Soil Biology & Biochemistry 62 (2013) 137–146

Contents lists available at SciVerse ScienceDirect

Soil Biology & Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Chars produced by slow pyrolysis and hydrothermal carbonization vary in carbon sequestration potential and greenhouse gases emissions

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ARTICLE INFO

Article history:
Received 6 September 2012
Received in revised form
10 March 2013
Accepted 12 March 2013
Available online 29 March 2013

Keywords:
Dry and wet pyrolysis
$\delta^{13}C$
GHGs
Laboratory incubation
Carbon sequestration
Bio-char
Soil organic matter

ABSTRACT

Bio-char, biomass that has been deliberately charred to slow its rate of decomposition, has been proposed as an amendment with the potential to sequester carbon and improve certain soil properties. Slow pyrolysis (temperature $\leq$500 °C) and hydrothermal carbonization (low temperature, high pressure) are two efficient methods to produce bio-char with high yield and are applicable to a broad range of feedstocks. Chars made using slow pyrolysis (PC) and hydrothermal carbonization (HTC) of the same feedstock material (corn, C4) differed in physical appearance, chemical properties and decomposition behavior. We added these HTC and PC chars as amendments to three soils with C3-derived organic matter that differed in clay content, pH, and land use (managed spruce forest, unmanaged deciduous forest and agriculture), and compared their impacts on carbon sequestration and net greenhouse gas (CO_2, $^{13}CO_2$, N_2O and CH_4) emissions. HTC addition (1% w/w) significantly increased CO_2 emissions in all three soils ($p < 0.001$), with much of the extra C derived from HTC decomposition. In contrast, PC addition (1% w/w) had almost no impact on deciduous forest soil and actually decreased CO_2 emission from the agricultural soil. HTC treatment resulted in increased CH_4 emission from all soils but reduced N_2O fluxes in the agricultural and spruce forest soils. PC amendment had no significant effect on CH_4 emission, and resulted in intermediate levels of N_2O emission (between control and HTC treatments). Although both HTC and PC chars were produced from the same feedstock, PC had markedly higher potential for carbon sequestration than HTC.

1. Introduction

The annual growth rate of atmospheric CO_2 was 1.9 ppm in the past decade (2000–2009), reflecting a continuing, large, imbalance between carbon (C) release to the atmosphere and removal by natural sinks (Peters et al., 2012). One proposed mitigation strategy is to enhance land C sinks by removing C from the atmosphere and storing it in a form that is stable over a long period of time. Soils provide a large global reservoir of C stabilized for decades to centuries (Schmidt et al., 2011) and therefore practices that increase soil C storage have received much attention. Recent attention has been to add charred biomass (bio-char), which has been demonstrated to persist in tropical 'anthroposols' for up to thousands of years, in order to store C in soils (Lehmann, 2007). Bio-char, resulting from pyrolysis of biomass that enriches overall carbon content and slows degradation, has been proposed as an amendment that can potentially sequester carbon and improve certain soil properties such as soil fertility (Sohi et al., 2010).

The concept of bio-char amendment was derived from the study of Amazonian dark earth soils, also known as anthroposols. These soils were managed by indigenous people living in the Amazon basin between 600 and 8700 years ago (Grossman et al., 2010), and are characterized by higher C content and greater microbial diversity compared to unamended adjacent soils with similar mineralogy (Grossman et al., 2010; Navarrete et al., 2010; O'Neill et al., 2009). The soils are also characterized by the presence of charred particles, suggesting that char lasts hundreds to thousands of years at these sites. Thus amendment with bio-char is widely hypothesized to increase carbon storage capacity, although this effect is largely unquantified and depends on many factors (Liang et al., 2010). One such factor is the method used for bio-char production. Large differences in the composition of bio-char produced using different methods can result in timescales for persistence in soils, ranging from millennia (Forbes et al., 2006; Liang et al., 2008) to decades (Steinbeiss et al., 2009).

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http://dx.doi.org/10.1016/j.soilbio.2013.03.013

Two thermal degradation processes, in the presence and absence of water, are most commonly used to carbonize biomass. Both methods efficiently produce large amounts of char, have high rates of carbon recovery, and can be applied to a broad range of feedstock. These properties make them optimal from a soil amendment point of view (Fuertes et al., 2010; Titirici et al., 2007).

The most efficient process for char production under dry conditions is slow pyrolysis. This method derives from methods for charcoal production used by mankind for millennia (Ogawa and Okimori, 2010). Slow pyrolysis uses moderate heating rates over a long period of time, and ultimately leads to 30–45% C yield as biochar (Bruun et al., 2012). However, this process is not suitable for carbonization of most agricultural wastes due to the requirement for drying of the feedstock prior to and/or during the reaction.

In contrast, hydrothermal carbonization (HTC) makes use of a range of unconventional biomass feedstocks, such as sewage sludge, animal wastes and compost (Titirici et al., 2007), without the need for drying prior to char production. Although HTC was discovered in the early 20th century during studies of natural coal formation, to date there are only a few studies about its potential use for C sequestration (Funke and Ziegler, 2010; Rillig et al., 2010; Schimmelpfennig and Glaser, 2012). In hydrothermal char production processes, the wet biomass mixture is heated to temperatures of up to 220–240 °C in a high-pressure reactor. Steam pressures reach up to 20 bar, and very little gas (1–5%) is generated, so that most organics remain either in dissolved form or transform into brown coal (Libra et al., 2010). Various carbonaceous materials with different sizes, shapes, and surface functional groups are synthesized during HTC but a large proportion of the initial carbon (40–54%) remains in soluble form (Hu et al., 2010). Among the advantages of the HTC process is the use of non-traditional feedstock that could provide a continuous feedstock stream for this process and less carbon losses during the char generations.

The net greenhouse gas effects of char amendment depend not only on the potential to sequester atmospheric CO_2, but also the changes in the overall consumption or emission of methane and nitrous oxide. Biophysical processes responsible for CH_4 and N_2O emissions from soils are considerably altered with incorporation of biomass, fertilizer or bio-char into soils. A large proportion of published literature agrees that pyrolysis char suppress N_2O emissions from soil majorly due to its effect on soil moisture, soil aeration and NO_3^- runoff/leaching (Kammann et al., 2012; Taghizadeh-Toosi et al., 2011; van Zwieten et al., 2010). The only reported results about HTC char showed initial decrease in N_2O emissions but this effect was not observed on later stages of field experiment (Kammann et al., 2012) In contrast, the reported impacts of pyrolysis char on CH_4 fluxes are inconsistent, with positive (Feng et al., 2012; Yu et al., 2012), negative (Spokas and Reicosky, 2009; Zhang et al., 2010), or neutral influence on emissions (Kammann et al., 2012; Yoo and Kang, 2012). These effects were highly moisture dependent, and a full explanation of the impact of HTC on CH_4 fluxes is lacking.

