Carbon sequestration in temperate grassland soil
-Risks and opportunities of biochar and hydrochar application-

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Dedicated to my family
and B.B.
with love and gratitude

Inmitten der Schwierigkeiten liegt die Möglichkeit.

(Albert Einstein)

Table of contents

List of Figures

List of supplementary Figures

List of Tables

List of supplementary Tables

List of abbreviations

a.s.l.	Above sea level
ABR	Absolute bacterial respiration
AFR	Absolute fungal respiration
AMF	Arbuscular mycorrhizal fungi
BBodSchG	Bundesbodenschutzgesetz (National soil protection act)
BET	Brunauer-Emmett-Teller
C	Carbon
CH_4	Methane
CI	Confidence Interval
C_{mic}	Microbial carbon
CO_2	Carbon dioxide
DIN	Deutsches Institut für Normung (German Institute for Standardisation)
DM	Dry matter
DOC	Dissolved organic carbon
EA-IRMS	Elemental analyser coupled to an isotope ratio mass spectrometer
EBC	European Biochar Certificate
ECD	Electron capture detector
EU-ETS	European trading system
FID	Flame ionization detector
GC	Gas chromatograph
GHG	Greenhouse gases
GWP	Global warming potential
H	Hydrogen
H/C	Elemental ratio of hydrogen and carbon
HTC	Hydrothermal carbonization
HTCs	Hydrothermal carbonization in a steam atmosphere
HTCw	Hydrothermal carbonization in a water atmosphere
IBI	International Biochar Initiative
IPCC	International Panel on Climate Change

ISO	International Organisation for Standardisation
$M_{(x)}$	molar mass of compound x
MRT	Mean residence time
N	Nitrogen
N_2	Di-nitrogen
N_2O	Nitrous oxide
NH_3	Ammonia
NH_4^+	Ammonium
NO_3^-	Nitrate
O	Oxygen
O/C	Elemental ratio of oxygen and carbon
O_2	Di-oxygen
PAH	Polycyclic aromatic hydrocarbons
PBR	Percentage of bacterial respiration
pF	Soil moisture tension
PFR	Percentage of fungal respiration
pH	potentia Hydrogenii
qCO2	CO_2 output per unit biomass
R_{eco}	Ecosystem respiration
SIR	Substrate induced respiration
SIRIN	Substrate induced respiration with inhibitors
SMB	Soil microbial biomass
SOC	Soil organic carbon
SOM	Soil organic matter
TOC	Total organic carbon
WHC	Water holding capacity
Wt%	Weight percentage

Summary

The discovery of Terra Preta soils and the identification of charcoal as an essential component therein initiated an ever-growing research field. Since then, many experiments on the production and application of carbonized plant material (biochar or hydrochar) to soil have been carried out. However, the integration of biochar/hydrochar into soil management systems requires comprehensive analyses to identify and evaluate possible risks and opportunities involved with biochar/hydrochar application. This thesis provides results on the influence of *Miscanthus* × *giganteus* feedstock, hydrochar and biochar application on the greenhouse gas balance, plant growth and nutrient status of an extensively managed grassland ecosystem in comparison with an unamended control. The results provide information on the recalcitrance of the carbon amendments against degradation under field conditions. The comprehensive field study was complemented by a greenhouse based plant growth experiment and additional incubation experiments. One of the incubation experiments focused especially on the degradation of the carbon amendments under simulated weather conditions fostering their break down and mineralization. The data gathered in the period of 2011-2014 show an increasingly recalcitrant behavior of the carbon amendments against degradation along their degree of carbonization. As a consequence, both N_2O and CO_2 losses from the hydrochar and biochar amended soil in the field and in incubation were significantly reduced as compared to the soils amended with uncarbonized feedstock material. Biochar even reduced the CO_2 emissions below the control treatment, leading to a significant soil organic carbon increase. The reduction in emissions could not be attributed to a decreasing abundance or activity of soil microorganisms; rather, the results suggested an interaction between biochar and soil- or plant-derived carbon compounds. Over a period of two years, none of the carbon amendments impaired plant growth or reduced the plant nutrient concentrations and nutrient removal. Biochar increased plant growth in a pot experiment in the greenhouse, while hydrochar induced short-term yield decreases in the field. The results suggest that especially and only biochar is suited for long term soil carbon sequestration without negatively affecting the soils' greenhouse gas balance or plant growth and plant nutrient concentrations.

Zusammenfassung

Die Entdeckung und Bestimmung von schwarzen, nährstoffreichen Terra Preta de Indio-Böden im Amazonasgebiet in den 80er Jahren, deren Fruchtbarkeit unter anderem auf Holzkohleanteile von bis zu 20 % zurückgeführt war der Auslöser für ein dynamisches Forschungsfeld. Dieses hat zum Ziel, den Einsatz von verkohltem Pflanzenmaterial als Bodenzusatz zu prüfen und zu bewerten. Die vorliegende Arbeit setzt an diesem Punkt an. Sie widmet sich vor allem den möglichen Risiken des Einsatzes verschiedener Biokohlen aus *Miscanthus* × *giganteus*-Ernteresten (hydrothermal karbonisiertes, halbverkohltes Material=hydrochar und pyrolysiertes, nahezu vollständig verkohltes Material=biochar) im Vergleich zu unverkohltem Ausgangsmaterial (Miscanthus-Stroh=feedstock) und einer Kontrolle ohne Kohlenstoff-Applikation. Dazu wurde im April 2011 ein Feldversuch in einem extensiv bewirtschafteten Grasland-Ökosystem angelegt. Zusätzlich durchgeführte Labor- und Gewächshausversuche ermöglichten zudem einen Einblick in das Abbauverhalten verschiedener Kohlen unter dem Einfluss forcierter Verwitterung z.B. anhand simulierter Wettereinflüsse. Die Ergebnisse der von 2011 bis 2014 erhobenen Daten zeigen eine eindeutige Erhöhung der Abbaustabilität von organischem Material mit dem Grad der Karbonisierung. So konnten die hohen CO_2- und Lachgasemissionen, die beim Abbau von unverkohltem, strohartigem Feedstock-Material im Freiland und im Labor zu beobachten waren, durch die Karbonisierung signifikant verringert werden. Vor allem die nahezu vollständige Karbonisierung (biochar) führte sowohl im Freiland als auch im Labor zu einer Verringerung der bodenbürtigen CO_2-Emissionen und zu einem signifikanten Kohlenstoff-Aufbau des Bodens, im Vergleich zur Kontrolle ohne Kohlenstoff-Applikation. Da der Kohlenstoff-Aufbau nicht mit einer Reduzierung des Bodenlebens erklärt werden konnte, erscheint eine Interaktion der biochar mit z.B. pflanzen- oder bodenbürtigen Kohlenstoffverbindungen wahrscheinlich. Keines der eingesetzten Materialien führte langfristig zu einer Verringerung des Pflanzenwachstums, der Pflanzengesundheit und der Qualität (Nährstoffgehalt) des Ernteguts. Biochar führte sogar zu einer Steigerung des Pflanzenwachstums in einem Kurzzeit-Topfexperiment, während nach Aufbringen von hydrochar mit geringem Inkohlungsgrad kurzfristige Ertragseinbußen im Feld zu beobachten waren. Die

Ergebnisse legen nahe, dass nur biochar langfristig Kohlenstoff im Boden festlegen kann, ohne das Pflanzenwachstum zu beeinträchtigen. Die Treibhausgasbilanz des Bodens konnte durch den Biokohleeinsatz sowohl in den Inkubationsexperimenten als auch im Feld verbessert werden.

1 Synopsis

1.1 Introduction

Carbonization or charring of plant material and subsequent soil amendment with the aim to improve soil fertility has been common agricultural practice in Europe and USA since the 19th century and is still applied in rural areas across India or Japan (Lehmann and Joseph, 2009; Ogawa and Okimori, 2010; Olarieta *et al.*, 2011). However, only the discovery of ancient Terra Preta soils led to renewed considerable scientific as well as public attention to the soil application of charcoal (Scheub, 2013; Chia *et al.*, 2014). Terra Preta, a tropical anthropogenic soil, owes its exceptional fertility *inter alia* to high amounts of charcoal (Glaser *et al.*, 2002). Likewise, the deliberate admixture of charred plant material to soil (biochar), aims at generating environmental benefits. Biochar is produced by heating dry biomass in an oxygen-limited atmosphere to temperatures of below 700 °C (Lehmann and Joseph, 2009). Creating biochar as a tool for climate change mitigation, sequestering carbon in soils, equally reducing greenhouse gas emissions from soils and/or improving soil fertility and plant growth is an attractive but not yet fully mastered challenge (Sohi *et al.*, 2010; Gurwick *et al.*, 2013; Lorenz and Lal, 2014; Mukherjee and Lal, 2014). The diversity of biochar production processes as well as the variety of suitable feedstock materials results in products that are not easy to classify. Efforts to provide a reliable biochar product have resulted in guidelines for the production of biochar and furthermore an US American (IBI certified biochar) and a European biochar Certificate (EBC). Both the guidelines for biochar production and the requirements for the Certificates are based on the scientific knowledge obtained so far (Montanarella and Lugato, 2013). Biochar therein is defined by its carbon (C) content, the elemental ratios of hydrogen:carbon and oxygen:carbon (H/C-O/C) and low heavy metal/polycyclic aromatic hydrocarbon/ polychlorinated biphenyl contents of the biochars, to guarantee ecotoxicological safety. These guidelines are voluntary and not legally binding. Biochar application to soil in Europe has to comply with EU and national legislation, mainly the fertilization act as well as the soil protection act. Up until now, only charcoal (=biochar from wooden feedstock) is allowed as soil amendment within the EU fertilization act, and a legislative initiative may

become necessary to include other feedstock for biochar production and soil application (Möller, 2014). To protect soils from the addition of potentially toxic substances such as polycyclic aromatic hydrocarbon compounds (PAH) or dioxins, all soil amendments have to comply with national soil protection acts such as the "Bundesbodenschutzgesetz" (BBodSchG) in Germany. The voluntary EBC and IBI Certificates meet these requirements by ensuring that a non-toxic biochar is (sustainably, EBC) produced, traded and used.

Legislation for horticulture and potted plants differs from agricultural legislation and might therefore offer other possibilities to use e.g. biochar substrates (biochar in mixture with other materials such as compost) or hydrochar, (material from wet pyrolysis, yielding a peat-like substrate) as potting substrate (Meinken, 2014). Hydrochar, a product from hydrothermal carbonization, became popular especially in Germany, with the rediscovery of a hydrothermal transformation process described and patented in the 19th century, converting biomass into a material similar to brown coal or lignite coal (Titirici *et al.*, 2007). Hydrothermal carbonization is suitable to process wet feedstock and thus offers possibilities to upcycle biomass waste with a high water content (biowaste, agricultural residues) to useful products in a very energy-efficient way (Schimmelpfennig and Glaser, 2012; Berge *et al.*, 2013; Yan *et al.*, 2014). In any case, prior to all usage, an understanding of the parameters shaping the end product of carbonization are essential for creation of the best possible biochar or hydrochar for a specific application as well as to prevent adverse effects. Besides the different production processes, feedstock and even more so temperature during the production process have been identified as the most influencing factors on biochar as well as hydrochar properties (Cao *et al.*, 2013; Angin and Sensoz, 2014; Sun *et al.*, 2014b; Yan *et al.*, 2014). A biochar/hydrochar product suitable for soil amendment, should comply with the earlier mentioned certificates and legislations. Also, high carbon yields, preferably produced with the highest possible energy efficiency, a high (aromatic) carbon content and low amount of toxic compounds are among the most desired features.

For biochar, micropore area, pH, the amount of aromatic carbon compounds and ash content generally increase with the pyrolysis temperature, with total surface area likely peaking at around 600 °C, slightly varying with the feedstock type (Schimmelpfennig and Glaser, 2012; Budai *et al.*, 2014; Rehrah *et al.*, 2014; Xiao *et al.*, 2014; Yan *et al.*, 2014).

Variations due to feedstock material are likely explained by the different carbonization behavior of the molecular compounds of the biomass, as e.g. polysaccharide components start to pyrolyze at 200-400 °C while lignin pyrolyzes at temperatures between 300 and 700°C (Cao *et al.*, 2014).

During hydrothermal carbonization, the process temperature was found to be of more influence on hydrochar properties than the feedstock. Highest carbon yields and amounts of aromatic compounds were found at temperatures around 230 °C (Gao *et al.*, 2013; Wiedner *et al.*, 2013).

Indicators such as elemental ratios (H/C_{org}, O/C) and volatile matter contents are suitable indicators for the processes dehydrogenation, decarboxylation and demethylation and thus, the carbonization grade of differently charred products (Schimmelpfennig and Glaser, 2012; Budai *et al.*, 2014). However, the same indicators did not correlate with properties such as cation exchange capacity or the surface area of charred products and thus, the prediction of the agronomic performance of charred products remains challenging (Budai *et al.*, 2014).

For heavy metal concentrations in both biochar and hydrochar, the feedstock was of major influence (Luo *et al.*, 2014). Heavy metals were found to be of major concern for biochar from some sewage sludge, depending on the region of collection, or for biochar from contaminated feedstock such as waste wood treated with preservatives or tannery waste (Chan and Xu, 2009; Jones and Quilliam, 2014; Song *et al.*, 2014; Van Wesenbeeck *et al.*, 2014). Hydrothermal carbonization of plant material and sewage sludge either reduced the heavy metal content in the hydrochars or lowered their bioavailability as compared to the feedstock material (Reza *et al.*, 2013; Zhang *et al.*, 2014a). Contrarily to being a source of heavy metals, biochar and hydrochar may also act as absorbents for heavy metals or other (soil) contaminants such as pesticide residues, depending on the presence of functional (carboxyl) groups on the biochar/hydrochar surface, their pH and polarity (Xue *et al.*, 2012; Houben *et al.*, 2013; Elaigwu *et al.*, 2014; Uchimiya, 2014). The environmental risk of polycyclic aromatic hydrocarbons (PAH) and dioxin contamination due to hydrochar amendment is low, since the low temperatures during production do not promote their formation (Wiedner *et al.*, 2013). Biochar produced from woody feedstock, especially by gasification technologies harbors the risk of tar volatilization and PAH generation during the heating phase and condensation during the cooling phase. Therefore, the heating and

cooling phases should be spatially separated in the reactor (Schimmelpfennig and Glaser, 2012; Wiedner *et al.*, 2013). In any case, the use of non-toxic feedstock is the first of all precautionary measures for the production of an environmentally safe hydrochar/biochar product (IBI, 2014; Luo *et al.*, 2014).

The agricultural benefits of biochar, such as a liming effect or improved water and nutrient retention are reportedly most effective in soils from subtropical or tropical regions. However, biochar could be favorable for temperate soils in terms of improving soil physical properties or fertility or simply could be "stored" by soil sequestration, increasing soil organic carbon (Downie *et al.*, 2011; Lorenz and Lal, 2014). Temperate soils are defined by the climate of temperate regions, with mean annual temperatures between -3°C and 18 °C (Kottek *et al.*, 2006). It is only recently, that several hydrochar/biochar field experiments have been installed in temperate regions, including temperate forest, agricultural soils and grassland (Karhu *et al.*, 2011; Hammond *et al.*, 2013; Domene *et al.*, 2014; Felber *et al.*, 2014; Schimmelpfennig *et al.*, 2014; Singh *et al.*, 2014; Sun *et al.*, 2014c).

Figure 1-1: Study site as viewed from above. The red rectangles highlight the four blocks of the biochar experiment, picture taken from google maps (https://www.google.de/maps; last access: 07.01.2015).

The Giessen Biochar Field Experiment is thereby among the first to elucidate the performance of differently carbonized material in comparison to uncarbonized feedstock

and a control (no carbon amendment) in undisturbed temperate grassland under slurry fertilization (Schimmelpfennig *et al.*, 2014).

1.2 General objectives and study site

The Giessen Biochar Field study was initiated in April 2011 at an experimental grassland site of the Justus-Liebig-University Giessen, located in Linden, Germany (50°32´N and 8°41.3´E, Figure 1-1) (Jäger *et al.*, 2003). The grassland site has been managed extensively for decades. In the area of the biochar plots, before the installation of the experiment, the grassland has been cut every time the height reached 10-15 cm and has not been fertilized since 1993.

In the experiment, the environmental performance of three different carbon amendments produced from *Miscanthus* × *giganteus* (feedstock, hydrochar and biochar) was compared to an unamended control (only grassland). The experiment was designed as a fully randomized fourfactorial block design, where four treatments (control/feedstock/hydrochar and biochar) were arranged randomly in each block. One block consisted of four 4x4 m sized plots, resulting in one treatment plot per block. The pure, uncharred *Miscanthus* chaff (feedstock) was included as a treatment to enable a direct comparison of the degradation and performance of the uncharred and the charred material (hydrochar and biochar, Figure 1-2 c) (Lehmann *et al.*, 2015). The *Miscanthus* chaff was provided by the agricultural centre Eichhof, Bad Hersfeld, Germany, where the field grown, senescent *Miscanthus* biomass had been harvested in winter 2009, when all aboveground plant material had receded. Hydrothermal carbonization of the chaff was carried out by the company Revatec, Geeste, Germany, at that time Hydrocarb GmbH, Ohmes, Germany. The feedstock was kept in a water vapor atmosphere for 2 hours at a temperature of 200 ± 3 °C under a pressure of 1.6 MPa and was air dried after the process. The biochar was produced by the company Pyreg GmbH, Bingen, Germany, using a pyrolysis unit fitted with a continuous flow reactor at temperatures of 550-600 °C and mean residence times of the material in the reactor of 15 min. All materials were ground to <10 mm before use in the field and laboratory experiments (SM 300, Retsch GmbH, Haan, Germany).

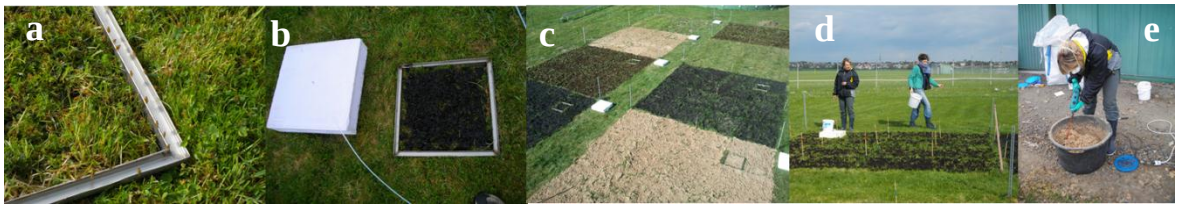

Figure 1-2: Initiation of the biochar experiment, showing the greenhouse gas measurement plots (one per plot, Figure 2a and b) the four treatment plots (control, feedstock, hydrochar and biochar) per block (Figure 2c), hydrochar amended plot with ongoing GHG measurement (Figure 2d) and the mixture of slurry with feedstock material (*Miscanthus* × *giganteus*, Figure 2e). Source: Christoph von Bredow, 2011, with permission.

The carbon amendments were applied on the basis of their C content, resulting in application amounts of 16 t ha^{-1} feedstock, 14.5 t ha^{-1} hydrochar and 9.3 t ha^{-1} biochar. With the deliberate choice of *Miscanthus* × *giganteus*, a C4 plant naturally enriched in ^{13}C, it was intended to ensure that differentiation of the specific carbon sources (soil/carbon amendment) in the soil and soil derived CO_2 is possible. To prevent substrate loss by wind erosion during application, as seen in other field studies (Major and Husk, 2010), and to promote positive side effects by potential binding of slurry nutrients to the carbon amendments, they were mixed with liquid pig slurry before application (Figure 1-2 e). Pig slurry was subsequently applied regularly in spring and autumn of each year, as to simulate common agricultural fertilization practice. Two supplemental incubation studies and an appending growth experiment in the greenhouse were carried out in parallel to the field study. These additional studies were conducted to test the performance of the carbon amendments under controlled environmental conditions in different soils (Chapter 2 and 5).

With this work, we aimed at elucidating the role of hydrochar and biochar in a semi natural grassland ecosystem with the focus on its degradation, effects on soil properties, plant growth and nutrient status as well as the ecosystem´s greenhouse gas (GHG) balance. It was hypothesized that the carbon amendments would degrade in the sequence of their carbonization grade: feedstock > hydrochar > biochar and that degradation would be measurable in the ecosystem respiration (CO_2 emissions, Chapter 2) (Qayyum *et al.*, 2012). Moreover, the hypothesis was tested if feedstock and hydrochar would lead to nitrogen immobilization and hence limitation, and therefore reduce plant growth (Gajić and Koch, 2012). Furthermore it was assumed that biochar would rather have positive effects on the plant nutrient status, reversibly binding nutrients from slurry on the biochar surface, thereby preventing nutrient loss via leaching or degassing (Chapter 3) (Taghizadeh-Toosi

et al., 2011a). With regard to the effect of carbon amendments on soil microorganisms, it was postulated that biochar would increase the abundance of both fungi and bacteria by providing a suitable habitat due to its porous nature (Thies and Rillig, 2009), that hydrochar would promote fungal growth due to its low pH (Abel *et al.*, 2013) and that both feedstock and hydrochar would support the growth of all microorganisms due to provision of a rather labile C-source (Chapter 4 and 5) (Bamminger *et al.*, 2014a). Additionally, the assumption that the degradation of carbon amendments and the GHG emissions following soil application could be related to their structural properties was investigated (Chapter 5) (Schimmelpfennig and Glaser, 2012; Budai *et al.*, 2014; Schimmelpfennig *et al.*, 2014).
The results obtained from both the field and the incubation studies are presented in context of the available literature. The methods of analysis are provided in the single Chapters.

