J Soils Sediments (2015) 15:578–583
DOI 10.1007/s11368-014-1029-7

SOILS, SEC 2 • GLOBAL CHANGE, ENVIRON RISK ASSESS, SUSTAINABLE LAND USE • SHORT ORIGINAL COMMUNICATION

Biochar modifies the thermodynamic parameters of soil enzyme activity in a tropical soil

Jorge Paz-Ferreiro · Shenglei Fu · Ana Méndez · Gabriel Gascó

Received: 30 July 2014 / Accepted: 9 November 2014 / Published online: 21 November 2014

Abstract
Purpose Temperature is a key determinant of soil microbial processes, including the decomposition of soil organic matter and nutrient cycling. There is an interest in obtaining information on how microbial processes will respond to global change and, in particular, warming. Biochar can modify soil biological properties, but there is a dearth of information about its influence on the temperature sensitivity of soil biological processes. This research question has important implications in relation to modeling land-atmosphere interactions in soils amended with biochar.
Materials and methods Enzyme activity was determined at 4, 18, 27, 37, 54, and 70 °C in a control soil and in a soil amended with biochar, in order to determine how biochar affects the temperature sensitivity of soil enzymes (CM-cellulase, β-glucosidase, β-glucosaminidase, phosphomonoesterase, and arylsulfatase). The activation energy (E_a) and the temperature coefficient (Q_{10}) were calculated.
Results and discussion In general, the values of E_a and Q_{10} were slightly but significantly lower for the soil with biochar. The results obtained are significant for modeling the carbon cycle and nutrient cycles in biochar-amended soils.
Conclusions The lower values for Q_{10} obtained for biochar-amended soil might be indicative of soil enzymes being underestimated by current enzyme assay conditions in biochar-amended soils.

Keywords Biochar · Q_{10} · Soil enzymes · Temperature sensitivity

Responsible editor: Chengrong Chen

J. Paz-Ferreiro (✉)
School of Civil, Environmental and Chemical Engineering, RMIT University, GPO Box 2476, Melbourne, VIC 3001, Australia
e-mail: jorge.paz-ferreiro@rmit.edu.au

S. Fu
Key Laboratory of Vegetation Restoration and Management of Degraded Ecosystems, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

A. Méndez
Departamento de Ingeniería Geológica y Minera. E.T.S.I. Minas, Universidad Politécnica de Madrid, C/Ríos Rosas n°21, 28003 Madrid, Spain

G. Gascó
Departamento de Producción Agraria, ETSI Agrónomos, Universidad Politécnica de Madrid, Avenida Complutense 3, Madrid 28050, Spain

1 Introduction

Soil enzymes catalyze the rate-limiting step in dissolved organic matter production and organic matter decomposition and are involved in the biogeochemical cycling of nutrients. Thus, soil enzymes are catalysts that play an important role in modulating ecosystem responses to changes in abiotic (temperature, humidity, changes in nutrient availability, or substrate quality) and biotic conditions. In addition, potential enzyme activities have been used for decades as indicators of soil quality and nutrient cycling Burns et al. (2013).

Enzymatic activity is typically measured only at a single temperature. However, enzymatic reactions are temperature sensitive. The temperature sensitivity of soil enzymes can be calculated from the activation energy (E_a). The E_a for enzyme-catalyzed reactions is lower than those for reactions not catalyzed by enzymes, as a consequence of enzymes diminishing the energy barrier that must be exceeded before a chemical reaction can occur. Values of E_a, and thus the temperature sensitivity of soil enzymes, are specific not only for enzymes but also depend on the edaphic medium and are prone to seasonal variations (Wallenstein et al. 2009).

Recently, increasingly realistic soil carbon dynamics and nutrient cycling models taking into account microbial processes were the object of high-profile research (Allison 2012; Lawrence et al. 2009; Schimel and Weintraub 2003). However, there are many obstacles hindering an accurate modeling of soil carbon and nutrient cycling. An important challenge concerns the integration of data regarding soil enzymatic reactions. Furthermore, as remarked by other researchers (Todd-Brown et al. 2012), these models usually utilize constant Q_{10} values, although some of them are starting to take into account the thermodynamic parameters characterizing soil enzymes (Allison 2012).

