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## Stabilizing effect of biochar on soil extracellular enzymes after a denaturing stress

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### HIGHLIGHTS

- We examined if biochar could stabilize soil enzymes following denaturing stress.
- Biochar amendment reduced potential enzyme activity prior to stress.
- Following stress, most enzyme activities decreased with increasing stress.
- Biochar can reduce evaporative losses and stabilize some enzymes.

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### ABSTRACT

Stabilizing extracellular enzymes may maintain enzymatic activity while protecting enzymes from proteolysis and denaturation. A study determined whether a fast pyrolysis hardwood biochar (CQuest™) would reduce evaporative losses, subsequently stabilizing soil extracellular enzymes and prohibiting potential enzymatic activity loss following a denaturing stress (microwaving). Soil was incubated in the presence of biochar (0%, 1%, 2%, 5%, or 10% by wt.) for 36 days and then exposed to microwave energies (0, 400, 800, 1600, or 3200 J g<sup>-1</sup> soil). Soil enzymes ( $\beta$ -glucosidase,  $\beta$ -D-cellobiosidase, N-acetyl- $\beta$ -glucosaminidase, phosphatase, leucine aminopeptidase,  $\beta$ -xylosidase) were analyzed by fluorescence-based assays. Biochar amendment reduced leucine aminopeptidase and  $\beta$ -xylosidase potential activity after the incubation period and prior to stress exposure. The 10% biochar rate reduced soil water loss at the lowest stress level (400 J microwave energy g<sup>-1</sup> soil). Enzyme stabilization was demonstrated for  $\beta$ -xylosidase; intermediate biochar application rates prevented a complete loss of this enzyme's potential activity after soil was exposed to 400 (1% biochar treatment) or 1600 (5% biochar treatment) J microwave energy g<sup>-1</sup> soil. Remaining enzyme potential activities were not affected by biochar, and activities decreased with increasing stress levels. We concluded that biochar has the potential to reduce evaporative soil water losses and stabilize certain extracellular enzymes where activity is maintained after a denaturing stress; this effect was biochar rate and enzyme dependent. While biochar may reduce the potential activity of certain soil extracellular enzymes, this phenomenon was not universal as the majority of enzymes assayed in this study were unaffected by exposure to biochar.

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### 1. Introduction

Extracellular enzymes are the primary means by which soil bacteria and fungi degrade insoluble macromolecules, including soil organic matter (SOM) and detritus, into smaller soluble molecules that can be microbially assimilated (Burns et al., 2013). Extracellular enzymes allow microbes to access unavailable carbon and nutrients in SOM by catalyzing the first step of decomposition

and nutrient mineralization, i.e., depolymerization of complex carbon substrates too large to enter microbial cells. Plant components such as cellulose, hemicellulose, and lignin, and microbial cell wall materials are among the more abundant soil organic compounds that are degraded enzymatically. However, extracellular enzymes may be found in different soil locations; they may be associated with biotic components such as proliferating and non-proliferating cells or with dead cells and cell debris, or sorbed to clay minerals or soil colloids (Burns et al., 2013). Extracellular enzymes associated with humic colloids and clay minerals may have a relatively long half-life (compared to enzymes in the soil aqueous phase), with these associations likely the best form of protection from the

Abbreviations: MW, microwave; SOM, soil organic matter.

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environment (Burns, 1982). Ladd (1978) demonstrated that many enzymes are capable of binding to humic material, giving some enzymes a persistence they would not otherwise display in the hostile extracellular environment of the soil.

Enzyme stabilization may maintain enzymatic activity and also protect against proteolysis and other denaturing events (Skujins, 1976; Nannipieri et al., 1988, 1996). Yet, we are still at the beginning of practical applications to manipulate stabilized enzymes for beneficial ecosystem services such as C sequestration, nutrient cycling, and bioremediation (Burns et al., 2013). Jastrow et al. (2007), for example, proposed that by modifying the soil physico-chemical environment, fungal growth and their extracellular enzymes could be promoted for C sequestration. Amonette et al. (2003) tested the ability of four alkaline fly ashes to stabilize tyrosinase enzymes and found that enzyme activity was stabilized and even enhanced by two (sub-bituminous and lignitic fly ash) of the four fly ashes. Others have found that another ashy material, biochar, has potential to sorb a wide range of organic and inorganic molecules and may affect enzymes (and inherently their activity) by sorbing them and/or their substrates (Bailey et al., 2010; Jin, 2010). In general, however, there is a poor understanding of the possible biochar effects on soil extracellular enzymes in biochar-amended soil, especially in the presence of stress.

