



# Influence of biochar on potential enzyme activities in two calcareous soils of contrasting texture



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## ABSTRACT

Application of pyrolysed feedstocks or biochar has the potential to affect soil enzyme activity and function. Nevertheless, our detailed understanding of the mechanisms responsible for biochar-enzyme interaction is limited in arid soils. The main aim of this study was to study how the potential activity of several extra- and intra-cellular enzymes involved in C and N cycling, and microbial metabolism would respond to addition of biochar in two calcareous soils with fine and coarse texture. Three corn stalk biochars were prepared at 200, 400 and 600 °C and added to sandy and clayey soils at 0.5 and 1% (w/w). Soils with uncharred feedstocks; and without biochar and feedstock additions as the control treatment were also included in the experiment. The potential activities of soil enzymes were assayed. Compared with the control, addition of uncharred and charred feedstocks significantly stimulated the activities of catalase (1.3- to 4.3-fold), dehydrogenase (1.2- to 3.1-fold), cellulase (1.1- to 1.7-fold), invertase (1.3- to 5.8-fold) and protease (1.03- to 2.9-fold), which varied with pyrolysis temperature and addition rate as well as soil texture. The positive effects of biochar addition on soil enzymes were much greater at 1% than 0.5% application rates for all the assayed enzymes and in sandy than clayey soils for catalase, dehydrogenase and invertase. The change in enzyme activity with biochar addition primarily attributed to the change in soil microbial biomass. However, enzyme activities were lower in the soils amended with charred than uncharred biomass, and decreased as pyrolysis temperature increased. Our results showed clearly that uncharred feedstock and lower temperature biochars gave higher benefits to both contrasting textured soils than higher temperature biochars, largely connected to changes in the physiochemical properties of biochars. Nevertheless; long-term field experiments are required to verify whether the beneficial effect of uncharred vs. charred feedstocks would be maintained over long timescales under the conditions of this study. We conclude that although biochar application may improve enzyme activities of calcareous soils with low organic matter content, increasing pyrolysis temperature adversely affects soil enzymatic functions, depending mainly on soil texture and application rate. The study evidently indicates that corn biochar addition to arid-soils may have a high potential for improving the enzyme activities as important indicators of soil quality, and subsequently carbon sequestration and biogeochemical cycles.

## 1. Introduction

Soil extra- and intra-cellular enzymes are important biocatalysts accelerating the biochemical reactions necessary for microbial life cycle and metabolism, soil organic matter (SOM) formation and decomposition, organic residue decomposition, nutrient cycling and degradation of hazardous organic pollutants (Burns et al., 2013). These biomolecules are the main driving force for microbial and biochemical processes that ultimately determine the soil function within ecosystems (Dick, 1994; Nannipieri et al., 2002). Soil enzymes are extensively used as potential microbial indicators of soil health and quality because they

are highly sensitive to soil management practices (Dick, 1997; Paz-Ferreiro and Fu, 2016). The activities of soil enzymes are largely influenced by abiotic and biotic factors that may impact its half-live times in the soil (Burns et al., 2013). In general, addition of organic amendments increases or maintains soil enzyme activity as a consequence of microbial proliferation, enzyme immobilization and protection from degradation and denaturation; and the enhancement of enzyme secretion (Gianfreda and Ruggiero, 2006). Among the organic amendments, pyrolysed biomass or biochar is also known to affect the activity of many soil enzymes and related microbial properties (Bailey et al., 2011; Masto et al., 2013; Demisie et al., 2014; Paz-Ferreiro et al., 2014).

