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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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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 CO_2. 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 CO_2, 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 (∅ > 630 μm), 222 mg g^{-1} silt (2–630 μm) and 66 mg g^{-1} clay (∅ < 2 μm). 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 CO_2 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 H_2O g^{-1} DM), using an automated system for continuous soil respiration measurements (Heinemeyer et al., 1989). The molar fraction of the emitted CO_2 (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

with a periodicity $\Delta t = 2\,h$ by using a Picarro G1101-i analyser (Picarro Inc., CA, USA) connected to the system via a T-pipe.

2.4. Extraction of DNA

For each treatment, one aliquot of each soil mixture was collected before onset of the incubation experiment and three (one per each replicate) at the end of the experiment.

Samples were thereafter stored at −20 °C until extraction of total genomic soil DNA using the NucleoSpin® Soil Kit (MACHEREY-NAGEL GmbH & Co., KG, Düren, Germany). For resulting DNA samples, DNA concentration and nucleic acid purity was assessed using NanoDrop 2000 Spectrophotometer (NanoDrop products, Wilmington, DE, USA) and nucleic acid agarose gel electrophoresis. DNA Samples were subsequently stored at 4 °C for further processing.

2.5. Amplification of DNA

qPCR (quantitative Polymerase Chain Reaction) assays were conducted in polypropylene 96-well plates on a QuantStudio™ 12 K Flex Real-Time PCR System (Life Technologies, Grand Island, NY, USA). Seven different primer pairs of taxa specific genes were separately used to quantify abundance of fungi and the bacterial taxa Firmicutes, Actinobacteria, Acidobacteria, Alpha-, Beta- and Gammaproteobacteria. The primers and corresponding standard microorganisms were chosen in agreement to Fierer et al. (2005). Besides the unknown samples, each plate included the appropriate standards in a 10 fold dilution series (from 10^{-1} to 10^{-7} in 3-fold replicate) to generate standard curves, as well as negative and positive controls. Each 20 µl reaction well contained: 4 µl of 5 × HOT FIREPol® EvaGreen® HRM Mix ROX (Solis Biodyne, Tartu, Estonia), 0.25 µl of each primer (10 pM, biomers.net), 14.5 µl of Millipore H_2O and 1 µl of template DNA. The performed runs consisted of an initial denaturation phase (15 min at 95 °C), followed by 40 amplification cycles (15 s at 95 °C, 20 s at 60 °C and 30 s at 72 °C). The progress of the amplification was tracked by means of an integrated optical detector which measured the fluorescence signal from the complete double strands over time. The quality of each run was assessed through a melting curve analysis of the PCR products.

2.6. Data analysis and statistics

The soil respiration response was quantified as cumulated CO_2 flux ($y(t)$, in mg CO_2-C g^{-1} soil) from each sample. First, the instantaneous CO_2-C flux (Φ_C, in mg CO_2-C min^{-1}) was calculated after correcting the CO_2 molar fraction by subtraction of a background: $\Phi_C = W \cdot (X - X_{bg}) \cdot \frac{MM}{VM}$, where $MM = 12.0107\ g\ mol^{-1}$ is the molar mass of carbon and $VM = 24.055\ L\ mol^{-1}$ is the molar volume of an ideal gas at 20 °C. Then the fluxes were cumulated and normalised by the total carbon amount (soil + substrate) in each container: $y(t) = \frac{1}{C_{tot}} \cdot \sum_{t'<t} \Phi_C(t') \cdot \Delta t$, where $t' = 0, \Delta t, 2 \cdot \Delta t, \ldots t$. An analysis of variance, followed by a Tukey test at significance level $\alpha = 0.05$, was conducted on the cumulated fluxes after day 2, 4, 6, 8 and 10 to determine significant differences among the treatments within each experiment by means of the software R, version 3.0.2 (R core team, 2012).