Microwave exposure can be used as a means to stress and denature soil extracellular enzymes because its radiation both heats and desiccates enzymes, its energy output can be accurately quantified and controlled to generate a gradient of stress, and has been successfully used by others (e.g., Islam and Weil, 1998; de Boer et al., 2003; Knight and Dick, 2004) to stress soil and denature enzymes in controlled experiments. Other methods required to denature extracellular enzymes in soil include autoclaving and gamma irradiation (Blankinship et al., 2014). These denaturing methods represent stresses unnatural to soil systems, but may be required to test the ability of biochar to prevent enzyme destruction.

There is an interest in biochar creation and land application for the purposes of bio-gas and bio-oil production, C sequestration, and increasing soil fertility (Lehmann et al., 2006). Because of its porous nature, researchers have speculated that biochar can provide habitats for bacteria and fungi (Thies and Rillig, 2009) and have shown that biochars can increase water storage capacity (Novak et al., 2012; Ippolito et al., 2014). If biochar can attract soil microbes and sorb extracellular enzymes, in conjunction with improvements in soil water relations, it is possible that biochar could stabilize enzymes and protect enzymes from degradation or denaturation during environmental stress. Biochar could thus be a useful material in cases where enzyme stabilization is desired. Therefore, the objective of this study was to determine the stabilizing effect of biochar on enzyme activities exposed to a denaturing stress, in this case microwaving, while concurrently determining the biochar effects on evaporative soil water losses due to microwaving.

## 2. Material and methods

### 2.1. Soil and biochar

Biochar-free soil (0–30 cm depth) was collected November 2012 from the border of a research field located near Kimberly, Idaho (42°31'N, 114°22'W, elevation of 1190 m). The soil was a Portneuf silt loam (coarse-silty, mixed superactive, mesic Durinodic Xeric Haplocalcids) with 20% clay, 56% silt, and 24% sand, 1.2% organic C, and an 8.8% calcium carbonate equivalency. The electrical conductivity (EC) of the soil was 0.50 dS m<sup>-1</sup> and its pH was 7.6 (saturated paste; Thomas, 1996; Rhoades, 1996). Prior to the study, the soil was air-dried and passed through a 2-mm mesh sieve.

Biochar was provided by Dynamotive Energy Systems (West Lorne, Ontario, Canada) and was marketed under the name CQuest™. It was derived from oak and hickory hardwood sawdust and created by fast pyrolysis at 500 °C. As described by Lentz and Ippolito (2012), the biochar had an ash content of 14%, an oxygen:carbon ratio of 0.22, a surface area of 0.75 m<sup>2</sup> g<sup>-1</sup>, and a pH of 6.8. Additional details regarding biochar chemical properties are listed in Table 1.

### 2.2. Incubation experiment

The incubation experiment was conducted with five biochar treatments (0%, 1%, 2%, 5%, and 10% biochar in soil by wt.), each replicated 20 times in glass scintillation vials. Final dry weight of soil plus biochar in each vial was 10.0 g. Soil and biochar were mixed by placing the vials on their sides and gently rolling the vials until biochar was mixed thoroughly with soil. After mixing, 1.8 ml of distilled water was added to each vial to achieve a moisture content of 18%, which was equivalent to 60% of the soil's water holding capacity. All vials were weighed, and then they were loosely capped and incubated for 36 days at 25 °C. The length of the incubation was arbitrarily selected to allow microbial biomass to recover from being air-dried and rewetted and to allow enzyme production. Water was added to each vial every two or three days to maintain constant water content.

