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Corn and hardwood biochars affected soil microbial community and enzyme activities

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Abstract

Biochar has gained interest as a soil amendment to improve soil quality and as means to help mitigate climate change. With the recent focus given to the soil as a living system and the essential functions it provides, knowledge of different effects of biochar on the microbial community is critical. A laboratory incubation (120 d) study was conducted on a Bennington silt loam (fine, illitic, mesic Aeric Epiaqualf) amended with corn (*Zea mays* L.) and hardwood biochars produced under slow pyrolysis. Biochars were analyzed for their chemical and physical properties and were added to the soil on a C content basis without exceeding 2.5% w/w. Microbial community abundance and composition were evaluated by phospholipid fatty acids (PLFA) analysis, and potential enzyme activities by β-glucosidase, and fluorescein diacetate (FDA) hydrolysis. There were no significant differences in the abundance of saprophytic fungi or bacteria in samples incubated with biochars when compared to the control. However, soils incubated with corn biochar had significantly ($P < .05$) higher abundance of Actinobacteria markers than hardwood biochar. The FDA hydrolysis did not show significant differences between soils incubated with biochar when compared with the control. Conversely, the β-glucosidase activity was significantly higher ($P < .05$) in soils incubated with either biochar than in control. Since biochar can influence changes in microbial community composition and enzyme activity it may influence cellulose degradation and soil organic matter dynamics in the agricultural soil evaluated.

1 | INTRODUCTION

Biochar, a carbonaceous material produced by pyrolysis, has received interest as a soil amendment to improve soil quality for the past decades to replicate the soil fertility and C sequestration observed in *Terra Preta* in the Amazon region (Glaser, Haumaier, Guggenberger, & Zech, 2001; Lehmann, Gaunt, & Rondon, 2006). Its potential to mitigate climate change due to its resistance to microbial degradation, limiting the amount of C released to the atmosphere as CO_2 has been highlighted (e.g., Lehmann et al., 2006; Sohi, Krull, López-Capel, & Bol, 2010). Furthermore, the effects of biochar on soil chemical and

Abbreviations: CB, corn biochar; EC, electrical conductivity; FDA, fluorescein diacetate; HB, hardwood biochar; PLFA, phospholipid fatty acids; PNP, *p*-nitrophenol; SEM, scanning electron microscopy; SOM, soil organic matter.

physical properties have been demonstrated. For example, it can improve soil fertility by increasing soil pH, cation exchange capacity (CEC), organic C, and reducing tensile strength (e.g., Chan, Van Zwieten, Meszaros, Downie, & Joseph, 2008; Liang et al., 2006; Novak et al., 2012) and change soil bulk density, improve saturated hydraulic conductivity and water infiltration (Major, Lehmann, Rondon, & Goodale, 2010). Furthermore, through designer biochar (Novak et al., 2009) this material could be tailored to ameliorate specific problems affecting soil quality (Novak, Cantrell, Watts, Busscher, & Johnson, 2014; Novak et al., 2019). However, much less is known about the influences of biochar on soil biology as both increases and decreases in microbial abundance and metabolic activity after the application of biochar have been reported. Furthermore, as recently reviewed by Palansooria et al. (2019), the biochar–microbe relationship has remained poorly integrated and knowledge is still limited despite the increasing number of studies.

Focus on the living component of soil by new soil health and conservation initiatives has brought attention to different management effects. For instance, biochar may increase abundance of microorganisms by serving as a habitat (Jaafar, Clode, & Abbott, 2014; Pietikainen, Kiikkila, & Fritze, 2000) where filamentous microbiota infiltrate via large pores (Hockaday, Grannas, Kim, & Hatcher, 2007), and providing surface area for colonization and allowing the utilization of labile C (Luo et al., 2017). However, this response depends on the type of biochar and pyrolysis conditions. Biochar addition has increased microbial abundance biomass C and enzyme activity (EA) in soil mesocosms treated with biochar when compared to the control (Ameloot et al., 2013; Luo et al., 2017). For example, Luo et al. (2017) reported increases in microbial abundance via phospholipid fatty acid analysis (PLFA) in soils treated with *Miscanthus* biochar produced at 350 °C, but not for the biochar that was produced at 700 °C. Ameloot et al. (2014) found a significant decrease in EA in soils treated with biochar, while O'Toole et al. (2018) found no changes in microbial biomass after 4 yr of application of *Miscanthus* biochar. Application rates of biochar to soil can adversely affect soil microorganisms by reducing both their activity and abundance (Ameloot et al., 2014; Palansooria et al., 2019). This is of concern because the mineralization of soil organic matter (SOM) is carried out by a large community of microorganisms and involves a wide range of metabolic processes. Thus, a decrease in microbial diversity may reduce the biological functionality of the soil (Coleman, 1993; Delgado-Baquerizo et al., 2016). As reviewed by Lehmann et al. (2011), the effects of biochar on soil biota may be driven by its physical and chemical properties suggesting that differences in the physical structure between biochar and soil matrix can alter soil properties such as tensile strength and transport of water and gas, all of which can impact soil microorganisms. Studies with biochar are needed due to the unique interactions associated to biochar type, pyrolysis conditions used, and rate of application, with each soil type and its inherent microbial community.

Core Ideas

- Soils with corn biochar had higher microbial abundance than with hardwood biochar.
- Corn biochar increased β-glucosidase activity by 25% when compared with the control.
- FDA hydrolytic activity was not affected by either biochar.