The total DNA in each sample was calculated on basis of the extracted DNA concentration reported by measurement of optical absorbance (Nanodrop, NanoDrop products, Wilmington, DE, USA), in ng µl^{-1}. The abundance of each microbial taxon in each sample was quantified as the DNA amount of the corresponding gene (in ng µl^{-1}), quantified by qPCR, obtained from the Ct value of the corresponding amplification curve. A variance analysis was performed on the average values by means of the software STATISTICA 10.

3. Results

3.1. Soil respiration response

During the incubation, a continuous emission of CO_2 was observed in all treatments. The cumulated CO_2 release at various time points is listed in Table 2. The maximum CO_2 release was induced by glucose, which at the end of the experiment (Day 10) was 2.4 times as high as compared to straw meal. Both soil-char mixtures emitted significantly less CO_2 compared to the soil-straw mixture (Fig. 1). The release of CO_2 from the treatment with pyrolysis char did not differ significantly from the control while CO_2 release from the HTC treatment was significantly higher. The combined addition of char (either pyrolysis or HTC) and glucose significantly increased CO_2 release but no difference was observed comparing both char treatments. In comparison to the glucose treatment, the combination of char (either pyrolysis or HTC) and glucose reduced soil CO_2 release between 35% and 39%, almost constantly over time.

3.2. Soil microbial community dynamics

The total soil DNA content in the extracted solutions was in a range between 90 ng µl^{-1} (in case of HTC treatment) and 140 ng µl^{-1} (in case of straw treatment), with no significant differences between all the treatments (data not shown). The incubation of the control soil induced an increased abundance for all microbial taxa under study, especially for Actinobacteria, whereas the addition of both chars induced a decrease in the abundance of all taxa under study (Table 3). The treatment with glucose, however, clearly increased the growth of Gammaproteobacteria, Actinobacteria, Alphaproteobacteria and Firmicutes and suppressed Acidobacteria and Betaproteobacteria. The combination of pyrolysis char with glucose enhanced the growth of Gammaproteobacteria, Actinobacteria, Alphaproteobacteria and Firmicutes and suppressed Acidobacteria. The combination of HTC char with glucose also greatly enhanced Gammaproteobacteria and decreased Acidobacteria in a similar extent to pyrolysis char, but in contrast clearly slowed down Betaproteobacteria, Acidobacteria and Firmicutes and stimulated greatly fungi. Based on these dynamics, a similarity analysis separated two major groups, cluster (I) including the variants control, straw and pyrolysis char and cluster (II) including all three variants with glucose addition while the HTC treatment was isolated, closer to the glucose variants (Fig. 2).

Table 2
Cumulated respiration over time for the chars with and without glucose. Pyro = pyrolysis char; HTC = HTC char; Gluc = glucose.

Treatment	Cumulated flux (mg CO_2-C g^{-1} sample-C)									
	2 days		4days		6 days		8days		10 days	
Control	0.50	e[a]	0.91	e	1.29	e	1.79	f	2.31	e
Straw	10.86	c	21.47	c	27.41	c	31.76	d	35.41	c
Pyro	0.87	e	1.37	e	1.81	e	2.20	f	2.56	e
HTC	1.66	d	3.19	d	4.81	d	6.26	e	7.81	d
Gluc	19.57	a	41.26	a	59.78	a	75.49	a	85.93	a
Pyro + Gluc	12.61	b	26.13	b	37.71	b	48.31	b	55.70	b
HTC + Gluc	12.46	b	25.58	b	36.59	b	46.02	c	53.77	b

[a] Values followed by the same letter in the same column are not significantly different at $P < 0.05$.