### 2.3. Microwave irradiation

Following the incubation period, soils were subjected to microwave (MW) stress using a 650 W household-type MW oven. The power output of the MW was determined according to Neas and Collins (1988), by measuring the rise in temperature of 1000 mL of distilled water (initial temperature 21 °C) in a 1-L beaker after microwaving at full power for two minutes. The power output was calculated as  $P = C_p K \Delta T m / t$ , where  $P$  is the apparent power absorbed by the water sample (J s<sup>-1</sup>),  $C_p$  is the heat capacity of water (J ml<sup>-1</sup> K<sup>-1</sup>),  $K$  is a factor (4.184) to convert thermal chemical cal ml<sup>-1</sup> K<sup>-1</sup> to watts (J s<sup>-1</sup>),  $\Delta T$  (°C) is the difference between final temperature and initial temperature of water,  $m$  is the mass of the water (g); and  $t$  is the duration (s) of MW energy application. Using this equation, the MW oven output was calculated as 675 W (J s<sup>-1</sup>).

The 20 replicates of each biochar treatment were divided into 5 stress levels and microwaved for different lengths of time to achieve microwave energy "stress" levels of 0, 400, 800, 1600, and 3200 J g<sup>-1</sup> soil. Soil temperature was measured in random vials immediately after microwaving, and a subsample of each vial was placed in a drying oven at 105 °C for 24 h to determine gravimetric water content.

**Table 1**

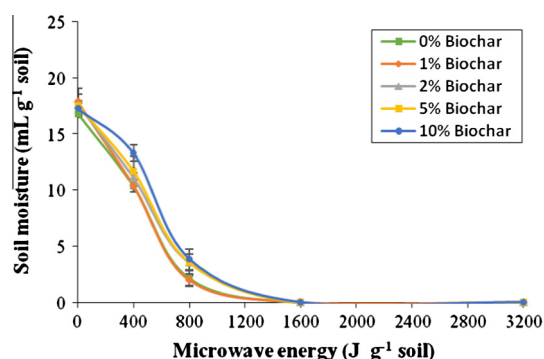
Selected chemical properties of a fast pyrolysis, hardwood-derived biochar (CQuest™) used in the laboratory incubation study. Data are from Lentz and Ippolito (2012).

Property	Units	Biochar
Surface area	m <sup>2</sup> g <sup>-1</sup>	0.75
pH		6.8
EC	dS m <sup>-1</sup>	0.7
Ash	%	14
Total C	%	66.2
Total N	%	0.32
Organic N	%	0.32
NO <sub>3</sub> -N	mg kg <sup>-1</sup>	1.5
NH <sub>4</sub> -N	mg kg <sup>-1</sup>	1.2
K	mg kg <sup>-1</sup>	3400
Ca	mg kg <sup>-1</sup>	3700
Mg	mg kg <sup>-1</sup>	1500
Na	mg kg <sup>-1</sup>	200
P	mg kg <sup>-1</sup>	300

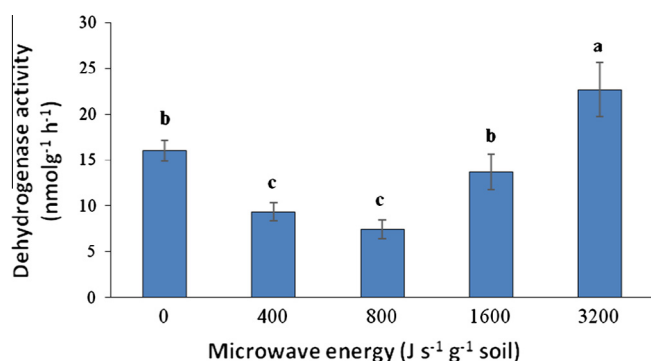
## 2.4. Enzyme assays

Dehydrogenase activity was measured immediately following the microwave stress application by following the method of Trevors (1984). As an intracellular enzyme, this enzyme was employed as an indicator of microbial biomass and activity and their response to microwaving. The potential activities of six extracellular enzymes were quantified according to fluorescence-based protocols as described in Steinweg et al. (2013) and Bell et al. (2013). The six enzymes included three C-cycling enzymes ( $\beta$ -D-cellobiosidase,  $\beta$ -glucosidase, and  $\beta$ -xylosidase), 1 C and N cycling enzyme (N-acetyl- $\beta$ -glucosaminidase), 1 N cycling enzyme (leucine aminopeptidase), and 1 P cycling enzyme (phosphatase).