Chars made from slow pyrolysis and HTC differ in physical appearance and chemical properties (Fuertes et al., 2010). The objective of this study was to evaluate the overall greenhouse effect of amendment with the two types of char by continuous monitoring of trace gases emissions from three different soils. The two chars used were produced from the same corn-based feedstock. We used differences in the natural abundance of $\delta^{13}C$ to track bio-char carbon (reflecting C4 origin of corn) from the organic matter in the amended soil, which reflected a pure C3 origin. In addition to CO_2, we monitored the effect of soil amendment on the production of CH_4 and N_2O. To our knowledge, this is the first study to compare the net greenhouse gas effect of soil amendment with slow pyrolysis and HTC chars.

2. Materials and methods

2.1. Soil sampling and characterization

Two forests soils (Cambisols) and one agricultural soil were collected from three different locations within the Thüringen state in Germany: a deciduous forest (DF) within the Hainich National Park; a spruce forest (SF) near Ölknitz village; and an agricultural soil (AG) from research plots located near the Max-Planck Research Institute for Biogeochemistry in the city of Jena. Together, these soils span a broad range of vegetation types and soil properties, including soil texture, which ranged from clay loam (DF) to sandy loam (AG). At each site 5–6 subsamples of the upper 15 cm of mineral soil were collected. The SF and AG soils were processed at field moisture. The DF soil was very wet when sampled, and was dried at room temperature to 20% of gravitational moisture content prior to processing. Soils were first passed through a 4 mm mesh size sieve to remove all plant material and large roots. Samples were then homogenized to produce a single, composite sample and stored at 4 °C prior to incubation. Sub-samples of the homogenized soils were dried at 40 °C for physical and chemical analyses.

Soil water holding capacity was measured by the volumetric method using char free soils (Livingston and Topp, 2007). Soil carbon and nitrogen concentrations were measured from ball-milled sub-samples by elemental analysis ("Vario Max", Elementar Analysensysteme GmbH, Hanau) before and after incubation. Organic carbon concentration was determined by calculating the difference between elemental analyses of the total carbon concentration and soil inorganic carbon concentration (Steinbeiss et al., 2008). Soil mineral N (NO_3^- and NH_4^+), dissolved organic carbon (DOC) and soil microbial biomass were determined by extraction before and after incubation (Karsten et al., 2007). For DOC analysis, 10 g of moist soil sample was suspended in 50 mL 0.05 M K_2SO_4 and shaken on a low speed reciprocal shaker for 1 h. Supernatant was filtered and analyzed ("high TOC" Elementar Analysensysteme GmbH, Hanau). Soil microbial biomass was determined by chloroform fumigation-extraction (Brookers et al., 2007). Unless otherwise stated, all measurements and are reported as the mean and standard variation of triplicate analyses.

The $\delta^{13}C$ of soil organic carbon was measured by a coupling an elemental analyzer to an isotope ratio mass spectrometer ("Bianca" Delta^{Plus}XL). Values are reported as $\delta^{13}C$ in per mill (‰) calibrated relative to the VPDB reference standard using NBS19 (Werner and Brand, 2001), and represent repeated measurements with a standard deviation of less than 0.3‰.

2.2. Bio-char production and characterization

Slow pyrolysis char (henceforth pyro-char, or PC) was produced from corn silage that was air dried (70 °C) and ground to less than 4 mm. The ground silage was sealed in aluminum foil to avoid aeration, with a small hole on one side of the foil to let gaseous products out. Samples were heated from room temperature to 500 °C at a rate of 10 °C per min and held at 500 °C for 2 h. After cooling, the resulting PC was passed through a 2 mm sieve and stored at 4 °C until the incubation experiment.

Hydro-char (HTC) from the same corn silage was obtained commercially from the carbon solutions Company Ltd, Kleinmachnow Germany. The delivered material was slurry (10% solids). Before we used it as an amendment, this slurry was freeze-dried at −50 °C and the resulting solid material was passed through a 2 mm sieve.

Elemental concentrations of C, N, and H in both types of char were measured from ball-milled subsamples using an elemental analyzer (VarioMax Elementar Analysensysteme GmbH, Hanau).

Oxygen was estimated as follows: O = 100 − (C + H + N + ash) (all expressed in weight %). Thermo gravimetric (TG) curves (Mettler Toledo, TGA/SDTA851) were used to characterize the relative lability of the char materials. Volatiles were assumed to equal the mass lost between 105 °C and 850 °C in an N_2 atmosphere, and the mass lost at 850 °C after introduction of O_2 was considered to be the stable fraction. Ash content was defined as the mass remaining after combustion in air. No inorganic C was detected in the chars. The $\delta^{13}C$ of both char and plant material was determined using same procedure as for soil samples.

2.3. Soil incubation

Prior to incubation, the approximatley15 kg of prepared soil sample from each soil was divided into three aliquots. The first aliquot was used as a control; the second received an amendment with PC (10 g/kg soil) and the third was amended with HTC (10 g/kg soil) and samples were homogenized to equally distribute the amendments. Each treatment (Control, +PC, +HTC) was then divided into four replicates of ~1 kg each and placed into incubation chambers (total 3 soils × 3 treatments × 4 replicates, or 36 chambers). The chambers used for incubation were constructed from PVC columns (10 cm diameter and 20 cm height).

Chambers were placed in a custom-built continuous flow incubation system, the details of which have been reported elsewhere (Thiessen et al., 2013). Briefly, this automated system continuously (~30 mL/min) flushes the chambers holding soil samples with CO_2-free synthetic air (20.5% O_2 and N_2). Fluxes of gases are determined from the concentration of gases in headspace air exiting the chambers and the rate of airflow. The outlet air sampling the headspace of the column was connected to an automated multiport stream selection valve (Valco) that directed the airstream from different columns sequentially to an infrared carbon dioxide analyzer ("LI-6262" LI-COR Biosciences Lincoln, USA). Other greenhouse gases and isotopes of CO_2 were sampled less frequently using flasks (see Section 2.4 below).

The chambers were installed in a temperature-controlled climate chamber, where we maintained temperatures at 20 °C throughout the incubation period. The soil water content of each column was initially adjusted to equal 70% water holding capacity by adding water to the field moist (SF and AG) or pre-dried (DF) soils, using the previously determined water holding capacity for each soil without char amendment to determine field capacity. Addition of 1% pyrochar char resulted increase in WHC of DF, AG and SF soils by 2.89%, 1.65% and 2.43% respectively. Similarly HTC-amended DF, AG and SF soils have 1.51%, 1.47% and 1.77% higher WHC respectively than respective controls. But this increase in WHC of bio-char amended soils was not accounted during initial soil moisture adjustment. The continuous stream of synthetic air that flowed through the incubation columns dried the soils at a rate of (~1 g H_2O/d), This water was replaced by sprinkling the surface of the column with distilled water every 4th day to replace the measured mass loss from the column.

