the influence of biochar on soil water relations.

The above findings may have important implications for arid ecosystems, as more than half the precipitation in arid and semi-arid regions can be directly lost to the atmosphere via soil surface evaporation (Brady and Weil, 1999). Thus, biochar's effect at improving the gravimetric soil water content may be of value to arid region crop producers where rainfall quantities are low and reliance on irrigation is high to meet evapotranspirational losses. Furthermore, as pointed out by Spokas et al. (2012), a single biochar application may provide long-term improvements in soil water content and a potential reduction in irrigation costs; the cost of biochar application could be amortized over several years.

3.3. Soil pH and plant-available nutrients

Data presented in Fig. 1A did not support our hypotheses that calcareous soil pH would decrease with increasing application rate of low pH biochar. Discounting the short-lived response in month 1, soil pH varied by only 0.2 units. Ippolito et al. (2012b) added a low pH biochar at 2% (by wt.; no steam activation) to two Aridisols, noting a significant decrease in soil pH. However, Ippolito et al. (2014a) observed a slight increase in soil pH with increasing hardwood biochar application rate in Portneuf topsoil. The authors suggested that the buffering capacity of soil from their research site prevented major pH changes; this likely occurred to some extent in the current study. Soil used in the study contained 2.02% inorganic C (Table 1), which would equate to 16.8% CaCO₃; similar to the suggestion of Ippolito et al. (2014a), this was likely enough to buffer the soil pH.

Increasing biochar rate increased both soil DTPA extractable Zn and Mn concentrations (Fig. 1B and C). Observations were likely due to biochar Zn and Mn being in readily available forms. In the case of Zn, however, biochar unfortunately did not raise Zn concentrations above that considered marginal for certain crops (1.0 mg kg⁻¹ available Zn; Davis and Westfall, 2009; Davis et al., 2009). Obviously, the change in soil pH did not positively affect Zn availability; this is especially noticeable for Zn as compared to soil pH in month 1. A previous study by Lentz and Ippolito (2012) observed increased Mn availability when biochar was applied to topsoil from this field location. Ippolito et al. (2012b) suggested that Mn was selectively sorbed on low pH switchgrass biochar exchange sites. Selective Mn sorption by biochar has also been suggested by others (Novak et al., 2009a). Over time, Zn

and Mn concentrations decreased; plant-available Fe and Cu content also decreased with time (data not shown). Over time decreases were likely due to mineral forms becoming less available (Ippolito et al., 2014a). DTPA-extractable Fe was not affected by biochar application rate; DTPA-extractable Cu did increase with increasing biochar application rate, but Cu concentrations rarely exceeded 0.90 mg kg⁻¹ (data not shown). Based on the pH, Zn, and Cu observations, it would not be recommended to apply this type of biochar to this soil, or similar soils, in order to reduce soil pH and improve Zn or Cu availability.

Olsen-extractable P concentration increased slightly, but significantly, with increasing biochar application rate (Fig. 1D). As with Zn and Mn, P must have been slightly available in the biochar. This finding supports previous research indicating that biochar-borne P can contribute to plant-available nutrient forms (Schnell et al., 2012; Ippolito et al., 2012b). As with Zn and Mn, the P content decreased over time for the 0%, 1%, and 2% biochar application rates likely due to mineral forms becoming less available. In contrast, the 10% application rate maintained or increased P content over time. Borchard et al. (2012) applied up to an equivalent of ~30 Mg ha⁻¹ of beech wood (*Fagus* sp.) biochar to two different soils, but did not affect available soil P; Jones et al. (2012) observed a similar response when hardwood biochar was applied at up to 50 Mg ha⁻¹. Laird et al. (2010), however, observed increases in plant-available soil P content with increasing hardwood biochar application rate, with greater biochar rates (equivalent of up to ~40 Mg ha⁻¹) reducing P leaching. The authors speculated that P was being bound by oxyhydroxides in the biochar. As compared to lower biochar application rates, the 10% application rate likely supplied greater quantities of bound, readily available P, that was easily removed with the Olsen extracting solution.