Biochar is a soil conditioner that alters soil physical, chemical, and biological properties, including nutrient cycling and organic matter decomposition (Biederman and Harpole 2013) but also soil microbial activity (Gomez et al. 2014). Biochar is also receiving an increased amount of attention by the scientific community due to its potential to increase agronomic yields (Liu et al. 2013). In the last years, as enzymological studies considering biochar addition to soil emerged, it has been found that biochar usually results in an increase in soil enzyme activity, although exceptions are present (Paz-Ferreiro et al. 2014). It also seems plausible that the effects of biochar in soil enzyme activity are more apparent in tropical compared to temperate areas (Paz-Ferreiro et al. 2014).

However, to date, no studies have explored the effect of biochar on the temperature sensitivity of soil processes, a topic that has profound implications, considering the present awareness surrounding the effects of climate change on soil organic matter decomposition. It is known that changes in the soil nutrient status (Stone et al. 2012) or in the quality of soil organic matter (Trasar-Cepeda et al. 2007) can lead to a profound effect on the thermodynamic parameters of soil enzymes. We hypothesized that this alterations would also be present following the addition of biochar to the soil, as both the nutrient and organic matter characteristics of the soil would be altered. Thus, the aim of our study was to study if biochar addition could alter the temperature sensitivity of soil microbial processes in a tropical soil. A wide range of temperatures was selected to perform our study, as it is a common practice in most of the studies involving thermodynamic parameters of soil enzyme activities (Trasar-Cepeda et al. 2007). Moreover, in our study area, soils may reach temperatures in excess of 60 °C as a consequence of direct radiative warming on recently tilled soil.

2 Material and methods

Soil (a Haplic Acrisol) was collected at Heshan Hilly Land Interdisciplinary Experimental Station, Chinese Academy of Sciences in Guangdong Province, China, located at 22° 41′ N and 112° 54′ E in September of 2013. The selected soil is very representative of the dominant soils in Southern China. The climate of the region is subtropical monsoon with a mean annual precipitation of 1700 mm and a mean annual evapotranspiration of 1600 mm. Precipitation mainly occurs in the rainy season from April to September. The mean annual temperature is 21.7 °C with a mean maximum monthly temperature of 29.2 °C in July and a mean monthly minimum of 12.6 °C in January. The soil has a sandy-clay-loam texture, with a sand content of 52 % and a clay content of 31 %. Soil pH value (in water) was 3.95, and C and N contents were 3.5 and 0.25 %, respectively.

Samples were taken from the 0–10-cm depth using a trowel and immediately passed through a 2-mm sieve in the field-moist state. It was then mixed and split into subsamples for a pot experiment.

The biochar was prepared from poultry litter at a final temperature of 400 °C and heating at rate of 10 °C min^{-1} as described in a previous article (Lu et al. 2014) where it has been fully characterized. This biochar was selected as previous experiments using the same material have shown an increase of agronomic yields in several plant species which was up to 200 % (Lu et al. 2014).

In September of 2013, a mesocosm using a fully replicated randomized experiment was set up in a greenhouse in South China Botanical Garden using four replications and two treatments (control and biochar addition at a rate of 3 % *w*/*w*). Each of the eight mesocosms involved in the experiment consisted of a pot filled with 500 g of soil. The soils were adjusted to a humidity content of 60 % of field capacity and watered daily to account for moisture losses.

Soil was incubated at room temperature for 4 months. After 4 months, soil was collected and stored in a fridge (4 °C) prior to soil analyses. Analysis of soil enzyme activity was done after a maximum of 1 week.

Enzyme activities were assayed in triplicate using published protocols which are described by Paz-Ferreiro et al. (2014) for β-glucosidase, β-glucosaminidase, phosphomonoesterase, and arylsulfatase but altering the temperature of the assay. CM-cellulase activity was determined after incubating the samples with carboxymethyl cellulose as substrate and incubating for 24 h in a 2 M acetate buffer (pH 5.5) and assessing the released reducing sugars following the method of Schinner and von Mersi (1990). All enzyme concentrations were determined by reference to standard curves as described by Paz-Ferreiro et al. (2014). In all cases enzyme activity was determined at 4, 18, 27, 37, 54, and 70 °C. In all the assays, we ensured that the reaction was not substrate limited.