All assays included appropriate blanks, where soil suspensions were incubated in the absence of enzyme substrate. To correct for quenching of fluorescence signals by soil and biochar, standard curves were prepared for each sample by incubating suspensions in the presence of increasing concentration of 4-methylumbelliferone (MUB) or 7-amino-4-methylcoumarin (MUC) standard.



**Fig. 1.** The effect of microwave energy stress on the moisture content of soil after 36 days of incubation with either 0%, 1%, 2%, 5%, or 10% biochar amendment to soil (by wt.). Error bars represent the standard error of the mean ( $n = 4$ ).



**Fig. 2.** The effect of microwave energy stress on dehydrogenase activity in soil after 36 days of incubation and averaged across biochar treatments ( $n = 20$ ). Histogram bars labeled with different letters are significantly different ( $\alpha = 0.05$ ) ( $n = 20$ ).

**Table 2**

The effects of microwave energy level on the mean ( $\pm 1$  standard error) of  $\beta$ -glucosidase (BG),  $\beta$ -D-cellobiosidase (CB) N-acetyl- $\beta$ -glucosaminidase (NAG), and phosphatase (PHOS) potential activity in soil ( $\text{nmol g}^{-1} \text{soil h}^{-1}$ ), averaged across biochar treatments.

Enzyme	Microwave energy ( $\text{J g}^{-1} \text{soil}$ )				
	0	400	800	1600	3200
BG	$36.0 \pm 5.76a^a$	$5.70 \pm 1.57b$	$2.76 \pm 0.75b$	$0.27 \pm 0.23b$	$0.15 \pm 0.14b$
CB	$4.11 \pm 0.80a$	$1.10 \pm 0.37a$	$0.38 \pm 0.20a$	$2.16 \pm 0.21a$	$0.00 \pm 0.00a$
NAG	$3.69 \pm 0.41a$	$0.80 \pm 0.27b$	$0.37 \pm 0.22b$	$0.41 \pm 0.29b$	$0.00 \pm 0.00b$
PHOS	$60.0 \pm 8.08a$	$23.6 \pm 2.27b$	$8.98 \pm 0.89bc$	$0.95 \pm 0.41c$	$0.21 \pm 0.15c$

<sup>a</sup> Within rows, means followed by different letters are significantly different at  $\alpha = 0.05$  ( $n = 20$ ).

Incubations were conducted at 25 °C. Fluorescence measurements of the plates were read on a Tecan Infinite® M200 microplate (Tecan, Mannedorf, Switzerland) at 365 nm excitation and 450 nm emission wavelengths.

## 2.5. Statistical analysis

Statistical analyses of the data were performed with SAS version 9.3 (SAS Institute, Cary, North Carolina) using the Proc Mixed procedure. Two-way factorial analysis of variance (ANOVA) tests were performed to determine the effect of biochar rate, stress level, and their interaction on enzyme activities ( $\alpha = 0.05$ ).

## 3. Results

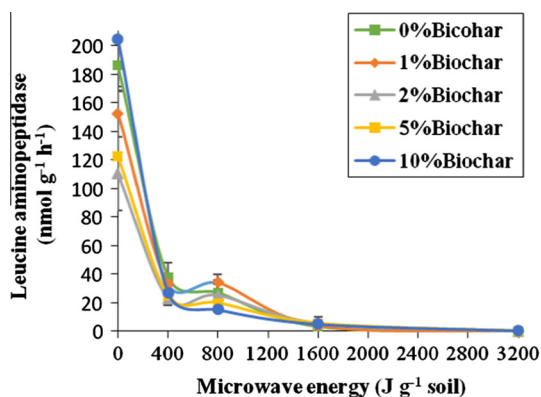
### 3.1. Stress effects on soil temperature, moisture, and microbial activity