2.4. Gas sampling and analysis

Other gases, including $\delta^{13}C$–CO_2, CH_4 and N_2O, were measured from flasks collected from the air exiting the chambers. The gas samples were taken on days 1, 5, 10, 20, 25, 35, 45, 55, 70, 85 and 100 after starting the incubation. Gases were collected using either 2.3 L or 1 L glass flasks (flushed with synthetic air prior to sampling) equipped with two stopcocks and connected via a capillary to the soil columns. The exhaust gas of each flask was passed through water filled diffusion traps to prevent back diffusion of atmospheric air, and the air was subsequently dried using magnesium perchlorate before entering the flask. After allowing ~2 h to flush the flasks with headspace air (~30 mL/min flow rate), the stopcocks were closed. Possible dilution from incomplete purging of synthetic air in the sampling flasks, especially the 2.3 L flasks, was corrected by comparing the CO_2 concentration in the flask with the simultaneously measured CO_2 measured by LiCOR. It was assumed that N_2O and CH_4 were diluted in the same ratio as CO_2 and no dilution correction was applied for $^{13}CO_2$ as there was no additional source of CO_2. Flasks where this dilution correction exceeded 25% were discarded and were not used in calculations.

The analysis of N_2O and CH_4 were made using gas chromatography (Agilent technologies 6890, Santa Clara, USA) equipped with an electron capture detector (ECD), and a flame ionization detector (FID). The $\delta^{13}C$ of CO_2 was determined by stable isotope ratio mass spectrometry (Finnigan MAT 252IRMS). Both analyses were carried out in laboratories at MPI-BGC (Jordan and Brand, 2001; Rothe et al., 2003). Cumulative emissions of CH_4 and N_2O were calculated by interpolating linearly between sampling events (see below).

To observe impact of char on N_2O emissions, we performed an additional experiment on the AG soil. Approximately 20 mL of fertilizer solution ($(NH_4)_2SO_4$ with a concentration of 200 mg-N/L) was added to AG soil after 51 days of incubation.

2.5. Calculations and statistics

The soils used in our incubations each had a long history of C3 vegetation inputs. In contrast, both PC and HTC were produced from corn silage, which is a C4 plant. We used two methods to quantify the contributions of HTC, PC and native soil organic matter to carbon mineralized during incubation period using the difference in ^{13}C from native SOC and amendment char. In both cases we used a two component mixing model to calculate the relative fraction of evolved CO_2 derived from bio-char (f_{char}) and from native soil organic matter (f_{soc}) in soil-bio-char mixtures (Balesdent and Mariotti, 1996; Gleixner et al., 2002):

$$\delta^{13}C\,(\text{soil bio}-\text{char mixture}) = f\text{char}*\delta^{13}C\text{char} + f\text{som}*\delta^{13}C\text{som} \quad (1)$$

Since: $f$soc + $f$char = 1

$$f\text{char} = \left(\delta^{13}CO_{2\ \text{teatment}} - \delta^{13}CO_{2\ \text{control}}\right) \Big/ \left(\delta^{13}CO_{2\ \text{char}} - \delta^{13}CO_{2\ \text{control}}\right) \quad (2)$$

To derive $\delta^{13}C_{char}$, we assumed that the $\delta^{13}C$ of CO_2 derived from decomposition of the char equaled that of the bulk $\delta^{13}C$ of the respective PC or HTC char. We tested this assumption by incubating pure char materials and found that the $\delta^{13}C$ of CO_2 evolved equaled the $\delta^{13}C$ of the initial char to ±1‰ (data not shown). However, we note that when we incubated pyrochar in ambient air, it showed net absorption of CO_2; to obtain a $\delta^{13}CO_2$ value, we therefore incubated the PC in air that was initially CO_2-free.

Our first method used direct measurements of CO_2 evolved and its $^{13}CO_2$ signature over the period of the experiment to derive $\delta^{13}C_{treatment}$ and $\delta^{13}C_{control}$. The automated incubation system measured CO_2 fluxes 2 times per day for each chamber. To calculate cumulative CO_2 fluxes, we interpolated fluxes linearly between these very frequent sampling times. Soil respiration results from days 41–45 were lost due to malfunctioning of the data logger, and we filled this data gap with linear interpolation.

The $\delta^{13}CO_2$ was sampled less frequently than CO_2 fluxes. The cumulative $\delta^{13}C$ signature of the evolved CO_2 was obtained by multiplying the amount of CO_2 evolved between ^{13}C sampling events

by the measured $\delta^{13}CO_2$ signature, summing over the entire period of the incubation, and dividing by the cumulative total CO_2 produced. By using the ^{13}C signature of the cumulative CO_2 evolved in the control as the "SOC" end member, we implicitly assume that any fractionation associated with mineralization of SOC carbon is the same with and without char amendment (Steinbeiss et al., 2009).

The second method we used compared the amounts and ^{13}C signatures of the carbon remaining in the incubated samples at the end of the experiment (C_{end}, $\delta^{13}C_{end}$) with those at the beginning of the experiment (C_{start}, $\delta^{13}C_{start}$) to estimate the amount and ^{13}C signature of the C mineralized (C_{min}, $\delta^{13}C_{min}$):

$$C_{min} = C_{start} - C_{end};$$

$$\delta^{13}C_{min} = \left(C_{end},\ \delta^{13}C_{end} - C_{start},\ \delta^{13}C_{start}\right) \Big/ C_{min}$$

We then use $\delta^{13}C_{min}$ calculated for treatment and control incubations as $\delta^{13}C_{treatment}$ and $\delta^{13}C_{control}$, respectively, in Equation (1) to estimate the fraction of organic matter and char mineralized in the incubations. We again implicitly assume that any fractionation associated with the mineralization of SOC will be the same in both control and amended soils, and that there is no isotopic fractionation on decomposition of the chars.

The two methods sometimes gave slightly different results, especially in terms of the significance when comparing control and amended incubations or soils. We have used the results using the method that yielded the greatest significance in the results and discussion, and also indicate where the two methods disagree. For example, a very small loss of char mass due to decomposition during the incubation will be difficult to detect by changes in mass and ^{13}C from beginning to end of the incubation (method 2), while it may be more sensitively measured in the relatively smaller amount of CO_2 evolved (method 1).

The amount of extra soil organic carbon released or suppressed as a result of the addition of char (primed carbon) was calculated as the difference between amounts of soil carbon respired from the pyrochar amended treatment (μg C/g dry soil) and soil carbon respired (μg C/g dry soil) in the control. For this calculation we report results using f_{char} calculating using both methods.

