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Activated biochar alters activities of carbon and nitrogen acquiring soil enzymes

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ABSTRACT

When biochar (BC) ages in soil, its properties change substantially: cation exchange capacity (CEC), surface area and porosity increase and water repellency decreases, consequently affecting the interactions with soil microorganisms. Activation of BC by organic acids may be regarded as artificial aging. Here, we study the effect of acid-activated BCs on soil microbial enzyme activities (EA) in comparison to several different control treatments without activated BC. A greenhouse pot experiment was conducted using a vineyard soil treated with multiple soil additives (four replications). In each pot, one grapevine and a selection of cover crops was grown. During incubation, the pots were sampled three times, one month, seven months and 22 months after establishment. Potential exoglucanase (EG) and ß-glucosidase (BG) activities were measured to assess possible effects on C dynamics, and exochitinase (NAG) and protease (LAP) activities for N dynamics. One month following incubation, the EAs of treatments with activated BCs increased for all four enzyme classes compared to controls; after seven months, C acquiring EAs were elevated; while after 22 months, LAP EA was elevated. Our results suggest that biochar oxidation with organic acids simulated biochar aging with distinct effects on EAs; however, further research is needed to identify the mechanisms that drive biological parameters such as enzymatic activity in response to activated BCs.

1. Introduction

Biochar (BC) has been found to positively affect ecosystem services such as soil fertility and biological activity (Lehmann et al., 2011; Sohi et al., 2010). Even though BC may provide soil microorganisms far less nutrients and mineralizable C than the bulk soil, its surface properties may represent niche for microbial colonization. From an ecological perspective, a niche provides both food supply and a physical habitat for organisms. Biochar characteristics such as porosity and surface charge facilitate the transfer of water and nutrients from the bulk soil to BC pores, thereby supporting microbial activity and growth (Gul et al., 2015; Jaafar et al., 2015; Quilliam et al., 2013). Changes in soil biochemical cycles appear also as shifts in the availability of nutrients and C to microbes and plants (Lehmann et al., 2011).

A central part of the soil biochemical cycle is the microbial degradation of plant residues and soil organic matter (SOM), which comprises C-rich macromolecules, like cellulose, hemicellulose, lignin, chitin, proteins, pectin and tannin (Demisie et al., 2014; Gessner et al., 2010). The major players in microbial driven decomposition are extracellular enzymes. Their production has evolved to align nutrient and energy supplies with demand and can be considered as a form of foraging strategy (Burns et al., 2013). Strategies that minimize nutrient and energy costs to the cell and maximize related benefits are promoted by natural selection (Allison et al., 2010). Cellular economics are reflected in enzyme activities as a balance between the profit of increased availability of energy sources, mineral and organic nutrients versus the cost of spending resources to produce enzymes (Burns et al., 2013).

Enzymatic activities are considered good indicators for soil quality because of their high sensitivity and fast response to changes in soil properties (Jain et al., 2016); SOM content was found to be one of the most important soil properties to affect enzymatic activities (Štursová and Baldrian, 2011). But also the properties of BC itself can influence

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enzyme activity. Considerable and wide variation has been reported among BCs not only in pH and nutrient contents (Lehmann, 2007), but also in physical (Downie et al., 2009) and organo-chemical properties (Nguyen et al., 2010). The main constituents of BC are recalcitrant C and minor fractions of leachable C and ash. It differs chemically from SOM especially in its much greater proportion of aromatic C, particularly fused aromatic C structures (that are in contrast to other aromatic C such as lignin) (Schmidt and Noack, 2000). A significant fraction of BC is not available to microorganisms as a source of energy or nutrients, as C, N and other elements are fixed within its complex structure. A smaller fraction that varies between BC types, is readily leached and mineralized (Lehmann et al., 2009) and may promote microbial activity and abundance (Lehmann et al., 2011; Steiner et al., 2008).

The surface charge on fresh BC can be net positive or negative depending on feedstock and production conditions, and the cation exchange capacity (CEC) is usually small compared to SOM (Lehmann et al., 2011). But as BC ages in soil, atmospheric oxidation and microbial activity cause the formation and attachment of negatively charged carboxyl-, phenolic-, carbonyl-, quinone- and hydroxyl groups, whereby CEC increases (Beesley and Marmiroli, 2011; Cheng et al., 2006; Gul et al., 2015; Lau et al., 1986; Lehmann, 2007; Zimmerman, 2010). Changes in BC properties through aging also cause alterations in microbial processes. For example, a former study reported up to 50% more volatile matter and ash contents after 100 days of incubation. This was attributed to high adsorption of low molecular weight substances by BC due to its increased oxidation over time and can substantially affect soil microorganisms (Gul et al., 2015; Spokas, 2013).