Microorganisms and the enzymes they produce, play an essential role in biogeochemical cycling, SOM dynamics, and overall soil health and productivity. For example, fluorescein diacetate (FDA) hydrolysis has been proposed as an indicator of overall enzymatic activity in the soil because it is mediated by different enzymes including esterases, lipases, and proteases (Green, Stott, & Diack, 2006; Prosser, Speir, & Sttot, 2011). Through these enzymes, different sources of C and N, as well as fats (lipids) become available nutrients for plants and soil microorganisms. Similarly, β-glucosidase has been a sensitive indicator of C cycling capable of responding to various management practices (Eivazi & Tabatabai, 1988; Deng & Popova, 2011). Thus, the objective of this study was to determine the effect of corn (*Zea mays* L.) and hardwood biochars (HB) on potential enzyme activities, microbial abundance, and community structure in an Ohio agricultural soil under laboratory conditions. This was achieved through the analysis of the enzyme activities of fluorescein diacetate hydrolysis and β-glucosidase, and by PLFA analysis.

2 | MATERIALS AND METHODS

2.1 | Soil description

The studied soil was a Bennington silt loam (fine, illitic, mesic Aeric Epiaqualf) with pH 7.6 and 1.8% C, from an agricultural farm located in eastern Delaware County, Ohio. The farm grows corn and glyphosate [*N*-(phosphonomethyl)glycine]-resistant soybean [*Glycine max* (L.) Merr.] rotation under no-till. Glyphosate is applied up to three times per year while growing soybean, and once per year when cultivating corn. The soil

TABLE 1 Physicochemical properties of corn and hardwood biochar

Biochar	Ash	C	N	EC	pH	SA	Yield
	%			mS cm^{-1}		10^3 m^2 kg^{-1}	%
Corn	28 (± 0.5)[a]	48 (± 4.8)	3.4 (± 0.2)	4.0 (± 0.2)	9.2	229 (± 2)	35
Hardwood	0.8 (± 0.03)	77 (± 2.9)	nd	0.2 (± 0.01)	8.2	388 (± 9)	27

Note. EC, electrical conductivity; SA, surface area; nd, not detected.
[a]Numbers in parentheses represent the standard deviation of $n = 3$.

samples were randomly collected after corn harvest at the 0- to 10-cm depth with probes (2.5 by 20 cm), composited, sieved (2 mm), and stored in sealed bags at 4 °C.

2.2 | Pyrolysis and physicochemical characterization of biochars

Biochars were produced from corn stover (corn biochar, CB) from Waterman Research Lab in Columbus, OH, and repurposed hardwood (HB). These materials were first dried at 40 °C for 1 wk, cut into 2.5-cm pieces and combusted at atmospheric pressure in a commercial electric furnace. They were charred at a heating rate of 5 °C min^{-1} up to 450 °C with furnace residence time of 5 h. After pyrolysis, biochars were kept in sealed containers. All analyses were performed in triplicate unless otherwise indicated. The biochar yield was determined as the proportion of the weight of pyrolyzed product to the original material. The pH of biochars was measured in deionized water from a 1% (w/v) mixture after shaking at 200 rpm for 24 h (Novak et al., 2009). Electrical conductivity (EC) was measured in a 1:10 water extract after a 24-h extraction (Kloss et al., 2012) using a YSI 3100 conductivity meter. The elemental composition of C and N was determined using a Carlo Erba EA 1108 elemental analyzer. Ash content was determined by the weight loss of dry biochar after combustion at 760 °C for 6 h (Novak et al., 2009), as the proportion of the weight of ash to the dry weight of biochar. Initial specific surface area (SA) analysis was done using the Brunauer–Emmet–Teller (BET) method. However, the results were not consistently reproducible. Thus, SA was determined via the modified ethylene glycol monoethyl ether (EGME) method of Amonette (2013), which has previously been used for surface area determinations of biochars (Amonette, 2013; Carter, Heilman, & González, 1965; Carter, Mortland, & Kemper, 1986; Cerato & Lutenegger, 2002). The EGME Method allowed for reproducible surface area determinations for all biochars (Table 1, standard deviation ≤ 2% for all biochars). Feedstock materials and their resulting biochars were analyzed using scanning electron microscopy (SEM). Additionally, at each sampling time, biochars retrieved from soil and biochar particles that were not incubated were imaged by SEM. Briefly, samples were coated with gold-palladium alloy for 30 s using a Pelco sputter coater and analyzed in a FEI Nova NanoSEM 400, using the Everhart–Thornley detector (ETD), with the microscope set at 0° tilt, and an accelerating voltage of 5 kV. No cells were observed for biochars that were not incubated in soil.

Fourier-transform mid-infrared (mid-IR) spectroscopy was performed using an Excalibur 3100 Fourier-Transform IR spectrometer (Varian) bearing a Michelson interferometer equipped with triple-reflection diamond ATR accessory, KBr beamsplitter and deuterated triglycine sulfate (DTGS) detector. Five independent spectra were collected for each biochar. The powder samples were pressed onto the diamond crystal using a pressure clamp with a slip clutch press. The spectra were collected using MicroLab software (Agilent Technologies) operating in the wave number ranges from 4,000 to 700 cm^{-1} with resolution of 4 cm^{-1}, and 64 co-added scans to increase signal/noise ratio. Subsequently, the analysis of the biochars was achieved by soft independent modeling of class analogy (SIMCA) using the chemometrics modeling software Pirouette 4.0 (Infometrix Inc.). A SIMCA model is a method based on principal component analysis (PCA). The latter was performed on each class in the data set, and a sufficient number of principal components were retained to account for most of the variation within each class. Consequently, a principal component model was used to represent each class in the data set and cross-validation was used to choose the optimal number of principal components for each model. Spectra were then transformed by using vector length normalization and a 15-point polynomial-fit Savitzky–Golay second derivative function.