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Biochar is the product of carbonized organic feedstocks (biomass) produced through pyrolysis or thermal conversion at high temperatures under low or zero oxygen concentrations (Sohi et al., 2010; Mukherjee and Lal, 2016). Biochar as a valuable soil amendment has received much attention from soil science community because of its beneficial effects on soil fertility and quality (Sohi et al., 2010; Paz-Ferreiro et al., 2014; Masto et al., 2013; Mukherjee and Lal, 2016), plant growth and production (Masto et al., 2013; Kumar et al., 2013; Xu et al., 2016), climate change mitigation through C sequestration in soil (Spokas, 2010; Ippolito et al., 2012). Generally, biochar addition to soil increases the activity activities of different extracellular enzymes involved in C, N, P and S cycles (Masto et al., 2013; Paz-Ferreiro et al., 2014; Demisie et al., 2014; Gascó et al., 2016) and intracellular enzymes involved in the life processes of soil microbes, in particular dehydrogenase and catalase (Kumar et al., 2013; Ouyang et al., 2014; Lu et al., 2015) in both long and short-term experiments conducted under field or laboratory conditions. The enhanced soil enzyme activities have been attributed indirectly to an improvement in soil physical and chemical properties, such as improved soil aeration, increased soil specific surface area, improved soil water holding capacity or the presence of different labile carbon (C) compounds in the biochar for enzymatic reactions (Lehmann et al., 2011; Ameloot et al., 2013; Demisie et al., 2014; Ouyang et al., 2014; Gul et al., 2015; Lu et al., 2015). Changes in enzyme activity as an indirect consequence of biochar addition would also occur through increased microbial activity and biomass as well as alterations in microbial community structure (Lehmann et al., 2011; Masto et al., 2013; Ouyang et al., 2014; Foster et al., 2016; Zheng et al., 2016); and directly through co-location of enzymes and their interaction with biochar surface (Lehmann et al., 2011; Gul et al., 2015). Nonetheless, biochar application can also have negative (Wu et al., 2013; Ameloot et al., 2014; Foster et al., 2016; Zheng et al., 2016) or no effect on soil enzyme activity (Bailey et al., 2011; Paz-Ferreiro et al., 2012; Ameloot et al., 2014; Elzobair et al., 2016). The discrepancies in biochar effects on soil enzymes are likely due to the type of soil and biochar (feedstock type), biochar production conditions (pyrolysis temperature and duration, particle size), application rate and the duration of the experiment (Bailey et al., 2011; Ameloot et al., 2014, 2015; Ouyang et al., 2014; Gascó et al., 2016). For instance, dehydrogenase activity increased in the presence of 350 °C biochar but decreased with 700 °C biochar addition in a temperate sandy loam soil (Ameloot et al., 2013). In a recent study, soil dehydrogenase activity increased only with addition of 300 °C manure biochar with no effect in the presence of the same biochar produced at 500 °C (Gascó et al., 2016). Low temperature biochars increased enzyme activity more than high temperature biochars, because of a lower content of aromatic structures and a higher amount of easily degradable compounds (Ouyang et al., 2014). Although the underlying mechanisms behind these effects are not fully understood and cannot be generalized, it is probably due to alterations in the soil pH and salinity (Paz-Ferreiro et al., 2012, 2014; Masto et al., 2013; Zheng et al., 2016), changes in biochar chemistry and properties including aromaticity and hydrophobicity after pyrolysis (Kumar et al., 2013; Ouyang et al., 2014) or the presence of toxic compounds in pyrolyzed feedstocks (Oleszczuk et al., 2014). It appears that the effect biochar on enzyme activities is not well appreciated across different soils under a variety of climatic conditions; and a better understating is essential for long-term and practical application of biochar as a soil amendment for the improvement of soil quality and fertility. Understanding the direct and indirect effect of biochar on the activity of soil enzymes has been acknowledged as a top research priority (Lehmann et al., 2011; Gul et al., 2015) because enzyme activity is often considered a sensitive indicator of early changes in soil degradation and quality (Paz-Ferreiro and Fu, 2016). Furthermore, although the response of soil enzyme activities to biochar addition is well documented (Masto et al., 2013; Zheng et al., 2016), their sensitivity to biochar obtained from different pyrolysis conditions (e.g., temperature) is poorly known (Ouyang et al., 2014; Ameloot

et al., 2015; Gascó et al., 2016). Yet, very little is known about the influence of biochar application on enzyme activity in arid and semi-arid soils with low organic matter content (Elzobair et al., 2016; Foster et al., 2016).

We tested the hypotheses that (1) biochar addition to the arid soils would stimulate enzyme activity and (2) the stimulating effects of corn biochar would vary with production temperature, application rate and soil texture. The objectives of this study were to (1) determine whether corn biochar produced at different pyrolysis temperatures has a significant impact on the activity of soil enzymes involved in C (invertase and cellulase) and N (protease) cycling, and microbial metabolism (catalase and dehydrogenase), (2) compare the levels of soil enzyme activities between the soils amended with corn biochar and soils amended with uncharred corn feedstock (not carbonized materials) and (3) establish whether the effect of corn biochar on soil enzyme activities is dependent on the application rate and soil texture in two arid soils.

## 2. Materials and methods

### 2.1. Soil sampling and biochar production

Detailed descriptions of soil sampling and analysis procedures can be found in the work by Khadem and Raiesi (2017). In brief, two calcareous soils with contrasting texture collected from the 0–20 cm of arable fields were used for this study. The basic soil properties before biochar amendment (Khadem and Raiesi, 2017) are reported in Supplementary Table S1. The biochar was obtained from corn feedstocks (mainly stalks) and produced using three different pyrolysis temperatures (200, 400 and 600 °C). Khadem and Raiesi (2017) reported the detailed descriptions of biochar production and chemical analysis. Biochar physical and chemical properties are listed in Supplementary Table S1.

### 2.2. Experimental design, soil incubation and enzyme assay

A completely randomized design with  $2 \times 4 \times 2$  full-factorial treatment combination was used in four replicates with the following factors: (1) soil texture (sandy and clay), (2) biochar temperature produced at 0, 200, 400 and 600 °C, and biochar application rate (0.5 and 1% w/w). A sample of each soil without addition of corn residue and biochar was also included as the control (C). A full description of the experimental setup has been reported elsewhere (Khadem and Raiesi, 2017). The soil samples were pre-incubated at 70% water holding capacity and 25 °C for 10 days to restore or reactivate microbial population and community and then incubated for 90 days. The activity of dehydrogenase (EC 1.1.1.30), invertase (EC 3.2.1.26), cellulase (EC 3.2.1.4) and protease (EC 3.4.4) was determined according to the procedures described by Alef and Nannipieri (1995), using fresh samples from the incubated soils. Catalase (EC 1.11.1.6) activity was measured based on its ability to hydrolyze hydrogen peroxide in the presence of potassium permanganate using the method described by Liu et al. (2008). All results were re-calculated on a 105 °C oven-dry weight basis. As a soil quality index, the geometric mean for enzyme activity (GME) was calculated according to Hinojosa et al. (2004) and García-Ruiz et al. (2008).

### 2.3. Statistical analyses

Differences in enzyme activity were characterized using a three-way ANOVA, considering pyrolysis temperature (0, 200, 400 and 600 °C), application rate (0.5 and 1%) and soil texture (sandy and clayey) as the factorial factors of the experiment. All statistical tests were carried out using the SPSS 17 software for Windows. The least significant difference (LSD) test was used to separate the means of the soil enzymes activities at  $P < 0.05$ , unless stated otherwise. Simple linear correlation and regression analyses were used to characterize the relationship between

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