3.4. Soil organic carbon, respiration, and nitrate-N

Biochar utilized in the current study had a C content of 88%, thus increasing biochar application caused a proportional increase in soil organic C content (Fig. 2A). Following the 6 month incubation period, soil organic C content for the 1%, 2%, and 10% biochar rates were 1.4-, 1.9-, and 6.0-times greater than that of the control. Results were similar to Ippolito et al. (2014a,b) who used hardwood biochar in a similar soil. Results were also comparable to others (e.g., Rogovska et al., 2011; Bolan et al., 2012). The majority (but not all) of biochar added C was either recalcitrant or binding to the natural soil organic C present, as soil organic C content did not decrease over time. This process has been suggested by Lentz and Ippolito (2012). However, opposite findings have been observed by Ippolito et al. (2014b), Wardle et al. (2008), and Hamer et al. (2004), implying that certain biochars may stimulate the decomposition of natural soil organic C.

Both disturbance and drying of the field soil caused some soil C to be utilized by microorganisms, or only a small fraction of C from the low pH switchgrass biochar was immediately available for microorganisms, because CO₂ concentrations at month 1 were greater than all other months (Fig. 2B). This result is consistent with Smith et al. (2010) who demonstrated that a labile C pool was rapidly consumed by microorganisms immediately following biochar application. Ippolito et al. (2014a,b) observed a longer lag response when hardwood biochar was added to a similar soil as used in the current study, suggesting greater recalcitrance. The CO₂ production was almost always greater in all biochar treatments as compared to the control, and similar to previous findings. Rogovska et al. (2011) also showed that biochar application (up to 2% by wt.) increased CO₂ production as compared to a control. As compared to control soils, Awad et al. (2012) measured a