2.3 | Laboratory incubations

The incubations consisted of triplicates for each biochar treatment. Additionally, two sets of controls were included using a soil without biochar (control) and another amended with corn stover (positive control). Biochars and corn stover materials were added to the soil on a C content basis. Corn stover had 38% C content, and was used as the baseline for adding C into the soil to a concentration of 2.5% w/w. Since corn and HBs had a higher C content

(Table 1), the addition of these materials was 1.9 and 1.2% w/w, respectively.

Fifty grams of oven dry equivalent of soil from each treatment were placed in glass sample jars (236 ml Ball mason jar, 6 cm diam.). Soil moisture was adjusted to 66% field capacity and maintained gravimetrically. Samples were incubated at 22 °C using a completely randomized design. Three replications were destructively sampled at 15, 30, 45, 60, 90, or 120 d.

2.4 | Microbial abundance and community composition

The microbial community of soil was characterized by PLFA analysis using the Bligh−Dyer method (Frostegård, Bååth, & Tunlid, 1993a; Frostegård, Tunlid, & Bååth, 1993b). This method has been used to assess changes in the abundance of soil microbial markers under different management practices (e.g., Moore-Kucera & Dick, 2008; Frostegård, Tunlid, & Bååth, 2011; Carlson et al., 2015). At each destructive sampling day (e.g., 15, 30, 45, 60, 90, or 120), total lipids were extracted from soil samples by incubating in the dark for 2 h at room temperature using a chloroform/methanol/citrate buffer (1:2:0.8). Samples were then treated with chloroform and citrate buffer, mixed by vortex, and centrifuged at 2,000 rpm for 10 min. The organic phase was transferred to a new tube and dried under N_2 in a 35 °C heating block. Samples were reconstituted in chloroform, and the lipids were separated into neutral, glycol-, and phospho-lipids with chloroform, acetone, and methanol, respectively, using silicic columns. The phospholipids were then subjected to alkaline methanolysis and dried under N_2 in a 35 °C heating block. Lastly, samples were reconstituted in 192 μl of 1:1 (v/v) hexane: methyl tert-butyl ether (MTBE), transferred to GC vials, and combined with 8 μl of internal standard (0.01 M C19:0ME in 1:1 hexane/MTBE). The latter is an analytical standard allowing GC peak areas to be converted to a molar basis. Biomarkers for PLFAs were detected and quantified using an Agilent GC 6890 (Agilent Technologies) equipped with ChemStation run by Sherlock Identification software (MIDI Inc.).

The absolute concentration of extracted PLFA (nmol PLFA kg^{-1} soil) was quantified and the sum of the identified PLFA used as an index of total biomass. For Actinobacteria the sum of 10Me16:0, 10Me17:0, and 10Me18:0 was used (Moore-Kucera & Dick, 2008). The sum of a15:0, i15:0, i16:0, a17:0, and i17:0 was used for Gram-positive bacteria, while 16:1ω7c, cy17:0, and 18:1ω7c were used for Gram-negative bacteria (Moore-Kucera & Dick, 2008; Zelles, 1997). The PLFA i14:0, 15:0, and 17:0 were used to quantify general bacterial markers, and 18:2ω6c and 18:1ω9c were used for saprophytic fungi (Frostegård & Bååth 1996; Moore-Kucera & Dick, 2008; Zelles, 1999). When determining the fungal/bacteria ratio, the sum of all PLFA for bacteria (i.e., Actinobacteria, Bacteria, Gram-positive and Gram-negative) and saprophytic fungi were used (Frostegård & Bååth, 1996).

2.5 | Enzyme activity and *p*-nitrophenol retention

All enzyme analyses were done in triplicate, with two technical replicates. Fluorescein diacetate hydrolysis was used as an indicator of overall enzymatic activity (EA) in the soil (Prosser et al., 2011) and it was determined as described by Green et al. (2006). β-glucosidase activity was determined based on *p*-nitrophenol (PNP) quantification at 415 nm (Eivazi & Tabatabai 1988). To correct for biochar interference (Jindo, Matsumoto, Izquierdo, Sonoki, & Sanchez-Monedero, 2014a) on the release of PNP, an additional set of samples was analyzed. Instead of the substrate (*p*-Nitrophenyl-β-D-glucopyranoside [PNG]), the reaction product (PNP) was added at the beginning of the incubation at concentrations of 100, 200, 300, and 500 nmol ml^{-1} (equivalent to 5×10^5 nmol PNP kg $soil^{-1}$). The PNP was added to controls after the incubation, prior measuring absorbance at 415 nm (Jindo et al., 2014a). The PNP retention was calculated by fitting to a linear equation the amount PNP from the enzyme analysis and the PNP added to samples (Jindo et al., 2014a). Lastly, the retained PNP was expressed as the percentage of the concentration measured from experimental samples divided by the concentration in the control.

