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Impact of pyrolysis and hydrothermal biochar on gas-emitting activity of soil microorganisms and bacterial and archaeal community composition

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ABSTRACT

Most biochar studies are focusing on the usage of char produced by pyrolysis (pyrochar). However, only dry biomass can be subjected to pyrolysis. It is beneficial to produce biochar by hydrothermal carbonization (hydrochar) from wet biomass to avoid energy use for drying. The objective of this study was to compare the effects of pyrochar and hydrochar on greenhouse gas-emitting activity, abundance and composition of the soil bacterial and archaeal community. Three different moisture contents (40%, 60% and 80% of water holding capacity) and two N fertilization steps (with and without N addition) were investigated. The microcosm study was conducted in 120 mL glass bottles with septum caps for periodic headspace gas analysis. N_2O and CO_2 emissions from pyrochar were in the same range as the char-free control. Hydrochar, however, caused high N_2O emissions in the fertilized high moisture treatment and significantly higher CO_2 emissions in all treatments compared to the control. Pyrochar increased CH_4 emission in the unfertilized treatments, whereas hydrochar had no effect except a small reduction in the fertilized and highest moisture treatment. Enzyme activity in all pyrochar microcosms was in the same range as the char-free control, but lower in unfertilized hydrochar microcosms. Pyrochar soil amendment did not change bacterial and archaeal abundance. Hydrochar decreased archaeal abundance in the majority of the treatments. T-RFLP analysis revealed that pyrochar, hydrochar and control each developed a distinct bacterial community. Pyrochar had no effect on archaeal communities, whereas hydrochar induced the formation of significantly different communities compared to the control. Furthermore, hydrochar reduced the abundance of *Acidobacteria* and *Firmicutes*, while it remarkably increased the abundance of *Bacteroidetes* and *Proteobacteria*. The results suggest that the addition of hydrochar induces considerably stronger effects on soil microbial communities than the addition pyrochar.

1. Introduction

Biochar has become an intensively discussed topic within soil science in the last few years due to its proposed beneficial impacts on soil health and agricultural productivity. The body of literature on soil application of biochar to improve soil quality (Atkinson et al., 2010), to enhance agricultural productivity (Biederman and Harpole, 2013; Jeffery et al., 2011; Liu et al., 2013) and to increase nitrogen retention (Ding et al., 2013; Taghizadeh-Toosi et al., 2012; Zheng et al., 2013) is growing rapidly. Furthermore, due to its recalcitrance against microbial degradation, addition of biochar to soil is a promising measure for long-term carbon storage in soil, and thus to mitigate climate change (Lehmann et al., 2006; Woolf et al., 2010).

Almost any kind of biomass can be thermochemically turned into biochar. Dry biomass can be converted by gasification or pyrolysis at high temperature and limited oxygen availability (Meyer et al., 2011; Wiedner et al., 2013) resulting in a biochar product referred to as pyrochar in this study. Moist biomass can be processed without drying by hydrothermal carbonization in an aqueous environment under high pressure (Libra et al., 2011; Reza et al., 2014) yielding hydrochar.

Compared to their precursors, pyrochar and hydrochar are relatively resistant to microbial attack in soil due to their more aromatic and condensed structure. However, pyrochar is with a mean residence time in soil of up to more than 1000 years (Cheng et al., 2008; Liang et al., 2008; Peng et al., 2011) much more recalcitrant than hydrochar. The latter exhibits a mean residence

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time of only 1.9–29 years (Bai et al., 2013; Gajić et al., 2012; Steinbeiss et al., 2009). Incorporation of pyrochar or hydrochar can alter the soil ecosystem by changing physico-chemical properties such as soil carbon content, pH (Van Zwieten et al., 2009), cation exchange capacity (Lehmann et al., 2003; Liang et al., 2008), soil aeration and water holding capacity (Case et al., 2012; Kammann et al., 2011; Karhu et al., 2011).

As one major goal of biochar soil amendment is to sequester carbon in order to mitigate climate change, numerous studies addressed the microbial response to biochar addition in terms of emissions of the greenhouse gases nitrous oxide (N_2O), carbon dioxide (CO_2) and methane (CH_4) from soil (Cayuela et al., 2014; Spokas and Reicosky, 2009; Thomazini et al., 2015). N_2O emissions are controlled by the balance of N_2O formation by denitrification, ammonia oxidation and dissimilatory nitrate reduction (Baggs, 2011) and the reduction by denitrification of N_2O to N_2. Reduction of N_2O emissions by biochar amendment was observed in field experiments (e.g., Liu et al., 2012) and confirmed by meta-analyses across laboratory and field studies (Cayuela et al., 2013, 2014).

So far, little is known about greenhouse gas emissions upon hydrochar addition. According to Kammann et al. (2012), hydrochar soil amendment resulted in higher N_2O emissions compared to unamended controls. However, Malghani et al. (2013) reported that hydrochar application resulted in higher but also in lower N_2O emissions depending on the soil type.

Comparatively few studies addressed CH_4 emission after biochar addition. Castaldi et al. (2011), Scheer et al. (2011) and Zhang et al. (2012) reported no significant effect of pyrochar addition on CH_4 emission. However, Spokas et al. (2009) as well as Spokas and Reicosky (2009) observed reduced CH_4 emissions. Further, Karhu et al. (2011) documented increased CH_4 uptake.