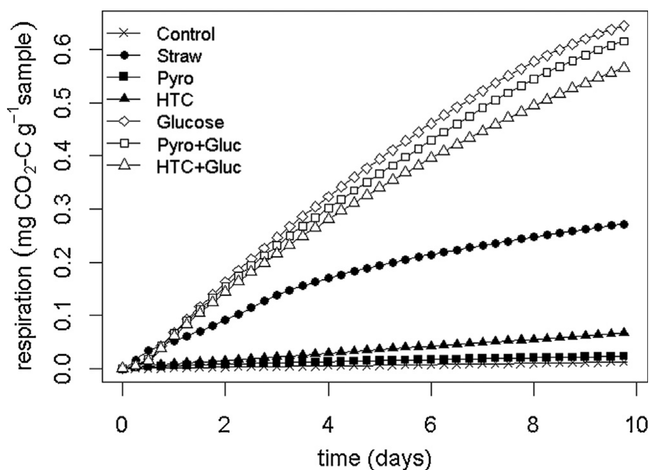

Fig. 1. Cumulated CO_2 release in control soil and soil-char mixtures, with or without glucose. Pyro = pyrolysis char; HTC = HTC char; Gluc = glucose.

Table 3
Dynamics of microbial taxa, expressed as relative changes to the corresponding initial values of each variant (in%); Pyro = pyrolysis char; HTC = HTC char; Gluc = glucose; Actino = Actinobacteria; Acido = Acidobacteria; Alpha = Alphaproteobacteria; Beta = Betaproteobacteria; Gamma = Gammaproteobacteria; Firmi = Firmicutes.

Treatment	Actino	Acido	Alpha	Beta	Gamma	Firmi	Fungi
Control	43.0	2.5	10.1	16.0	1.8	25.6	8.8
Straw	71.5	−12.7	31.2	14.2	46.9	27.5	−20.1
Pyro	−8.1	−10.5	−15.4	−2.3	−16.9	−17.7	−31.0
HTC	−25.5	−37.5	−5.7	−12.5	−23.3	−38.4	−7.3
Gluc	68.6	−43.2	23.9	−15.0	158.8	60.9	2.3
Pyro + Gluc	96.0	−38.4	36.8	−3.8	187.1	45.0	7.6
HTC + Gluc	17.3	−44.2	−4.8	−44.6	156.7	−51.5	60.0

3.3. Abundance of microbial taxa and community structure

Significant differences in microbial community among the variants were found at the end of the incubation experiment (Table 4). Compared to the control, the addition of either straw or glucose resulted in a higher abundance of Actinobacteria and a lower abundance of fungi by tendency. Pyrolysis char alone induced no significant changes of the taxa under study, while HTC char tended to increase the abundance of fungi, but only in combination with glucose. Both chars combined with glucose reduced the abundance of Acidobacteria and increased Gammaproteobacteria significantly. Alphaproteobacteria and Gammaproteobacteria were significantly reduced in the HTC + glucose treatment, what was not found for pyrolysis char.

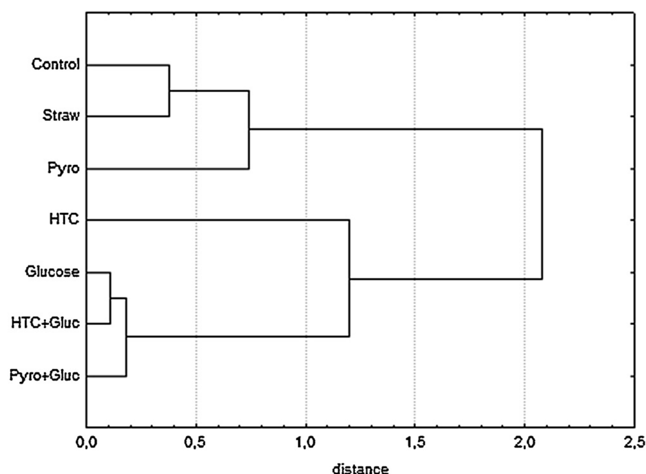

Fig. 2. Cluster analysis based on the dynamics of microbial taxa in the treatments (see Table 3). Pyro = pyrolysis char; HTC = HTC char; Gluc = glucose.