2.6 | Statistical analysis

All statistical analyses were performed using RStudio (RStudio Team, 2018). Exploratory data analysis for normality was assessed visually using *ggplot2* (Wickham, 2016). The absolute concentration of extracted PLFA (as individual markers and as groups) was used as an index of total biomass. Principal component analysis was performed to determine important predictors, using the function *prcomp*. Analysis of variance was conducted to determine the significant ($P < .05$) effect of treatment, the interactions between treatment and incubation day on soil microorganisms and potential enzyme activity. Least significant difference (LSD) was used for comparisons among the different treatments using package *agricolae* (Mendiburu, 2015).

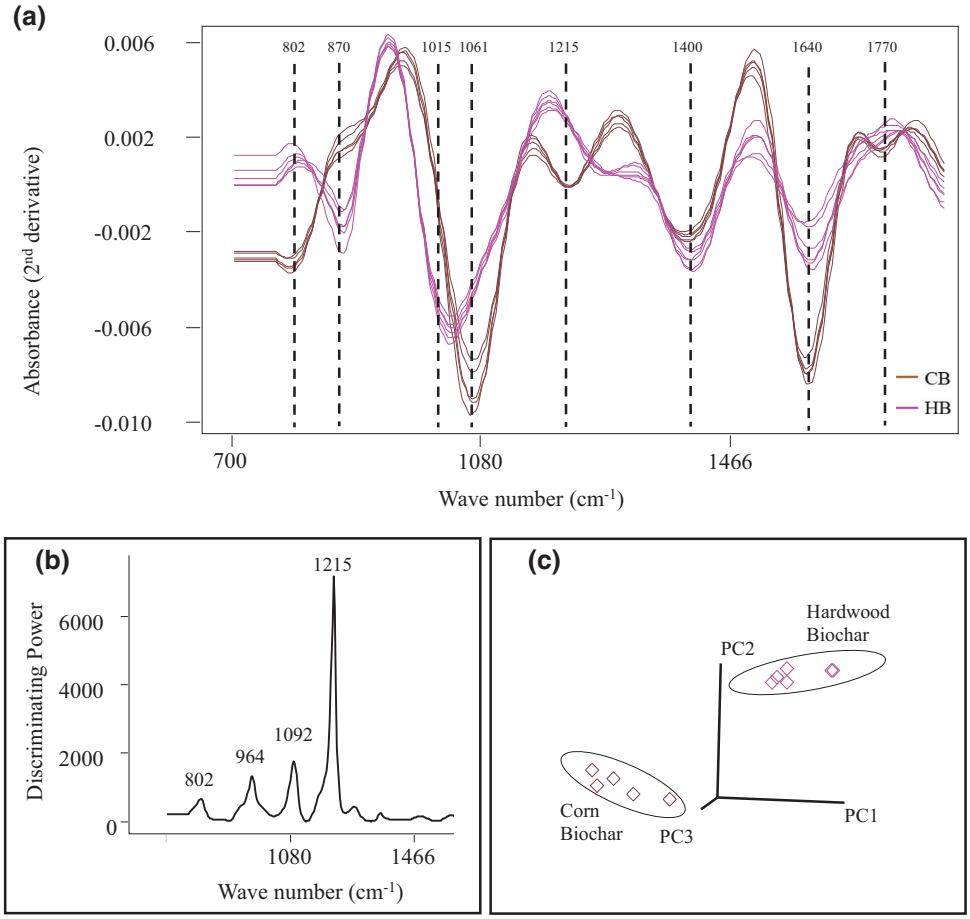

FIGURE 1 Mid-infrared spectra of corn and hardwood biochar. (a) Biochars shared functional groups, (b) but the discriminating power, (c) showed distinct bands responsible for the separation between the biochars. CB, corn biochar; HB, hardwood biochar

3 | RESULTS AND DISCUSSION

3.1 | Characterization of biochars used in this study

The two biochars evaluated in this study represent two feedstocks easily available and commonly used for assessing the impact of biochar in soils. These materials varied in their chemical and physical characteristics since the type of feedstock greatly affected biochar yields, nutrient and ash content, and surface area (Table 1, Figure 1). For example, CB had higher N content, pH, ash content, and EC than HB. Conversely, HB had the highest C content and surface area, but the lowest N and ash content when compared to CB. These are consistent with reports showing that feedstocks are good predictors for ash content, with non-wood derived biochars having higher ash content than wood derived (Brewer, Schimdt-Rohr, Satrio, & Brown, 2009; Mukome, Zhang, Silva, Six, & Parikh, 2013). For example, high ash content (e.g., >20%) from grasses has been attributed to compositional changes of organic and inorganic constituents during pyrolysis (Enders & Lehmann, 2012), while biochars from woody materials have higher C content (Yang, Yan, Chen, Lee, & Zheng, 2007; Jindo, Mizumoto, Sawada, Sanchez-Monedero, & Sonoki, 2014b). The soil in this study had a slightly alkaline pH (7.6) and was not affected by the application of either biochar.