Microwaving provided stress through heat and loss of soil water, but the effect of microwave stress on soil water loss was influenced by biochar treatment. At the lowest stress level ( $400 \text{ J g}^{-1} \text{ soil}$ ), loss of soil water was significantly reduced in soil amended with 10% biochar, compared to soil amended with 0%, 1%, or 2% biochar (Fig. 1). The attenuation of moisture loss by the 5% biochar treatment was marginally significant ( $P = 0.058$ ) at this stress level. A dramatic loss of soil water occurred when MW energy was  $800 \text{ J g}^{-1} \text{ soil}$ , at which more than 80% of the total soil moisture was lost in soil with 0% and 1% biochar (Fig. 1). At this stress level, soil temperature rose to approximately 70 °C in all soils. All soils reached 0% water content when MW irradiation applied was  $1600 \text{ J g}^{-1} \text{ soil}$ . Soil temperature reached 100 °C with energy applied at 1600 or  $3200 \text{ J g}^{-1} \text{ soil}$ .

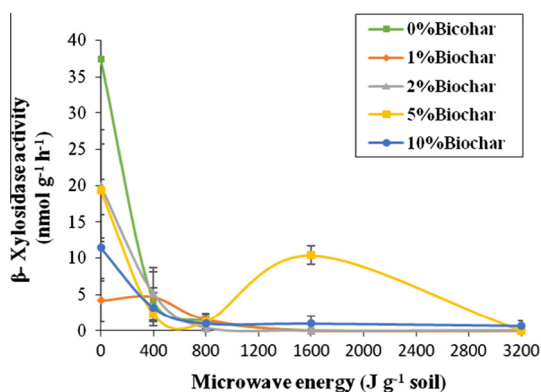
Microbial activity, as indicated by dehydrogenase activity, decreased with increasing MW energy up to  $800 \text{ J g}^{-1}$  but then increased at higher energy levels (Fig. 2). Dehydrogenase activity was significantly lower after exposure to  $800 \text{ J g}^{-1}$  soil than after exposure to 0, 1600, or  $3200 \text{ J g}^{-1}$ . At  $3200 \text{ J g}^{-1}$  of microwave energy, dehydrogenase activity was significantly greater than the other MW energies.

### 3.2. Stress effects on extracellular soil enzymes

In this study, extracellular enzyme activities were differentially affected by biochar rate, stress level, and their interaction. Main effect of stress level (but not biochar) was highly significant ( $P < 0.0001$ ) on the activities of  $\beta$ -glucosidase,  $\beta$ -D-cellobiosidase, N-acetyl- $\beta$ -glucosaminidase, and phosphatase. The potential activity of these enzymes significantly decreased with increasing MW energy, regardless of biochar rate. Potential activities of  $\beta$ -D-cellobiosidase and N-acetyl- $\beta$ -glucosaminidase fell below detection limits when MW energy was applied at  $3200 \text{ J g}^{-1}$ , whereas  $\beta$ -glucosidase and phosphatase enzymes retained some, but very low, levels of potential activity (Table 2).



**Fig. 3.** The effect of microwave energy stress on leucine aminopeptidase activity in soil after 36 days of incubation with either 0%, 1%, 2%, 5%, or 10% biochar amendment to soil (by wt.). Error bars represent the standard error of the mean ( $n = 4$ ).



**Fig. 4.** The effects of microwave energy stress on  $\beta$ -xylosidase activity in soil after 36 days of incubation with either 0%, 1%, 2%, 5%, or 10% biochar amendment to soil (by wt.). Error bars represent the standard error of the mean ( $n = 4$ ).

In contrast, a different pattern was observed for leucine aminopeptidase potential activity, which was significantly affected by biochar rate ( $P = 0.016$ ), stress level ( $P < 0.0001$ ), and their interaction ( $P = 0.0008$ ). Prior to stress exposure, leucine aminopeptidase potential activity was significantly reduced in soils receiving 1%, 2% or 5% biochar compared to control soil and soil receiving 10% biochar (Fig. 3). When soils were exposed to 400 or more  $J g^{-1}$ , potential activity declined in all soils to the point where no difference among biochar treatments existed. Cessation of the potential enzyme activity was observed in all soils when MW energy was applied at 1600 or 3200  $J g^{-1}$  soil.