All data were expressed as means of the four replicate incubations ± the standard error. Significance of differences among/between treatments was determined using one way analysis of variance (ANOVA). This was followed by a post-hoc test (Tukey, $\alpha = 0.05$). All statistical analyses were carried out using SPSS (PASW statistics-18) and graphs were prepared in SigmaPlot (Version 10.0) or MS-Excel 2010.

3. Results

3.1. Soil characteristics

Although all soils sampled for this study were classified as Cambisols, they differed in physical, chemical and biological properties (Table 1). The soil sampled in spruce forest (SF) had the highest sand content and lowest pH, with intermediate C content (4.59% by weight) compared to the other two soils. The deciduous forest (DF) soil had higher clay content and lower C content than the other soils (Gleixner et al., 2009; Tefs and Gleixner, 2012). The agricultural field soil (AG) had the highest overall pH and C content (Malik et al., 2012).

Microbial biomass, reported only for control soils, was highest in the DF soil (522.8 ± 27.43 μg/g soil) followed by SF and AG soils (355.8 ± 75.12 and 340.3 ± 32.92 μg/g soil respectively). Inorganic carbon contents were very small in all cases: zero in the spruce forest, and 0.09 and 0.03% in the deciduous and agricultural soils, respectively. Dissolved organic carbon content was lowest in AG soil. The ^{13}C signature of all three soils confirmed that the major source of C in all sites was C3 plants and $\delta^{13}C$ values ranged between −26.51‰ and −28.02‰ (VPDB).

Table 1
Soils characteristics; DF (deciduous forest, Hainich National Park), SF (spruce forest, Olknitz), AG (agricultural soil, in Jena).

Soils	Textural class	TOC (%)	TIC (%)	Total N (%)	DOC (ug/g)	Microbial biomass (ug/g)	pH	$\delta^{13}C$ (‰VPDB)
DF	Silty clay	3.61	0.03	0.29	14.2 ± 0.1	523 ± 27.4	6.26	−26.51
SF	Sandy loam	4.59	ND	0.19	39.4 ± 4.0	356 ± 75.1	4.70	−28.02
AG	Silty loam	5.11	0.09	0.27	21.3 ± 2.5	340 ± 32.9	6.68	−27.91

Values represent means ± S.D ($n = 3$) and are expressed on a dry weight basis.

3.2. Char characterization

Both char amendments used in this study were derived from the same feedstock (corn silage), with a $\delta^{13}C$ signature typical for C4 plants (Table 2). The PC (slow pyrolysis) and hydrothermal carbonization (HTC) chars produced differed in physical and chemical properties from each other as well as from the feedstock. Both types of bio-char had higher C and N concentrations and lower H and O concentrations than the feedstock. In particular, the PC had very low H:C and O:C atomic ratios compared to the HTC. The $\delta^{13}C$ signature of the char materials was depleted compared to the feedstock by ~0.5‰ (PC) to 1.3‰ (HTC). A large proportion of the HTC char was soluble, based on its high DOC content the fact that before freeze drying the HTC char was in the form of slurry with 10% (w/w) solid material. The HTC showed strong initial weight loss during thermogravimetry analysis (TGA) compared to PC (Fig. 1). Weight loss on heating followed the order feedstock > HTC > PC (Fig. 1). Ash content increased with the degree of charring; PC, HTC and corn had 11.39%, 21.35% and 3.07% of ash respectively.

3.3. Soil respiration

During the first 50 days of incubation, the hydro-char (HTC) amendment resulted in higher production of CO_2 ($p < 0.001$) compared to control or PC treatments. This effect was most pronounced during the first week of incubation where the rates of CO_2 production were 66.8 ± 2.8, 83.4 ± 15.6 and 62.0 ± 14.9 μg-C/g soil in SF, DF and AG soils, respectively (Fig. 2A, B and C). During the last 7 weeks of incubation, the increased respiration from HTC treated soils was statistically significant only in forest soils (DF and SF; $p \leq 0.01$). Amendment with slow pyrolysis (PC) char produced results that differed by soil type. The PC amended SF soil respired more than the control and this increase was significant during the first 50 days of incubation ($p < 0.01$), However, no increase was observed in DF soils, and overall caused a decline in the evolution of CO_2 from AG soils.

Overall, the CO_2 output over 105 days of incubation was significantly higher in all HTC amended soils (2.66, 2.11 and 1.89 times higher than the respective controls in AG, DF, and SF soil (Fig. 4A)). PC amendment increased CO_2 production in the SF soil specifically during the initial 7 weeks ($p \leq 0.01$), but this PC effect was not observed in another forest soil. Surprisingly, PC addition resulted in lower cumulative CO_2 production in the AG soil compared to unamended soil.

3.4. Source of respired CO_2 and char mineralization

The $\delta^{13}C$ of bulk SOM (DF, SF and AG) and bio-char (PC and HTC) reflected their respective C3 and C4 plant origins (Tables 1 and 2),

Table 2
Characteristics of bio-chars and its feedstock; PC (slow pyrolysis char), HTC (Hydrothermal char) and Corn silage (feedstock for both types of chars).

Char	Chemical composition (wt %)					Atomic ratio		mg/g	pH	(VPDB)
	C	H	O	N	Ash	O/C	H/C	DOC		$\delta^{13}C$ (‰)
PC	77.88	2.29	6.45	1.99	11.39	0.06	0.35	0.033	9.73	−13.11
HTC	51.63	5.70	19.42	1.90	21.35	0.28	1.33	12.99	4.15	−13.87
Corn silage	45.14	6.78	43.88	1.13	3.07	0.73	1.80	–	–	−12.69

Values represent means ($n = 3$) and are expressed on a dry weight basis.

with a 14‰ ± 1 difference in $\delta^{13}C$ between the soil and the char. The CO_2 respired from HTC treatment of all soils (Fig. 3A, B, C) was enriched in $\delta^{13}C$ compared to that respired from the respective unamended soils, indicating that the increased respiration from HTC-amended soils resulted at least in part from char decomposition. In contrast, no significant difference in $\delta^{13}C$–CO_2 was observed for PC-amended soils compared to their respective controls, except in the initial CO_2 effluxes from the spruce forest soil. Variability in CO_2 production rate as well as ^{13}C–CO_2 was largest in the agricultural soil, especially the difference between first and final date was 7‰ among replicates of control.

