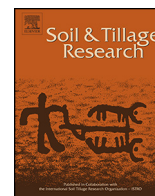




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Impact of chars and readily available carbon on soil microbial respiration and microbial community composition in a dynamic incubation experiment

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ABSTRACT

The carbonisation of biomass and organic residues is discussed as an opportunity to store stabilised carbon compounds in soil and to reduce mineralisation and the emission of CO₂. In this study, pyrolysis char (600 °C, 30 min) and hydrothermal carbonisation char (HTC char; 210 °C, 23 bar, 8 h), both derived from maize silage, were investigated in a short-term incubation experiment of soil mixtures with or without readily available carbon (glucose) in order to reveal impacts on soil microbial respiration and community composition. In contrast to pyrolysis char, the addition of HTC char increased respiration and enhanced the growth of fungi. The addition of glucose to soil-char mixtures containing either pyrolysis or HTC char induced an additional increase of respiration, but was 35% and 39% lower compared to soil-glucose mixtures, respectively, providing evidence for a negative priming effect. No significant difference was observed comparing the soil mixtures containing pyrolysis char + glucose and HTC char + glucose. The addition of glucose stimulated the growth of most microbial taxa under study, especially of Actinobacteria at the expense of fungi. Adding pyrolysis or HTC char to soil induced a decline of all microbial taxa but did not modify the microbial community structure significantly. Addition of pyrolysis or HTC char in combination with glucose however, increased the abundance of Actinobacteria and reduced the relative abundance of Acidobacteria and Betaproteobacteria while fungi were further increased in case of HTC char. We conclude that both chars hold the potential to bring about specific impacts on soil microbial activities and microbial community structure, and that they may compensate the variations induced by the addition of readily available carbon.

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1. Introduction

Char materials, which derive from thermochemical carbonisation of biomass, have been proposed as one option for long-term carbon storage and for the improvement of soil properties (Lehmann et al., 2006). The two main processes studied in recent years are pyrolysis and hydrothermal carbonisation (HTC), besides other techniques such as vapothermal carbonisation (Funke et al., 2013), gasification and fast pyrolysis (Libra et al., 2011). In contrast to pyrolysis, which is a dry process running under anaerobic conditions at temperatures between 200 °C and 900 °C (Lehmann et al., 2006), HTC is performed in aqueous systems under autogenous pressure of about 10–20 bar at temperatures between 180 °C and 250 °C (Libra et al., 2011). According to the different

process conditions, the products defined as pyrolysis char and HTC char, have completely different properties. Compared to pyrolysis chars, HTC chars have a lower carbon content and correspondingly higher contents of hydrogen and oxygen due to their lower carbonisation degree. The relationship between the carbon content of the char material and its stability against microbial decay has been described manifold (Bai et al., 2013; Busch and Glaser, 2015; Singh et al., 2012; Spokas, 2010).

The application of biochar, or carbonised organic matter to soils has been proposed as a method for the long-term storage of organic carbon in the environment, which at the same time will provide agronomic benefits due to the improvement of soil properties (Lehmann et al., 2006; Schulz and Glaser, 2012). Biochar in soil can increase the stability of soil aggregates and the availability of nutrients, which in turn have positive effects on plant growth and biomass yields (Biederman and Harpole, 2013; Lehmann et al., 2006). Moreover, variable effects on the abundance and composition of soil fauna and microflora were described,

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depending on environmental conditions (Atkinson et al., 2010; Lehmann et al., 2011). The extent, or the duration of the impacts of biochar is strongly dependent on its degradability, which results from complex biochemical mechanisms, which in turn depend on several external factors in the respective ecosystem, such as the physico-chemical and climatic conditions, the amount, quality and availability of carbon, the availability of nutrients and energy sources and the microbial abundance and activity and the influence of mycorrhiza and soil fauna. In order to understand the mechanisms of char degradation, and therefore the feasibility of its application in a soil amendment system, an inspection of its effects on soil microbial activity and community composition is crucial. Microbial activity can be assessed under defined experimental conditions by approaches based on respiration (Blagodatskaya and Kuzyakov, 2013; Lanza et al., 2015), while microbial community composition can be studied by DNA sequencing techniques such as qPCR (Fierer et al., 2005). Chars have shown to have several direct and indirect effects onto microbial communities. Addition of biochar to top soil may stimulate the activity of soil bacteria and fungi already on a short time scale (Ameloot et al., 2013; Bamminger et al., 2014), especially under stressful environmental conditions like during water scarcity (Liang et al., 2014). In a previous investigation with the same char materials as used in this study in presence of nitrogen fertiliser (Lanza et al., 2015) we did not find a significant response in soil respiration upon addition of pyrolysis char, but a significant increase upon addition of HTC char deriving from the same substrate (maize silage). Microbial community composition is also affected; recent studies reported an overall increase of various taxa of microorganisms after biochar addition to soil, such as Gram-positive and Gram-negative bacteria (Ameloot et al., 2013), Actinobacteria (Prayogo et al., 2014), or fungi (Steinbeiss et al., 2009), though in some cases growth was reduced during the first weeks (Mitchell et al., 2015) and the reaction differed depending on soil types (Chen et al., 2015). Several mechanisms behind impacts of biochars on the soil microflora were summarised and changes in microbial activity or community structure explained (Thies et al., 2015). Biochar may provide habitat or shelter for soil organisms (Quilliam et al., 2013) and promote soil ecological conditions, such as water holding capacity or buffer capacity (Karhu et al., 2011). Moreover, biochar may be source of energy (Watzinger et al., 2014) and nutrients (Warnock et al., 2007) and thus it may interact with soil trophic chains in the soil-plant system (McCormack et al., 2013).