Oxidation is the major cause of BC aging in soil, and surface activation of BC with organic acid produces similar effects (Mia et al., 2017; Uchimiya et al., 2010; Zimmerman, 2010). Acid-activated BCs show a similar increase in oxygen containing functional groups from partial oxidation during carbonization (Elsheikh, 2008; Klasson et al., 2009; Uchimiya et al., 2010). They also show a greater porosity and a larger surface area that is possibly biologically more active than that of fresh non-activated BC (Downie et al., 2009; Jaafar et al., 2015; Keech et al., 2005; Rillig et al., 2010). It should be noted that there are seemingly contradictory perspectives in literature regarding BC surface area changes through aging, most probably because of differences in production and activation methods (Uchimiya et al., 2012). Larger surface areas may enhance removal rates of pollutants, increase nutrient availability and promote microbial activities and respiration (Choppala et al., 2012; Cornelissen and Gustafsson, 2004; Foo and Hameed, 2010; Liu et al., 2008; Soleimani and Kaghazchi, 2007; Vithanage et al., 2015). The influence on microbial biomass and respiration seems to go in the same direction with non-activated BC and activated BC, even though the influence of activated BC is usually more pronounced (Anderson et al., 2011; Chan et al., 2008; Jaafar et al., 2015; Kolb et al., 2009).

To our knowledge, neither the short-term effects of carboxylic acid-activated biochar on enzyme activity nor its longer-term development has yet been investigated. Here, we used a greenhouse pot experiment that tested different acid-activated and non-activated BCs as soil amendments to evaluate the effects of carboxylic acid-activated BCs vs control treatments (including untreated soil, lime, compost or non-activated BC treatments) on microbial enzyme activities. For the present greenhouse pot experiment, compost was used in combination with biochar (BC) soil amendments as this has been shown to bring greater benefits for agriculture than pure BC treatments due to possible synergistic effects (Bamminger et al., 2014; Schulz et al., 2013).

2. Materials and methods

2.1. Experimental setup

2.1.1. Soil sampling and treatments

Soil samples were taken from the upper 15 cm of a vineyard in Styria/Austria. The soil is a sandy loam (sand, silt and clay content, 45%, 34% and 21%, respectively) with a pH (in 0.01 M $CaCl_2$) of 6.2, an organic matter content of 2.7% and a CEC of 18.5 $cmol_c\ kg^{-1}$ (Soja et al., 2017). The class A + quality compost was obtained from Bauernkompost FK Pixendorf and sieved < 2 mm. Wood-BC was obtained from SONNENERDE Gerald Dunst Kulturerden GmbH and pyrolized at 480 °C. A total amount of 1 kg of the wood chip biochar (BC) was mixed with 6.25 l of 1 M organic acid (citric or tartaric) and placed in a desiccator for 30 min. Vacuum was applied to the desiccator using a pump, which enabled the acids to pass through the pores of the BC. Afterwards, the slurry was shaken on an orbital shaker for 30 min and then rinsed with water. The acidified BC was dried at 50 °C overnight. The dried BC was rinsed with Milli-Q water over filter paper, until the filtrate was transparent. Subsequently the washed BC was again placed dried at 110 °C for two hours to obtain the activated BC (Zhu et al., 2008).

Table 1
Soil additives.

Group (Box plot)	Abbreviation	Treatment compositions
CONTR	C1	control [no additive]
	C2	lime [5 t ha^{-1}]
	C3	compost [40 t ha^{-1}]
	C4	compost + wood biochar 25:75 [40 t ha^{-1}]
ABC	ABC1	compost + tartaric acid activated biochar 25:75 [40 t ha^{-1}]
	ABC2	compost + citric acid activated biochar 25:75 [40 t ha^{-1}]
	ABC3	compost + citric acid activated biochar 25:75 [80 t ha^{-1}]
	ABC4	compost + citric acid activated biochar 75:25 [40 t ha^{-1}]

The soil was thoroughly mixed for two minutes with the respecitve additives using a concrete mixer (resulting in eight different treatments, Table 1) and filled into pots (Ø 20 cm, height 64 cm) designed as columns in a greenhouse with an irrigation system (Soja et al., 2017). A grape plant (*Vitis vinifera*) was planted and several cover crops (*Avena sativa, Medicago lupulina, Lathyrus sativus*) were sown. For each treatment (Table 1) four replicate pots were established.

An application rate of 4 kg m^{-2} (40 t ha^{-1}) corresponded to about 1.3% (m/m) assuming an incorporation depth of 0.2 m. This application rate agreed with the upper limit of compost application rate in the field (applied once in 3 years) according to the Austrian compost ordinance. The adherence to this upper limit was important to allow for a transfer of the results of the greenhouse study to field conditions. The treatment with double application rate (8 kg m^{-2}, abbreviated ABC3, Table 1) constitutes a case that does not exceed the upper limit for compost but adds a higher amount of BC (2 + 6 kg m^{-2}). Such treatment might become relevant in the future if carbon sequestration is an additional objective of such soil management measures.

Due to the liming effect of BC in acidic soils, a lime control (C2, Table 1) was included to monitor changes that can be attributed to a higher pH in the soil. We compared the compost treatment C3 with the mixed treatments ABC1 to ABC4 to study the interactions of compost and organic acid activated BCs in relation to compost as single additive. The comparison of ABC1 and ABC2 should reveal if the type of organic acid (tartaric acid vs. citric acid) had effects on the compost-BC mixtures in the soil. The comparison of ABC2 and ABC4 should show in how far the mixing ratio of activated BC and compost (1:3 vs. 3:1) changed the mixture effects. The comparison of C4 with ABC1 and ABC2 was chosen to investigate the efficacy of the organic acid activation of BC when the application rates and the ratios of compost to BC were kept constant.