For the two forest soils, PC amendments did not increase the respiration of native soil carbon ($p > 0.05$) calculated using either cumulative respiration (method 1) or mass balance of solids (method 2). The greatest effect of PC addition was observed in the AG soil, where native carbon mineralization decreased after PC addition and this effect was persistent for most of the measuring dates (method 1; $p < 0.05$). However, the HTC-amended AG soil showed accelerated native-soil SOM decomposition (priming); this effect was significant in cumulative CO_2 emissions (method 1; $p \leq 0.01$) though it was not detectable from comparing the amounts of HTC and SOC remaining in the bulk soil at the end of the incubation (method 2; Fig. 4B). During the initial days of incubation the effect of PC amendment on native soil carbon fluxes was inconsistent and varied among the three soil types. Initially there was positive impact and native (C3) soil organic carbon was respired at higher rates than the control treatments in both forest soils. In particular the SF soil showed positive priming for the first 25 days of incubation (Fig. 3B). However, given the errors involved, this positive priming effect was not detectable in the cumulative respiration (method 1) but it was in the mass balance of the residual soil material (Fig. 4B; method 2).

Based on the bulk soil carbon analysis at the end of the experiment (method 2), roughly 50% of the added HTC was decomposed into CO_2 during the incubation, and this mineralization rate was consistent irrespective of soil types (Fig. 4B). The loss of PC-C was only significant in the SF soil where a 7.07% (S.D = 1.35 %) decrease in added carbon was observed (Fig. 4B).

3.5 Methane and N_2O fluxes

No CH_4 was emitted in DF, DF + PC, SF and SF + PC treatments (Fig. 6); the use of zero-methane air as our inlet gas did not allow us

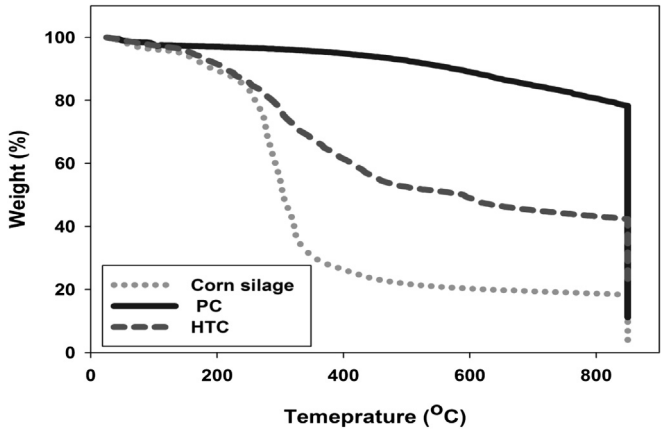

Fig. 1. TG curves for feedstock and bio-char samples (PC, slow pyrolysis char; HTC, hydrothermal char).

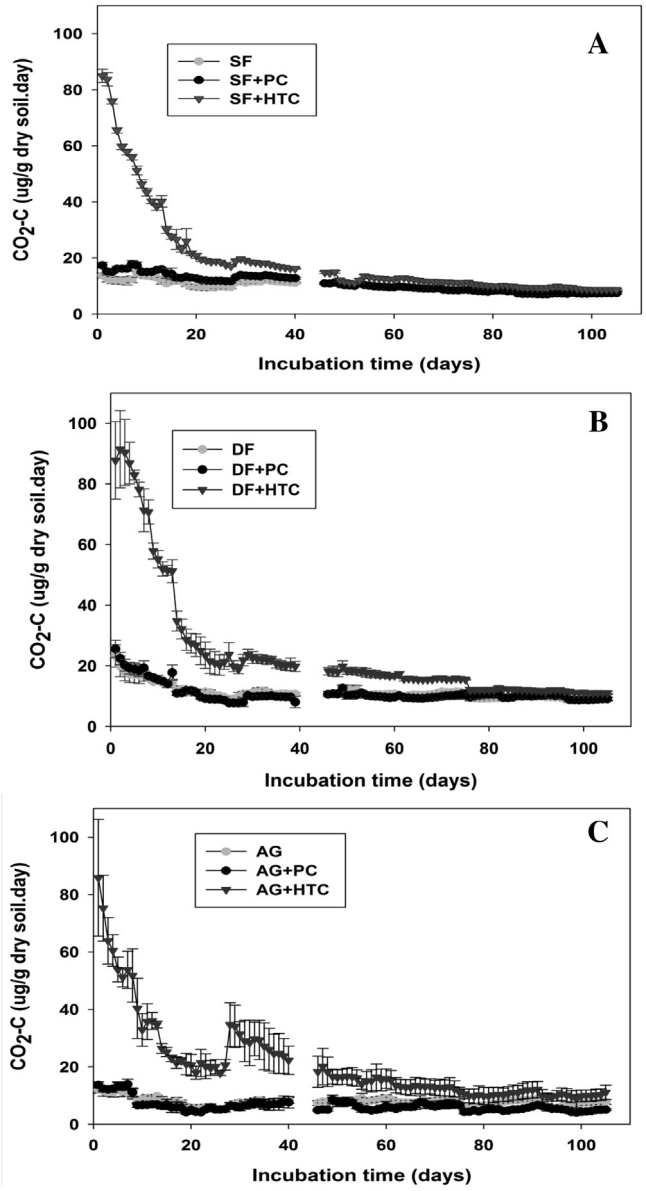

Fig. 2. Respiration rates in two forests soils and one agricultural soil; (A) respiration rates in DF soil treatments (B) respiration rates in SF soil treatments (C) respiration rates in AG soil treatments. Error bars represent standard deviation between four replicates.

to test whether these soils would have consumed methane. Although all treatments were incubated under aerobic conditions and at the 70% of their respective field capacities, CH_4 emissions were recorded in HTC treatments of all soils, with highest effluxes from the AG soil ($p < 0.01$). The only impact of pyrochar on CH_4 emissions was observed in the AG soil but there was large variability among replicates (Fig. 6).

Nitrous oxide emissions were suppressed in HTC and PC amended agricultural and spruce forest soils. This effect was enhanced in the AG soils following ammonium sulfate fertilizer addition, with reduced N_2O emissions from HTC and PC treatments compared to fertilized control soils (Fig. 5). This reduction was more pronounced for the HTC compared to the PC amended soils ($p < 0.01$). In contrast, the opposite was found for the DF soil, where HTC amendment increased N_2O emissions ($p < 0.05$). There were large variations in N_2O and CH_4 emissions among all replicates of AG soil treatments.