The response of enzyme activity to temperature alterations was described by means of the Arrhenius equation:

$$k = A\mathrm{e}^{(-E_a/RT)}$$

where A is the pre-exponential factor, E_a is the activation energy, R is the gas constant, and T is the absolute

temperature (K). Thus, E_a was calculated from the slope of the graphic obtained representing the Naperian logarithm of enzyme activity versus $1/T$ (see Fig. 1). The activation energy and Q_{10} are parameters mechanistically linking temperature response with enzyme kinetics, while providing a measurement of the sensitivity of the enzyme to warming.

We calculated the temperature sensitivity of enzyme activity by calculating the Q_{10} of the enzymes between 4 and 70 °C or between 4 and 54 °C, depending on the interval of temperature in which they followed Arrhenius equation, as in Wallenstein et al. (2009):

$$K = (\ln R70 - \ln R4)/66 \text{ or } K = \ln(R54 - \ln R4)/50$$
$$\text{and } Q_{10} = \exp(10 \times K)$$

where R4, R54, and R70 represent the activities of the enzymes and 4, 54, and 70 °C.

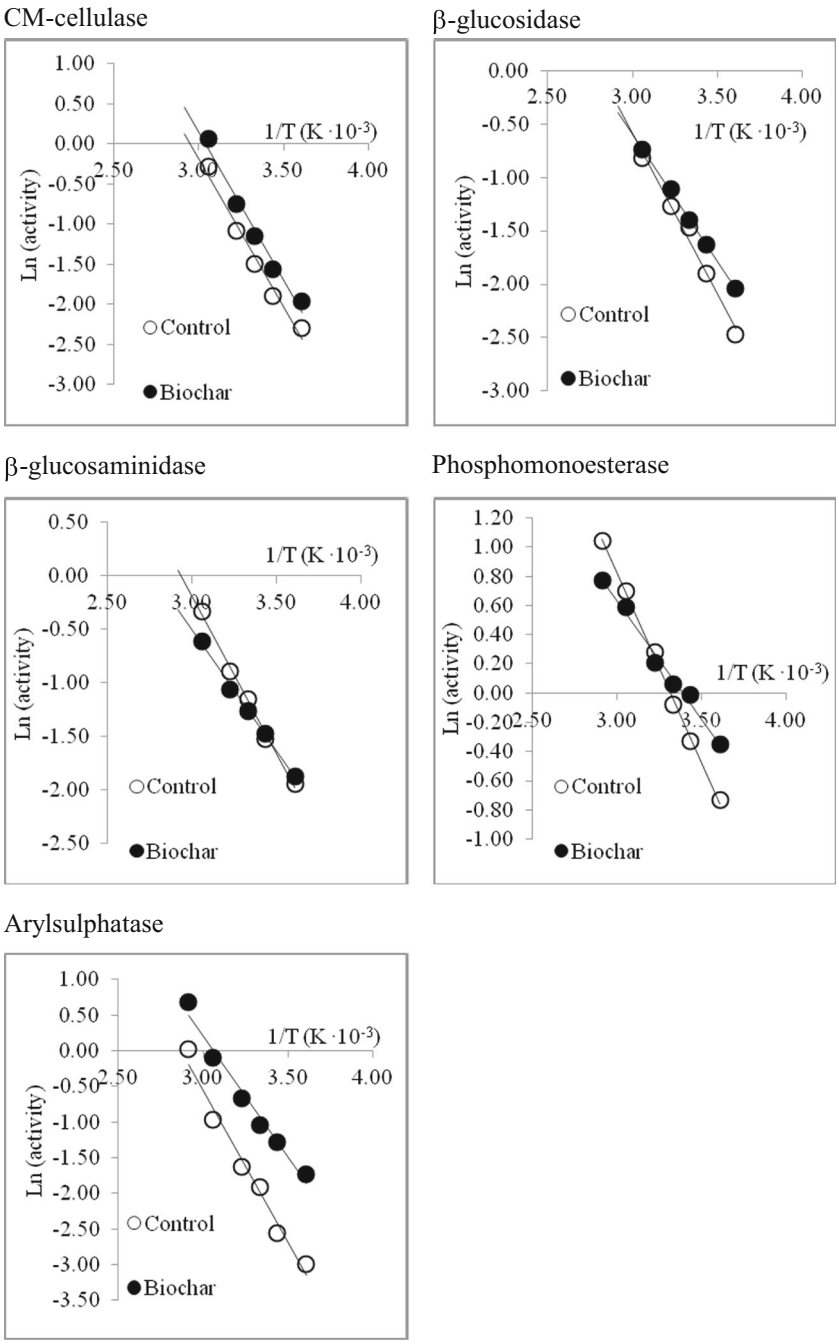

Fig. 1 Arrhenius plots for enzyme activities

All statistical analyses were done using SPSS 15.0. A one-way ANOVA was carried out to test the effect of biochar use on Q_{10} and E_a. The differences were considered to be significant at $P<0.05$ level.

3 Results and discussion

Data of enzyme activity are shown in Fig. 2. Biochar addition increased soil enzyme activity for cellulase, β-glucosidase, and arylsulfatase in the temperature range studied (see Table 1). For all hydrolases, we observed the same pattern of changes in activity with temperature. The rate of substrate hydrolysis increased up to 70 °C, except for CM-cellulase, where the rate decreased for temperatures higher than 54 °C. In general, this behavior is consistent with that found in other studies (Trasar-Cepeda et al. 2007).

While Q_{10} and E_a values are mathematically related, they are discussed separately in this section as in most of the articles in the bibliography, only one of these values are provided. Our results for E_a were higher for cellulase (values of 30.56 and 30.69 kJ K^{-1} mol^{-1} for soils without and with biochar, respectively) and arylsulfatase (values of 35.51 and 28.29 kJ K^{-1} mol^{-1} for soils without and with biochar, respectively) than for other enzymes (see Table 2). All our values agree with the range of values found by other authors (Trasar-Cepeda et al. 2007; Stone et al. 2012; Steinweg et al. 2013) and are in the lower range of those reviewed by Sinsabaugh and Shah (2012). However, our values for the E_a of β-glucosaminidase were lower than those reported by Parham and Deng (2000). We were able to estimate the temperature