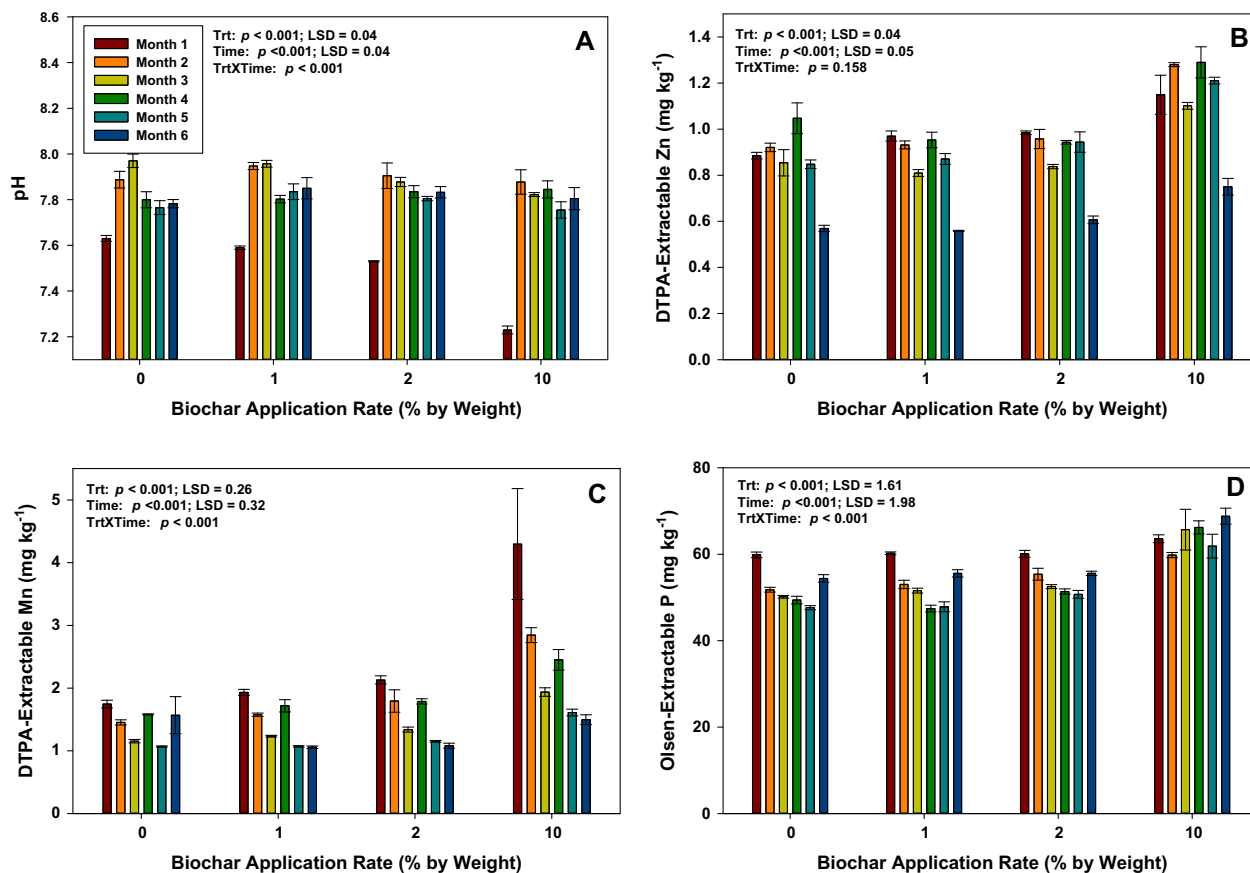


Fig. 1. The effect of increasing biochar application rate and time since application on soil (A) pH, (B) diethylenetriaminepentaacetic acid (DTPA)-extractable zinc, (C) DTPA-extractable manganese, and (D) Olsen-extractable P. Error bars represent one standard error of the mean ($n = 4$). LSD = Fisher's Protected Least Significant Difference.

cumulative CO₂ increase of ~8–18% even with biochar applied at a rate equivalent to 0.22% by wt. to sandy and sandy loam soil.

Soil NO₃-N concentrations significantly decreased with increasing biochar application rate (Fig. 2C). Biochar contained relatively low concentrations of total N and NO₃-N (Table 1) and may have caused microorganisms to immobilize NO₃-N from the soil. In support of this contention, the biochar C/N ratio was ~130/1, much greater than the assumed 20/1 to 30/1 ratio where immobilization and mineralization responses are assumed equal. Biochar-induced immobilization has also been suggested by others when using comparable biochar application rates (Ippolito et al., 2014a,b; Sarkhot et al., 2012; Lentz and Ippolito, 2012; Shenbagavalli and Mahimairaja, 2012). Ippolito et al. (2012b) showed that a 2% (by wt.) application of low pH (5.4) switchgrass biochar reduced NO₃-N leaching in two Aridisols as compared to high pH (8.0) switchgrass biochar. The authors suggested that the ease at which microorganisms could utilize the added C source was much greater with the lower pH biochar, causing greater immobilization. In addition to the potential for immobilization occurring, denitrification may have also been responsible for the reduction in soil NO₃-N with increasing biochar application rate. Ducey et al. (2013) used qPCR to measure gene abundances associated with N cycling in biochar-amended soils from the current study. The authors showed that the 10% biochar application rate caused a significant increase in relative abundance of genes associated with denitrification as compared to lower biochar rates. Further research is obviously needed to identify whether immobilization or denitrification dominates in this and potentially other systems.

It was obvious that the 10% biochar application rate was excess (Fig. 2C); this has also been observed for hardwood biochar applied

at identical rates to a similar soil (Ippolito et al., 2014a). This finding suggests that over-application would not be suitable for crop growth in N limited systems (Ippolito et al., 2014a). However, greater biochar application rates could lead to greater N use efficiency when applied in combination with N fertilizers (Chan et al., 2007; Kammann et al., 2011; Kameyama et al., 2012). Excess biochar application rates may also suggest its use for reducing excess NO₃-N in the environment, when applied in controlled, concentrated locations. For example, biochar could potentially be used in a fashion similar to that shown by Moorman et al. (2010) where a C source (wood chips) was buried near tile drains, promoting denitrification and thus preventing NO₃-N from entering water bodies.