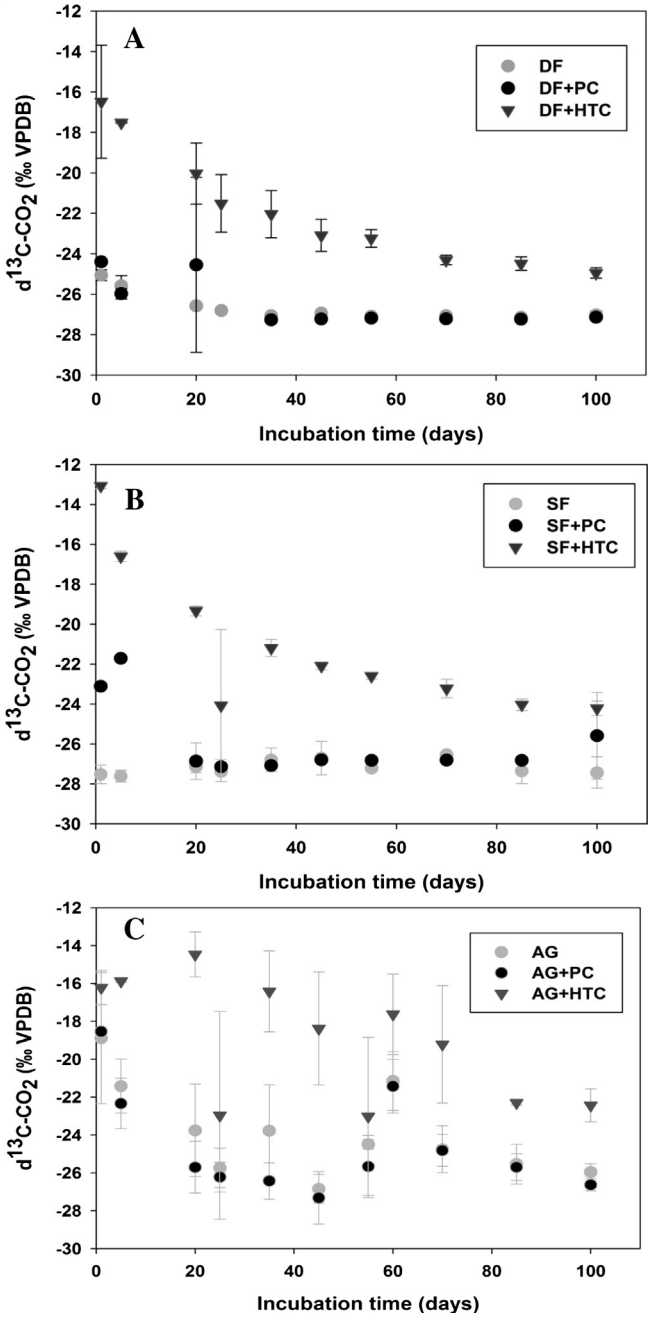

Fig. 3. Stable carbon isotope signature in aerobic laboratory incubations of two forest soils and one agricultural soil. Shown are the $\delta^{13}C$-signatures of CO_2 emitted from soil column. Vertical bars represent standard deviation of the mean ($n = 3$). A; DF soil treatments, (B) SF soil treatments and (C) AG soil treatments.

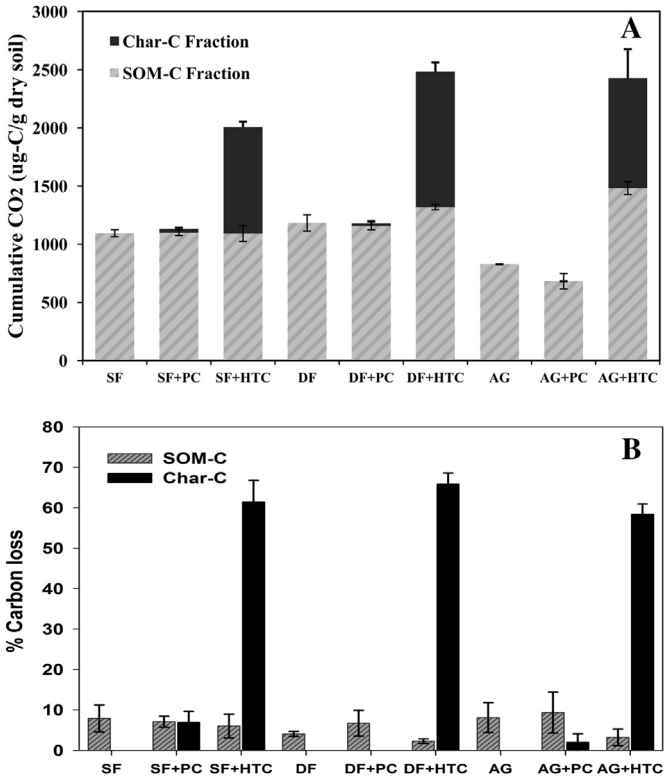

Fig. 4. (A) Cumulative respirations (CO_2–C ug/g dry soil) from different treatments and relative carbon losses of bio-char and soil organic matter after 105 days of laboratory incubation. Vertical bars represent standard deviation among replicates ($n = 3$). (B) Losses of bio-char carbon and soil organic carbon after 105 days of incubation given relative to the respective initial amounts in the treatments (bulk soil). Vertical bar represents deviation among replicates ($n = 4$).

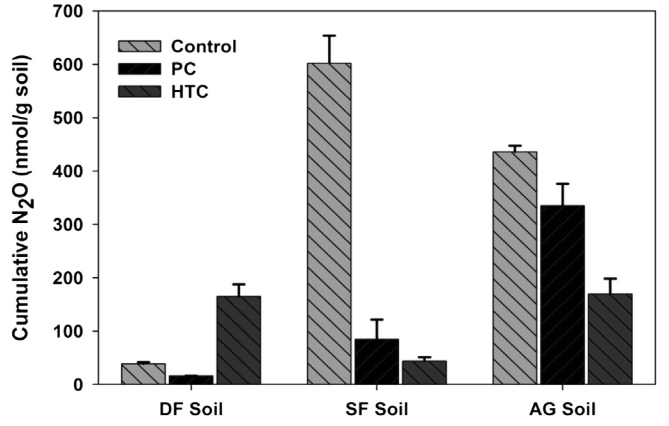

Fig. 5. Cumulative emissions of N_2O different treatments of two forest soils and one agricultural soil after 105 days of aerobic incubation. Error bars represents standard deviation ($n = 3$).

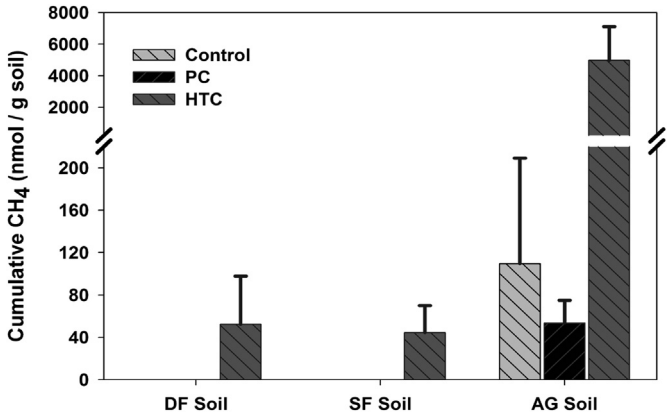

Fig. 6. Cumulative emissions of CH_4 different treatments of two forest soils and one agricultural soil after 105 days of aerobic incubation. Error bars represents standard deviation ($n = 3$).