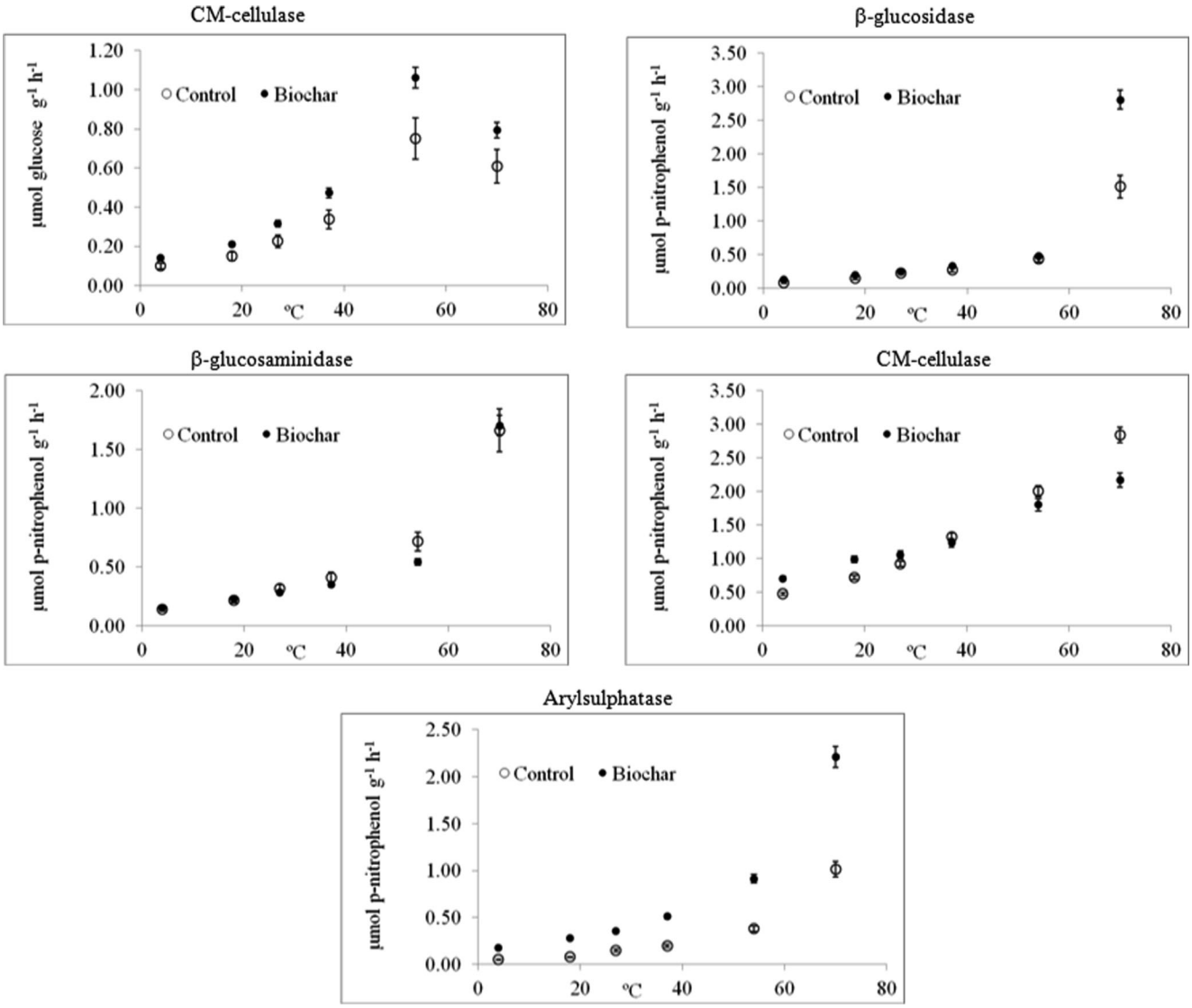

Fig. 2 Enzyme activity in soils with and without biochar

Table 1 Effect of temperature and treatment (biochar vs control) and their interaction on soil enzyme activity

Enzyme	Temperature	Treatment	Temperature × treatment
Cellulase	2508.7 (<0.001)	725.4 (<0.001)	64.4 (<0.001)
β-Glucosidase	810.3 (<0.001)	122.6 (<0.001)	88.4 (<0.001)
β-Glucosaminidase	669.2 (<0.001)	3.6 (0.064)	3.1 (0.020)
Phosphomonoesterase	272.1 (<0.001)	2.6 (0.115)	16.3 (<0.001)
Arylsulfatase	614.2 (<0.001)	532.5 (<0.001)	77.3 (<0.001)

F values are shown with *P* values in brackets

sensitivity of several hydrolases, which is an essential control of in situ enzyme activity (Wallenstein et al. 2009). Our values for Q_{10} were also in the lower range compared with other studies (Sinsabaugh and Shah 2012) which was expectable as cold-adapted enzymes are generally more responsible to increases in temperature than warm-adapted enzymes (Koch et al. 2007).

We found that with the exception of CM-cellulase ($F<0.01$, $P=9.54$), the Q_{10} of soil enzymes diminished in soils amended with biochar. In particular, this was true for β-glucosidase ($F=6.57$, $P<0.05$), β-glucosaminidase ($F=12.29$, $P<0.05$), phosphomonoesterase ($F=9.12$, $P<0.05$), and arylsulfatase ($F=11.90$, $P<0.05$). There are several possible explanations for this result. First, this could be due to changes in the microbial community which implies the release of a different set of isoenzymes (enzymes differing in their amino acid sequence but catalyzing the same chemical reaction). This mechanism has