1.3 Effects of biochar/hydrochar on soil properties

1.1.1 Soil chemistry

Soil properties have considerable influence on the GHG balance of an ecosystem and microbial abundance and activity in soils. They are therefore important background parameters and indicators for changes. Intrinsic nutrient contents as well as the pH and the liming potential of biochar/hydrochar materials are among the most dominant chemical properties influencing the properties of the amended soil. Biochar is mostly alkaline, especially if produced at temperatures >350 °C, whereas hydrochar mostly exhibits acidic pH values, as a consequence of decarboxylation and dehydration during the production process (He *et al*., 2013; Parshetti *et al.*, 2013; Novak *et al.*, 2014). Biochar often led to a significant increase of the soil´s pH, both in field and laboratory, or greenhouse based studies. Most distinct changes were found for biochar application rates > 30t ha^{-1}, likely depending on the buffer capacity of the soil used (Kloss *et al.*, 2014a; Kloss *et al.*, 2014b; Rogovska *et al.*, 2014; Soinne *et al.*, 2014). However, also the management of the site of biochar application or the experimental setup in the laboratory matters, as pH changes are most apparent if the biochar is mixed into soil (Rogovska *et al.*, 2014; Schimmelpfennig *et al.*, 2014). Concomitantly, in the Giessen Biochar field experiment, no pH changes have been observed during the three years following the top-dressing of biochar and hydrochar

onto the grassland surface (Chapter 2). In the upper 2 cm however, there was a strong tendency ($p < 0.055$) towards a higher pH in the biochar plots, assumedly triggering short-lived, high CH_4 and NH_3 emission peaks directly after slurry amendment (see Sections 1.5.3 and 1.5.4). In the incubation experiments, where the carbon amendments were mixed into the soil, biochar did initially increase the soil pH; however the changes leveled out through the experimental period of one year (Chapter 5).

1.3.1 Soil physics

Physical soil parameters such as bulk density and water holding capacity (field capacity), pore volume and pore distribution are key factors to soil fertility and plant growth and can be altered significantly by biochar and hydrochar amendment (Mukherjee and Lal, 2013). Biochar's usually large surface area can lead to an increase in total soil-surface area with positive effects on soil water- and nutrient-retention (Laird *et al.*, 2010b; Manyà, 2012). Especially pores in the size of >0.2 µm on the inner biochar surface may host and retain plant available water and therefore increase the field capacity of the amended soil, resulting in a higher resilience of a soil against drought (Kammann and Graber, 2015). Indeed, biochar has been found to improve water retention in sandy soil while improving drainage of clayey soil, by water movement through biochar pores or creation of interstitial biochar-soil space (Barnes *et al.*, 2014). However, blocking of pores by particulate soil organic matter may sometimes overlay these benefits (Prost *et al.*, 2013). Reductions in the bulk density of soil following biochar amendment have been shown to be most distinctive in loamy or clayey soil, prone to soil compaction (Laird *et al.*, 2010b), with positive effects on the resilience of vineyards to drought (Baronti *et al.*, 2014). In the Giessen biochar field study, bulk density, water holding capacity and soil aggregate formation two years from biochar/hydrochar application remained unchanged most likely because integration into the soil matrix was low due to top-dressing (Figure 1-3)(Rex *et al.* (2015) Chapter 4, Schimmelpfennig *et al.*, in preparation).

Figure 1-3: Soil core from a biochar plot. Personal photograph by author. October 2012. The black particles show the biochar incorporation into the root zone of the grassland vegetation, as highlighted by the arrow

The surface area of hydrochar is with 8-25 m^{-2} g^{-1} generally rather low (Schimmelpfennig and Glaser, 2012; Kalderis *et al.*, 2014). Nonetheless, Kammann *et al.* (2012) reported increases in soil water holding capacity following hydrochar soil amendment, compared to an unamended control. This can likely be ascribed to the presence of small-sized pores in the hydrochar mainly in the size of <10 µm (Abel *et al.*, 2013; Conte *et al.*, 2014). Approvingly, a decreased bulk density and an increased available water capacity (the amount of water held by a soil between field capacity, pF 1.8 and the permanent wilting point, pF 4.2) has been reported for hydrochar amended sandy soil low in organic matter, both in incubation and field studies with best results for hydrochar application amounts of 2.5 wt%. Furthermore, hydrochar improved the water repellency of the same soil indirectly via the colonization with fungi, (Abel *et al.*, 2013). In the Giessen incubation experiments, all carbon amendments (feedstock, two hydrochars and biochar) increased the water holding capacity of both a sandy and a loamy soil. These results are promising; however, they still have to be confirmed by the ongoing Giessen field experiment in the future.

1.1.2 Soil biology

Soil provides a habitat for millions of microorganisms, mostly in the upper soil horizons. Biochar and hydrochar application can affect the habitat conditions for microorganisms directly by provision of living space inside the biochar pores or indirectly by altering the pH, supply of nutrients or inhibitory effects (Thies and Rillig, 2009). The majority of biochar pores were found to be in the macropore range, with a pore diameter ranging from 0.1-1000 µm, which includes pore sizes hosting plant available water (> 0.2 µm) as well as fungal hyphae or bacteria (2-10 µm, Figure 1-4) (Warnock *et al.*, 2007; Brewer *et al.*, 2014; Kammann and Graber, 2015).

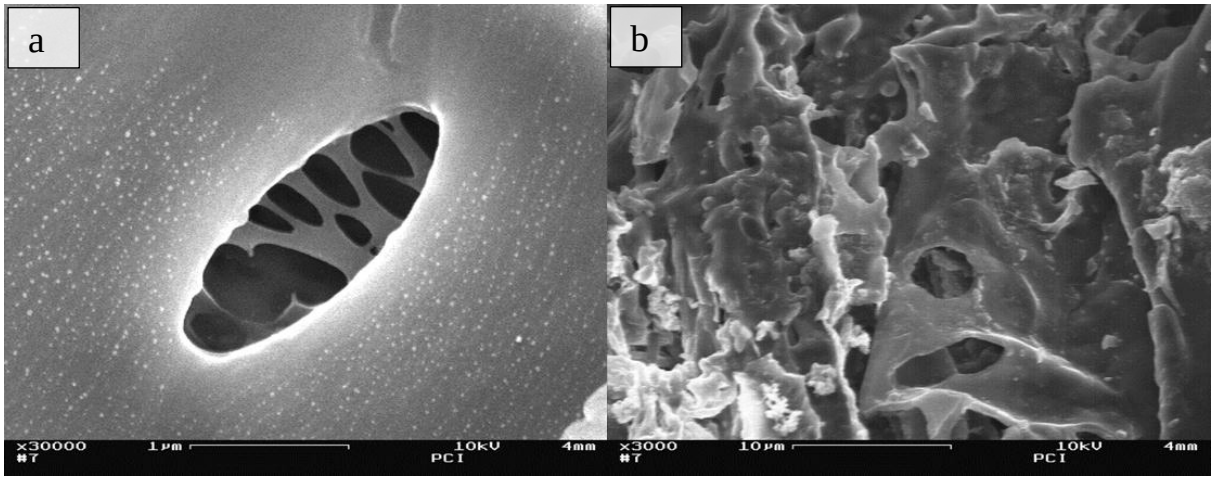

Figure 1-4: Inner surface of a biochar (left) and hydrochar (right) particle, potential habitats for soil microorganisms. Scale is indicated by bars (Figure 4a: 1 µm, Figure 4b: 10 µm). Scanning electron microscopic pictures taken by author. December 2011.

The promotion of habitat space for microorganisms by biochar has been reported by several authors, with deliberate inoculation in the laboratory, or incubation in the mix with microbial active soil or compost resulting in quicker colonization of the biochar pores compared to field studies (Pietikäinen *et al.*, 2000; Quilliam *et al.*, 2013; Hammer *et al.*, 2014; Jaafar *et al.*, 2014). Even after three years in the field, the internal and external surfaces of biochar particles were only little colonized by microorganisms (Quilliam *et al.*, 2013). The authors assume that sorption of nutrients to the biochar surface limited their bioavailability and thus impaired the growth of microorganisms.

The effects of biochar on arbuscular mycorrhizal fungi (AMF) also differ depending on experimental duration and integration of biochar into the soil matrix. In the short term,

biochar reportedly led to lower or equal AMF abundance in soil, whereas in the long term, AMF colonization of the soil increased in the presence of biochar, compared to unamended control soil (Warnock *et al.*, 2007; Warnock *et al.*, 2010).

Regardless of the provision of habitat space, biochar amendment to soil often led to short-term increases of microbial activity, sometimes accompanied by a microbial community shift (Bamminger *et al.*, 2014b; Watzinger *et al.*, 2014). Labile carbon compounds on the biochar surface remaining from the production process, may promote microorganisms adapted to degrade such biochar components, especially gram positive bacteria (Ameloot *et al.*, 2013b; Bamminger *et al.*, 2014b). In the long term, biochar is assumed to be recalcitrant against microbial decay; however experimental proof is still scarce and mostly based on the extrapolation of results from incubation studies (Kuzyakov *et al.*, 2009; Kuzyakov *et al.*, 2014; Zhang *et al.*, 2014c). Long-term shifts in the microbial community composition may not be similar to short term changes following biochar admixture to soil.

In the Giessen biochar field study, 1.5 years from initiation, biochar treatments exhibited higher total microbial biomass, with the promotion of fungi being the most prominent difference (Rex *et al.* (2015), Chapter 4). Also Zhang *et al.* (2014c) reported a significantly increased soil microbial biomass from a four-year-old field study where biochar had been applied to a coarse-textured, agriculturally managed soil. This result was corroborated by Domene *et al.* (2014), who also observed a biochar-induced increase of soil microbial biomass after 4 years from application, most likely by indirect effects such as increased soil moisture and pH. However, in contrast to the Giessen field trial, a change in microbial community composition had not been observed in their studies.

Hydrochar is likely not as recalcitrant to microbial degradation, due to its structure and chemical composition (Schimmelpfennig and Glaser, 2012; Eibisch *et al.*, 2013). Approvingly, hydrochar is composed of mostly labile carbon compounds, readily available for assimilation by microorganisms, especially if nitrogen is not a limiting factor (Gajić and Koch, 2012; Bargmann *et al.*, 2014a). Hydrochar led to a microbial community shift in the Giessen field study 1.5 years after initiation, with higher relative fractions of both fungi and bacteria compared to the control treatment, whereas the total microbial biomass remained unchanged. This points to the development of a specific microbial community, adapted to the degradation of hydrochar (Rex *et al.* (2015), Chapter 4). In the incubation study, the same hydrochar in the same soil increased the growth of all microorganism

groups and especially fungi, following the addition of glucose, highlighting the bioavailability of hydrochar material. Also Steinbeiss *et al.* (2009) report a promotion of different microbial groups by different hydrochars. In their study, yeast-derived hydrochar promoted fungi, while glucose-derived hydrochar was mostly metabolized by bacteria. Other studies report positive effects of hydrochar on growth, root colonization and spore germination of mycorrhizal fungi (Rillig *et al.*, 2010; Salem *et al.*, 2013a) as well as on the activity and abundance of total SMB (Bargmann *et al.*, 2014a). Nonetheless, also detrimental effects of hydrochar on mycorrhiza have been reported. These effects have been found to relate with the occurrence of toxic compounds, mostly present in the water soluble carbon fraction of hydrochar (George *et al.*, 2012). Contrarily, collembola ingested and digested unwashed hydrochar without being negatively affected, likely even gaining nutritional benefits from it (Salem *et al.*, 2013b).

Neither hydrochar nor biochar application induced any adverse effects on the soil microbial biomass in both the Giessen incubation and field studies. However, while an increased microbial biomass was accompanied by a higher degradation of the hydrochar material in the unvegetated incubation study (Chapter 5), a higher microbial biomass in the biochar treatments did not involve biochar degradation. Contrariwise, the biochar plots emitted less CO_2, as compared to the control in the field trial (see Section 1.5.1), while the metabolic efficiency of the microorganisms was unaffected and similar to the control. This points to interactions e.g. sorption of plant root exudates or labile soil organic carbon, that need further investigation. Taken together, biochar increased SMB, especially fungi, in the field, while we observed no changes in the incubation experiment. Hydrochar led to a community shift in the field and increased total SMB during incubation with the same soil, following the addition of glucose.

1.4 Effects of biochar/hydrochar on plant growth and nutrient concentrations

Two recent meta-analyses revealed an overall 10-12 % increase in plant and crop productivity due to biochar addition to soil with the most positive results for soils with a coarse texture and neutral to acidic pH (Jeffery *et al.*, 2011; Biederman and Harpole, 2013). This is consistent with other findings, indicating that biochar has the most positive

effects on plant yield in soils exhibiting a low cation exchange capacity, low carbon content and low pH (Crane-Droesch *et al.*, 2013). However, most results are based on studies conducted in the greenhouse. Results on the effects of biochar and even more so hydrochar on plant growth and nutrient status in temperate soils, especially from field experiments, are still scarce.

Within the Giessen field trial on fertilized grassland, biochar had no effect on biomass yield, supposedly because of the "slow" downward migration and bioturbation of the top-dressed biochar (Chapter 2, Schimmelpfennig *et al.* (2014)). Concomitantly, in a greenhouse based pot experiment, biochar increased the biomass of *Lolium perenne* plants significantly when it was mixed into the soil and pre-incubated with pig slurry before sowing (Chapter 2). Slow bioturbation, especially in extensively managed soils, may delay some interactions of biochar and the plant-soil matrix, such as the bioavailability of nutrients contained in or sorbed to the biochar (Rajkovich *et al.*, 2012; Gueerena *et al.*, 2013). Other authors reported that specifically maize did not respond to biochar application on intrinsically fertile temperate soil after one year and during four years from biochar application with and without fertilization (Jones *et al.*, 2012; Gueerena *et al.*, 2013; Borchard *et al.*, 2014a). The authors assume that maize as a deep rooting crop did not benefit from e.g. improved water retention or decreased bulk density in the upper, biochar amended soil layer. Conversely, biochar increased the dry matter and N-content of grass linearly with a biochar application rate of up to 50 t ha^{-1} in a field study on sandy clay loam soil two and three years from application (Jones et al., 2012). The authors ascribe this effect to sorption of residual herbicides in the soil onto the biochar surface, as hydrological explanations seemed unlikely due to the soils´ good intrinsic water holding capacity. Concomitantly, sorption of allelochemical compounds of plant residues onto biochar likely improved grain yield of maize in a field study on Mollisol (Rogovska *et al.*, 2014). Furthermore, biochar, especially if produced from *Miscanthus* or corn digestate at high temperatures (750 °C), decreased the bioavailability of isoproturon (an herbicide) in Cambisol. This effect was likely caused by physical adsorption of the herbicide within the nanopores of the biochars (Eibisch *et al.*, 2015).

On a Mediterranean silty loam soil, biochar increased the yield of durum wheat during two consecutive years after biochar application both with application rates of 30 and 60 t ha^{-1}, likely due to increasing the pH of the slightly acidic soil and soil temperature during the

seed germination period of the experiment (Vaccari *et al.*, 2011). A meta-study of biochar field application across the UK with different soils and crops revealed diverse results with mostly no effects on crop yield compared to unamended controls, however also very large significant yield increases (+116 %, Spring barley) and yield deficits (- 8.23 %, Spinach) have been reported (Hammond *et al.*, 2013). Biochar had positive effects on the available soil water content in a Mediterranean vineyard field experiment, reducing the drought stress of Vitis vinifera in the whole experimental period of two years. However, biochar had mostly neutral effects on vine growth and health as well as grape quality in a vineyard field experiment in Switzerland over an experimental period of three years (Schmidt *et al.*, 2014). During early stages of plant growth, especially during germination, biochar application also had negative effects, partially inhibiting germination, particularly if the seeds were exposed to the pure material (Borchard *et al.*, 2014a; Buss and Masek, 2014). Experiments with contaminated biochar revealed that re-condensated vapors from a miscarried pyrolysis or from wood gasification (likely phenolic compounds) may be the underlying cause of plant growth or germination reductions following biochar exposure, highlighting the importance for a controlled, clean pyrolysis process for biochar production (Buss and Masek, 2014). More indirect biochar induced effects on plant growth include changes in the soils' enzyme activity, build-up of soil organic N (improving fertilizer efficiency), provision of nutrients contained in the biochar or positive effects on soil biology (Rajkovich *et al.*, 2012; Du *et al.*, 2014; Prommer *et al.*, 2014).

While short- and long-term negative impacts of biochar on plants are rather the exception than the rule, for hydrochar, most of the available studies report negative (short term) results on seedling germination and plant growth both in incubation studies and in the field (Busch *et al.*, 2012; Gajić and Koch, 2012; Busch *et al.*, 2013; Jandl *et al.*, 2013; Bargmann *et al.*, 2014b; Wagner and Kaupenjohann, 2014). Toxic compounds from the production process, of which some can be volatile, are more likely to remain on the hydrochar compared to biochar, where volatiles mostly evaporate. Such components were found to be responsible for germination reductions following seedling exposure to hydrochar (Bargmann *et al.*, 2013; Jandl *et al.*, 2013). However, the carbonization conditions, especially the process duration and the feedstock may determine the degree of the toxicity, as not all hydrochars exhibited negative effects on germination (Jandl *et al.*, 2013; Sun *et al.*, 2014b). In the field and in pot experiments, plant growth reductions

following hydrochar amendment were ascribed to N-limitation of the plants due to microbial or physical N-immobilization (Gajić and Koch, 2012; Bargmann *et al.*, 2014c; Borchard *et al.*, 2014a). However, apparently some of the negative effects are transient, as was revealed by the Giessen field study with a temporary decline of biomass in hydrochar amended plots, leading to a significant yield loss two months after application but to a "normal" yield again after an additional three months, compared to the control. Additionally, hydrochar that was pre-incubated with soil three months before its use as a growth medium did not trigger adverse effects on plant growth (Chapter 2, Schimmelpfennig *et al.* (2014)). This observation has been confirmed by Bargmann *et al.* (2013), reporting that storing and/or washing of hydrochar alleviated the negative impacts on plant germination and growth.

Apart from effects on plant growth, biochar triggered shifts in the plant community composition in the Giessen field trial, where the growth of forbs was favored over grasses (Chapter 3, (Schimmelpfennig *et al.*, 2015a)). Biochar induced plant community shifts have been found by others, where legumes were favored over grasses, especially in N-limited environments (Oram *et al.*, 2014; van de Voorde *et al.*, 2014). Moreover, changes in plant nutrient concentrations following biochar but also hydrochar application to temperate soil have been observed in the Giessen field trial. The plant biomass contained significantly more potassium, likely induced by the release of potassium contained in the biochar and hydrochar or sorption of potassium from the soil solution or slurry onto the biochar or hydrochar surfaces (Chapter 3) (Schimmelpfennig *et al.*, 2014). Higher potassium concentrations in the soil (solution) following biochar soil application were also reported from another field study and a greenhouse based study, however effects on plant potassium concentration were not significant there (van de Voorde *et al.*, 2014; Wagner and Kaupenjohann, 2014).

1.5 Effects of biochar/hydrochar on greenhouse gas emissions

Soils, especially agriculturally managed, fertilized soils as well as grazing land and associated livestock breeding contribute significantly to the global GHG emissions (IPCC, 2013). An increase of GHG emissions following biochar/hydrochar amendment to soil is neither desirable with regard to climate change nor would it fit the concept of carbon

capture and long term soil sequestration by biochar/hydrochar application. On the contrary, strategies and tools to decrease GHG emissions from soils by e.g. improving the fertilizer efficiency or increasing the soils´ methane oxidation potential are clearly necessary and biochar might be promising especially in regions with livestock production (Jones *et al.*, 2014; Van Zwieten *et al.*, 2015).

From previous studies, it can be assumed that biochar/hydrochar amendments have the potential to alter the GHG budget of soil by interactions with both the carbon and the nitrogen cycle or by changing the physical, chemical or biological parameters of soils (Lehmann, 2007; Clough and Condron, 2010; Van Zwieten *et al.*, 2015). Furthermore, there is some evidence that biochar and to a certain extent also hydrochar can function as a stable carbon pool in the soil, while simultaneously improving soil functions (see above), affecting both biotic and abiotic mediated GHG emissions. However, there are certain trade-offs for both sides, with a highly recalcitrant biochar exhibiting a low mineralization rate and few nutrient sorption sites and vice versa (Chan and Xu, 2009; Hale *et al.*, 2011; Song and Guo, 2012; Spokas, 2013). Hydrochar has been reported to be less effective in both long term carbon stability and/or sorption of plant available nutrients; however it may lead to a short- to medium term increase of soil fertility, with diverse effects on GHG emissions (Kammann *et al.*, 2012; Bargmann *et al.*, 2014a).

1.5.1 CO_2 emissions

Carbon dioxide emissions from soils with and without carbon amendment can be both of abiotic (chemical decomposition of carbonates) or biotic origin, such as microbial respiration. If the soil is vegetated, plant root respiration as well as root associated mycorrhizal respiration additionally contribute to the soil CO_2 flux. Together, these components can be summed under the term ecosystem respiration. Gas measurements in the field were made using static dark chambers to exclude CO_2 depletion by plant assimilation. In the incubations, where the soil was unvegetated, the measurements allowed only for the acquisition of microbial respiration.

Degradation of carbonates contained in biochar was found to lead to short term increase in CO_2 emissions right after application (Jones *et al.*, 2011; Bruun *et al.*, 2014). In the Giessen biochar field and laboratory studies, abiotic factors played a minor role, since both

the carbonate content of the soil and the carbon amendments were negligible (Schimmelpfennig *et al.*, 2014). However, an immediate peak in CO_2 emissions following slurry application in the unvegetated incubation experiment and to a lesser extent in the field suggests that slurry-carbonates dissolved during and after application, however with little treatment effects. Both in the field and in the incubations, most distinct before any fertilization, biochar amendment led to decreased CO_2 emissions as compared to the control soils (Chapter 2 and 5) (Schimmelpfennig *et al.*, 2014; Rex *et al.*, 2015). This likely indicates a negative priming effect of biochar on soil or plant root derived carbon. Biochar-induced negative priming of SOC, leading to lower CO_2 emissions compared to unamended control soil was confirmed by other authors, using isotopically labeled biochar or soil organic matter (Cross and Sohi, 2011; Jones *et al.*, 2011). Sorption of extracellular enzymes, SOC or NH_4^+ on the biochar surface, reducing microbial mineralization and (pH induced) shifts in microbial metabolism assumedly trigger negative priming effects but evidence is still scarce (Ding *et al.*, 2010; Jones *et al.*, 2011; Borchard *et al.*, 2012). Moreover, the interactions of soil, biochar and plant roots are not fully understood. Ventura *et al.* (2014) report an increased mineralization of biochar-C in the presence of plant roots, indicating co-metabolism induced by plant root exudates. On the other hand, Whitman *et al.* (2014) observed that in the presence of biochar, the priming effect of root exudates on SOC mineralization was reduced significantly. In the data obtained in the Giessen field and incubation studies, the "negative priming" effect (lower CO_2 emissions from biochar amended plots compared to unamended control soil) was more distinct in the vegetated field but still significant in the non-vegetated incubation, especially in sandy soil (Chapters 2 and 5). These results are in agreement with the observations of Whitman *et al.* (2014).