For  $\beta$ -xylosidase potential activity, the interaction effect of biochar and stress level was marginally significant ( $P = 0.066$ ). Without any microwave stress, biochar reduced potential activity of this enzyme regardless of application rate (Fig. 4). When exposed to a stress of 400  $J g^{-1}$ , potential activity remained steady in soil receiving 1% biochar, whereas potential activity declined in all other treatments. After a stress exposure of 1600  $J g^{-1}$ ,  $\beta$ -xylosidase potential activity increased in 5% biochar amended soil and was significantly greater than the activities of the other soils.

#### 4. Discussion

In this study, dehydrogenase activity, an enzyme that only functions inside the cell, decreased with increasing MW irradiation up to 800  $J g^{-1}$  and then increased with increasing MW irradiation up to 3200  $J g^{-1}$  (Fig. 2). The measurement of this enzyme was used

to confirm that the stress applied was strong enough to kill living microbes and denature their intracellular enzymes, but not necessarily affect stabilized extracellular enzymes. We observed that soil temperature rose to approximately 100 °C when MW stress applied was  $\geq 1600 J g^{-1}$ , and this might have stimulated the abiotic reduction of tetrazolium salt utilized in the assay and subsequently affected the colorimetric measurement of dehydrogenase activity. A similar result was found by Ciardi (1998) when dehydrogenase activity was greater at 200 °C than at 150 °C in both fresh and air-dried soils. It is likely that with temperatures of 100 °C, as observed in the current study, the measured activities were mainly driven by abiotic reactions such as hydrolysis, oxidation, and reduction that were masked at lower temperature. Such results demonstrate the need to develop a new method to accurately evaluate dehydrogenase activity in soil affected by high temperature stress.

Soil enzymes are active in different soil locations. For example, enzymes might be associated with biotic components such as proliferating and non-proliferating cells (spores, cysts, etc.) or with dead cells and cell debris, or stabilized on clay minerals and humic colloids (Burns et al., 2013). Stabilization of extracellular enzymes on soil colloids can possibly maintain their activity for extended periods of time, as long as enzyme active sites remain functional (Burns, 1982; Nannipieri et al., 1996; Knight and Dick, 2004). In the present study, we tested if potential enzyme activity would be protected from microwave stress after incubation in the presence of biochar. The results demonstrated that the ability of biochar to stabilize enzymes was dependent on the biochar application rate and the enzyme itself.

After a 36-day incubation period, biochar amendment did not affect potential activities of  $\beta$ -glucosidase,  $\beta$ -D-cellobiosidase, N-acetyl- $\beta$ -glucosaminidase, or phosphatase, suggesting that biochar did not sorb these enzymes or their substrates/products during the enzyme assay. This contrasts with findings of Bailey et al. (2010) and Jin (2010), who noted reduction of potential enzyme activities in biochar-amended soil. Jin (2010) examined the effect of corn stalk biochar (slow pyrolysis at 550 °C) at rate of 0, 1, 12, and 30  $Mg ha^{-1}$  (~0%, 0.05%, 0.5%, and 1.3% by wt.) on potential activity of two C-cycling enzymes ( $\beta$ -D-glucosidase and  $\beta$ -D-cellobiosidase), and found that activities decreased after biochar additions to soils. Bailey et al. (2010) tested the effects of fast-pyrolysis biochar (0% or 2% by wt.) produced from switchgrass on the potential activity of purified enzymes, and observed decreases in glucosidase potential activity. In the current study, biochar-induced reduction of enzyme potential activities occurred only for leucine aminopeptidase and  $\beta$ -xylosidase. Leucine aminopeptidase is an enzyme that preferentially catalyzes the hydrolysis of leucine residues at the N-terminus of peptides and proteins (Rawling and Barrett, 2004; Matsui et al., 2006).  $\beta$ -xylosidase is essential for the complete breakdown of xylans (the major hemicellulose component in plant cell walls) and is produced by plant, animals and microbes (Poutanen and Puls, 1988; Nanmori et al., 1990; Saha, 2002). This study showed that all biochar treatments reduced potential activity of xylosidase enzyme in non-stressed soil, whereas intermediate rates (1–5%) of biochar amendment reduced the potential activity of leucine aminopeptidase. The reductions were likely due to the sorption of enzymes by biochar and subsequent masking of active sites, rather than sorption of substrates or products as the assay corrected for quenching, presumably due to biochar porosity and reactive surface area (Thies and Rilling, 2009; Jindo et al., 2012). In contrast, the highest biochar application rate (10%) resulted in leucine aminopeptidase potential activity equivalent to the control treatment, which suggests that at high enough rates, biochar might stimulate enzyme production, protect enzymes from degradation, or facilitate substrate-enzyme reactions by acting as a platform so that higher activities are detected.