Changes in community structure after incubation are shown in Fig. 3, expressed as relative abundance of microbial taxa, referred to the control. The addition of glucose and straw decreased the relative portion of fungi, but fungi were enhanced after addition of HTC or HTC + glucose. In the variants combining char and glucose, the relative proportions of Acidobacteria and Betaproteobacteria were decreased, as well as the proportion of Alphaproteobacteria in the HTC + glucose treatment.

4. Discussion

4.1. Effects of chars on soil respiration and microbial community composition

During our short-term incubation study, the addition of pyrolysis char had no impact on soil respiration, whereas the addition of HTC char increased soil respiration, as reported previously (Bai et al., 2013; Lanza et al., 2015). This finding can be explained by the different amounts of recalcitrant structures and is in accordance to other reports, e.g. (Bai et al., 2013). Furthermore, Mitchell et al. (2015) reported that initially unfavourable changes in microbial habitat or the introduction of compounds associated with biochar such as polycyclic aromatic hydrocarbons, residual pyrolysis oils and polar pyrolysis condensates may be the reason for toxic effects on microorganisms and their activity (Hale et al., 2012; Spokas et al., 2011). A trend of decreased microbial biomass in soils amended with biochars produced from feedstocks with high lignocellulosic content has been reported by Gul et al. (2015). This finding corresponds to a previous study of Gomez et al. (2014) who reported concentration-dependent changes in microbial activity in response to biochar addition but without major changes in community composition at the lowest application rates, which were even higher compared to concentrations used in our study.

The abundance of all microbial taxa under study did not significantly differ after the addition of chars in our short-term incubation experiment. However, the analysis of community structure indicated an enhanced proportion of fungi after HTC treatment in relation to the control but, with respect to the significantly increased respiration, this change is considered to be just a hint for structural changes within this taxon. An increased growth of the fungi after char addition and a change in their proportion of the total community is in agreement with other publications (Steinbeiss et al., 2009; Titirici et al., 2012) and may be explained by a benefit for fungal proliferation in nutrient poor and acidic environments, especially in the presence of volatile organic carbon compounds such as furfurals, phenols and also organic acids, which are known to be sorbed on HTC chars (Hale et al., 2012; Spokas et al., 2011). These compounds may undergo volatilisation and decomposition in the course of time, due to the extracellular enzymatic activity of fungi (Nichols et al., 2008). Similar processes obviously do not occur for pyrolysis char, which is carbonised to a higher degree and which furthermore may induce an increase in soil pH (Cayuela et al., 2014). Such conditions are known to be detrimental for fungal growth (Gul et al., 2015). At the beginning of the incubation experiment, microbial abundance tends to be higher in the char variants; thus the dynamics of all microbial taxa towards the endpoint followed a negative trend, matching more or less the control levels, except for fungi in the

Table 4
Relative abundance of microbial taxa at day 10 of the incubation (in %); Pyro = pyrolysis char; HTC = HTC char; Gluc = glucose; Actino = Actinobacteria; Acido = Acidobacteria; Alpha = Alphaproteobacteria; Beta = Betaproteobacteria; Gamma = Gammaproteobacteria; Firmi = Firmicutes.

Treatment	Actino	Acido	Alpha	Beta	Gamma	Firmi	Fungi
Control	8.8 a[a]	8.7 bc	2.7 ab	8.3 ab	0.4 a	0.2 ab	70.9 ab
Straw	14.7 b	7.7 bc	2.9 bc	9.5 b	0.7 a	0.3 b	64.0 a
Pyro	9.3 a	9.2 c	2.5 ab	8.0 ab	0.4 a	0.2 ab	70.4 ab
HTC	8.9 a	6.6 ab	2.8 ab	8.3 ab	0.4 a	0.1 a	72.8 b
Glucose	17.2 b	6.8 ab	3.5 c	8.1 ab	1.9 c	0.2 ab	62.3 a
Pyro + Gluc	14.7 b	4.9 a	2.9 bc	6.1 a	1.5 bc	0.2 ab	69.5 ab
HTC + Gluc	10.9 a	4.9 a	2.2 a	5.9 a	1.3 b	0.1 a	74.6 b

[a] Values followed by the same letter(s) in the same column are not significantly different at $P < 0.05$.