Contrarily to biochar, hydrochar had a neutral effect on the ecosystem respiration in the Giessen field trial (top-dressed). However, it led to higher CO_2 emissions in all incubation studies, compared to the control, where it was mixed into the soil (Schimmelpfennig *et al.*, 2014). Approvingly, hydrochar application increased CO_2 emissions from a plowed field experiment and in incubation in the mix with arable soil (Kammann *et al.*, 2012; Eibisch *et al.*, 2013; Bamminger *et al.*, 2014a; Bargmann *et al.*, 2014c). The additional CO_2 emissions originated both from hydrochar mineralization as well as from additional SOC degradation as a result of positive priming. In hydrochar amended arable soil, Bamminger *et al.* (2014a) found increased activities of enzymes that are involved in the N-cycle. This

effect could imply that microorganisms react with additional enzymatic activity to bridge the N-immobilization involved in hydrochar mineralization (Gajić and Koch, 2012). Such a reaction could explain the overall increased microbial activity and additional CO_2 emissions from hydrochar amended soils, found in the Giessen incubation experiments and elsewhere (Bamminger *et al.*, 2014a). Taken together, biochar generally improved the CO_2 balance of the soils used in the Giessen experiments, while hydrochar had neutral effects or led to increased emissions.

1.5.2 N_2O emissions

A meta-analysis obtained from all available studies published between 2007-2014 concerning N_2O emissions from biochar amended soils revealed overall N_2O emission reductions ranging from 10 to 90 % (Cayuela *et al.*, 2014). However, even if the overall effect is an emission reduction, also increased N_2O emissions following biochar amendment were reported, e.g. from biochar enriched with compost high in N or from biochar-soil mixtures subsequent to N-fertilization (Spokas *et al.*, 2009; Kammann *et al.*, 2012). Proposed reasons for the (predominant) reduction of N_2O emissions from soils mixed with biochar are improved aeration (Yanai *et al.*, 2007; Van Zwieten *et al.*, 2010b), adsorption of NO_3^-, NH_3 or NH_4^+ (Singh *et al.*, 2010; Van Zwieten *et al.*, 2010b; Taghizadeh-Toosi *et al.*, 2011b) or an increase of the soil pH (Wang *et al.*, 2012), all of which were disproved as single causes by others (Bruun *et al.*, 2011; Case *et al.*, 2012). Furthermore, also immobilization of nitrogen (Case *et al.*, 2012), inhibition of one of the steps during nitrification or denitrification (Spokas *et al.*, 2009; Van Zwieten *et al.*, 2009; Clough *et al.*, 2010) or an increase in the gene expression and hence abundance of the enzyme N_2O reductase (Harter *et al.*, 2014) were identified as potential causes. Moreover, the function of biochar as electron shuttle (Cayuela *et al.*, 2013) may have contributed to N_2O emission reductions.

Van Zwieten *et al.* (2015) identified the biochar feedstock as important influencing factor on N_2O emissions from the biochar-soil system, with woody feedstock and feedstock from crop residues decreasing emissions, while biochars from other feedstock (manure, biosolids, processing wastes, paper mill residues) lacked any significant effect. Looking specifically on N_2O emissions from biochar amended temperate soils, the majority of the

studies reported a decrease of N_2O emissions from biochar amended soil compared to unamended control soil, with most distinct reduction effects from high temperature biochars and subsequent N-fertilization (Zheng *et al.*, 2012; Ameloot *et al.*, 2013a; Malghani *et al.*, 2013; Stewart *et al.*, 2013; Bamminger *et al.*, 2014b; Felber *et al.*, 2014; Harter *et al.*, 2014; Nelissen *et al.*, 2014; Sun *et al.*, 2014a). However, biochar had mostly no effects on N_2O emissions if top-dressed or following fertilization with sewage sludge (Diaz-Rojas *et al.*, 2014; Schimmelpfennig *et al.*, 2014). Only two studies reported higher N_2O emissions, which were accompanied by increased crop biomass, indicating synergistic effects (Saarnio *et al.*, 2013; Verhoeven and Six, 2014). Recently, Van Zwieten *et al.* (2015) provided a comprehensive update on the current understanding of underlying mechanisms of decreased N_2O emissions from biochar amended soil. Evidently, biochar may also catalyze chemo-denitrification, providing iron or tin which are concentrated in the biochar during the production process, participate in redox reactions or change the bioavailable C supply in soil with consequences for N_2O emissions.

Some incubation studies showed that biochar alleviated the effect of weather events with a strong environmental impact (N_2O emissions outbursts) such as frost-thaw cycles or strong precipitation, leading to water logging (Kettunen and Saarnio, 2013; Schimmelpfennig *et al.*, 2015b) (Chapter 5). These findings are supported by the Giessen field study, where N_2O emissions tended to be reduced during a freeze-thaw period (Schimmelpfennig *et al.*, 2014) (Chapter 2).

Hydrochar amendment had no significant effect on N_2O emissions in the combined incubation and field study in Giessen, even after frequent additions of liquid pig slurry (Chapter 2, Schimmelpfennig *et al.* (2014)). However, in the longer-term incubation study with several degradation-promoting experimental conditions including frequent N-fertilization, two hydrochars from *Miscanthus* × *giganteus* (a weekly and a strongly carbonized one) led to significantly higher N_2O emissions from both a Cambisol (clayey sand) and a Haplic Stagnosol (clayey loam) (Chapter 5). Further studies dealing with the effects of hydrochar on N_2O emissions from the soil report diverse results. Nitrous oxide emission increases have been reported as well by Kammann *et al*. (2012), following (mineral) fertilization to bark-or beet- hydrochar (produced at 203 °C) amended soil in an incubation experiment with Luvisol (loamy silt). Contrastingly, Malghani *et al.* (2013) reported decreased N_2O emissions following the application of hydrochar produced from

corn silage to spruce forest Cambisol (sandy loam) and agricultural soil (silty loam) receiving no fertilization. However, increased N_2O emissions compared to unamended control soil were reported from deciduous forest soil (Cambisol, silty clay) in the same experiment. This increase was ascribed to higher water rich microsites in the silty clay soil. Dicke *et al.* (2014) also reported decreased N_2O emissions from hydrochar (produced from wheat straw or digested wheat straw) amended sandy topsoil (Cambisol, silty sand), before and after N-fertilization, but did not find any treatment effects in the subsoil. The authors ascribe the observed emission reduction to the relatively high HTC production temperature of 230°C, resulting in HTC material with a high sorption potential for N compounds. Moreover, improved soil aeration by hydrochar could also have caused the observed N_2O emission reductions compared to the unamended control soil, since the WHC had not been adjusted (Dicke *et al.*, 2014). These results suggest that the mechanisms involved in N_2O emissions following hydrochar application to soil are still unclear. Nonetheless, it seems likely that fine textured soil is prone to higher N_2O emissions compared to coarse textured soil. Lastly, the feedstock and production temperature of the hydrochar may also determine its degradability and sorption capacity Generally, N_2O emission reductions might be temporary, suggesting that the experimental duration might also be an important influencing factor to the evaluation of N_2O emissions. Both hydrochar and biochar amendment led to lower N_2O emissions compared to feedstock amended soil. However, compared to the unamended control soil, hydrochar amendment had either neutral effects and led in some cases also to higher N_2O emissions, depending on the experimental conditions. Contrastinigly, biochar reduced the N_2O emissions even below control soil emission levels. The results obtained in the Giessen field and incubation studies thus agree with the general trend of N_2O emission reductions following biochar amendment to soil.

1.5.3 Methane fluxes

Biochar may be an interesting management option for agricultural systems prone to high methane emissions such as paddy fields, and much research has been carried out accordingly in the last years (Zhang *et al.*, 2010; Liu *et al.*, 2011; Xie *et al.*, 2013; Singla and Inubushi, 2014). In paddy soils, biochar was found to decrease methane emissions by reducing the abundance of methanogenic archaea (Dong *et al.*, 2013) or improvement of

habitat conditions for methanotrophic bacteria. The habitat conditions can be improved by an increase of the soil pH (Shen *et al.*, 2014) or soil aeration (Kammann *et al.*, 2012), the retention of CH_4 in biochar pores (Feng *et al.*, 2012) or provision of potassium, stimulating the methanotrophic bacterial populations (Barbosa de Sousa *et al.*, 2014). However, also increased CH_4 emissions from biochar amended paddy soils were reported. The increase in CH_4 emissions is possibly caused by carbon compounds available to microorganisms and interactions with slurry during fertilization (Zhang *et al.*, 2010). Fewer studies investigated the effects of biochar on methane fluxes from temperate soils and the results are diverse, strongly depending on the experimental design, e.g. the soil type, soil water status and soil management. However, the majority of the studies in temperate climates report an increase of the methane oxidation, i.e. methane uptake into the biochar amended soil. Biochar e.g. induced an increase in the gene abundance of methanotrophic bacteria in landfill cover soil (Reddy *et al.*, 2014). Also, sorption of organic compounds to the biochar surface, providing substrates for methanotrophs (Borchard *et al.*, 2014b) reportedly improved methane oxidation. Furthermore, biochar was found to mitigate soil moisture fluctuations that are generally associated with high CH_4 emissions (Castro *et al.*, 1994; Spokas and Bogner, 2011). Moreover, also the adsorption of fertilizer-borne ammonium onto the biochar surface could prevent the inhibition of methane oxidation by NH_4^+ especially in fertilized soils (Karhu *et al.*, 2011).

Soils with a low background methane oxidation potential i.e. methanotrophic activity responded little to biochar amendment (Van Zwieten *et al.*, 2015). Correspondingly, all carbon amendments improved the methane oxidation capacity of the grassland soil used in the Giessen field trial and in the incubations; the grassland soil exhibited an intrinsically high CH_4 oxidation potential, which is typical for semi-natural grassland. Accordingly, in the incubation using an agriculturally managed sandy soil with a negligible methane oxidation potential, none of the carbon amendments induced any methane flux changes in the soil (Schimmelpfennig *et al.*, 2014; Schimmelpfennig *et al.*, 2015b) (Chapter 2 and 5). This interaction was confirmed by other authors, although fertilization can mask the sole influence of the carbon amendments (Zheng *et al.*, 2012; Angst *et al.*, 2014; Diaz-Rojas *et al.*, 2014). Correspondingly, fertilization with pig slurry led to higher short-term methane emission outbursts from biochar amended soil in the Giessen biochar field experiment as well as in the incubation study compared to all other treatments and the control. These

emission outbursts may derive from the slurry itself and could relate to the slurry infiltration rate or redox reactions with the biochar surface (Chapter 2) (Chadwick and Pain, 1997; Schimmelpfennig *et al.*, 2014).

Hydrochar amendment increased the methane oxidation capacity of soils in all the Giessen incubation studies, possibly caused by an improved aeration (Chapter 2 and 5). Soil flooding did not trigger methane emissions here, which is in contrast to results reported by (Kammann *et al.*, 2012). The different results may likely be ascribed to the four times higher amount of hydrochar applied in their study, compared to the Giessen incubation experiments, providing larger amounts of labile carbon compounds for the metabolic activity of methanogens. Generally, in the incubation experiments, all carbon amendments increased the methane fluxes into the loamy soil under study, increasing its initial methane oxidation capacity. No effect was found in agriculturally used, sandy soil and in the field.

1.5.4 Ammonia emissions

Ammonia (NH_3) emissions from agricultural areas are major drivers of soil acidification and eutrophication of ecosystems (Umweltbundesamt, 2011). Up to 28 % of the annual N input can be lost as NH_3 emissions from grasslands, depending on farm management practices (Ball and Keeney, 1981; Pain *et al.*, 1989). Thus, reducing NH_3 emissions from agriculturally managed soils is of both environmental and economic interest. Several field and laboratory experiments dealing with the influence of biochar or hydrochar on processes prone to NH_3 emissions have been conducted so far, including slurry application to the field or slurry/manure storage (Zhao *et al.*, 2013b; Sun *et al.*, 2014a; Schimmelpfennig *et al.*, 2014). The results suggest the possibility of biochar to decrease NH_3 emissions from slurry, if the biochar is incorporated into the soil prior to slurry application (Taghizadeh-Toosi *et al.*, 2012). Ammonia emission reductions of up to 63 % have been reported following the admixture of *acidified* biochar to poultry litter manure, compared to a control without biochar, suggesting a strong relation of the biochar pH and urea hydrolysis (Vandré, 1997; Steiner *et al.*, 2010; Doydora *et al.*, 2011). Concomitantly, as an adverse effect, increases in NH_3 emissions due to biochar amendment have been observed when alkaline biochar was mixed with liquid slurry (without soil or top-dressed onto soil), especially at excessive urea fertilization rates (250 kg N ha^{-1}) (Zhao *et al.*,

2013b; Schimmelpfennig *et al.*, 2014). Contrarily, ammonia emissions following slurry admixture to the mostly slightly acidic hydrochar were negligible, confirming the dominance of the pH effect (Schimmelpfennig *et al.*, 2014). Thus, biochar and hydrochar may offer the option to decrease NH_3 emissions from slurry. However, this effect is clearly linked to the pH of the carbon amendments.

1.6 Stability of biochar/hydrochar with regards to long term carbon storage in soil

The stability or recalcitrance of carbon amendments against degradation is an important factor for evaluating their suitability for long term carbon sequestration and potential integration in carbon trading systems such as the European Emissions Trading System (EU- ETS).

From both Terra Preta sites and environments affected by natural (forest or steppe) fires, the age of pyrogenic carbon compounds in soil has been estimated to range between 500-7000 years, suggesting a similar long mean residence time for biochar (Neves *et al.*, 2003). While evidence from long term biochar field experiments is still scarce, extrapolating the degradation rates obtained by month-or year long incubation and field experiments provides first insights into the degradation behavior of various carbon amendments. Also, some important influencing factors on the degradation behavior have thus been identified. The mean residence time (MRT) of biochar, obtained from one of the incubation studies performed in this study, 30-90 years (Chapter 5), was rather low compared to results reported by others. This can easily be explained by the highly degradation promoting conditions applied in this experiment. Besides the experimental conditions, factors like biochar/soil properties and soil biota, the stage of the experiment at the time of sampling as well as the model used for calculation, may also explain differences in the MRT of different studies (Lehmann *et al.*, 2015).

In any case, the long-term stability of hydrochar was less promising (2-12 years), however hydrochar still exhibited a significantly longer MRT than uncarbonized material (0.3-2 years) (Chapter 4), which is in line with results reported by others (Qayyum *et al.*, 2012; Bai *et al.*, 2013). Generally, the degradation of organic material in soils depends on both its intrinsic chemical structure and the environmental conditions, especially temperature, soil

type and soil moisture. Both the carbon content as well as the cluster size of the aromatic ring structures increases with pyrolysis temperature, particularly above 360 °C, leading to the recalcitrant chemical structure of biochar (Mimmo *et al.*, 2014; Lehmann *et al.*, 2015). Moreover, also the size matters, as biochar dust was mineralized faster compared to pellets (Sigua *et al.*, 2014). On the other hand, smaller sized biochar particles are more likely stabilized by soil aggregates and mineral coating, depending on the clay content of the soil (Joseph *et al.*, 2013; Bruun *et al.*, 2014). Interestingly, evaluating the stability of *Miscanthus* × *giganteus* biochar in two different soils revealed a higher stability of biochar in the biologically less active sandy soil, compared to the loamy grassland soil (Chapter 4). These results indicate that biochar integration into soil aggregates or organo-mineral complexes is a rather long-term process, likely depending on the soil's aluminum and calcium content, as was also reported by other authors (Lin *et al.*, 2012; Singh and Cowie, 2014). Biological mineralization, especially of recalcitrant compounds, generally increases with temperature. However no direct comparison of the influence of soil temperature on mineralization between uncharred and charred organic matter is presently available and warrants further research (Lehmann *et al.*, 2015). In spite of its intrinsic recalcitrance, biochar was susceptible to co-metabolism following the addition of a labile carbon source such as glucose (Chapter 4). These results were corroborated by findings from other authors, especially if N was not a limiting factor (Kuzyakov *et al.*, 2000). On the contrary, glucose addition had little influence on the degradation of feedstock and hydrochar after about one year of incubation, suggesting that these materials were already strongly degraded without an additional labile C-source (Rex *et al.*, 2015)(Chapter 5). Generally, any kind of carbonization (vapo-, hydrothermal carbonization as well as pyrolysis) increased the stability against degradation as compared to the feedstock material. The recalcitrance increased with the carbonization grade, leading to highest stability of biochar material in both sandy and loamy soil.

1.7 Conclusion

The results obtained in the Giessen field and laboratory studies so far confirm the assumption that the degradation of carbon amendments in soil can be related to their carbonization grade. The degradation of feedstock material in the soil was associated with

detrimental GHG emissions in the incubation (CO_2 and N_2O) and in the field (N_2O). Carbonization of *Miscanthus* straw clearly reduced its GHG emission impact: Hydrochar led to lower CO_2 and N_2O emissions compared to feedstock material in the incubation studies. However, higher CO_2 emissions as compared to the control in both incubation experiments still indicate that quite a large amount of hydrochar was prone to mineralization during the experimental periods. Hydrochar additionally reduced plant growth in the field over the period of 1.5 years, indicating the presence of toxic compounds. A better understanding of the carbon compounds created during the hydrothermal carbonization process seems crucial before any larger scale soil application of hydrochar can be recommended. Additionally, long-term carbon sequestration can likely be not achieved with hydrochar.

Rather unexpectedly, biochar amendment did not only lead to lower N_2O and CO_2 emissions compared to the feedstock treatment in the incubation (especially before slurry amendment) and to lower CO_2 emissions in the field; biochar amendment also reduced the CO_2 emissions compared to the pure control soil. Interestingly, these CO_2 emission reductions were neither accompanied by a lower soil microbial biomass nor reduced plant growth or plant nutrient concentrations. Quite contrarily, biochar led to significant yield increases (29 %) of *Lolium perenne* in a greenhouse based pot experiment. Also, the total microbial biomass was significantly higher in the biochar field plots after 1.5 years, compared to the control soil. Rather, the results point to sorption interactions of biochar with root- or soil borne organic carbon and/or slurry compounds. This assumption is corroborated by the fact that the influence of hydrochar and biochar on GHG emissions was always more distinct in the unvegetated incubation experiments compared to the vegetated field, implying interactions of the carbon amendments with plant roots and/or slurry. Based on the results, biochar can be recommended for long-term soil application in temperate grasslands without adverse effects on soil microorganisms and plants.

1.8 Outlook

Information on the chemical composition and the results from the incubation studies suggest that mineralization of hydrochar can likely be expected in the coming years of the field study. From the state of knowledge on biochar, it can be expected that ongoing

bioturbation will lead to an increased mixing of the carbon amendments with the soil matrix, facilitating interactions with the plant roots, soil organic and mineral compounds as well as microorganisms. Long term stabilization of the carbon amendments in organo-mineral complexes or in soil aggregates and increased plant growth due to a higher microbial activity and/or sorption of slurry-borne nutrients are some of the potential results that may be expected for the coming years in the field study. The future trend of the "negative priming", as observed both in biochar amended vegetated and un-vegetated soil, will be particularly worth to investigate in more detail. On the one hand, biological and physical breakdown of biochar particles might generate new sorption sites for SOC, root exudates or nitrate, extending the biochar-induced "negative priming" effect. On the other hand, complete coating of the biochar surface with minerals and soil particles could lead to saturation of the sorption sites, eliminating the CO_2 efflux reductions. Fertilization with slurry as a fixed factor of the study design may interfere with the positive effects of biochar on the GHG budget of the grassland, as it leads to short term methane or N_2O emission peaks. However, ongoing integration of biochar into the soil matrix may induce a reduced bulk density, followed by improved soil aeration and/or sorption of N-compounds onto the biochar surface. These effects could reduce N-losses and increase methane oxidation, leading e.g. to the frequently reported biochar-induced N_2O emissions reductions.

Taken together, biochar seemed to be a suitable mitigation option for "extreme" weather event-related GHG emission outbursts, such as heavy rainfall or severe, immediate freezing, as shown in the incubation study. A more detailed understanding of the performance of biochar under "extreme" weather conditions in the field warrants further investigations with specifically designed experiments.

2 Biochar, hydrochar and uncarbonized feedstock application to permanent grassland - effects on greenhouse gas emissions and plant growth

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Abstract

Both reductions of greenhouse gas emissions and carbon sequestration have the potential to reduce global climate warming and avoid dangerous climate change. We assessed the sequestration potential as well as possible risks and benefits of carbon amendments (16 ± 4 % of soil organic C) from *Miscanthus* × *giganteus* in different carbonization stages of a temperate grassland soil together with pig slurry: 1) untreated dried biomass (feedstock), 2) hydrothermally carbonized biomass (hydrochar) and 3) pyrolyzed biomass (biochar) in comparison to a control (only pig slurry application). The field study was complemented by a laboratory incubation study, followed by a growth experiment with *Lolium perenne*. In the field, greenhouse gas emissions (CO_2, N_2O, and CH_4) were monitored weekly over 1.5 years and over three months in the lab. Initial nitrogen losses via ammonia emissions after substrate-slurry application were assessed in an additional greenhouse study.