In general, the addition of readily available organic matter to soil has shown to increase microbial activity and also to induce changes in the microbial community composition (Cleveland et al., 2007). However, simultaneous addition of chars and readily available organic carbon sources can lead to interaction effects on soil community composition, as well as modification of the degradability of both additives, so called priming effects (Kuzyakov, 2010). Both positive (Hamer et al., 2004; Jones et al., 2011) and negative (Whitman et al., 2014) priming effects of chars on the decay of soil organic matter have been reported and discussed (Kuzyakov, 2010; Woolf and Lehmann, 2012). Even in some cases, the priming was either positive or negative at different points in the course of time (Maestrini et al., 2014). Against this background we performed incubation experiments with chars and glucose, intending to amplify any char-induced impacts and to inspect possible interactions between these two different carbon sources in terms of availability.

According to a previous study (Lanza et al., 2015), pyrolysis char and HTC char made from the same feedstock, i.e. maize silage, were tested in a 10 day incubation besides the feedstock itself and a soil control without any substrate addition. The aims of the present study were to test the following hypotheses:

- (1) Chars, being mostly inert material, do not impact overall soil microbial activity and microbial abundance;
- (2) Different chars promote differences in soil microbial respiration and shifts in microbial community composition;
- (3) Addition of a readily available carbon source to soil-char mixtures promotes additional soil respiration and shifts in microbial community composition.

2. Materials and methods

2.1. Preparation of the chars

Maize straw samples were taken from an experimental field site located in Braunschweig, Germany (Becker et al., 2014), ground in an ultra-centrifugal mill (0.75-mm sieve) and stored until used. All other substrates tested in our study were produced from maize silage. Pyrolysis char (REW, Quakenbrück, Germany) was produced in a continuous reactor (600 °C, 30 min) and quenched by means of water sprinkling. HTC char (AVA CO₂, Karlsruhe, Germany) was produced in a one-pot batch reactor (210 °C, 23 bar, 8 h) and separated by means of a chamber filter press. After production, all chars were stored at –20 °C. A few weeks before the experiments started, the samples were unfrozen, oven-dried for 48 h at 105 °C, ground up to a fine powder and stored at 4 °C. The pH value of straw and chars was measured 1:5 in distilled water. The straw and the carbonised products were analysed for total C and N content with an elemental analyser (Vario EL III, Elementar, Germany). The chemical properties of the substrates used are listed in Table 1.

2.2. Preparation of soil-char mixtures

The soil used was taken from the top layer (0–15 cm) of an experimental field located in Berge (Kreis Havelland, Brandenburg, Germany, 52°63'N, 12°80'E), which represents a typical site of the glacial landscape of North-eastern Germany. It was a loamy sand (Haplic Cambisol) with the following texture: 712 mg g⁻¹ sand ($\phi > 630 \mu\text{m}$), 222 mg g⁻¹ silt (2–630 μm) and 66 mg g⁻¹ clay ($\phi < 2 \mu\text{m}$). The chemical properties of the soil are also included in Table 1.

The field-moist soil (dry mass = 93%) was sieved up to a particle size <2 mm and stored at 4 °C in a container until analysis. After equilibration (2 d, 20 °C), soil was mixed with either straw meal or char (5 mg DM g⁻¹ soil, corresponding to 2–4 mg C g⁻¹ soil) using a kitchen mixer. D(+)-glucose, anhydrous (Merck, Germany) was added to half of the samples also in the amount 5 mg DM g⁻¹ soil, corresponding to 2 mg glucose-C g⁻¹ soil.

2.3. Incubation design and CO₂ measurement

Soil-substrate mixtures (100.5 g FM per sample) were incubated in three replicates in Plexiglas tubes (4 cm diameter) for 240 h at 20 °C at constant soil moisture (75 mg H₂O g⁻¹ DM), using an automated system for continuous soil respiration measurements (Heinemeyer et al., 1989). The molar fraction of the emitted CO₂ (X, in ppm) was measured in a continuous flow of W = 80 ml min⁻¹

Table 1

Physico-chemical properties of the substrates used. FM = fresh mass; DM = dry matter; oDM = organic dry matter; Pyro = pyrolysis char; HTC = HTC char.

Substrate	pH	DM mg g ⁻¹ FM	oDM mg g ⁻¹ DM	C mg g ⁻¹ DM	N mg g ⁻¹ DM
Soil	4.72	929	14.7	6.26	0.55
Straw	6.29	939	926	464	14
Pyro	9.72	973	837	756	17
HTC	5.18	984	966	636	23

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