4. Discussion

4.1. Links between chemistry and decomposition rates for PC and HTC

The major goal in char production is to increase the carbon content in products compared to the original biomass (Sohi et al., 2010). To be the most beneficial for carbon sequestration, however, the char produced must not only be stable (not rapidly decomposed) when added to soil, but also must use the less energy during production the carbonization (production) process. Slow pyrolysis char and hydrothermal char are both methods known to have high rates of carbon recovery from biomass but also use less energy. HTC does not require wet biomass pre-drying, and so saves additional energy in production (Meyer et al., 2011). In our study, the carbon recovery (percentage of C originally contained in the raw material that is retained in the carbonized sample) of dry pyrolysis process was 44.88 %, similar to other PC chars reported (Brown, 2009; Fuertes et al., 2010; Meyer et al., 2011).

Chars produced from various pyrolysis techniques differ in their physical structure and chemical composition even when the same feedstock was used in their preparation (Brewer et al., 2009; Bruun et al., 2012). In our study, the physical structure of the corn silage feedstock was maintained until the final product during slow pyrolysis. For the HTC, however, the end product was a slurry and no structures from the original corn vegetation were visible. These characteristics likely resulted from the very high pressures reached during the hydrothermal processes.

The PC and HTC chars differed not only in physical form but also chemical composition. The carbon and ash content of HTC was much higher than PC, and had higher hydrogen and oxygen concentrations and were similar to those were recorded by other studies (Cao et al., 2010; Fuertes et al., 2010; Schimmelpfennig and Glaser, 2012).

The biggest differences between the two chars were in pH and in the amount of soluble/volatile carbon. The PC and HTC chars had pH's of 9.89 and 4.70, respectively. The hydrothermal carbonization reactions are sensitive to pH and generally, an acidic pH (<7) is a pre-requisite for the HTC method (Meyer et al., 2011). The end products usually have pH similar to the liquid added to biomass prior to carbonization (Liang et al., 2011). Lower pH is achieved with addition of low strength acids (e.g. citric acid) which also enhance dehydration and improve the overall reaction rate of hydrothermal carbonization (Cao et al., 2010; Hu et al., 2010). In contrast, slow pyrolysis produces char with pH values generally above neutrality, and these generally increase, along with ash content, with the temperature of pyrolysis (Ueno et al., 2008). With pH values above 9, there may be some carbonates that add inorganic C with the PC to our soil amendments.

The more rapid decomposition of HTC compared to PC is in accord with the differences in elemental composition and volatile content of the two chars. Elemental ratios i.e. O:C, C:H and C:N provide a reliable measure of the stability of chars in soil and correlate with initial decomposition rates of substrates (Novak et al., 2010; Singh et al., 2010a). A correlation between O:C ratio and char stability has also been reported, and it has been suggested that the half-life of the char with O:C molar ratio lower than 0.2 could be more than 1000 years (Spokas, 2010).

It has been recommended that char with O/C ratio <0.4, H/C ratio <0.6 and black carbon >15% is best suited for soil application as a method for sequestering C (Schimmelpfennig and Glaser, 2012). While we did not measure black carbon, the O/C of both our HTC and PC fall within the range of suitability; for our materials the O:C ratio declined from 0.73 to 0.28 to 0.06 for corn silage feedstock, HTC and PC chars, respectively (Table 2).

The HTC char also had much higher volatile compound content, as measured by thermogravimetry, compared to the PC in this study. It also contained large amounts of soluble carbon (DOC), which is presumably more available for microbial degradation. Pyrolysis–gas chromatography–mass spectrometery (PY–GC–MS) analysis of HTC and PC (Julia Baumert and Gerd Gleixner, MPI-Biogeochemistry, personal communication 2012) showed that the char prepared from pyrolysis (PC) had higher aromatic C content (benzene, styrene, phenol etc.), where the char produced from HTC was mainly comprised of heteroatomic compounds like furans, pyrans, dihydropyranones, pyrroles, imidazoles suggesting biomass contribution. These results are consistent with previous reports that found slow pyrolysis produced char rich in aromatic compounds, whereas HTC contained precursors of cellulose, hemicellulose and lignin (Fuertes et al., 2010; Schimmelpfennig and Glaser, 2012; Titirici et al., 2008).

4.2. Behavior of amendments in different soils

The three soil types in our study differed considerably in their characteristics, including control (unamended) respiration rates. The two forest soils emitted significantly higher amounts of carbon than the agricultural soil ($p \leq 0.001$; method 1), although more CO_2 was emitted from the DF soil than the SF soil ($p < 0.05$; method 1). Microbial biomass was the only characteristic that showed the same pattern as the respiration rates (Table 1). Soil microbial biomass is closely related to soil fertility and is considered as sensitive indicator of soil quality (Iqbal et al., 2010). Lower respiration rates in AG soil might be impact of low available carbon pool (Table 1) as substrate limitation may cause stability in SOC (Marschner et al., 2008).

During 105 days of incubation, we observed more than 50% loss of the HTC char (Fig. 4B; method 2), and this loss was slightly higher in DF soil followed by SF and AG soil respectively. In contrast, the pyrolysis char (PC) was either inert or its mineralization rate was too low to be measured by either mass balance method we used (cumulative CO_2 loss or comparison of initial and final organic matter), except for a 7.07% (s.d 1.35%; method 2) loss from the SF soil (Fig. 4B). Instability of HTC char in soil was found in number of recent studies (Kammann et al., 2012; Qayyum et al., 2012) and would be predicted from its chemical content as described above.

The enhanced respiration in PC amended SF soil could arise from abiotic as well as biotic factors (Jones, et al. 2011). The high pH of PC may result in inorganic C release during the incubation (Jones et al., 2011), and soils with lower pH showed higher PC mineralization in

other studies (Luo et al., 2013). The observed short-term acceleration of PC mineralization in acidic SF soil could result from acidification of PC inorganic C, but could also result from the effect of pH on microbial communities. Soil pH is the major driver of microbial community structure, (Gleixner et al., 2009; Griffiths et al., 2011; Thoms et al., 2010) and microbial communities in spruce forests are specialized to degrade complex and aromatic compounds (Carletti et al., 2009) whose growth might be initially stimulated by the addition of PC (Luo et al., 2013).