We found that biochar reduced soil and ecosystem respiration in incubation and in the field respectively. Additionally, biochar improved methane oxidation, though restricted by emission outbursts due to slurry amendment. It also reduced N_2O emissions significantly in the lab study but not in the field. Hydrochar and feedstock proved to be easily degradable in incubation, but had no effect on ecosystem respiration in the field. Feedstock amendment significantly increased N_2O emissions in incubation and one year after application likewise in the field. In a growth experiment subsequent to the incubation, only biochar amendment increased *Lolium perenne* biomass (+29 %) significantly, likely due to N retention. In the field, biochar caused a significant shift in the plant species composition from grasses to forbs, whereas hydrochar significantly reduced yields within two growth periods (2011 and 2012). Ammonia emissions were significantly higher with feedstock and biochar compared to the control or acidic hydrochar. The overall results indicate that biochar is better suited for C sequestration and GHG mitigation in grasslands than hydrochar or the uncarbonized feedstock. However, NH_3 emission reductions may only occur when the biochar is neutral or slightly acidic.

Keywords

Biochar, Hydrochar, *Miscanthus* × *giganteus*, Grassland, NH_3 emissions, GHG emissions

2.1 Introduction

Biochar, an organic carbon soil amendment, has great potential to alleviate the CO_2 accumulated in the atmosphere by sequestration of recalcitrant carbon into the soil (Lehmann, 2007; Glaser *et al.*, 2009). Such a biological sequestration of CO_2 would be cost-effective (Schellnhuber *et al.*, 2006) and serve as a fast action strategy for climate change mitigation (Melino, 2009). Positive effects of biochar amendments on crop yields (Jeffery *et al.*, 2011; Biederman and Harpole, 2013) would provide an additional incentive for its agricultural use. However, before using biochar as a carbon sink and environmental management tool, it must be proven that it remains stable after soil application and that such application does not create adverse effects, e.g. increased greenhouse gas emissions (GHG). Greenhouse gases such as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4), increase the radiative forcing of the Earths´ atmosphere (Houghton *et al.*, 1997) by contributing to ozone depletion (N_2O) (Ravishankara *et al.*, 2009) and by interaction with aerosols (CH_4) (Shindell *et al.*, 2009). Possible positive feedback effects of biochar or biochar-slurry mixtures on GHG emissions would be detrimental for the field use of biochar as a carbon sequestration tool. To date, the effects of biochar on GHG emissions are rather diverse. They depend on the biochar production process parameters, the feedstock used, the ecosystem and soil properties to which biochar is applied, and the strategy of application and (agricultural) management.

Biochar could be beneficial as a soil conditioner in degraded or naturally poor soils by improving nutrient availability and mycorrhiza abundance (Chan *et al.*, 2008; Alvum-Toll *et al.*, 2011). Although it might not be needed as a soil conditioner in fertile temperate soils, an increment of the grassland carbon stocks by carbon amendment may act as carbon sink due to long C turnover times (Scurlock and Hall, 1998). Biochar use in grasslands may even be based on ancient soil types in temperate climates, i.e. chernozems, of which some are assumed to have developed under grassland (steppe) vegetation (Eckmeier *et al.*, 2007). For anthropogenically used grasslands, which are typically used for livestock breeding with considerable amounts of manure and urine accumulation, positive biochar-slurry interactions may offer new ways for reducing GHG emissions (Winsley, 2007). Indeed, Bruun *et al.* (2011) showed in an incubation study that the addition of 3 % fast

pyrolysis biochar on a mass basis reduced CO_2 and N_2O emissions from a slurry amended soil significantly. Biochar and slurry can also reduce the wind erosion of biochar during application and alleviate the odor of the slurry (Blackwell *et al.*, 2009). However, the promising idea of charging biochar with the nutrients contained in the slurry still has to be proven effective. Experiments with biochar and slurry showed that biochar can bind ammonia by surface interactions (Spokas *et al.*, 2012). Furthermore, biochar reduced NO_3 and total N leaching from manure-amended soil significantly (Laird *et al.*, 2010a; Ventura *et al.*, 2013), with subsequent positive effects on plant-available nitrogen and thus plant growth. Concerning the N-efficiency of ecosystems, ammonia and denitrificatory N losses (including N_2O emissions) are very important factors, as well as losses of NO_3-N by leaching, the main pathways for losses of N from an ecosystem. NH_3 losses from grasslands can account for up to 28 % (grazed pasture) or 27 % (grassland fertilized with pig slurry) of the annual N input (Ball and Keeney, 1981; Pain *et al.*, 1989), depending on farm management practices. Nitrous oxide emissions can add up to 2 -2.2 % total N loss of added fertilizer of a grassland ecosystem (Velthof *et al.*, 1996; Clayton *et al.*, 1997).

However, results of biochar effects on ecosystems in temperate climates are still scarce, and the interactions of different biochar-slurry mixtures in the field still have to be elucidated. Consequently, the background of this study was to assess possible risks and chances of carbon amendment co-applied with slurry to a temperate grassland site with a focus on GHG- and ammonia emissions. We hypothesized that, firstly, the materials would degrade in the sequence of their carbonization grade: feedstock > hydrochar > biochar and that degradation would be measurable in the ecosystem respiration. To assess possible priming effects of biochar on hydrochar or vice versa, we introduced a mixed treatment in the incubation study. Secondly, we hypothesized that biomass growth will be negatively impacted by hydrochar application, as reported by others who found negative effects on plant germination and growth with hydrochar use in soils (Bargmann *et al.*, 2012; Gajić and Koch, 2012). Thirdly, that biochar will reduce N_2O and CO_2 emissions (Augustenborg *et al.*, 2012; Case *et al.*, 2012; Dempster *et al.*, 2012), improve CH_4 oxidation (Liu *et al.*, 2011), and that hydrochar will have rather adverse effects on the GHG balance (Karhu *et al.*, 2011; Kammann *et al.*, 2012), as shown by incubation studies so far.

2.2 Material and methods

2.2.1 Laboratory incubation

A laboratory study was carried out with the same parameters as the field experiment but under controlled conditions. Soil for incubation was taken from the top 15 cm of the experimental field site prior to initiation of the field experiment. The grassland site in Linden, near Giessen, Germany (50°32'N und 8°41.3'E) has been managed extensively for decades as grassland with two cuts per year (Jäger *et al.*, 2003). The soil, a Haplic Stagnosol (WRB, 2006), has a soil texture of 25 % sand, 28 % clay, 47 % silt and a pH of 5.8 - 6.0. For the incubation study, 500 g of the field-fresh soil (or 373 g of dry soil) with 3.5 % total organic carbon (TOC, see Table 2-1) was mixed with carbon substrates. All carbon amendments originated from *Miscanthus* × *giganteus* and were applied non-carbonized (feedstock) hydrothermally carbonized in a steam atmosphere (hydrochar) or pyrolyzed (biochar). *Miscanthus* straw had been harvested in winter 2009, when all aboveground plant material had receded. The hydrothermal carbonization was produced by keeping the feedstock in a water vapor atmosphere for 2 hours at a temperature of 200 ±3 °C under a pressure of 1.6 MPa (Revatec, Geeste, Germany, at that time Hydrocarb GmbH, Ohmes, Germany).

Biochar was produced using a pyrolysis unit with a continuous flow reactor at 550-600 °C (Pyreg GmbH, Bingen). Soil and substrate characterization parameters are given in Table 2-1.

Table 2-1: Key characteristics of the soil and C-substrates used. Numbers behind plus minus signs represent the standard deviation (n=3 for feedstock and hydrochar, n=30 for biochar).

	pH (H_2O)	**C[%]**	**N [%]**	**Ash content [%]**	**C/N ratio**	**O/C ratio**	**H/C ratio**	**BET surface area [m^2*g^{-1}]**	**P-content [mg/kg]**	**Liming equivalence [%$CaCO_3$]**
Soil	5.8	3.5±0.01	0.33±0.01	n.d.	10.6	n.d.	n.d.	n.d.	n.d.	n.d.
Feedstock	6.8	47.94±0.41	0.12±0.02	2.04±0.69	399.5	0.71	1.56	1.1	n.d.	-1.02
Hydrochar	5.1	50.47±1.04	0.19±0.02	3.13±0.63	265.6	0.55	1.28	3.5	0.44	-2.77
Biochar	10.1	60.8±14.54	0.4±0.09	34.93±15.17	152.0	0.07	0.11	864.2	2.98	0.21

All materials were ground to <10 mm before admixture with the soil (SM 300, Retsch GmbH, Haan, Germany). The amount of the substrates applied to the incubation jars and the field was equivalent to an increase of the soil organic carbon (SOC) content (3.5 %) of 16 ± 4 %, with total amounts of 16 t ha^{-1} feedstock application, 14.5 t ha^{-1} hydrochar application and 9.3 t ha^{-1} biochar application, respectively.

In the incubation, we introduced a new treatment where biochar was mixed with hydrochar in equal shares, depending on the C content from each source. The soil-substrate mixtures (n=4 per treatment) were placed in 1100 ml incubation jars (WECK GmbH u. Co. KG, Wehr, Germany) and incubated in the lab at 21 ± 1 °C for 125 days. Soil moisture was controlled gravimetrically by adjusting it weekly to the initial field-fresh soil conditions at the start (WHC 31-37 %); soil moisture raised to WHC 38-46 % with slurry addition, depending on the treatment. WHC_{max} was determined following the DIN ISO 11274 guideline with slight modifications due to the increased soil sorptive capacity after biochar application. In brief, field fresh soil was mixed with the substrates, put into small cylinders prepared with a filter paper on gauze at the bottom to prevent soil and biochar particle runoff and left to soak immersed in water for 24 hours. Subsequently, the samples were taken out of the water, put on a rack in a closed box (to prevent evaporation), left to drain for 24 hours, and re-weighed.

To separate priming effects due to soil disturbance during preparation of the experiment from priming effects of fertilization, we started GHG-flux measurements (CO_2, N_2O and CH_4) three days after initiation of the experiment but added pig slurry in the equivalent to 40 m^3 slurry ha^{-1} to each incubation jar after 36 days of incubation. The slurry had a pH of 7.7 and a dry matter content of 0.81 %. The majority (75 %) of the N in the slurry (1.34 kg m^{-3}) was in form of ammonium (NH_4^+-N), the rest in organic forms. The slurry was pipetted onto the surface of the substrate mixtures in the incubation jars (as would be done in the field). At the end of the GHG measurement period, the soil mixtures were analyzed for their mineral N (N_{min}) contents. For that, 40 g of fresh soil mix was suspended in 200 ml of 2M KCl, shaken for two hours at 200 rpm, then filtered and analyzed colorimetrically for nitrate and ammonium using an auto analyzer (Seal Analytical, Norderstedt, Germany). The pH was measured with a pH meter (InoLab; WTW, Weilheim, Germany) at the beginning and end of the GHG measurement period using a soil: water

ratio of 1:4. For determination of the pH values of the pure C-substrates we used a substrate water relation of 1:20.

A subsequent growth experiment was performed to investigate if an overall N retention might have occurred that may increase plant growth. Thus, the soil mixtures from the incubation experiment were put into flower pots (9 cm diameter) and were adjusted to 60 % WHC. Ten *Lolium perenne* seedlings were transferred and planted to each pot and were kept at constant moisture level in the temperature-controlled greenhouse (day: 20-24 °C night: 14 °C, humidity: 60-70 %, length of day: 12h). The plants were cut for the first time after three weeks to determine above-ground biomass, left for regrowth for another two weeks and were cut a second time. The biomass was dried at 105 °C and weighed.

2.2.2 Field experiment

The study was initiated in April 2011 with application of C-substrates in equal amounts as in the incubation study (16 t ha^{-1} feedstock, 14.5 t ha^{-1} hydrochar and 9.3 t ha^{-1} biochar).

To prevent substrate loss during biochar application by wind erosion as seen in other studies (Major and Husk, 2010), the substrates were mixed with pig slurry (40 m^3 ha^{-1}) before application to promote positive side effects by potentially binding slurry nutrients to the added substrates. Pig slurry was subsequently applied twice a year, in spring and in autumn. In 2011, we applied, besides the initial amount during C-substrate application, 30 m^3 ha^{-1} in autumn, followed by 50 m^3 ha^{-1} in spring 2012 and 70 m^3 ha^{-1} in autumn 2012. The nitrogen (N) amounts applied as well as N (NH_4^+-N and total N) contents together with the dry matter contents and pH of the different pig slurries applied are provided in Table 2-2. Differences in the N contents of the slurries were due to storage methods (covered/uncovered) and the type of feed the animals received.

Table 2-2: Properties and amounts of the pig slurries applied to the soil in the field experiment.

Date	N-content of the slurry [kg m^{-3}]	NH_4^+-N [kg m^{-3}]	N amount applied on field [kg ha^{-1}]	Dry matter content of the slurry [%]	pH
April 2011	1.34	1.0	53.6	0.81	7.7
October 2011	0.7	0.6	21.0	0.25	8.0
May 2012	2.2	1.6	110.0	4.5	8.3
October 2012	0.81	0.7	56.7	0.22	7.4

The four treatment plots (control (slurry only), feedstock+slurry, hydrochar+slurry and biochar+slurry) of 16 m² (4 x 4 m) in size were arranged randomly in rows with four replications. The substrates-slurry mixes were applied as top dressings on 11th April 2011 before the spring growth period.

Three subplots (60 x 60 cm) per plot were harvested in June and October 2011 and in May and September 2012 to evaluate biomass growth. The harvested biomass was separated into functional groups (grasses, herbs and legumes) and dried at 105 °C. To evaluate possible differences in biomass growth and ecosystem respiration, the vegetation in the GHG plots was kept at a height of about 10 cm and harvested three times a year.

2.2.3 NH_3-emission experiment

To simulate the conditions at the beginning of our field experiment (direct mixing of slurry and C-substrates), we performed a separate NH_3 emission study. For this, 200 g of unvegetated, field fresh grassland soil were transferred into flowerpots with a diameter of 15 cm and placed in the greenhouse at 20 °C. Based on the soil surface area in the flowerpots, we added the carbon substrates in the same amount as in the field, as top dressing (28.4 g feedstock, 25.7 g hydrochar and 16.5 g biochar), mixed with pig slurry (0.13 % N). Each treatment (control, feedstock, hydrochar and biochar) was set out in four replicates in the greenhouse and measured in a random order at eight subsequent time points. Since the majority of the NH_3 emission sum was expected in the first 24 hours after application, our measurements took place from 0.5 up to 28 hours after carbon-slurry application.

2.2.4 Analyses

Carbon, nitrogen and hydrogen contents of the soil and carbon material were determined using a CNH Macro Elemental Analyzer (Hanau, Germany), while the oxygen content was determined as difference of the measured elements and ash. Phosphorous content was analyzed following the DIN EN ISO 11885 guideline. The specific surface area (BET surface) of the carbon amendments was determined using an Autosorb-1 (Quantachrome)

with automated gas (N_2) absorption and was calculated from the BET equation (DIN ISO 9277). The results of the analyses are summarized in Table 2-1.

Weekly measurements of the emissions of the greenhouse gases CO_2, N_2O and CH_4 from the field plots were carried out following the closed static chamber method (Hutchinson and Livingston, 1993; Kammann *et al.*, 2008), using dark chambers of 10 x 40 x 40 cm size (stainless steel, insulated). Consequently, measured CO_2 effluxes represent the ecosystem respiration, including above- and below-ground (= roots, rhizosphere) plant respiration, bulk soil respiration and eventually C additive decomposition. Frames of the same size were installed into the soil to a depth of 10 cm before the start of the experiment. During flux measurement, the chambers were placed on the frame in a water-filled trench. Gas samples were taken after 0, 20 and 40 minutes with a 60 ml syringe (Ecoject Plus, Gelnhausen, Germany) through a rubber septum inserted in the top cover of the chamber.

In the lab, GHG fluxes were analyzed weekly following a closed static chamber method as well (Hutchinson and Livingston, 1993; Kammann *et al.*, 2012). Briefly, for starting a measurement, the jars were closed with glass lids and sealed air-tight with moistened gaskets and three metal clips. Gas samples were taken at three consecutive time points by piercing through a rubber septum (see above). To ensure taking a representative gas sample and to prevent O_2 deficiency, the syringe was flushed twice with the air inside the chamber/vessel before sampling. The gas samples were analyzed within 24 hours after sampling on a GC (HP 6890 or Shimadzu 14B, Japan) coupled with an Electronic Capture Detector (ECD, detection of CO_2 and N_2O) and a Flame Ionization Detector (FID, detection of CH_4) run by the automatic sample injection and peak integration software ´Probe65` (LAL, Göttingen, Germany; (Loftfield *et al.*, 1997)). GHG fluxes were calculated by linear regression, considering the ideal gas law as well as average air pressure and temperature during the cover period. Only measurements with an R^2 of ≥ 0.85 and higher or within the daily accuracy of the GC, defined as the standard deviation of n=6 repeated measurements of an atmospheric standard gas, were considered to be fluxes. Otherwise, the fluxes were considered to be zero.

To obtain information on total amounts of GHG lost during the incubation experiment, cumulative fluxes were calculated using linear interpolation (Bruun *et al.*, 2011).We therefore separately cumulated fluxes before and after slurry application, using the six

measurement time points for the period before slurry application and the six (N_2O and CH_4) and eight (CO_2) time points respectively for the period following slurry application. In order to compare the calculated cumulative carbon losses with analytical data for a mass balance, soil carbon contents of the different treatments were analyzed at the beginning and the end of the experiment using a CN analyzer (VarioMax, Elementar Analytical systems, Hanau, Germany).

Soil pH (determined in H_2O) from the field plots was measured once a year with the first measurement in March 2011, before biochar and slurry application and the second measurement one year later. Soil samples (three subsamples per plot) were taken with a core sampler (Ejkelkamp, Giesbeek, The Netherlands) from depths of 0-7.5, 7.5-15, 15-22.5 and 22.5-30 cm. For pH analyses, the three subsamples per plot were pooled according to depth, dried at 105 °C, and ground to 2 mm. Since one year after initiation of the experiment the substrates were mostly in the upper root zone of the grass cover, we sampled and analyzed the upmost 2 cm of the soils separately.

Slurry was analyzed for total N, following the Kjeldahl method including digestion of the samples with sulfuric acid, subsequent distillation with NaOH, and titration against 0.1M HCl and for NH_4^+ by distillation with MgO and subsequent titration (Amberger *et al.*, 1982).

Ammonia measurements were done with a closed dynamic chamber method using Dräger tubes for ammonia detection 2a, 5a and 0.25a, depending on the expected NH_3 concentration (Drägerwerk AG, Lübeck, Germany) following Pacholski et al. (2006) with slight modifications. We worked with one chamber stocked with one gas inlet and one gas outlet. The gas outlet was attached to a hand pump, by which ambient air was sucked into the chamber to generate a steady state situation. Subsequently, the air mix inside the chamber was analyzed for its ammonia content by sucking the air mix into a Dräger tube attached to the hand pump. Each measurement took between 40 and 90 seconds and N fluxes were calculated using the formula $FNg = volume * conc. * 10^{-6} * \rho NH_3 * U_N * U_F * U_Z$, with FNg being the NH_3 flux (mg N m^{-2} h^{-1}), the volume the amount of air sucked through the chamber (in L), conc. meaning the volumetric concentration of NH_3 and ρNH_3 the temperature sensitive ammonia density (mg l^{-1}), U_N is the conversion factor from NH_3 to

N, U_F the factor for upscaling the chamber covered soil surface to m^2 and U_Z the time conversion factor from seconds to hours.

2.3 Statistics

Statistics were carried out using Microsoft Excel 2010, SigmaPlot 11.0 and 12.0 and IBM SPSS Statistics Versions 19 and 20. The differences in the cumulated gas fluxes before and after slurry addition, as well as differences in N_{min} concentrations among the treatments, were analyzed by one way Anova. Potential differences of cumulated (linearly interpolated) vs measured carbon contents from the incubation were determined using a paired t-test and differences in pH and WHC were determined using a two-sided t-test. Biomass data (incubation) were analyzed via a two-way ANOVA (factors ´harvest´ and ´C-additive´), significant differences between the treatments were determined using the SNK-test. The dependence of biomass growth and nitrate concentrations was determined via linear regression analysis. If data were not normally distributed or lacked heterogeneity of variances, data were log- or square-root- transformed to achieve normality. The Kolmogorov-Smirnov test (with Lilliefors' correction) was used to assess normality of the data or the residues of the General Linear Models (GLM). Outliers were determined using the Grubb´s outlier test (Grubbs, 1950). For the GHG flux data from the field we used GLMs with time as random variable. In case of the time series of N_2O and CH_4 fluxes from the field experiment, normal distribution could not be achieved. Nonetheless, we postulated the statistical data to be reliable due to the high number of cases for the long time series. Differences in field biomass data (total yield and the yield divided into the three subgroups grasses, forbs and legumes) were determined using a three-way ANOVA and the LSD and SNK post hoc- tests with the factors `char treatment´, `harvest year after start´ and `harvest season´.

2.4 Results

2.4.1 Laboratory incubation

2.4.1.1 Water holding capacity

Generally, the addition of C-substrates led to an improvement of the soils´ WHC. The addition of hydrochar alone (+14 %) and mixture with biochar (+25 %) led to significant increases of the WHC (t-test, $p < 0.05$).

2.4.1.2 CO_2 emissions and carbon balance

Carbon dioxide (CO_2) effluxes during the three months incubation period were in the order: feedstock > hydrochar > hydrochar-biochar mix > control ≥ biochar, $p = 0.001$) (Figure 2-1 A and B). In the period prior to slurry application, CO_2 emissions were significantly reduced in the biochar treatment (81 % of the control). After slurry application, no differences were observed between biochar and the control treatment. Highest emissions (587 % of the control) were found in the feedstock treatment. After slurry amendment, the CO_2 losses from feedstock and hydrochar treatments had accumulated to 465 and 304 % of the CO_2 emissions from the control. With 198 % of the control, the emissions from the hydrochar-biochar mix were in between the pure biochar and hydrochar treatments (Figure 2-1).