HTC treatment. In contrast to the chars, a completely different reaction of the microbial community was detected when straw was applied to soil, especially with respect to the Actinobacteria, which gained a predominant role at the expense of fungi. These bacteria are well-known for their capacity to metabolise recalcitrant substrates such as ligno-cellulose (McCarthy and Williams, 1992; Jiang et al., 2016), which makes up the main straw component.

4.2. Combined effects of glucose and chars on soil respiration and microbial community

Addition of glucose as a readily available carbon source to soil-char mixtures induced an additional increase of respiration and promoted evident changes in microbial community structure. The respiration response upon glucose addition was similar for both chars, indicating that char-derived carbon did not play the major role as a carbon substrate for microbial activity, although differences were detected comparing pyrolysis and HTC char without glucose addition.

However, compared to the respiration response to glucose addition in the absence of chars, the presence of either char remarkably reduced glucose respiration in soil, as well as the amplitude of variation in the relative abundance of microbial taxa, particularly of Acidobacteria, Betaproteobacteria, Gammaproteobacteria and fungi. Based on these findings, the chars are considered to have exerted a negative priming effect. Since HTC char is known to have a lower stability compared to pyrolysis char (Lanza et al., 2015), the over-all respiration in absence of a priming effect should be higher for HTC char + glucose compared to pyrolysis char + glucose. However, there was no significant difference between both treatments and it can be assumed that HTC char exerts a higher negative priming effect than pyrolysis char, which is in agreement with recent studies using chars from the same feedstock (Bamminger et al., 2014; Malghani et al., 2013). In order to differentiate and quantify the mineralisation of distinct carbon sources (glucose, char, or soil organic carbon), measurements of the isotopic composition of CO_2-C would be required as shown by Kuzyakov et al. (2009) by using ^{14}C-enriched pyrolysis char derived from ryegrass. The authors reported a glucose-induced increase in soil respiration in a similar extent compared to our results and calculated a char decomposition rate of 0.5% per year.

With respect to soil microbial communities, the abundances of Actinobacteria, Alphaproteobacteria and Gammaproteobacteria at the end of the experiment were increased in all treatments with a high respiration rate (straw, glucose), while fungi showed a reversed tendency. A correlation analysis between respiration and DNA abundance of the single taxa yielded a high correlation coefficient for the abundance of Gammaproteobacteria (R^2 = 0.95) and Actinobacteria (R^2 = 0.71), confirming that these taxa play an important role in the degradation of soil organic carbon compounds. The decreased glucose-induced respiration response in the presence of chars does not correspond to a general decline of microbial taxa, but is considered an adaptation effect which is specific for each char, but nevertheless results in the same soil respiration activity. For both chars, the relative abundance of Betaproteobacteria and Acidobacteria was reduced and the relative abundance of fungi, which was declined by glucose, was restored in case of HTC addition. In general, our results show that the main effect of both chars with respect to microbial communities is