Mid-infrared spectra from the biochars showed high reproducibility within samples, and that while sharing some functional groups, there were differences in the 700–1,800 cm^{-1} region (Figure 1a). The classification plots generated by the SIMCA displayed well-separated clustering between the biochars (Figure 1c). The discriminating power used to identify the variables (i.e., wavenumbers) responsible for the separation among the biochars, showed four distinct bands associated with functional group vibrations in the 800–1,500 cm^{-1} region (Figure 1b). For example, the peaks in the 1,000–1,100 cm^{-1} region, which were common between the biochars, have been associated with C–O stretching of polysaccharides and the bending of Si–O stretching (Hsu & Lo, 1999; Jindo et al., 2014b; Uchimiya, Orlov, Ramakrishnan, & Sistani, 2013). However, the second derivative of the absorbance showed

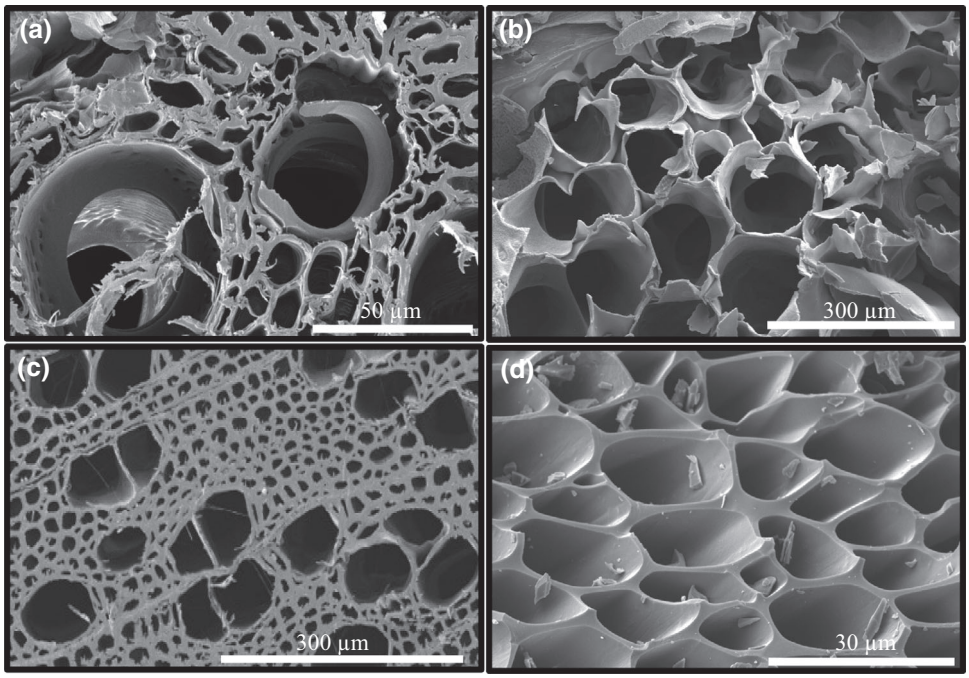

FIGURE 2 Scanning electron microscopy analysis of feedstocks and biochars. Panels a and b show corn stover and corn biochar, respectively. Panels c and d show hardwood and its resulting biochar, respectively

stronger peaks for CB than for HB (Figure 1a). A possible explanation is that silica (SiO_2) in hardwood is generally present in trace amounts (Pettersen, 1984), while corn plants are rich in this mineral (Brewer et al., 2009). Bands in the 1,400 and 1,640 cm^{-1} regions were also present in both biochars, and have been assigned to C−C stretching vibrations in aromatic rings, and C = C aromatic rings, respectively (Hsu & Lo, 1999; Liu, He, & Uchimiya, 2015) which are associated with the oxidation of species during thermal degradation.

Although both materials increased porosity after pyrolysis, CB was very brittle while HB retained its structure (Figure 2). During pyrolysis, biomass undergoes several physical, chemical, and molecular changes. Typically, pyrolysis leads to structural modifications including shrinkage and loss of volatile organics (Chia, Downie, & Munroe, 2015) with higher temperatures decreasing the yield (Lehmann et al., 2006) while increasing C concentration (Lehmann et al., 2006) and surface area (Lehmann et al., 2006; Novak et al., 2009). The latter was higher in HB than CB, nonetheless the values were similar to those reported in the literature for wood-derived biochar (e.g., Sun et al., 2014) and plant materials (e.g., O'Toole et al., 2018).

At each destructive sampling day, some biochar particles were recovered from soils and visualized using SEM. Corn biochar was structurally brittle and broke easily thus making its removal from the incubated soils, and subsequent imaging analysis challenging. However, after 120 d, SEM analysis showed organic material inside the pores of CB, and microbial cells in samples amended with HB (Figure 3). When mixed in the soil, biochars generate very different living conditions, thus proximity of the microbial cells to the biochar's surface may indicate favorable conditions for colonization. For example, studies have shown microbial colonization and utilization of labile C in biochar (Luo et al., 2013; 2017) while others have suggested that pore spaces as well as changes in pH may influence microbial abundance (Lehmann et al., 2011; Pietikainen et al., 2000). Although the pH of the soil studied here did not change with either biochar, it is possible that biochar particles created microenvironments suitable for bacterial growth.