The comparison of the carbon losses determined by the measurement of the bulk C differences and the losses calculated from the cumulative CO_2 emissions are given in Table 2-3.

The cumulative CO_2-C-loss was not significantly different from the C loss based on the difference in bulk-C measurements during the experiment (Table 2-3). Averaged over both methods, the carbon losses from the feedstock treatment were highest, amounting to 11.7 % loss of the initial carbon (SOC+amended carbon). Compared to the feedstock (set to 100 %), the C loss rates were reduced in the other treatments. In the hydrochar treatment, the carbon losses were only 56.1 % of the initial carbon, while no C was lost in

the biochar treatment. The C-losses from the hydrochar-biochar mix treatment lay with 44 % in between the losses of the pure hydro-and biochar treatments.

2.4.1.3 N_2O emissions

Nitrous oxide emissions generally were much lower before slurry addition than after, but they followed a similar order being highest from the feedstock treatment, followed by the hydrochar and the hydrochar-biochar mixes and the control treatment. Biochar was the only C-additive which significantly reduced N_2O emissions, compared to all other treatments (Figure 2-1 C and D). Slurry application caused N_2O emission peaks in all the treatments, with biochar and control showing the largest peaks (8 and 12 µg N kg^{-1} h^{-1}) and hydrochar showing the lowest emission peak (2 µg N kg^{-1} h^{-1}) (Figure A.2-1). The hydrochar-biochar mix´ peak was only slightly larger than that of the pure hydrochar treatment.

Observation of the cumulative emissions revealed that feedstock increased the N_2O emissions of the soil by about 600 % compared to the control. Biochar amendment led to a significant reduction of N_2O emissions (54 % of control); even when biochar was mixed with hydrochar, the reduction persisted so that cumulative N_2O emissions amounted to only 58 % of those from the control treatment (Figure 2-1 D).

2.4.1.4 CH_4 uptake and emissions

At the beginning of the incubation experiment, the grassland soil exhibited a CH_4 oxidation capacity, which was enhanced after C additions. This behavior was most prominent in the biochar and biochar-hydrochar treatments (Figure 2-1E). Slurry addition caused short CH_4 emission outbursts, especially from the hydrochar treatment, and subsequently had an effect on the oxidative capacity of the pure soil in the period after slurry addition (Figure A.2-1). Independently of the type of C addition, cumulative CH_4 oxidation rates were on average in all treatments more than twice the oxidation rates in the control treatment (229 % on average, Figure 2-1 E and F).

Table 2-3: Carbon contents [g kg-1 dw] of the soil and the soil-C-substrate mixtures in the incubation experiment

Treatment	C-content beginning of experiment (calculated)	C-content end of experiment (measured as bulk C)	Difference (carbon loss during the experimental	C-losses. calculated from the cumulative CO_2-losses	Mean	T-test level of significance	C-loss in % of initial C (mean of the two methods)	C-loss [g of added substrate]	% C-loss in comparison with feedstock
Soil	36.0	33.7 ± 1.65	2.3	1.0	1.7	n.s.	4.6	-	
Soil+feedstock	44.6	38.9 ± 12.0	5.6	4.8	5.2	n.s.	11.7	3.8	100.0
Soil+HTC	44.3	42.3 ± 5.71	1.9	3.1	2.5	n.s.	5.7	2.1	56.1
Soil+BC	43.8	42.1 ± 0.55	1.7	1.0	1.4	n.s.	3.1	0.0	0.0
Soil+HTC+BC	44.0	42.1 ± 2.94	1.9	2.0	2.0	n.s.	4.5	1.0	26.9

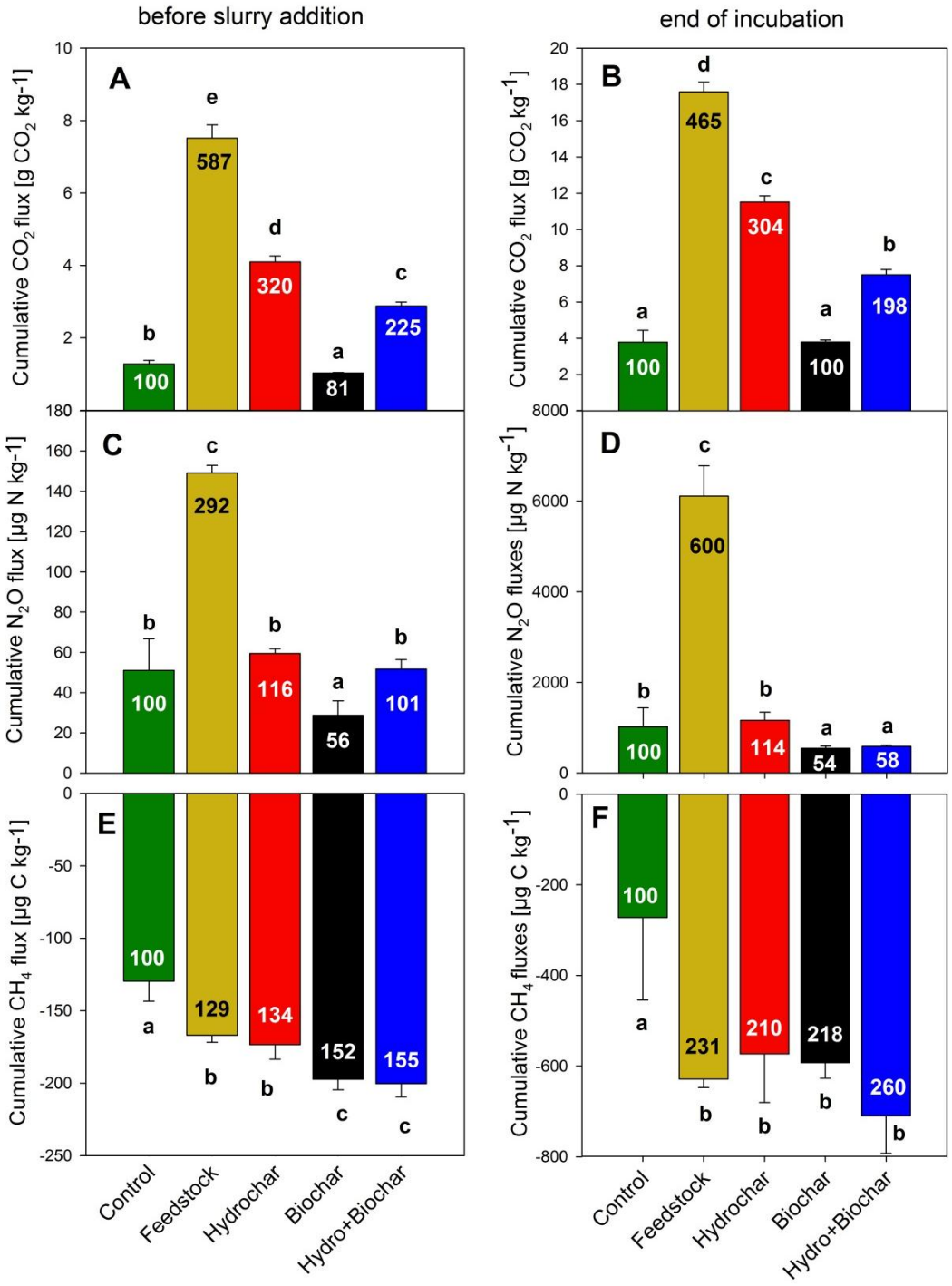

Figure 2-1: Cumulative GHG fluxes (CO_2, N_2O, CH_4) from the soils with standard deviation in the incubation experiment, before (left) and after (right) slurry amendment. Numbers mark percental differences in the cumulative flux compared to the control. Letters mark significant differences between the treatments.

2.4.1.5 Nmin concentrations and plant growth experiment

At the end of the incubation period and before *Lolium* growth, the N_{min} concentrations of the incubated soils and soil mixtures were 187.5 ± 43.9 µg NO_3-N g^{-1} soil (-mix) on average, with no significant differences between the treatments, and nearly zero (3.98 ± 3.96 µg NH_4^+-N g^{-1} on average) for NH_4^+-N, with significantly higher ammonium concentrations in the hydrochar-biochar mix treatment ($p < 0.001$, Table 2-4).

Biomass growth (*Lolium perenne* yield) was stimulated by all substrate amendments up to the first harvest but was significantly higher only in the biochar and hydrochar treatments compared to the control (+42 % and 32 %, respectively). The second harvest showed an equally strong biomass growth which was similar for all treatments. Overall, only biochar led to a significant improvement of biomass growth, resulting in yield increases of +28.9 %, compared to the control soil.

A positive correlation was observed ($R^2 = 0.338$, $p < 0.007$) between the initial NO_3^- concentration and the biomass yields, with highest NO_3^- concentrations being associated with pure hydrochar (189 mg N kg^{-1}) and biochar treatments (223 mg N kg^{-1}).

2.4.1.6 pH values

The pH values of the pure C-substrate materials were very diverse, with hydrochar being slightly acidic (pH 5.15), biochar alkaline (pH 10.12) and the feedstock about neutral (pH 6.82). Mixed with soil (before slurry application), biochar slightly increased the pH of the soil-substrate mix (pH 6.2) compared to the control (5.81), even if biochar was mixed with hydrochar (hydrochar-biochar mix, pH 6.2). Slurry amendment lowered the pH values by about 1.5 units (paired t-tests, $p<0.01$) (Table 2-5).

Table 2-4: Soil Nmin [mg/kg soil mix] at the end of the laboratory incubation experiment (mean ± standard deviation, n=4). The asterisk marks a significant difference compared to the control (p<0.001).

Treatment	NH_4^+-N [mg kg soil mix^{-1}]	NO_3^--N [mg kg soil mix^{-1}]
Control	1.71 ± 1.33	164.6 ± 19.8
Soil+feedstock	3.28 ± 2.2	177.7 ± 76.0
Soil+hydrochar	1.19 ± 0.26	189.1 ± 17.0
Soil+biochar	1.7 ± 1.91	223.5 ± 12.8
Soil+hydro+biochar	10.38 ± 2.65*	182.7 ± 55.0

Table 2-5: Mean pH values, (H_2O) of the substrates, substrate-soil-mixtures before and after slurry addition in the laboratory incubation experiment (n=4).

Materials	pH	The beginning of experiment	pH	The end of the experiment	pH
Soil	5.8	Soil	5.8	Soil+slurry	4.5
Feedstock	6.8	Soil+feedstock	5.9	Soil+feedstock+slurry	4.5
Hydrochar	5.2	Soil+hydrochar	5.8	Soil+ hydrochar +slurry	4.6
Biochar	10.1	Soil+biochar	6.2	Soil+biochar+slurry	4.8
Hydrochar -biochar-mix	7.3	Soil+ hydrochar +biochar	6.2	Soil+ hydrochar +biochar+slurry	4.7
Pig slurry	7.7				

2.1.1 Ammonia emissions

The NH_3-N emission losses from the feedstock treatment were highest, with 22 % of the NH_4^+-N applied being lost as NH_3 in the first 30 hours after slurry amendment. The biochar mixture had N losses summing up to 12 %. Emissions from the control and hydrochar treatments did not differ significantly and were low with 0.8 and 3.74 % of NH_4^+-N lost as NH_3, respectively. Beside the amount of NH_3-N emissions, also the duration of the emissions differed among the treatments; most of the NH_3-N emissions from the hydrochar and control treatments occurred during the first five hours after slurry application; and were close to zero afterwards. Emissions from the biochar treatments were initially very high with an emissions peak in the first hour after slurry application, followed by a rapid decline to the control soil level within three hours. The emissions from the feedstock treatment, however, showed a different behavior, with a rather slow increase and decrease of the emissions, peaking after about three hours (Figure 2-2).

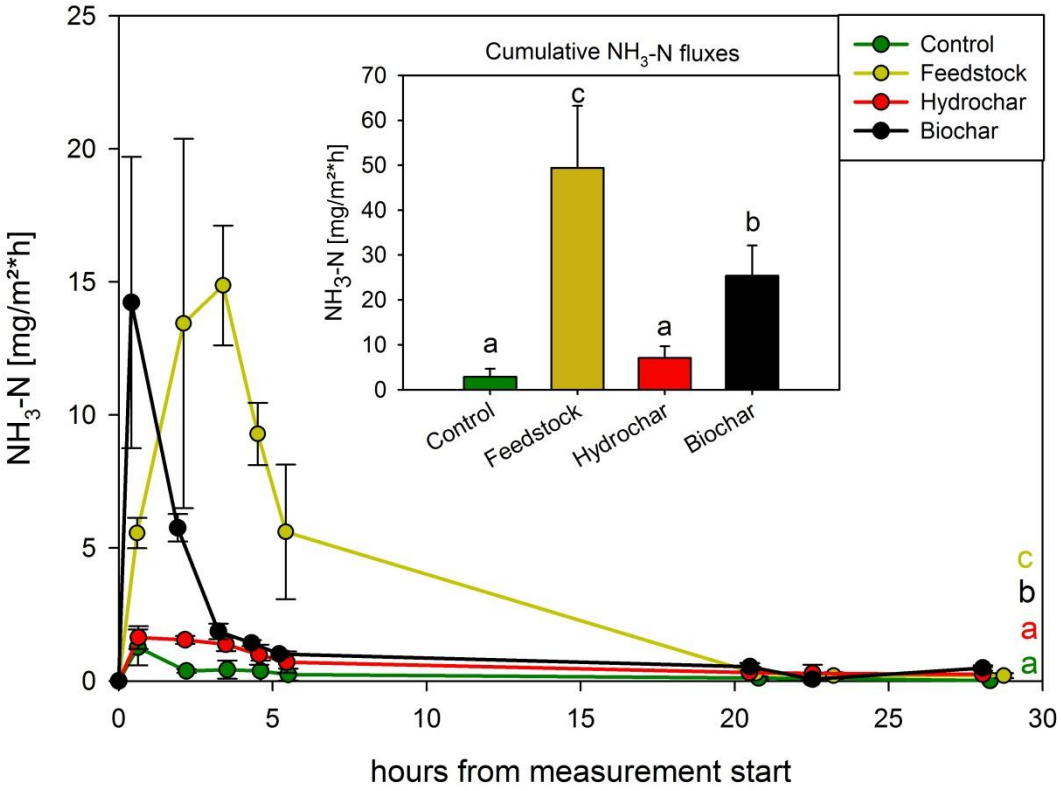

Figure 2-2: NH_3-fluxes from the soils in the greenhouse, measured with a closed chamber after slurry application.

2.4.2 Field experiment

2.4.2.1 Ecosystem respiration: CO_2 emissions in the dark

Ecosystem respiration (R_{eco}) over 18 months was lowest in the biochar treatment, followed by fluxes from the hydrochar treatment, and the feedstock and control treatments (Figure 2-3 B). Slurry application events were not followed by CO_2 emission outbursts in the field, in contrast to the incubation experiment. Additionally, biomass in the GHG plots was not different between treatments, though showing the same tendencies as did the larger harvest plots (data not shown). We therefore argue that biomass in the GHG measurement plots can likely be ruled out as a major influencing factor on the ecosystem respiration in the field experiment.

A polynomial correlation was observed between R_{eco} and air temperature ($R^2 = 0.403$, $r = 0.755$). Low temperature in winter caused a period with low R_{eco} values from October 2011 to March 2012 (winter dormancy). Extreme events with exceptional high or low fluxes were associated with high and low precipitation events, but a lack of extreme event data limits statistical evidence.

2.4.2.2 Nitrous oxide emissions

N_2O emissions of the feedstock-amended plots were significantly higher than emissions from all other treatment plots ($p < 0.0001$), despite existing block effects, as indicated by large standard errors. No significant difference between the other treatments was found (Figure 2-3 C). Frost-thaw events or recurrent strong precipitation events stimulated N_2O emissions in all treatments; during a frost-thaw event in winter 2011/2012, N_2O emissions were highest from the hydrochar plots (158.9 % of control), followed by emissions from the feedstock plots (119.2 %) and biochar-amended plots (65.8 %) but the differences were not significant.

2.4.2.3 CH_4 emissions

As in the incubation study, methane fluxes in the field were dominated by net microbial methane oxidation i.e. net CH_4 uptake into the soil. Methane emissions were observed

from all treatments within 1-2 days of slurry application from all plots. The extent of these peaks was dependent on slurry composition (dry matter content), as well as abiotic factors such as temperature, affecting microbial activity. Therefore, CH_4 emissions after slurry amendment were higher in spring than in autumn (Figure 2-3 D). Methane emissions were highest from biochar amended plots after the slurry applications, creating the lowest overall net methane oxidation values (insert in Figure 2-3 D). However, excluding the upper and lower 1 % off the data set (i.e. extreme outgassing events), the CH_4 flux characteristics were different, with biochar showing significantly higher mean CH_4 oxidation rates (-35.3 µg m^{-2} h^{-1}) than the feedstock (-32.7 µg m^{-2} h^{-1}), hydrochar (-31.4 µg m^{-2} h^{-1}) and control plots (-33.1 µg m^{-2} h^{-1}), see Figure A.2-3.

2.4.2.4 pH

The carbon amendments caused pH changes among treatments only in the upmost two cm of the soil layer. Due to top dressing, the additives were not mixed with the soil at the start of the experiment, though they had mixed a little in May 2012 (caused by the vegetation growing through the C substrate layers) leading in tendency to increased pH values in the biochar plots (6.1 ± 0.4) compared to the control plots at the soil surface (5.4 ± 0.2) ($p<0.05$, two-sided t-test). A pH decline, as in the lab experiment, was thus not observed in the field.

2.4.2.5 Biomass yield and composition from the field experiment

The three-factorial ANOVA showed significant differences in the overall biomass yield, due to treatment ($p<0.036$), harvest season (on average 284.4 g m^{-2} in spring and 253.3 g m^{-2} in autumn, $p<0.001$) and harvest year (on average 348.77 g m^{-2} in 2011, and 726.63 g m^{-2} in 2012). A Post-hoc-test (Fisher LSD) revealed significant differences ($p<0.006$) between biomass of the hydrochar and control treatments for both years (1014 g m^{-2} vs 1137.8 g m^{-2}, i.e. -10.0 % with hydrochar, Figure 2-4 C). The lowest biomass yields in 2011 were measured from the hydrochar amended plots, which was a result of a significant reduction of grass (but not of forbs or legumes) biomass on these plots compared to the others (Figure 2-4 A). In the second year 2012, grass biomass in all C-amended plots was

significantly reduced compared to the control plots, but was lowest in the biochar plots, amounting to only 60 % of the grass yield from the control plots. Interestingly, in the biochar plots, the reduced grass biomass was more than compensated for by forb biomass, being significantly higher than in all other treatments (Figure 2-4 B), resulting in an overall (2011+2012) significant positive growth effect of biochar on forbs and an impeding effect of carbon amendment on grass growth (Figure 2-4 C). Leguminous biomass was low (on average 1 % of the total yield), and was not significantly affected by any of the treatments.