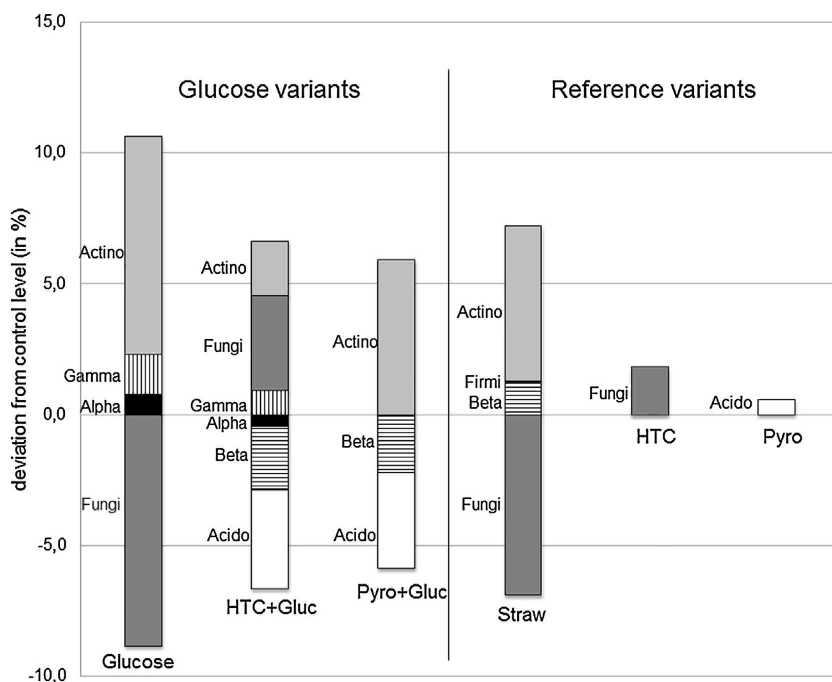

Fig. 3. Differences in the relative abundance of microbial taxa, referred to the control after 10 days of soil incubation (in %). Pyro = pyrolysis char; HTC = HTC char; Gluc = glucose; Actino = Actinobacteria; Acido = Acidobacteria; Alpha = Alphaproteobacteria; Beta = Betaproteobacteria; Gamma = Gammaproteobacteria; Firmi = Firmicutes.

manifested in taxa-specific abundance and structure, and furthermore these effects are char-specific.

In more detail, the adaptation of Betaproteobacteria spans from a significant enhancement after addition of straw to a striking reduction after the addition of chars + glucose. It was shown previously (Parales, 2010) that Betaproteobacteria play an important role in the degradation of aromatic hydrocarbons, also Eilers et al. (2010) reported a significant dominance of Betaproteobacteria in a coniferous soil, which could explain an adaptation to recalcitrant organic carbon compounds. The decrease of the common soil taxon Acidobacteria (Dunbar et al., 2002) was significantly enhanced by pyrolysis char and was reduced in all other treatments, particularly after the addition of both chars in combination with glucose, which seems to be not only an adaptation to soil pH-values (Kishimoto et al., 1991), but rather an adaptation to more oligotrophic conditions (Eichorst et al., 2007; Koch et al., 2008) after addition of char. The reduction of Acidobacteria abundance by about one half is also seen in bulk Terra Preta soils characterised by highly increased amounts of stabilised organic compounds, charcoal, bone, and pottery sheds as compared to the corresponding non-anthropogenic adjacent soil (Barbosa Lima et al., 2015). The abundance of bacterial taxa that preferred nutrient-rich environments, such as Actinobacteria, showed a similar trend after the addition of straw or glucose as found in a field study about adding maize residues (Ramirez-Villanueva et al., 2015). Surprisingly, the treatment HTC + glucose with a per saldo similar nutrient level as the treatment without HTC char reduced the abundance of Actinobacteria significantly in favour of fungi, which are best adapted to low-pH conditions and to the possible occurrence of volatile organic carbon compounds, thus suppressing bacteria. Further studies are required to resolve such dynamics, both in the short and the long term.

5. Conclusions

Our study showed that the addition of chars, especially in the presence of readily available carbon, modifies soil conditions in terms of microbial respiration response and microbial community composition. In contrast to pyrolysis char, the addition of HTC char stimulated microbial activity and enhanced the growth of fungi. Upon addition of chars to a system enriched with glucose, respiration rates were significantly reduced and shifts in microbial community composition were detected. We conclude that chars hold the potential to bring about specific impacts on soil microbial activities and microbial community structure already in the short term, and may compensate or counteract the variations induced by the addition of readily available carbon. Thus the decision to use biochar as a soil amendment must carefully weigh the proposed benefits such as an increased nutrient availability or carbon sequestration potential against non-predictable changes in biotic processes in soil. Future work should consider in more detail the composition or fractions of soil organic matter and substrates added to soil, as well as the reactions of soil microbial communities in response to biochar amendment.

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