Previous studies have showed that the enzymes in soils are resistant to denaturation by heat and other stresses when associated with abiotic fraction such as soil colloids and clay minerals (Hayano and Katami, 1977; Miller and Dick, 1995; Nannipieri et al., 1996; Deng and Tabatabai, 1997). To date, this is the first study we are aware of that examines the potential application of biochar for the purposeful stabilization of extracellular enzymes in soil. This study found that biochar had variable effects on soil enzymes in terms of protecting enzymes from a denaturing stress. When exposed to microwave stress, leucine aminopeptidase potential activity declined with increasing stress levels, although at 800 J g<sup>-1</sup>, potential activity was somewhat (but not significantly) maintained to a greater degree in soil amended with 1% biochar compared to the other treatments (Fig. 3). Interestingly, the 1% biochar treatment also maintained  $\beta$ -xylosidase potential activity when soil was exposed to 400 J g<sup>-1</sup> of microwave stress, while a greater concentration of biochar (5%) protected  $\beta$ -xylosidase potential activity upon exposure to an even greater stress level (800 J g<sup>-1</sup>).

Very little is known about the stabilization of extracellular enzymes interacting with biochar. It is likely that the size matching between pore size of biochar and the molecular diameter of enzymes will play a key role in achieving high enzymatic stability (Klibanov, 1983). Many other factors such as the temperature stability range and isoelectric point might also play an important role in enzyme stability, especially at high temperature. Both leucine aminopeptidase and  $\beta$ -xylosidase are relatively small in size (28–400 kDa and 20–120 kDa, respectively) and have a wide temperature stability range (25–100 °C and 30–95 °C, respectively) (Schomburg et al., 2013). Leucine aminopeptidase also has a high isoelectric point (8.2), meaning that this enzyme carries a net positive surface charge below pH of 8.2. Thus, this enzyme could have been attracted to negative sites present on biochar surfaces and subsequently sorbed. Hardwood biochars which have undergone fast pyrolysis at 500 °C have an average of 28.8 mmolc kg<sup>-1</sup> of negative sites (Ippolito et al., 2015). In comparison,  $\beta$ -D-cellobiosidase and  $\beta$ -N-acetylglucosaminidase have relatively lower ranges of temperature stability (40–80 °C and 20–37 °C, respectively) and lower isoelectric points (3.8 and 4.6, respectively) (Schomburg et al., 2013). These enzymes would carry net negative surface charges, which would have been repelled by negative biochar sites, affecting their ability to be sorbed and stabilized by the biochar used in this study. Others have found that enzyme size was important for enzyme stabilization on nanostructures such as mesoporous silica (Diaz and Balkus, 1996), but further research is necessary to understand the mechanisms of enzyme stabilization on biochar.

## 5. Conclusions

Stabilization of soil extracellular enzymes may maintain enzymatic activity for ecosystem services such as C sequestration, nutrient cycling, and bioremediation, while protecting enzymes from proteolysis and denaturation. A laboratory experiment was conducted to determine if biochar added to soil would stabilize soil extracellular enzymes so that enzymes would retain potential activity after a denaturing stress. This study found that biochar has the potential to prevent evaporative losses of soil water to some degree and stabilize certain extracellular enzymes such as  $\beta$ -xylosidase so that activity is maintained after a denaturing stress. This effect was dependent, however on biochar application rate and the enzyme itself. Furthermore, while biochar may reduce the potential activity of certain extracellular enzymes in soil, this phenomenon was not universal as the majority of enzymes assayed in this study were unaffected by exposure to biochar.

More research is needed to understand the mechanism(s) by which biochar stabilizes some extracellular enzymes but not others, and how stabilization is affected by different biochars and biochar concentrations in soil.

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