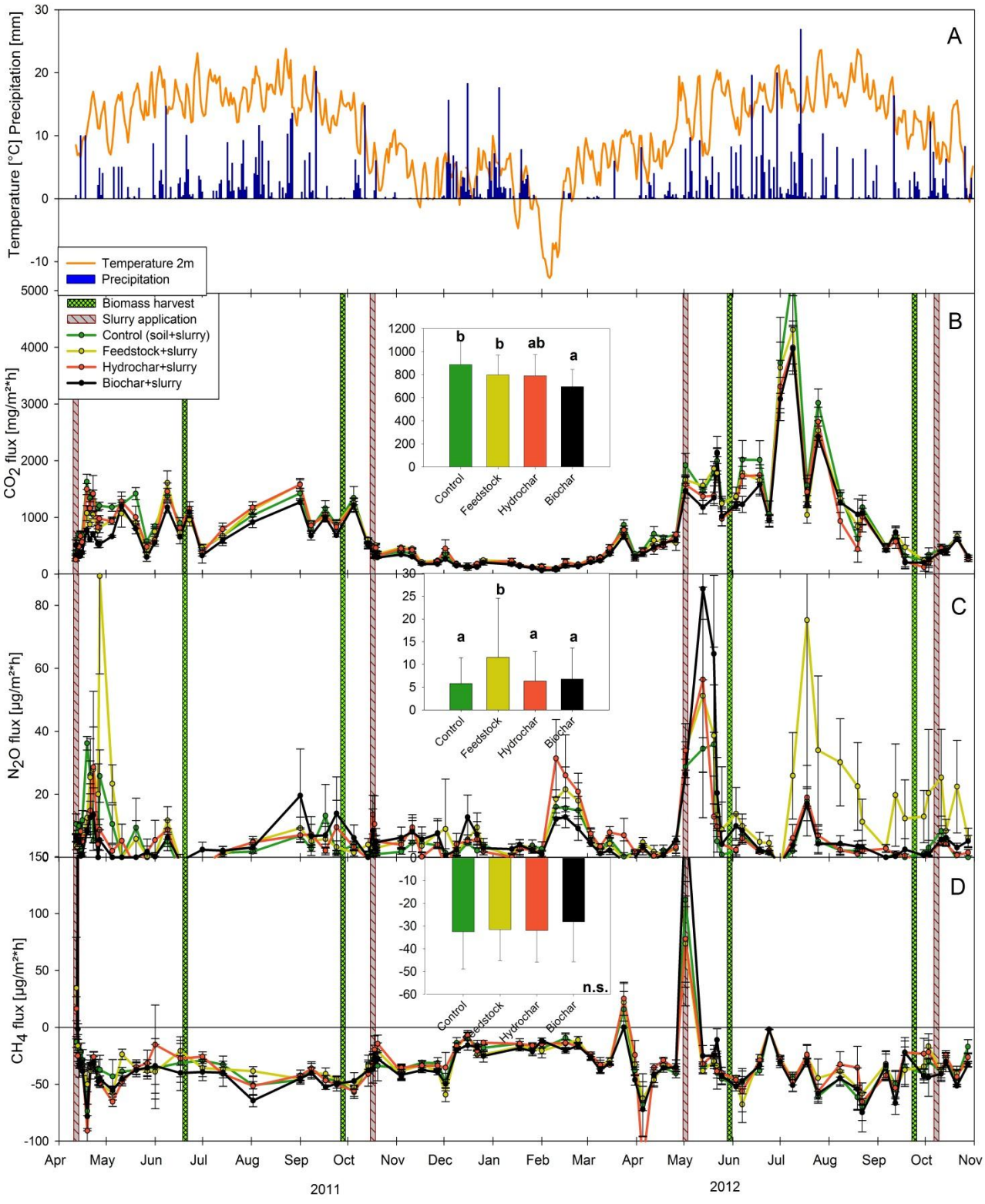

Figure 2-3: GHG fluxes from the soils in the field experiment. A: air temperature at 2m above ground and precipitation, B: CO_2 emissions, C: N_2O emissions and D: Methane fluxes. Bar graphs (green and gray) mark slurry application events and biomass harvests. Error bars mark the standard error. The inserts show the mean of the whole experimental period with standard deviation and labels for significant differences ($p<0.05$)

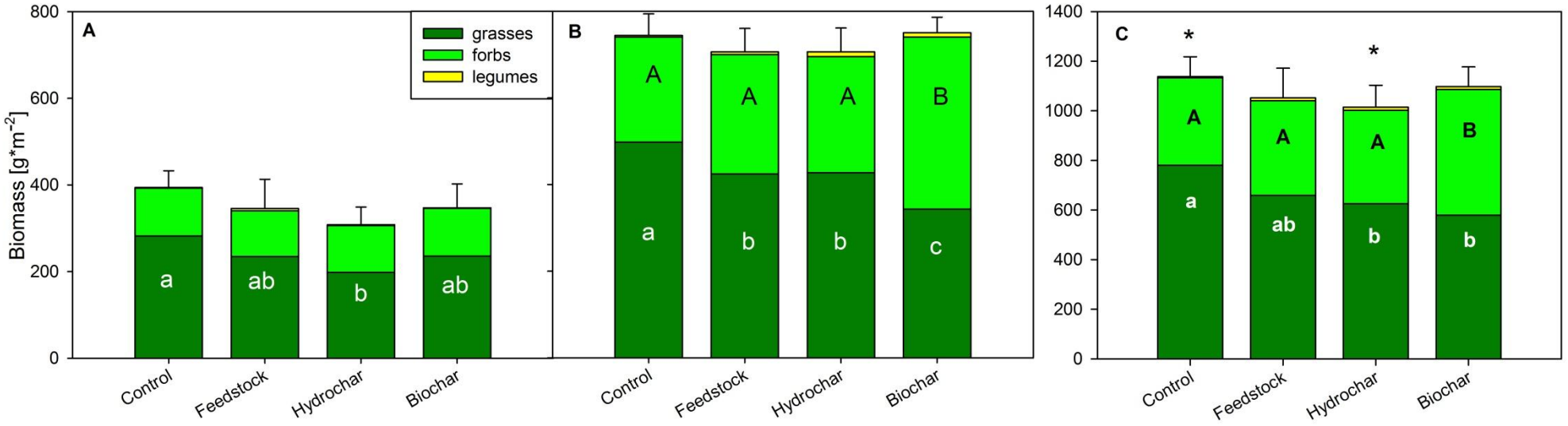

Figure 2-4: Illustration of the biomass harvests from the field plots in A=year 2011, B=year 2012 and C=sum of both years (2011+2012, note different scale). Letters mark significant differences between the plant functional groups (grasses, forbs and legumes) according to the different treatments. Asterisks mark significant overall differences between the treatments over the two years 2011 and 2012, as well as across all three plant functional groups.

2.5 Discussion

2.5.1 Soil and ecosystem respiration and C stability

We measured soil respiration in the incubation (experiment without plants) and ecosystem respiration (R_{eco}) in the field (experiment with plant cover). In the field, plant growth in the GHG measurement plots was not significantly different among treatments. Thus, we argue that significant effects observed in the field were predominantly related to differences in soil respiration.

In the incubation study, soil CO_2 efflux was reduced in the biochar treatment before slurry application, whereas hydrochar and feedstock increased the emissions from the incubated soil mixtures. These results are in line with other biochar incubation studies where a reduction of CO_2 emissions has been identified (Van Zwieten *et al.*, 2010b; Liu *et al.*, 2011). Moreover, the study by Van Zwieten *et al.* (2010b) showed, like our study, increased CO_2 emissions from soil mixed with more labile C additives, such as green waste or, in our case, *Miscanthus* feedstock or hydrochar material. In particular, the H/C ratio may serve as a good indicator of stability, with high ratios pointing to low stability against degradation (Van Zwieten *et al.*, 2010b; Schimmelpfennig and Glaser, 2012). Evidently, the H/C ratio of the green waste used as feedstock by Van Zwieten *et al.* (2010b) (1.35) and our feedstock and hydrochar material (1.62 and 1.35), are in good agreement, indicating quick degradability of the substrates.

No significant changes in soil CO_2 emissions due to biochar amendment have been found in incubation experiments by Kammann *et al* (2012), Spokas and Reicosky (2009) or Zavalloni *et al*. (2011), whereas significantly higher emissions were generated by hydrochar-soil mixtures compared to the pure soil control (Kammann *et al.*, 2012). The lack of an effect from biochar on soil respiration could be due to the soil types used in the cited studies and may be related to the SOC contents, being much lower (1.0 / 2.2 / 2.6 %) compared to the grassland soil used in this study (SOC 3.5 %).

The degradation sequence of the materials (feedstock > hydrochar > biochar) observed in our incubation study is in line with results of Qayyum *et al.* (2012) with wheat straw showing highest CO_2 emissions, followed by bark-hydrochar and finally biochar (charcoal) which was similar to pure soils (an Oxisol and Alfisol, respectively). Short-term increases in soil respiration after biochar amendment to soil, as reported in many other studies (Luo *et al.*, 2011) have not been observed here but could have been masked by the high CO_2 peak emission events of all treatments following slurry addition. Despite the observed overall reduction of CO_2 emissions in our laboratory experiment, biochar did not reduce the degradation of hydrochar, if both materials were mixed at equal shares in the incubation study. Results of Zavalloni *et al*. (2011) are in agreement with this behavior: in their study, degradation of wheat straw could also not be decelerated by the addition of biochar.

A reduction of the overall CO_2 emissions from soil amended with biochar, measured using stable isotope techniques (i.e. a negative priming of SOC by biochar amendment) has also been found by Cross and Sohi (2011) who compared the effects of sugarcane bagasse biochar, produced at different temperatures, on the SOC priming of fallow soil, agricultural soil and grassland soil. Only in the grassland soil with the highest SOC content of 3.64 % they found a negative priming effect of biochar on SOC, comparable to our results. Also, Jones *et al.* (2011) incubated a grassland soil (SOC 3.5 %) and found a negative priming of SOC after hardwood biochar amendment. Reasons for negative priming effects by biochar have been discussed as (1) pH-induced change in the microbial community, although such shifts occur rather as long-term effects, as (2) sorption interactions of biochar with extracellular enzymes which play an important part in SOM breakdown or (3) as shifts in microbial metabolism (Jones *et al.*, 2012). Moreover, (4) an inhibition of nitrifying bacteria due to ammonium adsorption to biochar surfaces (Ding *et al.*, 2010; Taghizadeh-Toosi *et al.*, 2011b) may explain reduced soil respiration. Such a behavior was also found with reduced N_2O emissions, as observed in the incubation study. Furthermore, (5) adsorption of native SOM on biochar surfaces has been reported (Borchard *et al.*, 2012) and seems to be a function of native soil organic carbon content as well as of biochar production temperature and feedstock, which essentially control the surface area of the biochar (Cross and Sohi, 2011; Schimmelpfennig and Glaser, 2012).

In the field study, biochar amendment caused a significant reduction in R_{eco} emissions during the experimental period of 1.5 years. No data on the effects of top-dressed biochar on GHG fluxes, or from other field studies in temperate grasslands are available so far. However, biochar field studies with agricultural crop use or plowing reported no effects on soil respiration at levels up to 20 t/ha (Castaldi *et al.*, 2011; Karhu *et al.*, 2011; Zhang *et al.*, 2012), or increased CO_2 emissions from soils amended with 40 t biochar ha^{-1} (Zhang *et al.*, 2012). In addition to the amount of biochar applied, the feedstock and its elemental composition and the production temperature, and thus volatile matter content, seem to predominantly influence the degradation dynamics (Zimmerman *et al.*, 2011; Ippolito *et al.*, 2012; Singh *et al.*, 2012; Ameloot *et al.*, 2013a). For the amended soil, next to its TOC content, texture has been proposed as an important variable influencing biochar decomposition and stabilization (Cross and Sohi, 2011; Hilscher and Knicker, 2011; McCormack *et al.*, 2012).

In the field experiment, the two more labile carbon substrates, feedstock and hydrochar, did not promote higher R_{eco} (i.e. mineralization rates). This is in contrast to Gajić and Koch (2012) who found that soil respiration was stimulated due to fast degradation of hydrochar material in the first year of a plowed field experiment, especially after mineral nitrogen supply. Since our carbon substrates have been top dressed onto the field, the fast degradation of the labile carbon substrates as seen in the incubation and found by Gajić and Koch (2012) could have been limited because of spatial separation of the substrates and the active soil layer.

Slurry amendment stimulated CO_2 emissions in the incubation and field experiment, leading to CO_2 emission peaks. Emission peaks were likely induced by carbon compounds readily available for microorganisms contained in slurry, enhancing metabolic turnover rates and soil respiration (Focht *et al.*, 1979; Chantigny *et al.*, 2001; Bol *et al.*, 2003). However, these stimulating effects were found to be less prominent in the field. Slurry may have infiltrated the soil profile in the field, which is impossible in a closed incubation vessel. Increases in ecosystem respiration following slurry application were observed in spring 2011, and especially in spring 2012. This was likely a result of the varying dry matter- and thus C-content of the slurry (Table 2-2). Interactions of the carbonaceous additives with slurry at initiation of the experiment (outgassing of CO_2 due to slurry

carbonates) could have caused immediate CO_2 losses during mixing prior to application, thus measured fluxes in spring 2011 likely underestimate the real emissions.

2.5.2 Nitrous oxide emissions

In our study, a strong reduction of N_2O emissions was observed when biochar was *mixed into* the soil, but not when it was *top-dressed* (for density of biochar cover see Figure A.2-2).

Significant reduction in N_2O emissions with biochar addition, as found in the incubation study, is now a widely reported phenomenon. It was observed with very different biochars in controlled incubation studies with various soils (Spokas and Reicosky, 2009; Cayuela *et al.*, 2010; Bruun *et al.*, 2011; Case *et al.*, 2012; Cheng *et al.*, 2012; Kammann *et al.*, 2012; Yoo and Kang, 2012; Ameloot *et al.*, 2013a; Cayuela *et al.*, 2013), also in comparison with other C additives (Cayuela *et al.*, 2010), in greenhouse experiments with plant presence (Aguilar-Chávez *et al.*, 2012; Kammann *et al.*, 2012; Saarnio *et al.*, 2013), and even in the presence of N_2O-producing earthworms (Augustenborg *et al.*, 2012). The relative reduction of about 50 % observed in this study is comparably large in relation to the biochar addition rate (1.3 % w/w) when compared to the above-mentioned studies. Emissions continued to be reduced throughout the incubation period, although the extractable N contents of the biochar-soil treatment were not different from the other treatments at the end of the incubation period. As reasons for reduced N_2O emissions, physico-chemical sorption of N_2O on biochars (Van Zwieten *et al.*, 2009; Cornelissen *et al.*, 2013), or N immobilization/adsorption in a non-extractable form, reducing the available mineral N for N_2O production (Hua *et al.*, 2009; Ding *et al.*, 2010) have been discussed, although the exact mechanisms are not understood yet (Cornelissen *et al.*, 2013).

Moreover, a more complete denitrification towards N_2 can cause reduced N_2O emissions (Sahrawat and Keeney, 1986), although changes in soil pH as underlying mechanism have not been observed (Table 2-5). Nonetheless, either small scale pH effects in the vicinity of the biochar particles, provision of electrons from biochar to denitrifying bacteria, or

increased NosZ gene expression in denitrifiers are all potential mechanisms leading to a complete reduction towards N_2 (Cayuela *et al.*, 2013; Harter *et al.*, 2014).

NH_3 or NH_4^+ sorption onto biochar as reasons for N_2O emissions reductions, as observed by Taghizadeh-Toosi *et al.,* (2011b) are not likely in our experiments, since we observed a NH_3 emission outburst from biochar-slurry mixtures, likely due to the alkaline pH of the biochar. Generally, only a total N balance including NO_x- N_2 or NH_3 losses could elucidate all effects of biochar on the N-cycle.

In the field experiment, the lack of N_2O emission reductions with biochar amendment is likely due to top-dressing. Reduction mechanisms involving the soil-biology matrix have thus not evolved so far. Evidently, in all studies from which reduced N_2O emissions from field measurements have been reported so far, biochar was mixed (plowed) into the soil (Liu *et al.*, 2011; Taghizadeh-Toosi *et al.*, 2011a; Zhang *et al.*, 2012). In the incubation, feedstock started to degrade substantially after slurry application, triggering N_2O emissions. In the field, significantly increased N_2O emissions were only observed after more than one year, however, without indications for increased decomposition. Physico-chemical weathering of the *Miscanthus* straw, downward migration to the microbially active root-mineral soil interface zone, combined with slurry addition and thus varying soil moisture conditions, finally may have initiated its degradation when it was warm in summer 2012. Generally, slurry application has been found to promote denitrification by creating anaerobic environments and providing an energy source to denitrifying bacteria or co-denitrifying fungi (Beauchamp *et al.*, 1989; Laughlin and Stevens, 2002; Laughlin *et al.*, 2008). A correlation of denitrificatory N_2O losses and labile carbon (i.e. water soluble carbon) availability may explain the emissions from the feedstock treatment (Burford and Bremner, 1975; Laughlin and Stevens, 2002; Laughlin *et al.*, 2008).

In the incubation and the field study, hydrochar had no effect on N_2O emissions before or after N-fertilization with slurry. This is in contrast to observations by Kammann *et al.* (2012) where bark and beet hydrochars significantly increased N_2O emissions after mineral N fertilization, but not before. This discrepancy might be due to differences between the two hydrochars (beet and bark, vs. *Miscanthus*) or, more likely, to the moisture regimes. In our experiment, aeration of the soil due to carbon amendments is not likely an explanation,

because hydrochar and feedstock would have caused a higher aeration than biochar, but in these treatments no N_2O reduction was observed.

The hydrochar-biochar mixtures' N_2O emission sum before slurry application was similar to that of pure hydrochar or the control. After slurry application it was similar to the pure biochar amended treatment. The difference in behavior before and after slurry application may be related to mineral N adsorption by biochar, nitrate in particular (Kammann *et al.*, 1998; Clough *et al.*, 2013; Kammann *et al.*, 2013; Prost *et al.*, 2013), reducing denitrificatory N_2O emissions. Adsorption of labile carbon compounds e.g. from hydrochar onto biochar, as observed during composting (Prost *et al.*, 2013), or quick degradation of hydrochar during the first weeks (Libra *et al.*, 2011) is no likely explanation for the observed reduction in hydrochar-biochar N_2O emissions here, since the mineralization rate as measured by CO_2 emissions remained unchanged.

N_2O peaks due to slurry amendment, frost-thaw events and varying moisture regimes of the soil as observed in the field study over 1.5 years are well known from previous studies (Clayton *et al.*, 1997; Flessa *et al.*, 1998; Kammann *et al.*, 1998; Allison, 2005; Matzner and Borken, 2008; Zimmerman *et al.*, 2011). N_2O peak emissions due to slurry amendment in May 2012 are in a typical range for grassland on clayey soil with a duration of up to three weeks after application (Monaghan and Barraclough, 1993; Allen *et al.*, 1996). A positive correlation of N_2O emissions from nitrification with soil temperature has been reported (Sahrawat and Keeney, 1986; Maag and Vinther, 1996), and together with the high ammonium content and dry matter value of the slurry in May 2012 might be the reason for the prominent peak at the time. N_2O emissions from frost-thaw events though were found to originate mostly from nitrate leaking out of frozen cells, readily available for denitrification (Müller *et al.*, 2002).

2.5.3 CH_4-fluxes

Results from the incubation experiment show that various carbon additions to a clayey loam grassland soil can enhance the potential for methane oxidation. These results point to the well-known aeration effect demonstrated for biochar (Case *et al.*, 2012), leading to an improved oxygen diffusion into the soil and thus a better oxygen and atmospheric methane

supply for methanotrophic bacteria (Castro *et al.*, 1994; Castro *et al.*, 1995; Czepiel *et al.*, 1995). Nevertheless, the expected sequence of a pure aeration effect, simply due to the amount of substrate added to the soil (control < biochar < hydrochar+biochar < hydrochar < feedstock), differs from the sequence that was actually measured (control < hydrochar < biochar < feedstock < hydrochar+biochar); all C additives had nearly the same CH_4-consumption promoting effect. The second explanation might be NH_4^+ adsorption by hydrochar and biochar, or N immobilization by a larger soil microbial population with feedstock and hydrochar. This may have allowed CH_4 consumption activity to continue while methanotrophs were likely inhibited by the slurry-applied NH_4^+ in the control (Bédard and Knowles, 1989; Gulledge *et al.*, 1997).

Under field conditions, only the biochar top-dressing resulted in an overall increase in methane oxidation (after exclusion of the methane outbursts due to slurry application which may have been derived from the slurry itself, Figure A.2-3). This is in line with the findings of Karhu *et al.*, (2011). Besides the aeration effect or adsorption of NH_4^+ to biochar surfaces (Ding *et al.*, 2010), further mechanisms for an increase in CH_4 oxidation may be adsorption of CH_4 to biochar surfaces, metals potentially contained in biochar catalyzing CH_4 oxidation, or growth stimulation of methanotrophic populations (Van Zwieten *et al.*, 2009; Feng *et al.*, 2012).

Short-lived CH_4 emission peaks from temperate soils due to slurry amendment as observed in the incubation and field study likely originate from dissolved methane contained in the slurry (Hütsch, 2001; Sherlock *et al.*, 2002). Furthermore, short-chained volatile fatty acids (C2-C6) from slurry are easily available to methanogenic Archaea (Hrapovic and Rowe, 2002) and can result in short-term methane emission outbursts (Sherlock *et al.*, 2002). The CH_4 emission peaks after slurry amendment in the field occurred only in spring and were especially large in spring 2012. This may have been related to the high dry matter content of the slurry applied at that time (Table 2-2). Slurry with low dry matter content can infiltrate quickly and thus keep CH_4 emissions from the slurry itself low, whereas a high dry matter content and a thereby low infiltration rate generates higher emissions from the slurry itself (Chadwick and Pain, 1997; Amon *et al.*, 2006).

In the field, the observed pH increase may explain the enormous emission peak in the biochar plots at the beginning of the field experiment and, weakened, in May 2012. The pH

of 6.1 from the biochar plots, compared to a pH of 5.4 in the control plots may have promoted methanogenic Archaea with a pH optimum of 7 (Amaral *et al.*, 1998). Thus, a larger Archaeal population may temporarily have increased CH_4 emissions in the biochar treatment (Clemens and Wulf, 2005), until it declined due to the oxic environment. This theory is supported by the fact that the (liquid) slurry: biochar ratio was higher in the biochar treatments and thus the obligatory anaerobic methanogens had better conditions to subsist.

Contrariwise, methanotrophic bacteria existing especially in slurry crusts could have led to an increase of the population of methanotrophs in the soil (Petersen *et al.*, 2005), leading to the increase in methane oxidation after the slurry initiated emission peak, as observed in the incubation study (Figure 2-1 E and F).

2.5.4 NH_3-emissions

A high pH buffer capacity of the slurry amended soil as well as low ammonia and CO_2 concentrations in the surrounding atmosphere have been described as the main factors for evolvement of NH_3 emissions from slurry application (Vandré, 1997).

Slurry usually contains urea as the dominant N form. During storage, urea is converted via hydrolysis by H_2O and the enzyme urease to NH_4^+ and CO_3^- , until a chemical equilibrium is reached (Vandré, 1997). This equilibrium is preserved until slurry comes in contact with the soil-atmosphere system. With rising pH, an acceleration of the reaction towards NH_3 and CO_2 outgassing can occur. As CO_2 release consumes protons and NH_3 release dispenses protons these two processes trigger each other. Moreover, degradation of slurry-borne volatile fatty acids by soil microorganisms leads to a pH increase and could also stimulate NH_3 emissions (Sørensen, 1998).

The largest NH_3 emissions observed from the feedstock treatment may be explained by the large surface exposure of the slurry to the surrounding ammonia-depleted atmosphere with the larger mass of *Miscanthus* feedstock applied compared to the masses of the other C-additives (Sommer and Hutchings, 2001). Another factor for higher gaseous losses especially from the feedstock treatment could be the retarded infiltration rate. In the control treatment (pure soil), slurry infiltrated quickly and likely was absorbed by clay mineral

surfaces so that the lowest overall NH_3 emissions were observed. The hydrochar caused the lowest NH_3 losses of all three C additives, possibly due to its acidic pH of 5.1 or microbial N immobilizing capacity (Gajić and Koch, 2012).

In general, the amount of nitrogen lost from the feedstock and biochar treatments (22 and 12 % of the NH_4^+-N) in the form of NH_3 is not extraordinarily high and is consistent with other studies in which pig slurry was broad-spread on grassland, although field and lab studies cannot be compared directly. The reported amounts of NH_3-N lost in % of total N applied range from 8 % to 35 % (Pain *et al.*, 1989; Sommer *et al.*, 1997; Sommer and Hutchings, 2001; Misselbrook *et al.*, 2002). Under favorable conditions though, NH_3-emissions after pig slurry application only amounted to about 5 % (Gronauer, 1993), which is in line with our control and hydrochar treatment results (0.8 and 3.7 %). Thus, the use of all three C additives neither resulted in detrimental NH_3 outbursts, nor can the interactions be judged as beneficial in reducing NH_3 losses below the control. However, using acidic rather than alkaline chars produces lower NH_3 losses, as described by Chen *et al.* (2012) and Taghizadeh-Toosi (2012). Moreover, incorporation of biochar into soil with subsequent slurry application resulted in significant NH_3 emissions reductions (Taghizadeh-Toosi *et al.*, 2012), indicating the importance to consider possible differences of biochar-slurry and biochar-soil-slurry matrices. More high-frequency measurements in the field are clearly necessary to assess chances and risks regarding NH_3 emissions after slurry-char and slurry-char-soil applications.