been suggested to explain seasonal differences in Q_{10} for a same set of soils (Wallenstein et al. 2009) and for soils under different land use (Khalili et al. 2011). However, different isoenzymes have also been related to the same microbial community transcribing alternate genes (Loveland et al. 1994). In fact, biochar has been shown to alter soil microbial community composition (Gomez et al. 2014), although depending on the particular biochar and soil the dominance could be shifted toward more dominated bacterial or more dominated fungal channels (Liang et al. 2014). These changes alone could explain a release of a different set of isoenzymes. Another mechanism that we suggest to account for these differences would be the conformational changes that alter the active site in the enzyme which could be due to adsorption into biochar particle, a mechanism that has been reported previously in clay particles (Quiquampoix 2000). Finally, pH increased after biochar addition to a value of 6.39. There is a scarce literature on the effect of pH on Q_{10} values, but other studies found no relation for a set of four enzymes (Steinweg et al. 2013). It is difficult to quantify how much of the observed changes in temperature sensitivity were due to an alteration of the edaphic chemical conditions and, in particular, soil pH. However, we should bear in mind that biochar addition in this kind of highly weathered tropical soils will always lead to a sharp increase in soil pH. In this sense, the dose of biochar used in this soil is highly relevant for agronomic improvement (Lu et al. 2014).