3.5. Microbial enumeration

Utilizing DNA as a measure of microbial biomass has been previously reported (Marstorp et al., 2000; Taylor et al., 2002), showing a strong correlation with other microbial biomass calculations when performed in agricultural soils with traditionally low organic C (Leckie et al., 2004), such as the Portneuf soil examined here. Mean values of DNA ($\mu\text{g g}^{-1}$ soil) extracted from soils amended with varying rates of biochar are shown in Fig. 3. The DNA amounts extracted from biochar amended soils exceeded that of the control, by 1.4 \times (1% biochar), 1.6 \times (2% biochar), and 2.1 \times (10% biochar). Higher yields of extractable DNA from soils with increasing rates of biochar amendment suggest a comparative stimulation of microbial growth by these additions. This parallels results of Kolb et al. (2009) who reported a correlation between microbial biomass and charcoal application rates. These findings are also

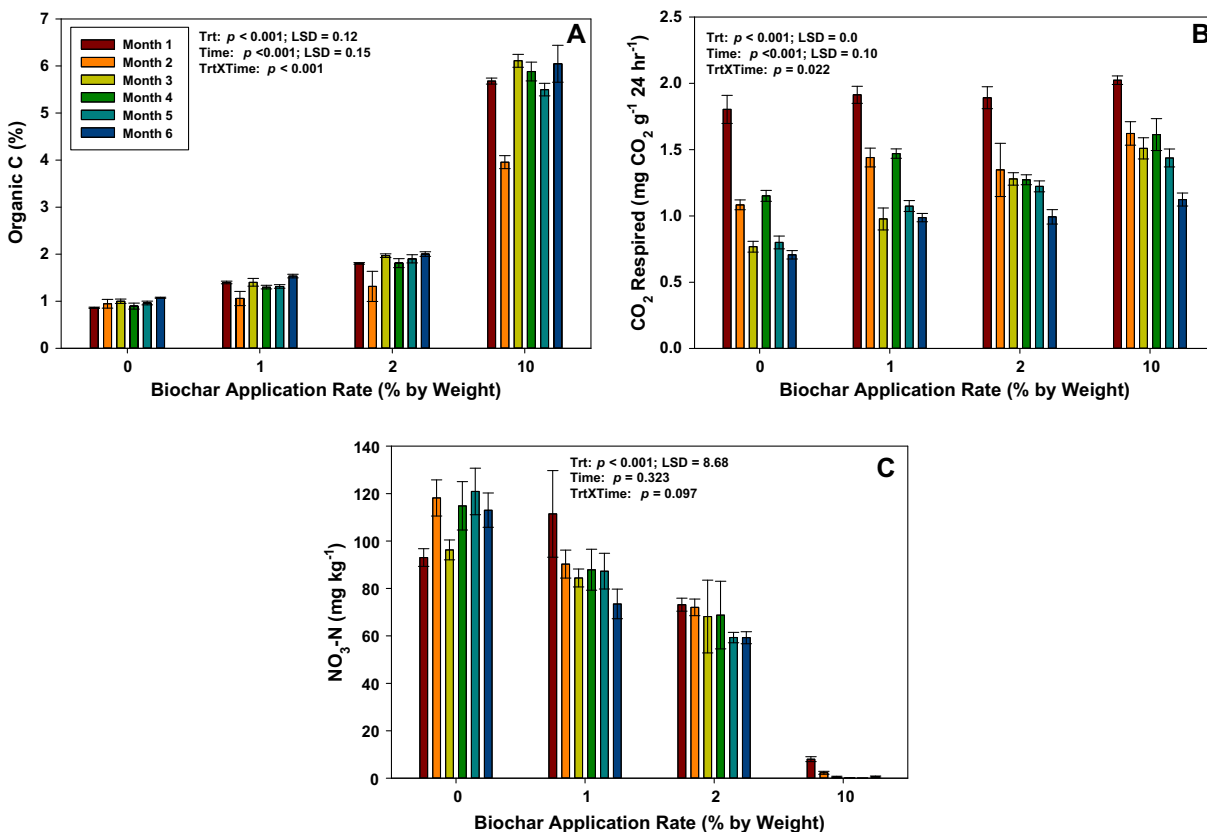


Fig. 2. The effect of increasing biochar application rate and time since application on soil (A) organic C, (B) respired CO₂, and (C) NO₃-N. Error bars represent one standard error of the mean (n = 4). LSD = Fisher's Protected Least Significant Difference.