3.2 | Microbial abundance and community composition

Our study demonstrated the capacity of these materials to significantly ($P < .05$) modify components of the microbial community abundance and composition of this agricultural soil during the 120-d incubation. Principal component analysis of the microbial profile via PLFA, showed a distinct clustering in samples treated with corn stover suggesting that treatment was a strong predictor, explaining 97.5% of the variability (Supplemental Figure S1). However, it did not discriminate between the biochars and control. The ANOVA on the effect of treatment, showed similar trends (Table 2). There was significantly ($P < .05$) higher

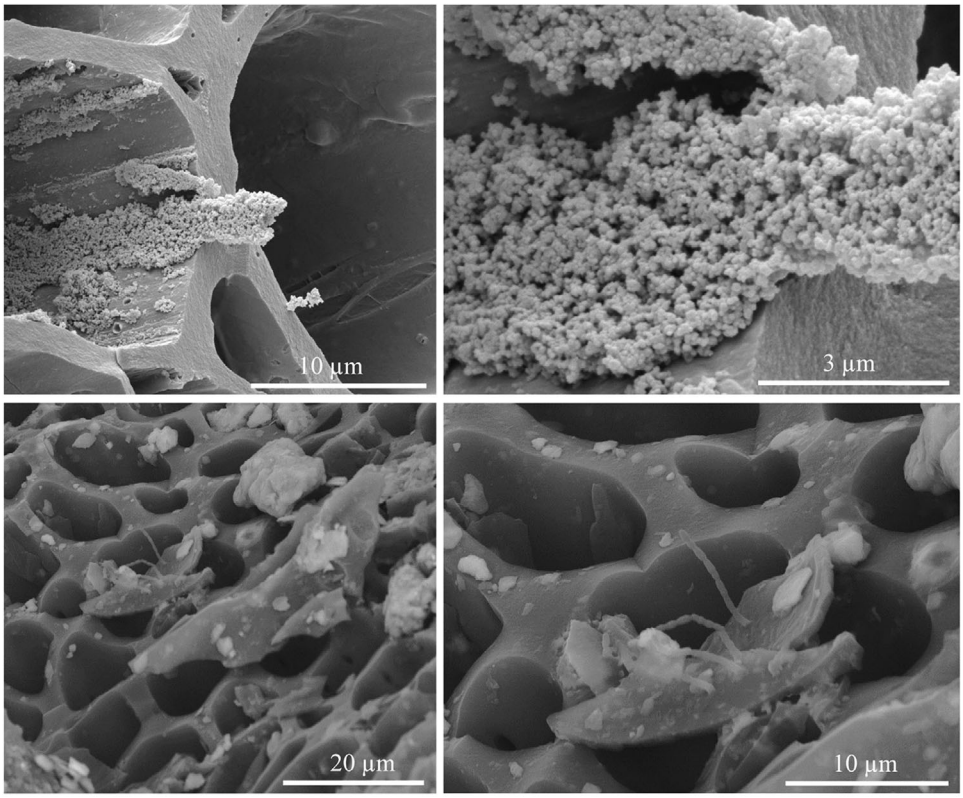

FIGURE 3 Biochar particles retrieved from soils after 120 d. Top and bottom panels show corn and hardwood biochar, respectively

TABLE 2 Effect of biochar type on microbial abundance during a 120-d incubation study

Treatment	Actinobacteria	Bacteria	Gram positive	Gram negative	Saprophytic fungi	F/B
	10^3 nmol PLFA kg^{-1} soil					
Control	9.2[a] (1.9)[b]c[c]	1.3 (0.4)b	12.5 (3.5)b	15.2 (3.8)b	6.7 (1.5)b	0.18 (0.01)b
Corn stover	11.5 (2.0)a	2.8 (0.7)a	19.5 (4.6)a	31.1 (7.1)a	19.6 (4.3)a	0.32 (0.01)a
Corn biochar	9.9 (1.5)b	1.3 (0.3)b	12.9 (3.0)b	16.1 (3.1)b	6.8 (1.1)b	0.17 (0.01)b
Hardwood biochar	8.7 (1.8)c	1.2 (0.3)b	12.2 (3.3)b	14.6 (3.5)b	6.3 (1.4)b	0.17 (0.02)b

Note. F/B, fungi/bacteria ratio.
[a]Numbers are the mean of $n = 18$.
[b]Numbers in parenthesis represent the standard deviations.
[c]Means within a column that do not share a letter are significantly different at $\alpha = .05$.

abundance of all microbial groups in soils incubated with corn stover when compared to the biochars. This suggests the preference of microorganisms for utilizing more readily available C (i.e., plant residue) over biochar, which is more refractory than the feedstocks used to make it (Lehmann et al., 2011).

Most microbial groups from soils incubated with biochar showed no significant change when compared to control (no treatment). Overall, microbial abundance in CB was higher than that from hardwood, but it was not significant. However, abundance of Actinobacteria was significantly ($P < .05$) higher in CB when compared to control (no treatment) and HB (Table 2). Previous studies have reported increases in Actinobacteria with different types of biochar using both PLFA (Luo et al., 2017) and sequencing techniques (Khodadad, Zimmerman, Green, Uthandi, & Foster, 2011; Sheng & Zhu, 2018). The use of DNA-based approaches along with analysis of changes in C dynamics have suggested active roles of these bacteria in metabolic degradation of recalcitrant polymers such as pyrogenic C (Khodadad et al., 2011; Sheng & Zhu, 2018). However, this response was not observed in HB. Contrary to other studies (e.g., Dai et al., 2018; Luo et al., 2017), fungal abundance did not increase with either biochar. As recently reviewed by Palansooria et al. (2019) neutral impacts of biochar on soil biological properties have been reported and attributed to the biochar type, application rates, and soil type. For example, Luo et al., 2013, reported increased