Previous studies (Steinweg et al. 2013) have shown that enzymes depolymerizing high molecular weight compounds, such as CM-cellulase, require more enzymatic steps and have higher E_a and Q_{10} values than enzymes that break down simpler compounds (such as β-glucosidase). This suggest that reactions taking place at the later stage of soil organic decomposition are more favored than those occurring at earlier stages and is confirmed by the higher E_a and Q_{10} values of β-glucosidase, compared to cellulose, found in our study. It is interesting to observe that biochar addition has altered the relative value of the parameters E_a and Q_{10} for cellulase and β-glucosidase. Higher values of E_a are indicative of a lower

Table 2 Activation energy (E_a) and Q_{10} of the hydrolases investigated, calculated from the temperature range in which Arrhenius equation was followed

Enzyme	Treatment	Temperature range (°C)	E_a (kJ K^{-1} mol^{-1})	Q_{10}	*r*
Cellulase	Control	4–54	30.56±3.12	1.50±0.14	0.98
	Biochar	4–54	30.69±1.22	1.50±0.06	0.98
β-Glucosidase	Control	4–54	24.91±1.08	1.39±0.06	0.99
	Biochar	4–54	19.70±0.61	1.30±0.04	0.99
β-Glucosaminidase	Control	4–54	24.35±0.71	1.38±0.04	0.99
	Biochar	4–54	18.60±0.45	1.29±0.03	0.99
Phosphomonoesterase	Control	4–70	25.70±0.97	1.32±0.05	0.99
	Biochar	4–70	13.51±0.79	1.19±0.07	0.99
Arylsulfatase	Control	4–70	35.51±0.45	1.58±0.02	0.99
	Biochar	4–70	28.29±1.15	1.47±0.06	0.98

substrate affinity, and our results are suggesting that, in thermodynamic terms, the enzyme hydrolyzing low molecular weight substrates (β-glucosidase) is more favored than the enzyme hydrolyzing a higher molecular weight substrate (cellulase). This effect was more pronounced in biochar-amended soils than in the control soils.

Our results show that biochar-amended soils have a lower temperature sensitivity, which could result in an underestimation, at field temperatures, of soil enzymatic activity in biochar-amended soils. This can be shown in the values of enzyme activity in Fig. 2.

With regard to the effect of climate change, our data (higher E_a and Q_{10} in the control soils) show that biochar-amended soils are less likely to be affected by the increase in temperature. Models of enzymatic decomposition need to account for the different interaction with temperature between the biochar soil and the soil systems. This can only be achieved through a better understanding of the interaction between soil and biochar and of enzyme kinetics.

4 Conclusions

We found lower values for Q_{10} in biochar-amended soil. Owing to differences between field temperatures and temperatures for soil enzymatic assays, this might be indicative of soil enzymes being underestimated by current enzyme assay conditions in biochar-amended soils. From our findings, it emerges clearly that are necessary further studies regarding the temperature dependence of hydrolytic enzymes in soils from different latitudes. This can improve our understanding and capability to predict the effects of warming on soil organic matter decomposition and nutrient cycling in biochar-amended soils.