2.5.5 Biomass yield and composition

In the greenhouse pot experiment following the incubation, each C-additive stimulated *Lolium perenne* growth above the control; this was significant for hydrochar and biochar at the first harvest. The stimulation may be explained by higher SOC contents and consequently increased water retention, proven by higher WHC of all C-amended treatments compared to the control. Furthermore, since higher nitrate concentrations mostly in the hydrochar and biochar treatments significantly correlated with the yield increases, it is likely that reduced N losses during the incubation may have promoted plant growth. N retention and increased nitrate amounts are in agreement with other studies on

reduced nitrogen leaching from biochar amended soils (Beck *et al.*, 2011; Bell and Worrall, 2011; Knowles *et al.*, 2011; Ventura *et al.*, 2013). However leaching did not play a role here in the closed incubation vessels, and in the pot study care was taken not to drain the pots; thus only reduced gaseous losses can explain the differences. For hydrochar, reduced NH_3 losses (compared to feedstock and biochar) and for biochar halved gaseous N_2O losses could possibly serve as indications for an improved N retention.

Detrimental growth effects by the use of hydrochar, as observed by Gajić and Koch (2012), Pielert *et al*. (2012), Rillig *et al*. (2010), Busch *et al*. (2012) or Bargmann *et al.* (2012) were absent after the incubation. These results point to the decomposition of inhibitory or toxic substances over time, as observed earlier by Busch *et al.*, (2012). In the field study, in contrast, hydrochar significantly reduced biomass growth within 18 months; the reduction was most prominent at the harvest in the first year after application. Production-derived, phytotoxic (volatile) organic components (Bischoff *et al.*, 2012*;* Becker *et al.*, 2012; Busch *et al.*, 2012) and/or microbial immobilization of nitrogen resulting in N-limitation of plants (Nelson *et al.*, 2011; Bargmann *et al.*, 2012; Gajić and Koch, 2012) have been discussed as reasons for the adverse effects of hydrochar on plants. Our results indicate that detrimental compounds reduced plant growth even via top-dressing in the field experiment. N immobilization effects, as proposed by Gajić and Koch (2012), are less likely after four repeated slurry applications during the experimental period and two consecutive growing seasons. Grasses were stronger reduced by hydrochar than forbs, especially in the first year of the experiment; other studies report reduced growth for species of all plant functional groups (*Taraxacum sect. ruderalia, Trifolium repens, Allium ampeloprasum, Beta vulgaris subsp. vulgaris* or *Zea mays*) (Rillig *et al.*, 2010; Bargmann *et al.*, 2012; Busch *et al.*, 2012; Gajić and Koch, 2012; Pielert *et al.*, 2012). More research is urgently needed to identify the inhibiting mechanisms and substances and possible risks associated with hydrochar.

In the biochar treatment, the reduction of grass biomass growth was more than compensated for by increased forb growth. Van de Voorde *et al.* (2014) observed something similar: In their 2012 established field experiment, a grassland seed mixture of 18 species was sown into biochar amended soil, resulting in higher biomass of individual plants as well as increased abundances of legumes compared to the control plots. Improved

availability of micronutrients by biochar as Ca, K and Mg rich ashes, or pH induced changes could have changed plant community composition (Laser, 2007). Further harvests in the field study, as time progresses, will reveal if the shift is a lasting phenomenon.

2.6 Conclusions

Our hypothesis of a degradation rate in the order biochar < hydrochar < feedstock was confirmed by the results of the incubation study and priming effects of biochar on hydrochar degradation or vice versa were not observed. In the field, increased CO_2 emissions from the degradation of the carbon amendments have not been observed; biochar even lowered the emissions significantly compared to the control plots.

The hypothesis that hydrochar would reduce plant growth was confirmed by results from the field study. Surprisingly, this detrimental effect was alleviated after pre-incubation with slurry for three months prior to sowing, probably due to degradation of toxic components by microbes. Biochar amendment significantly increased *Lolium* yields in the pot experiment and shifted the plant community towards forbs in the field. The appraisal of such a shift however, will depend on the needs of the farmer and intended land use management.

Our third hypothesis, that biochar would reduce CO_2 and N_2O emissions while improving methane oxidation, was observed, though more pronounced in the incubation than in the field study. Further downward migration of the carbon amendments, by bioturbation in the field, will elucidate if the interactive effect of biochar and the soil matrix will lead to reduced N_2O emissions from the field site, as in the incubation study. Ammonia measurements revealed a risk of NH_3 losses from feedstock and biochar-slurry mixtures, while results with the more acidic hydrochar suggest that this risk can be avoided either if a biochar is acidic or acidified before it is mixed and applied with slurry. Moreover, the risk of ammonia emissions is probably curbed if biochar is mixed with soil. Taken together, by the results obtained here, we identify biochar as the most suitable soil C amendment, compared to untreated feedstock and hydrochar, when C sequestration is the central aim (Table 2-6): it was most recalcitrant, had a positive effect on GHG fluxes and had

beneficial rather than unwanted effects on plant growth. Thus, biochar has the potential to become a climate mitigation tool integrated in grassland management.

Table 2-6: Overview on the effects of carbon amendments (feedstock, hydrochar and biochar) on GHG and ammonia emissions as well as on plant growth in the incubation (left) and the field experiment (right). Effects are shown in comparison to the control treatment. Plus stands for beneficial effects, minus for adverse effects on the measured parameters. The plus in brackets marks the positive effect of biochar on methane emissions after cutting the upmost and lowest 1% of the data. The minus in brackets stands for significantly higher NH_3 emissions from the biochar treatment compared to the control but at the same time significantly lower emissions compared to the feedstock treatment.

	Incubation study				Field study		
	Feedstock	Hydrochar	Biochar	HTC+BC mix	Feedstock	Hydrochar	Biochar
CO_2	–	–	~	–	~	~	+
N_2O	–	~	+	~	–	~	~
CH_4	+	+	+	+	~	~	**(+)**
NH_3	–	~	**(-)**				
Plant growth	~	~	+	~	~	–	~

2.7 Acknowledgments

We thank the agricultural centre Eichhof, Bad Hersfeld, Germany, for providing us with Miscanthus feedstock from a long term field experiment. Hydrothermal carbonization was kindly carried out by Julian and Anke Schwark. The production of the biochar material was carried out by Helmut Gerber, Pyreg GmbH, Bingen. Slurry was kindly provided by the Upper Hardthof Farm, (Giessen University, institute for animal breeding and domestic animal genetics). We acknowledge the support of R. Pfanschilling and Prof. Steffens with slurry analyses and the help of S. Ratering (JLU) with the GC facility management. We are indebted to A. Pacholsky, University Kiel for lending us the NH_3 emission measurement device and thank Anne Wagner, TU Berlin, for analysis of the BET surface areas. We would like to thank the Hessian Agency for Environment and Geology for funding this research.

2.8 Appendix

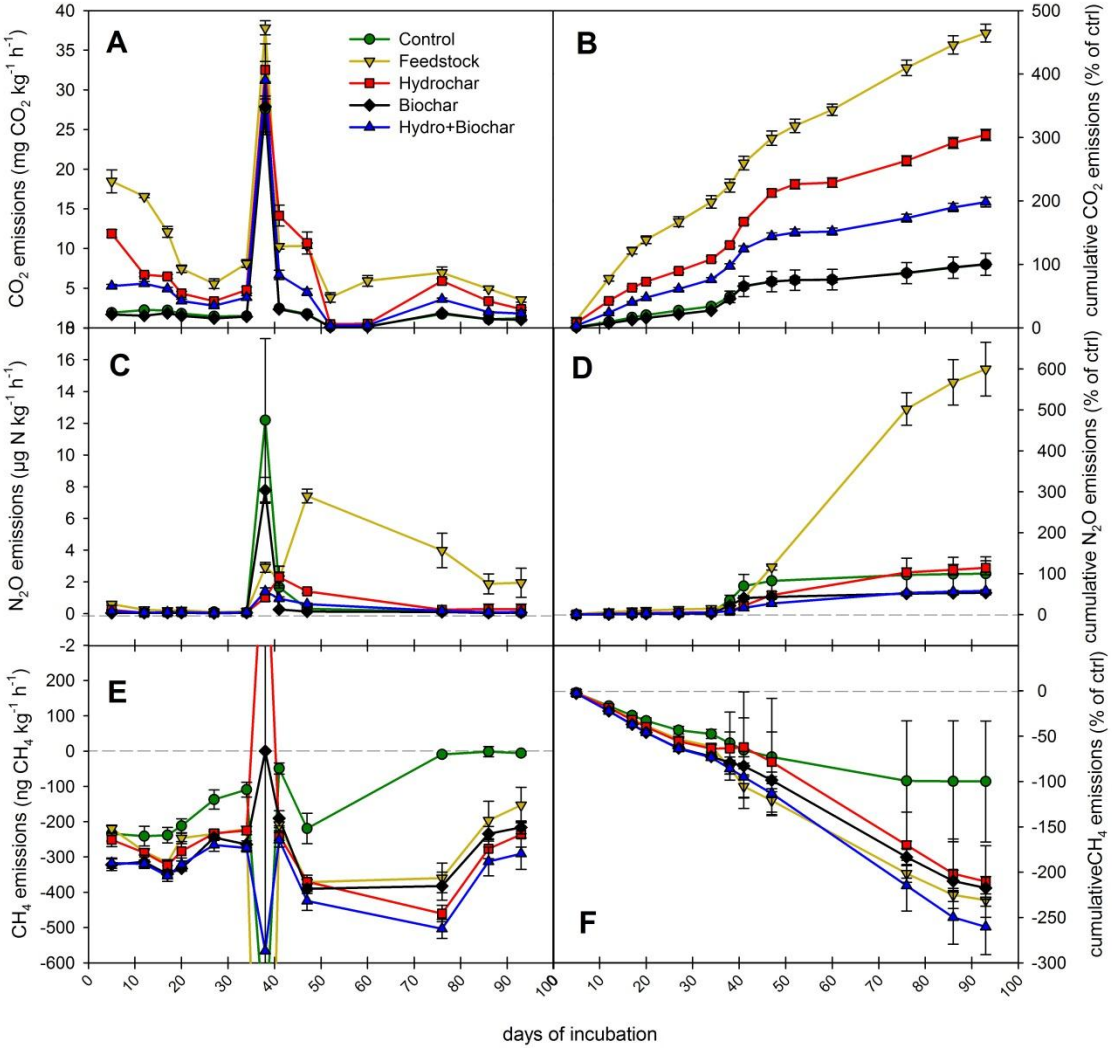

Figure A. 2-1: GHG emissions (CO_2, N_2O and CH_4) from the incubation experiment (92 days). A,C and E depict means and standard deviation of the fluxes as measured at the different time points, B,D and F show the cumulated fluxes in % of the control.

Figure A. 2-2 : The experimental biochar field site Linden, Germany one day after initiation of the experiment in April 2011.Treatments: control (no carbon substrate application), feedstock (uncarbonized *Miscanthus x giganteus* chaff), hydrochar (hydrothermally carbonized Miscanthus chaff) and biochar (pyrolyzed Miscanthus chaff)

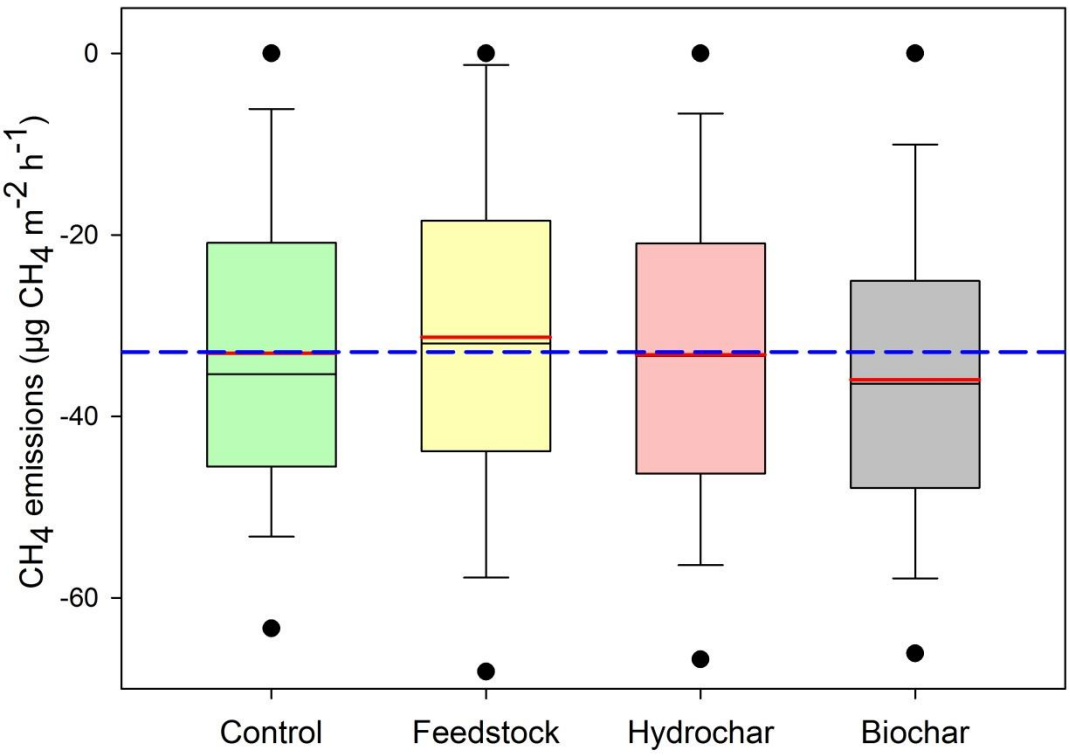

Figure A. 2-3: Box- and whisker plot illustrating the methane emissions from the field over 1.5 years, after exclusion of the upmost and lowest 1% of the data (outlier in the data due to methane emissions outbursts after slurry amendment). The dashed line marks the mean of the control treatment.

3 Changes in macro- and micronutrient contents of grasses and forbs following *Miscanthus × giganteus* feedstock, hydrochar and biochar application to temperate grassland

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Abstract

Biochar and hydrochar application to soil holds promise for climate change mitigation. This study provides first insights into the nutrient concentration and removal of grassland vegetation after addition of various carbon compounds together with pig slurry. Four treatments: control (no carbon application), feedstock, hydrochar and biochar from *Miscanthus* × *giganteus* were applied at a permanent grassland site near Giessen, Germany. We monitored changes in plant functional groups, biomass production and nutrition status over two years. Total biomass production was not affected by the carbon amendments. However, biochar favoured growth of forbs over grasses, while legume growth was increased by all carbon amendments. The initial nutrient concentrations of the carbon compounds were enriched according to their degree of carbonization, potentially providing nutrients to plants. We found that the biomass from hydro- and biochar amended plots, added up over two years, exhibited higher potassium concentrations compared to biomass from feedstock and control plots. All carbon amendments led to lower sodium concentrations in total biomass, compared to the control. Uncarbonized feedstock led to increased manganese concentrations in total biomass, while the concentrations of all other heavy metals were not influenced by any carbon amendment, compared to the control. From a plant and animal nutritional point of view, none of the carbon amendments reduced grassland fodder and yield quality. The study suggests that hydrochar and even more so biochar may provide a source of potassium to plants.

Keywords: biochar, hydrochar, plant growth, grassland, plant nutrients, nutrient concentration

3.1 Introduction

Carbon amendments such as hydro- and biochar are currently being studied as an option for climate change mitigation (IPCC, 2007; Woolf *et al.*, 2010), with positive side effects on physico-chemical as well as biological soil properties (Lehmann *et al.*, 2006; Atkinson *et al.*, 2010; Titirici, 2013). Depending on feedstock and process production conditions, biochar was found to improve the nutrient status of the amended soil directly by its nutrient content (Chan and Xu, 2009), direct or indirect pH effects (Hossain *et al.*, 2011; Lehmann *et al.*, 2011), or impacts on soil nutrient cycling due to biochar-fertilizer surface interactions (Clough *et al.*, 2013). Two meta-studies on the effect of biochar on plant growth revealed an overall positive influence, with yield increases of 10-12 % (Jeffery *et al.*, 2011; Biederman and Harpole, 2013), largely depending on the plant species. In contrast hydrochar has often proved to be detrimental to plant growth and germination, even generating genotoxic effects, assumedly due to N-limitation or labile carbon fractions attached to the hydrochar as remains from the production process (Gajić and Koch, 2012; Bargmann *et al.*, 2013; Busch *et al.*, 2013; Wagner and Kaupenjohann, 2014).

Results on the long-term effects of carbon amendments on the soil-plant matrix in temperate soils are still scarce (Mukherjee and Lal, 2014). The opportunities for biochar to create soils of high fertility require more attention (Ponomarenko and Anderson, 2001). Since most of the worlds´ grasslands are grazed with continuous application of nutrients from animal faeces, possible interactions of animal manure/urine and carbon amendments need to be identified. Biochar reportedly reduced NH_3^+ losses from urine patches in grazed land and from slurry during storage, possibly due to reversible sorption mechanisms, providing a nitrogen source to plants (Haeni *et al.*, 2012; Taghizadeh-Toosi *et al.*, 2012). Thus, the main aim of this study was to identify the effects of three different carbon amendments on the plant nutrient composition of grassland undergoing repeated slurry fertilization (Scurlock and Hall, 1998). We hypothesized that feedstock and hydrochar would lead to a plant growth reduction due to nitrogen limitation and expected biochar to have rather positive effects on plant nutrient availability due to bonding of nutrients from slurry on the biochar surface.

3.2 Material and Methods

We analysed the effect of *Miscanthus* × *giganteus* chaff (uncarbonized = feedstock, hydrothermally carbonized at 200 °C for 2h = hydrochar and pyrolyzed in a continuous flow reactor at 550-600 °C = biochar) on the plant biomass and its nutrient concentrations from temperate grassland. A grassland field experiment had been installed in Linden-Leihgestern, near Giessen (50°32'N, 8°41.3'E, at 172 m a.s.l.) in spring 2011. The N-limited, extensively managed grassland (Haplic Stagnosol, 25 % sand, 28 % clay, 47 % silt and 3.5 % soil organic carbon) with an annual mean precipitation of 586 mm and a mean air temperature of 9.6°C (1997-2004) had received no fertilizer since 1993 (Janze, 2006). For more information on the site see Jäger *et al.* (2003).

In the experiment, the carbon amendments were applied in four random repetitions (4×4 m) as top dressing (see also Schimmelpfennig *et al.* (2014)). The substrates were applied to achieve equal carbon amendment (+20 % of soil organic carbon, as calculated for the upper 10 cm of the soil with a bulk density of 1g cm^{-3}) for all treatments except the control, leading to an application of 1.6 kg m^{-2} feedstock, 1.45 kg m^{-2} hydrochar and 0.93 kg m^{-2} biochar. After the initiation of the experiment, the plots were fertilized with pig slurry twice a year (control = no *Miscanthus* amendment, normal slurry amendment). The N-amounts given with the slurry were 53.6+21.0 kg N ha^{-1} in 2011, 110.0+56.7 kg N ha^{-1} in 2012 and 60.7+63.6 kg N ha^{-1} in 2013 (spring and autumn, respectively).

Plant biomass was cut twice a year (spring and autumn) from three predefined harvest subplots (60×60 cm) per plot, sorted by the plant functional groups grasses, forbs and legumes, dried, quantified and ground to ≤ 1mm (SM 300, Retsch GmbH, Haan, Germany). The three samples from the harvest subplots were pooled to one sample per plot and plant functional group (grasses/forbs) for further analysis. Legumes were not considered for analysis of trace elements and minerals because they accounted for only 1.3 % on average of total biomass, providing not enough material for analysis. Likewise, plant biomass from 2011 was not sufficient for the analysis. This resulted in n=64 for grasses and forbs, and grasses+forbs, respectively (16 samples per season per group). The single grass and forb species are provided in Table A.3-1.

Biomass carbon (C) and nitrogen (N) concentrations were analysed using an elemental analyser (Vario Max, Hanau, Germany). For the analysis of phosphorus (P), potassium (K), sulphur (S), calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), sodium (Na) and chlorine (Cl), the plant samples were pressed in a ring vessel to produce a pellet which was then dried to a residual moisture of <3 % in 3-4 hours at 60°C before X-ray fluorescence analysis. The analysis was carried out by the Hessian Federal laboratory, accredited by the German National Accreditation Body in 2013. The measurement procedure is validated by ring trials, including 40 laboratories across Europe to approve the reference material. See Table A.3-2 for illustration of detection limit, range limit and measurement uncertainty.

Initial macro- and micronutrient contents of hydrochar and biochar were determined using wet chemistry and atomic emission spectrometry after pressure digestion by nitric acid and hydrogen peroxide. *Miscanthus* × *giganteus* feedstock was analysed with the plant material as described above (Table 3-1). Soil pH values (in H_2O) were determined yearly from three pooled samples per plot (0-30 cm).

Statistics were performed using Sigma Plot 11.0 and IBM 20. Effects on biomass yield (dry matter), macro- and microelement concentration and removal were determined by a four factorial univariate ANOVA, followed by post-hoc-tests (Tukey HSD, CI = 95 %) where plant functional group (grasses/forbs), harvest year (2012/2013), harvest season (spring/autumn), plot replication (1,2,3,4) and treatments (control, feedstock, hydrochar and biochar) were modelled as factors. Normal distribution of the residues was tested by a Kolmogorov-Smirnov-test with Lilliefors correction of significance. Differences in the N : P ratio due to C-treatment were determined by a one way ANOVA. Differences in nutrient removal according to the plant functional groups grasses and forbs were determined by t-tests. If data were not normally distributed, we log-10-transformed the data to achieve normal distribution. If unsuccessful, medians were tested with the Mann-Whitney-U median test. Correlations between the nutrient concentrations of the carbon amendments and the harvested biomass were tested using Pearson Product Moment Correlations. The nutrient use efficiency was determined by calculating the individual nutrient-to-C ratio (g/g) of the samples (Chapin III *et al.*, 2011).