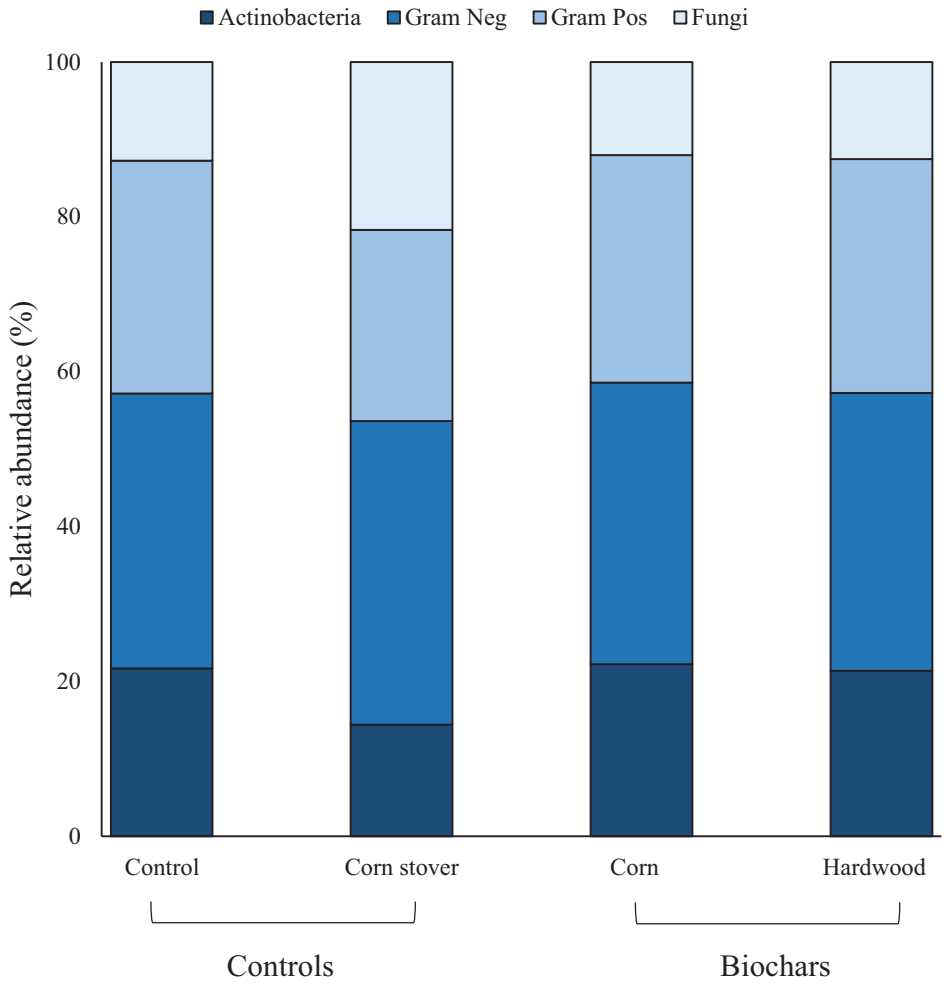

FIGURE 4 Relative abundance of microbial groups during a 120-d incubation. Shifts in microbial groups were only observed in soils incubated with corn stover. However, there were no differences in soils incubated with biochar when compared to control (no treatment); $n = 18$

microbial abundance after application of biochar, in soil of low pH (3.7) but not for the soils with higher pH (7.6) such as the one used in this study. Gómez, Denef, Stewart, Zheng, and Cotrufo (2014) found significant increases in microbial abundance and activity with increasing biochar rates while Luo et al. (2017) showed significant increases in microbial abundance via PLFA associated to labile C fractions from biochar produced at 350 °C but not at 700 °C. Thus, it is possible that the types of biochars used in this study could not provide sufficient labile C or N substrates or the short incubation period did not impact soil properties to cause changes in the abundance and community structure of the biological component.

Samples incubated with corn stover increased fungal markers thus, increasing the fungal/bacterial ratio. Furthermore, during the incubation period there were shifts in the community composition of the PLFA profiles. For example, the relative abundance of Actinobacteria decreased while saprophytic fungi markers increased significantly when compared to control (Figure 4). However, since both microbial groups are involved in decomposition, the shift in communities may not affect overall decomposition processes.

3.3 | Enzyme mediated reactions differed based on type of biochar

Soil enzymes are considered important indicators of changes in management practices, climate and land use, and have been shown to provide sensitive assessments of soil health (Acosta-Martínez, Moore-Kucera, Cotton, Gardner, & Wester, 2014; Dick, Breakwell, & Turco, 1996; Lehman et al., 2015; Stott, Andrews, Liebig, Wienhold, & Karlen, 2010). Both enzyme activities evaluated here, fluctuated during the incubation period with most treatments having the lowest activity on Day 120. However, the two enzyme activities responded differently to CB and HB.