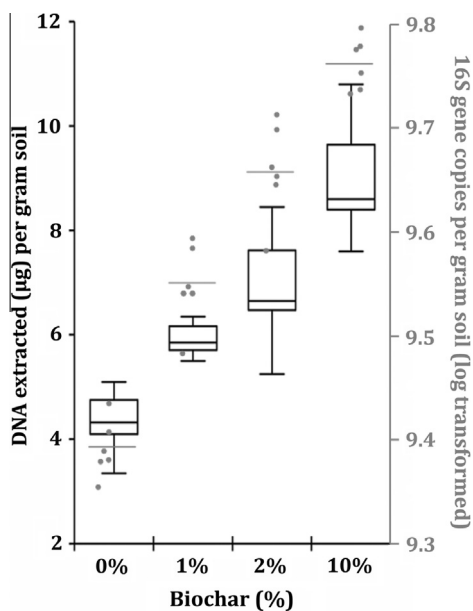


Fig. 3. Box and whisker plot showing concentration (µg) of extracted DNA/g⁻¹ soil (primary Y axis), and dot plot showing 16S rRNA gene copies/g⁻¹ soil (secondary Y axis) for each biochar amendment. All treatments are statistically significant (P < 0.001). Mean lines were calculated for DNA concentrations (n = 12) and 16S rRNA gene copies (n = 144). For 16S rRNA gene copies, each dot represents an averaged value for one month of the study (n = 24).

reflected in the previously reported abundances of 16S rRNA gene copies (Ducey et al., 2013), and are shown in Fig. 3. Abundances for the 16S rRNA gene increased 1.4-fold for 1% biochar, 1.9-fold for 2%

biochar, and 2.7-fold for 10% biochar over the control soil. As previously reported (Ducey et al., 2013), statistically significant separation occurred between all treatments by the first month, which suggests a burst of activity upon biochar addition. This hypothesis is supported by the reported respiration rates (Fig. 2B) which showed the highest CO₂ emissions in month one of the study.

4. Conclusions

The research goal was to design a biochar to improve exposed calcareous subsoil physicochemical characteristics. Specifically, a switchgrass biochar was designed to lower soil pH and increase micronutrient availability, and improve the soil water status. The use of this specific biochar partially met some of these objectives by slightly lowering soil pH and initially increasing micronutrient availability; thus, we accepted both of our original hypotheses. However, pedogenic free carbonates in the soil strongly buffered biochar's influence on soil pH regardless of biochar rate. This likely diminished the beneficial effects of biochar on soil micronutrient availability. Based on the application rates and considering a production agricultural setting, applying up to 10% biochar would not be recommended due to drastic reductions in soil NO₃-N. However, this application rate may help reduce excess NO₃-N concentrations in areas where NO₃-N is in excess. In situations where irrigation or rainfall may be limited, it appears that a low temperature switchgrass biochar application may be beneficial for improving the gravimetric soil water content, and we accept our original hypothesis. The change in soil water status may be the single most important aspect of applying biochars in arid, calcareous systems. In the future, it may be prudent to utilize a different designer biochar in exposed, eroded calcareous subsoils, or a

different calcareous soil for biochar application. Regardless, this research shows that the concept of designing specific biochars for specific uses has merit.

Acknowledgements

This publication is based on work supported by the US Department of Agriculture, Agriculture Research Service. The authors thank all parties responsible for biochar manufacturer, characterization, and laboratory analyses.

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