Microbial activity is often estimated by linking the emission of CO_2 to microbial respiration. Several studies report an initial increase of CO_2 emissions after pyrochar incorporation in laboratory incubation and soil column experiments (Kammann et al., 2012; Singh et al., 2010) as well as in field trials (Castaldi et al., 2011) and link this effect with the turnover of the pyrochar's labile carbon fraction. Other field experiments, however, showed no initial CO_2 emission increase (Liu et al., 2014). It is likely that increased microbial activity leading to higher CO_2 emissions co-occurs with changes in the microbial community composition.

Several studies dealt with the impact of biochar on the microbial community in soils. It was observed that biochar can increase the soil microbial activity and nutrient retention (Chen et al., 2013; Lehmann et al., 2011). Several investigations showed fluctuations in composition and abundance in gram-positive and gram-negative *Bacteria* and arbuscular mycorrhiza fungi using pyrochar (Ameloot et al., 2013a; Anderson et al., 2011; Ding et al., 2013; Khodadad et al., 2011). Like *Bacteria*, also *Archaea* are contributing to the soil's carbon and nitrogen cycle (Offre et al., 2013). However, the influence of hydrochar and pyrochar on the archaeal soil community has to the authors' knowledge not been studied so far and information about microbial community change after hydrochar addition to soil is scarce. So far, only Steinbeiss et al. (2009) is known to have reported a link between hydrochar treatment and shifts in the microbial communities. In the strive towards clarification of the underlying mechanisms, recent studies indicate that short-term changes of the microbial structure depend on the volatile organic carbon (VOC) content of pyrochars (Sun et al., 2015). For highly recalcitrant pyrochars, contradictory effects have been reported ranging from no impact on microbial abundance and activity (Zhang et al., 2014) to strong according effects (Chen et al., 2013, 2015). Systematic knowledge about biochar–microflora interaction, however, is still scarce but urgently needed for further scientific and practical advances in soil application of biochars (Lehmann et al., 2011). Several factors have been reported to influence biochar–soil interactions such as temperature (Fang et al., 2015) and nitrogen availability (Clough et al., 2013). With respect to soil moisture, biochar was shown to increase the water holding capacity (Yu et al., 2013). The literature, however, lacks systematic knowledge about the impact of soil moisture on the interactions of biochar and soil microflora.

In light of the state of the art, the overall objective of this study was to yield new knowledge on the impact of two vastly different biochars (pyrochar and hydrochar) on the greenhouse gas-emitting activity, abundance and composition of the bacterial and archaeal community in a laboratory microcosm. Further individual aims were to measure GHG emissions and microbial activity based on a fluorescein diacetate assay (Schnürer and Rosswall, 1982), to assess the abundance and community composition of *Bacteria* and *Archaea* by quantitative PCR, T-RFLP analysis and clone libraries, and to determine the influence of different moisture levels (40%, 60% and 80% of the soil water holding capacity WHC) and nitrogen (non-fertilized and fertilized approach).

2. Material and methods

2.1. Soil and char

Fresh standard soil no. 2.3 was obtained from LUFA Speyer (Speyer, Germany) representing a silty sand (uS) or sandy loam according to German DIN or USDA, respectively. The soil has been cultivated under agricultural use without application of pesticides, biocidal fertilizers or organic manure for at least 5 years. Application of mineral fertilizers was stopped 3 months before sampling. The soils are normally sampled from 0 to 20 cm depth, prepared and sieved with a 2 mm screen. The chemical properties determined at ATB Potsdam are shown in Table 1. Further characteristics stated by LUFA are (means and standard deviations): CEC 10.1 ± 0.5 meq/100 g, particle size (UASD) <0.002 mm 8.5 ± 1.7%, 0.002–0.05 mm 28.4 ± 4.5%, 0.05–2.0 mm 63.1 ± 5.0%, WHC 37.3 ± 1.8 g/100 g, bulk density 1282 ± 30 g/L.

Pyrochar and hydrochar were produced from typical types of feedstock considering their moisture level. Hydrochar was produced by hydrothermal carbonization from corn silage (AVA-CO_2, Karlsruhe, Germany; 8 h, 23 bar, 210 °C) and subsequent separation from the HTC process liquor by means of a chamber filter press. Pyrochar was produced by Pyreg (Dörth, Germany) from a mixture of deciduous and coniferous wood chips. According

Table 1
Biochar and soil starting properties (n.d. = not detected)

Parameter	Unit	Soil	Pyrochar	Hydrochar
DM	%FM	92.87	55.09	49.27
Ash	%DM	97.72	16.64	4.28
pH	–	6.30 (H_2O)	9.35 ($CaCl_2$)	5.25 ($CaCl_2$)
NH_4^+–N	mg kg^{-1} FM	0.32	0.64	n.d.
NO_3^-–N	mg kg^{-1} FM	49.59	0.88	n.d.
N_{tot}	%DM	0.09	0.71	1.67
C_{tot}	%DM	1.06	76.99	62.59
S	%DM	0.02	0.24	0.35
H	%DM	0.20	1.39	4.62
O[a]	%DM	0.91	4.03	26.49
O/C	atomic ratio	0.64	0.04	0.32
H/C	atomic ratio	2.28	0.22	0.89
Ca	g kg^{-1} DM	5.32	26.22	3.03
Fe	g kg^{-1} DM	9.46	3.07	10.58
Mg	g kg^{-1} DM	2.12	3.05	0.28
K	g kg^{-1} DM	4.33	5.75	0.57
P	g kg^{-1} DM	1.08	2.26	2.68

[a]Calculated (O = 100—ash–N–C–S–H).