References

Allison SD (2012) A trait-based approach for modelling microbial litter decomposition. Ecol Lett 15:1058–1070

Biederman LA, Harpole WS (2013) Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. GCB Bioenergy 5:202–214

Burns RG, de Forest JD, Marxsen J, Sinsabaugh RL, Stromberger ME, Wallenstein MD, Weintraub MD, Zoppini A (2013) Soil enzymes in a changing environment: current knowledge and future directions. Soil Biol Biochem 58:216–234

Gomez JD, Denef K, Stewart CE, Zheng J, Cotrufo MF (2014) Biochar addition rate influences soil microbial abundance and activity in temperate soils. Eur J Soil Sci 65:28–39

Khalili B, Nourbakhsh F, Nili N, Khademi H, Sharifnabi B (2011) Diversity of soil cellulase isoenzymes is associated with soil cellulose kinetic and thermodynamic parameters. Soil Biol Biochem 43: 1639–1648

Koch O, Tscherko D, Kandeler E (2007) Temperature sensitivity of microbial respiration, nitrogen mineralization, and potential soil enzyme activities in organic alpine soils. Glob Biogeochem Cycles 21:GB4017

Lawrence CR, Neff JC, Schimel JP (2009) Does adding microbial mechanisms of decomposition improve soil organic matter models? A comparison of four models using data from a pulsed rewetting experiment. Soil Biol Biochem 41:1923–1934

Liang CF, Zhu X, Fu S, Méndez A, Gascó G, Paz-Ferreiro J (2014) Biochar alters the resistance and resilience to drought in a tropical soil. Environ Res Lett 9:064013

Liu X, Zhang A, Ji C, Joseph S, Bian R, Li L, Pan G, Paz-Ferreiro J (2013) Biochar's effect on crop productivity and the dependence on experimental conditions- a meta-analysis of literature data. Plant Soil 373:583–594

Loveland J, Gutshall K, Kasmir J, Prema P, Brenchley JE (1994) Characterization of psychrotrophic microorganisms producing beta-galactosidase activities. Appl Environ Microbiol 60:12–18

Lu H, Li Z, Fu S, Méndez A, Gascó G, Paz-Ferreiro J (2014) Can biochar and phytoextractors be jointly used for cadmium remediation? PLoS ONE 9:e95218

Parham JA, Deng SP (2000) Detection, quantification and characterization of β-glucosaminidase activity in soil. Soil Biol Biochem 32: 1183–1190

Paz-Ferreiro J, Fu S, Méndez A, Gasco G (2014) Interactive effects of biochar and the earthworm *Pontoscolex corethrurus* on plant productivity and soil enzymes activities. J Soils Sediments 14:483–494

Quiquampoix H (2000) Mechanisms of protein adsorption on surfaces and consequences for extracellular enzyme activity in soil. In: Bollag J, Stotzky G (eds) Soil biochemistry. Marcel Dekker, New York, pp 171–206

Schimel JP, Weintraub MN (2003) The implications of exoenzyme activity on microbial carbon and nitrogen limitation in soil: a theoretical model. Soil Biol Biochem 35:549–563

Schinner F, von Mersi W (1990) Xylanase-, CM-cellulase- and invertase activity in soil: an improved method. Soil Biol Biochem 22: 511–515

Sinsabaugh RL, Shah JJF (2012) Ecoenzymatic stoichiometry and ecological theory. Annu Rev Ecol Evol Syst 43:313–343

Steinweg JM, Jagadamma S, Frerichs J, Mayes MA (2013) Activation energy of extracellular enzymes in soils from different biomes. PLoS ONE 8:e59943

Stone MM, Weiss MS, Goodale CS, Adams MB, Fernandez IJ, German DP, Allison SD (2012) Temperature sensitivity of soil enzyme kinetics under N-fertilization in two temperate forests. Global Change Biol 18:1173–1184

Todd-Brown KEO, Hopkins FM, Kivlin SN, Talbot JM, Allison SD (2012) A framework for representing microbial decomposition in coupled climate models. Biogeochemistry 109:19–33

Trasar-Cepeda C, Gil-Sotres F, Leirós MC (2007) Thermodynamic parameters of enzymes in grassland soils from Galicia, NW Spain. Soil Biol Biochem 39:311–319

Wallenstein MD, McMahon SK, Schimel JP (2009) Seasonal variation in enzyme activities and temperature sensitivities in Arctic tundra soils. Global Change Biol 15:1631–1639