Both char amendments impacted native soil carbon mineralization rates, the effect known as priming. The potential for priming is of considerable interest in bio-char research (Keith et al., 2011). However, it is still unclear which basic processes might explain the role of char in priming, and this makes it is difficult to predict how different types of char will behave in a range of soils. If we relate priming (short term acceleration or decline of native carbon decomposition (Kuzyakov, 2010)) to the presence of a labile pool in heterogeneous pyrolysed biomass, we would predict that the char with the highest amount of labile material would enhance degradation not only of SOM but also of recalcitrant components of the char itself. The HTC char used in this study had high amounts of DOC and volatile constitutes (more than 50%, based on TGA), and its high degree of decomposability could be easily observed in the incubation results (Fig. 4). A decrease in SOC-derived respiration compared to the unamended control (negative priming) was observed initially when HTC was added (data not shown). This initial negative impact of HTC char on SOC mineralization might be related to shift of soil microbes from less available SOC to more labile HTC. Several studies showed phytotoxic effects of HTC due to higher PAH content and presence or emission of toxic volatiles (Busch et al., 2012; Jandl et al., 2013; Rogovska et al., 2012; Schimmelpfennig and Glaser, 2012) but there is no study related to toxic effect of HTC on soil micro-organisms. However, later in the incubation, SOM mineralization was enhanced by HTC amendment (positive priming), and this increase was greatest in the AG soil (Fig. 4A). However, this positive priming was not measureable in bulk soil carbon, which could be due to either to our assumption that the $^{13}CO_2$ from HTC char reflects its bulk $\delta^{13}C$ content, or to the fact that the C overall was greatest (and therefore the HTC addition proportionally the smallest) in the AG soil. On the contrary, bulk soil carbon measurements confirmed the considerable negative impact of HTC on AG native soil carbon mineralization ($p < 0.05$) (Fig. 4B). To our knowledge there is no previous report on HTC addition on native soil carbon mineralization.

The impact of PC amendment on native soil was overall smaller, inconsistent and varied with soil type. These inconsistencies may reflect the practical limitation of two pool model used in this study where one end member was assumed (PC $\delta^{13}C$ was assumed not to fractionate on decomposition). The AG soil, the most carbon rich soil in our experiment, respired less native soil-CO_2 when amended with PC, but no effect was found in forests soils. In summary, the priming effect of HTC and PC in forest soils was not consistent and it was so small in size that we were not able to measure it in bulk soil carbon (method 2). Our results agree with other field and incubation studies that showed PC amendment had either no effect or negative priming (Major et al., 2010; Zimmerman et al., 2011).

4.3. Effect of Char amendments on non-CO_2 greenhouse gases

All three native soils produced nitrous oxide. Nitrous oxide fluxes were significantly higher from SF soil compared to DF soil (Fig. 5). These results are in contrast with studies that showed lower N_2O emissions from spruce forest soils compared to deciduous forest soils (Ambus et al., 2006) Application of bio-char can affect the fate and transformation of N in soils (Singh et al., 2010b; van Zwieten et al., 2010) and hence the emission of N_2O. Rondon et al. reported a 50–80% reduction in N_2O emissions following bio-char addition to tropical soils (Rondon et al., 2006). We found similar results, but observed greater suppression of N_2O emissions with HTC compared to PC (Fig. 5). This suppression in N_2O flux with HTC amendment was most prominent in the AG soil to which N-fertilizer was applied. Kammann et al. (2012) also found that HTC amendment suppressed N_2O emissions, but this effect was short-lived and opposite when nitrogenous fertilizer was applied (Kammann et al., 2012). Increased N_2O emission from HTC treatment of DF soil might be due to its role on water holding capacity of clay rich soil. Higher hydrophilicity of HTC was recorded compared to PC (Schimmelpfennig and Glaser, 2012) and this may resulted in water rich microsites in DF soil. Amendment with PC always decreased N_2O efflux regardless of soil type or fertilization, in agreement with other studies (Kammann et al., 2012; Libra et al., 2010).

Since the incubation system used air with no methane as the inlet gas, it was not possible to measure any impact of char on methane uptake in our soils. In our unamended control soils, the AG soil was the only one to emit CH_4 while no emissions were observed from either forest soils (Fig. 6). Globally, forest soils are considered to be natural sinks for atmospheric CH_4, while agricultural practices tend to reduce the soils' ability to take up CH_4 (Guckland et al., 2009; Mancinelli, 1995). However, with the addition of char we found measureable methane production from certain treatments. The addition of HTC resulted significant CH_4 emission ($p < 0.05$) from HTC treatments of DF and SF soils, and stimulated CH_4 emissions ($p < 0.01$) from the AG soil (Fig. 6). The increased in CH_4 emission could result from single or combined factors, including water content, soil type and char type. Although we kept the total moisture content constant, it was practically impossible to distribute moisture evenly throughout the soil column and anaerobic microsites must have been produced in the soils. Addition of the relatively hydrophobic HTC char could enhance anaerobicity and increase CH_4 emissions. In a field study, HTC char amendment was observed to increase CH_4 emission (Kammann et al., 2012). The net emission of CH_4 includes the balance of methanogenesis and methanotrophy and our experimental set-up was not well equipped to address this balance. This study was able to demonstrate that char amendment influences not only C sequestration but other greenhouse gas fluxes. Further research will be needed to fully investigate the mechanisms involved in the role of char amendment on N_2O and CH_4 emissions.

5. Conclusion

Slow pyrolysis and hydrothermal carbonization are two of the most efficient methods to produce bio-char in terms of carbon yield and utility to a broad range of feedstocks. The HTC method especially is proposed as a useful way to increase soil C sequestration as it uses less energy than PC production and can use unconventional wet biomass sources such as sewage sludge, city wastes, animal and human excreta without requiring additional pretreatment such as drying. Both PC and HTC have been suggested as mitigation options for carbon sequestration to help offset increasing atmospheric concentrations of CO_2. Hydrothermal char decomposes rapidly (50% in 100 days regardless of soil type) and can stimulate emissions of GHG like CH_4 and CO_2 derived from priming of native organic matter. Slow pyrolysis char is more stable in soil and had consistent effect on GHG emissions in this study. Therefore PC would seem to have the greatest climate mitigation potential, though its overall emissions during production are potentially greater because of higher temperature combustion and the need for drying feedstock also need to be taken into account. Properties of

the amended soils seem to be less important than the method of char production, though they are not insignificant. Because much of the bio-char mitigation is suggested as an amendment for agriculture, the observed reduction in decomposition rates of native OM needs careful consideration as it may impact the supply of nutrients to plants.

Acknowledgments

The authors are thankful to research training group GRK1257 'Alteration and Element Mobility at the Microbe–Mineral Interface and International Max-Planck Research School for Global Biogeochemical Cycles for funding. The authors are also thankful to the stable isotope and gas analysis facilities at MPI-BGC, and to three anonymous reviewers whose comments improved the manuscript considerably.

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