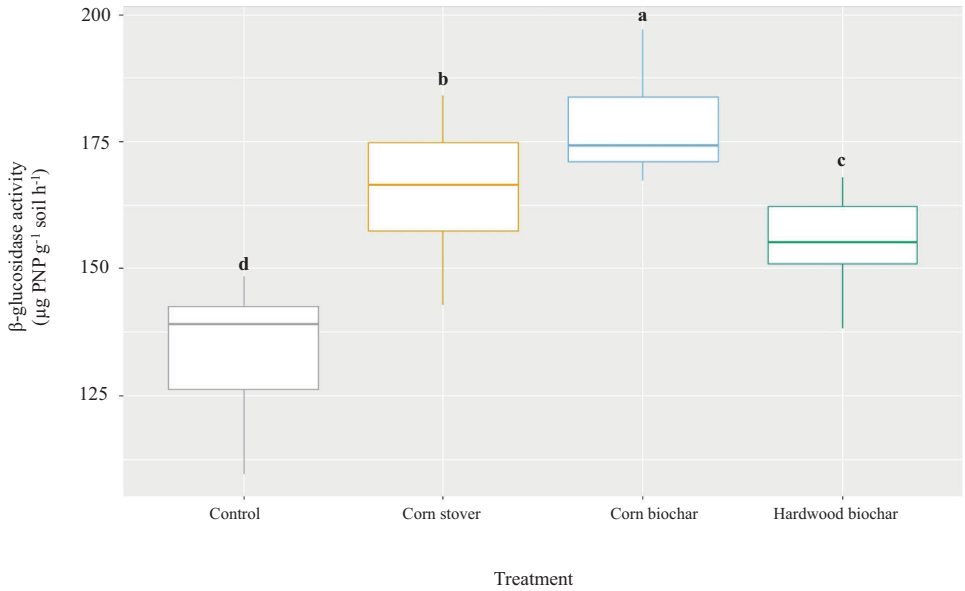

FIGURE 5 Effect of treatment on β-glucosidase activity

Fluorescein diacetate hydrolytic activity is present in primary decomposers (e.g., fungi and bacteria) in the soil and is mediated by different enzymes such as lipases, proteases, and esterases (Lundgren, 1981; Schnurer & Rosswall, 1982). Thus, FDA can be valuable in determining several reactions occurring in soil. Overall, the highest hydrolytic activity of FDA was in soils incubated with corn stover, but ANOVA showed no significant differences between biochars and control (Supplemental Figure S2). The significantly higher activity in stover could be attributed to highly decomposable material in the presence of decomposers (Schnurer & Rosswall, 1982), which is supported by the high abundance of different microbial groups in samples incubated with corn stover when compared to the other treatments (Table 2). Although studies have shown increases in FDA hydrolysis in soils amended with biochar, the response (e.g., increase or no change) has varied due to type of biochar, application rates, and the complex interactions of soil type with the biochar (Bu, Su, Xue, Zhao, & Wang, 2019; Tan et al., 2015). In our study, FDA hydrolysis was significantly lower in all treatments on Day 120 (Supplemental Figure S3). For HB, the highest activity was measured on Day 90, while there were no significant differences between the other incubation days.

β-Glucosidase catalyzes the hydrolysis of water-soluble oligosaccharides, specifically the last step in cellulose degradation to release monosaccharides. Its activity in soil is important because it provides labile C and energy sources to support microbial life and is currently used as an index of soil quality (Stott et al., 2010). It also plays a major role in the mineralization and degradation of organic compounds and development of SOM (Deng & Popova, 2011). During the day of analysis, a PNP-spiking assay was conducted simultaneously because it has been reported that biochar may interfere with some enzyme assays, especially those that involve measuring *p*-nitrophenol (Jindo et al., 2014a; Foster, Fogle, & Cotrufo, 2018). The spiking analysis showed that PNP retention increased in soils amended with biochars (Supplemental Figure S4). From the biochars, up to 45% of PNP was retained by CB, thus showing the lowest activity. The difference in retention could be attributed to the different chemical and physical properties of the biochars. Alternatively, it could also be related to the amount of biochar added to the soil. Since biochars were added on a C content basis, more CB material was added to the soil when compared to HB. The excess material could have interfered by retaining more PNP. It was recently reported that addition of biochars resulted in decrease in the activities of β-glucosidase and phosphatase due to direct sorption to biochar, with approximately 40% of the enzymes being retained (Foster et al., 2018).

After correcting for the PNP retained by the samples, the potential enzyme activity from soils incubated with either biochar was significantly higher ($P < .05$) than that measured from control (Figure 5). From all treatments, CB resulted in the highest β-glucosidase activity followed by corn stover > HB > control. The two enzyme activities measured in this study show that the effects of biochar on soil enzymes are highly variable. Although previous studies have shown increases in enzyme activities with increasing soil microbial populations (Sekaran, McCoy, Kumar, & Subramanian, 2019; Tabatabai, 1994), the highest β-glucosidase activity was not in soils with corn stover which had the highest microbial abundance. Nonetheless,

as reviewed by Lehman et al. (2015) it is possible that although soil microbial biomass may contribute to the observed soil functions, it is challenging to determine whether they respond in unison to environmental changes.

In conclusion, the biochars derived from corn stover and hardwood, influenced soil microbial communities and activities differently during our 120-d incubation study. Although biochars were added to the soil on a C content basis, their chemical and physical properties might have played a major role in modifying the microbial abundance and enzyme activities. The abundance of some microbial groups was higher with CB but not with HB, while others were not impacted by either biochar. The two potential enzyme activities responded differently. For instance, both biochars increased β-glucosidase EA when compared to control but caused no differences in FDA hydrolytic activity when compared to control. However, FDA showed fluctuations throughout the incubation period indicating the potential of biochar for impacting some components of the soil microbial community. Our study demonstrated different responses of the microbial community composition and enzyme activity to two biochar types, which can represent shifts in essential functions in the soil. However, our study was limited, and we cannot extend the conclusions to other soil types. Additionally, this microcosm study was conducted without plants in the soil, thus excluded important plant–microbe interactions that could have impacted microbial communities and their activities. It is also possible that extending the incubation period could show other shifts in the biological parameters measured. Nonetheless, our study showed that biochar can influence the soil microbial community in the absence of plants and under controlled conditions.

CONFLICT OF INTEREST

No conflict of interest is declared.

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SUPPORTING INFORMATION

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