Table 3-1: Elemental contents (macro and micro elements) of the carbon substrates applied to the field

Element / Material	C [%]	N [%]	P [%]	K [%]	S [%]	Ca [%]	Mg [%]	Fe [mg kg^{-1}]	Mn [mg kg^{-1}]	Cu [mg kg^{-1}]	Zn [mg kg^{-1}]	Na [%]	Cl [%]
Feedstock	47.94	0.12	0.05	0.55	0.03	0.73	0.38	241	44.5	3.0	10.8	0.02	0.08
Hydrochar	50.47	0.19	0.04	0.61	0.00	1.04	0.56	1450	58.3	0.9	13.3	0.02	0.00
Biochar	60.80	0.40	0.24	1.56	0.00	1.14	0.52	3090	434.0	21.7	88.5	0.08	0.00

For evaluation of the fodder quality of the plant biomass, measured nutrient concentrations (g or mg kg^{-1}, respectively) were compared with the recommended intake as well as minimal requirements of micro- and macronutrients as given by the German Agricultural Society (DLG) (Flachowsky *et al.*, 2001), and values from reference grassland, taken from the DLG database (mean values of available data n = 304, from German grassland 1935-2014). Differences between the experimental biomass in this study and the values from the reference grassland from the DLG table were determined by one-way ANOVAs.

3.3 Results

Macro- and microelement contents of the carbon substrates generally increased with degree of carbonization (feedstock < hydrochar < biochar) (Table 3-1). Total biomass dry matter (DM) (g m^{-2}) increased significantly from 2012 to 2013 (726.0 g m^{-2} vs. 839.8 g m^{-2}, $p < 0.001$, n = 64, Figure 3-1 A and B) but differed not with treatments. Nevertheless, we found significant treatment effects over the two years in the biomass of the plant functional groups grasses ($p < 0.001$, n = 64), forbs ($p < 0.001$, n = 64) and legumes ($p = 0.006$, n = 64) (Figure 3-1 C). Grass biomass was highest in the control plots and lowest in the biochar plots (67 vs. 50 % of the total yield) and vice versa for the forbs (31 vs. 47 % of the total yield). All carbon amendments led to an increased growth of legumes ($p = 0.006$, n = 64). The results of the biomass yield remained unchanged if tested without legumes (no treatment differences in the grass+forb yield).

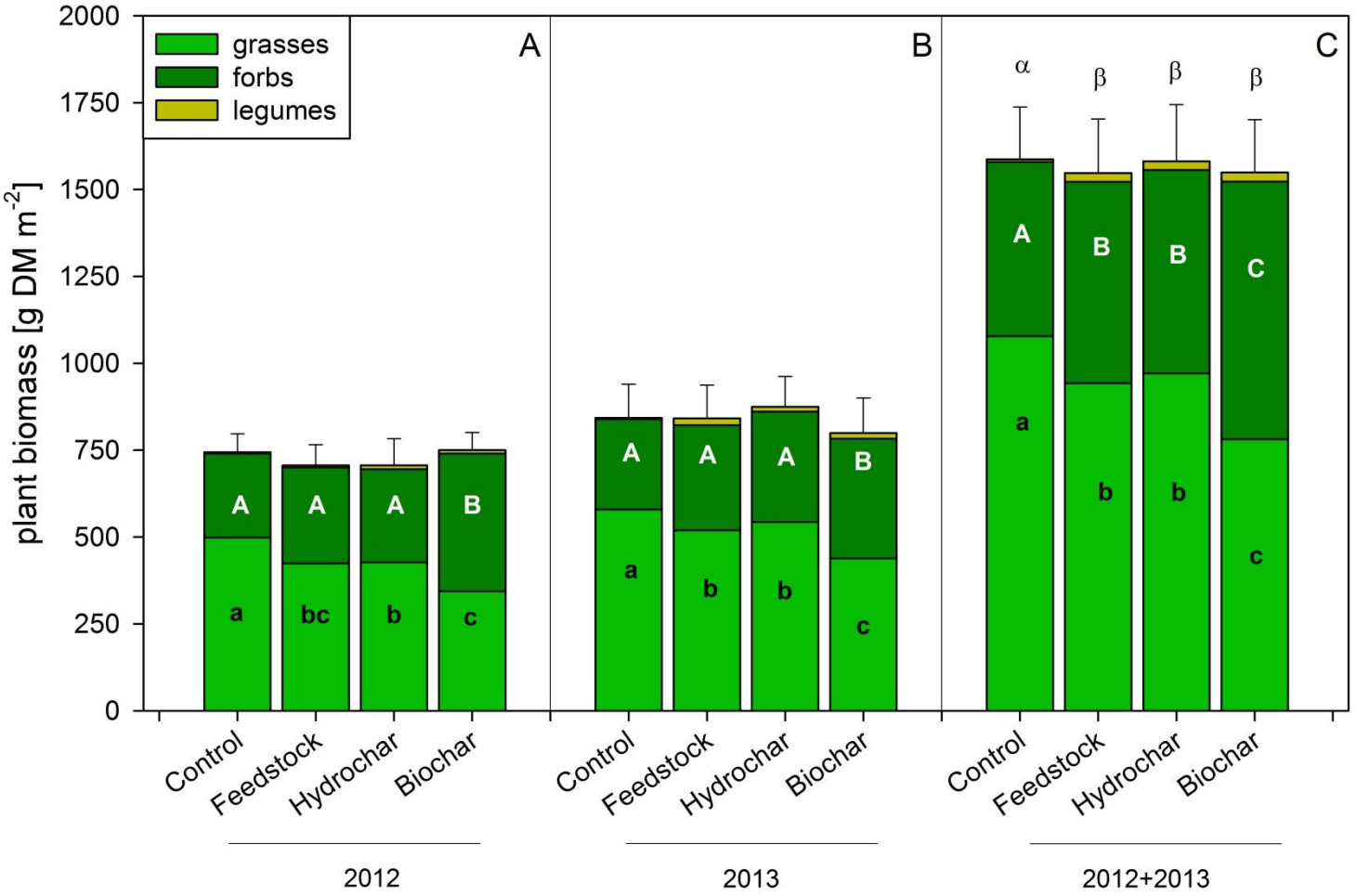

Figure 3-1: Mean and standard deviation of the biomass dry matter (DM) of the treatment plots in the years 2012 (A), 2013 (B) and both years added (C), n=64. Big Latin letters depict significant differences in grass biomass yield, small letters differences in forb biomass and Greek letters show significant differences in legume biomass over the two years 2012 and 2013.

The concentration of macro- and microelements (in % and mg kg^{-1}) in the biomass differed according to harvest year, harvest season, plant functional groups (grasses and forbs) and treatments (Figure 3-2 and 3-3). Concentrations were almost always higher in 2012, except for Ca, Mn and Na, where harvest year had no effect, and Fe, where concentrations were higher in 2013. Nutrient concentrations were mostly higher in spring than in autumn, though not for Ca, Mg, Fe and Mn, where concentrations were higher in autumn and Zn, where there was no difference between seasons. Almost all elements were found in significantly higher concentration in forbs, except K, Cl and Mn where the concentrations were higher in grasses, and Fe, where there were no effects (Figure 3-2 and 3-3).

Treatment effects were found for K, Mn and Na concentrations of the total harvested biomass (Figure 3-4), but without a distinctive pattern. K concentrations increased regardless of carbon amendment type, compared to the control, with the highest concentrations in biomass from hydrochar and biochar plots. In contrast, Na concentrations were decreased by all carbon amendments, compared to the control. Mn concentrations increased by about one third due to feedstock application, compared to the biomass from all other treatment plots. The initial nutrient concentrations of the carbon amendments correlated positively with K concentrations ($r = 0.805$, $p < 0.001$, $n=16$) and negatively with Mg concentrations ($r = -0.531$, $p < 0.03$, $n=16$) of the total biomass (grasses+forbs, 2012+2013) from the treatment plots.

For the evaluation of the nutrient status of the grassland under study, we calculated the N : P ratio as it can serve as indicator for N-or P-limitation of ecosystems (Koerselman and Meuleman, 1996; Güsewell, 2004). The average N : P ratio of the total biomass under study was 4.9 and showed no significant differences between grasses and forbs. Among the carbon amendments, biomass from feedstock amended plots exhibited a significantly higher N : P ratio than biomass from the other plots (5.6).

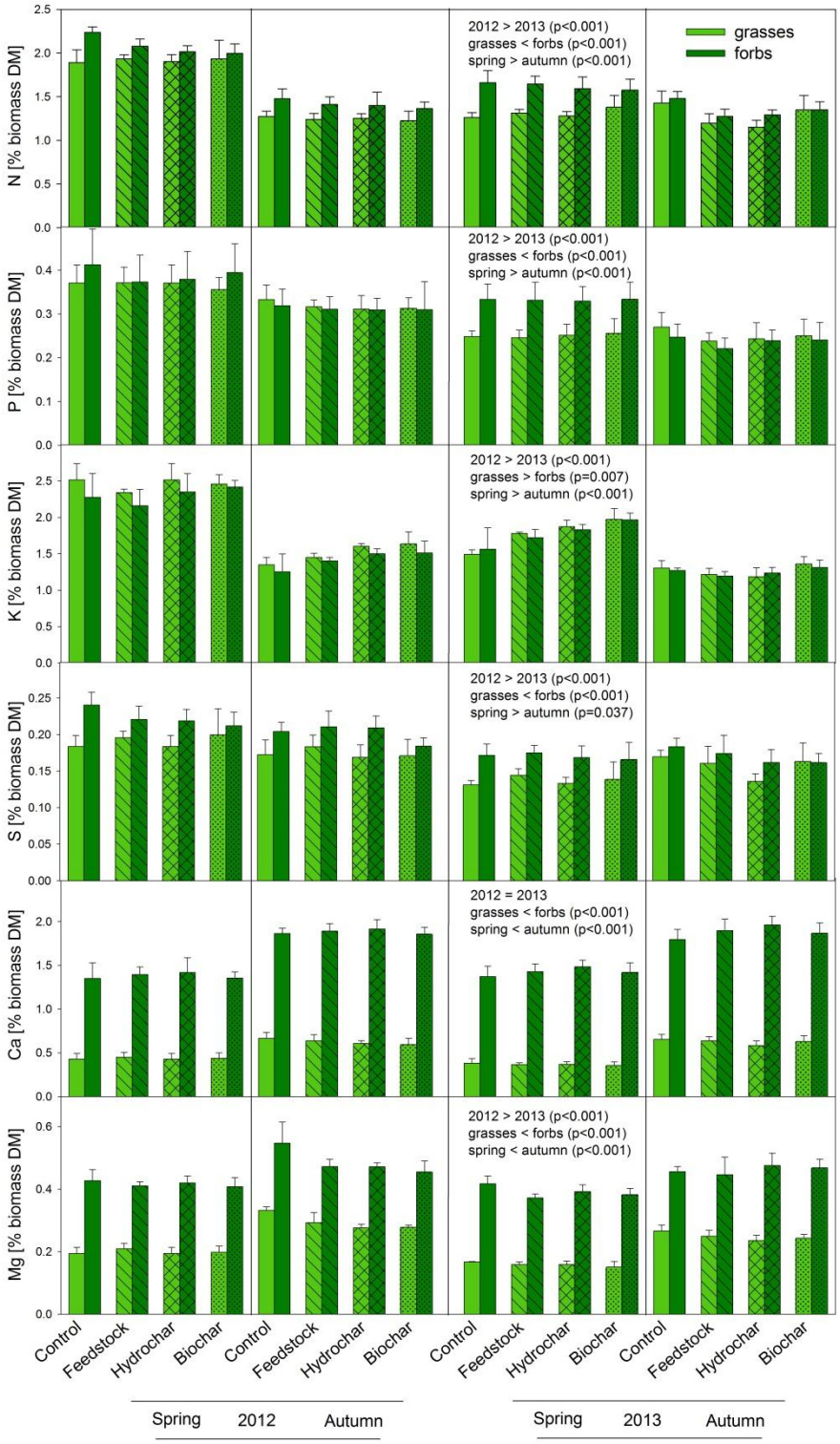

Figure 3-2: Mean macronutrient concentrations of the biomass dry matter (DM), sorted by treatment (control, feedstock, hydrochar, biochar), season (spring/ autumn), plant functional group (grasses/forbs) and year (2012/2013), n=64. Significant differences within the several factors, as determined by UNIANOVA, are given in the graphs.

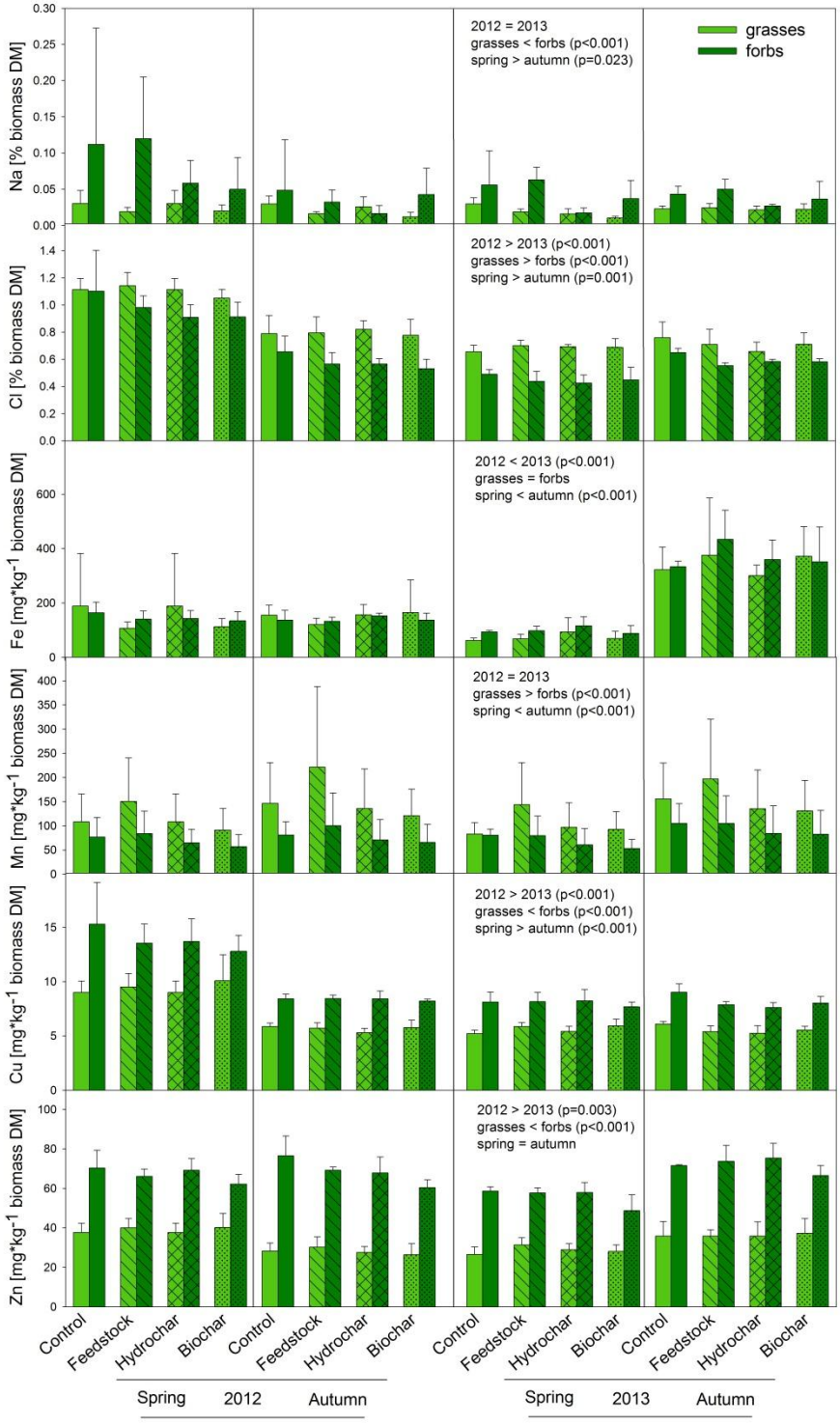

Figure 3-3: Mean micronutrient concentrations of the biomass dry matter (DM), sorted by treatment (control, feedstock, hydrochar, biochar), season (spring/ autumn), plant functional group (grasses/forbs) and year (2012/2013), n=64. Significant differences within the several factors, as determined by UNIANOVA, are given in the graphs

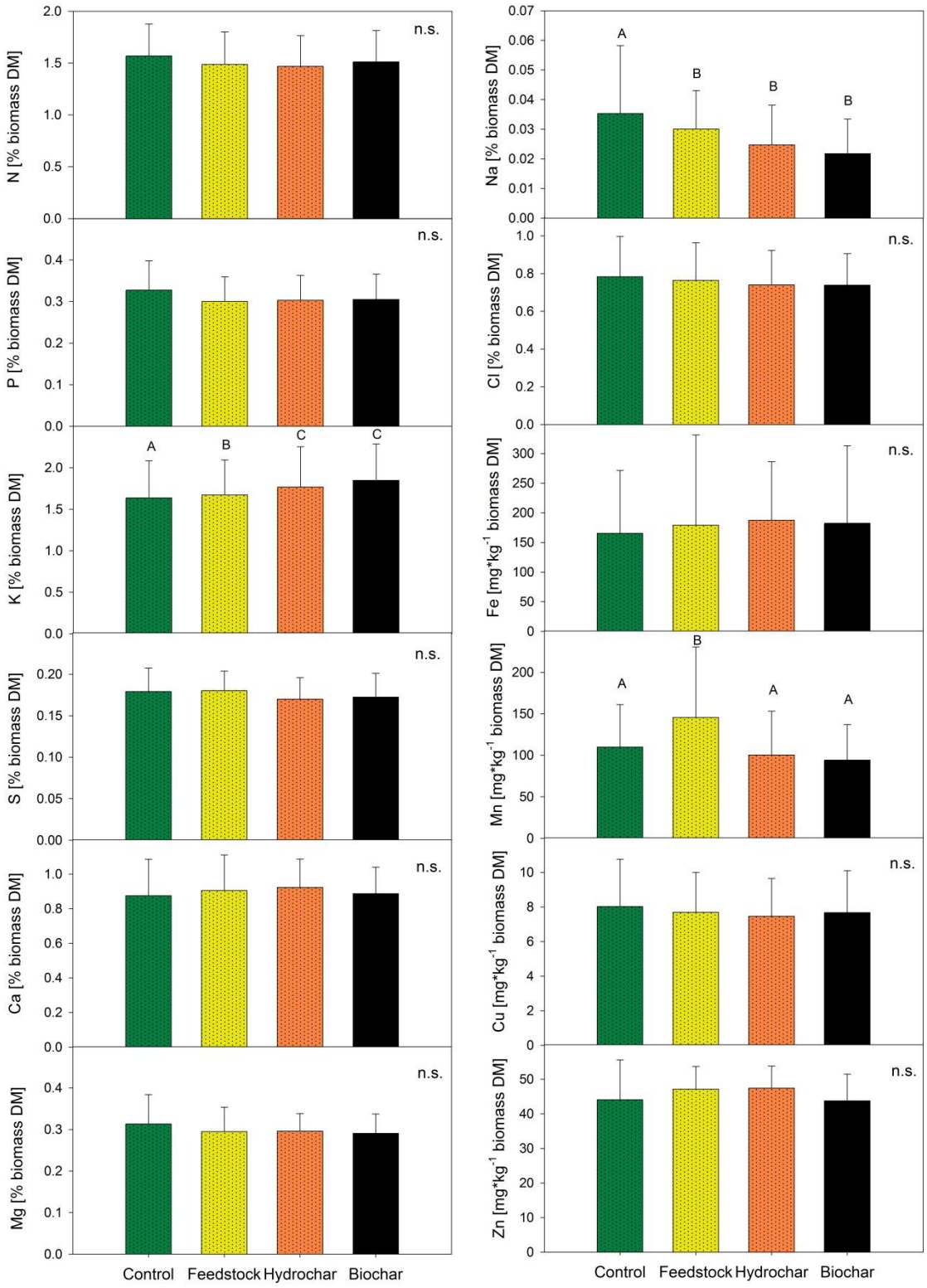

Figure 3-4: Mean weighted concentrations of the macro- and micronutrients in % or mg kg-1 biomass dry matter (DM) (biomass yield and composition were considered). Letters mark significant differences between the treatments (n=64).

In addition to the trace element and mineral concentrations in the plant biomass, we determined the total nutrient removal in the biomass per square meter (g m^{-2}) (Figure A.3-2 and A.3-3. The total nutrient removals differed according to harvest year, plant functional group, season and treatment, though not consistently for all nutrients. Nutrient removal of N, Mg, Zn and Na was equal in both years, higher in 2012 for P, K, S, Cu and Cl and higher in 2013 for Ca, Fe and Mn. Higher total amounts of most nutrients were taken up in spring, except Ca, Mg, Fe and Mn, where removal was higher in autumn in both years. Concerning the plant functional groups, removal of N, P, K, S and Mn was higher by grasses, removal of Ca, Mg, Cl, Fe and Zn higher by forbs and removal of Na and Cu was similar for both groups. Treatments affected total removal of K, Ca, Na and Mn. Potassium removal was higher in hydrochar and biochar plots, compared to feedstock and control plots. Vice versa, Na removal was lower in hydrochar and biochar plots compared to feedstock and control plots. Calcium removals were increased by all carbon amended plots compared to the control, whereas Mn removal was highest in feedstock amended plots compared to all other plots. Furthermore, removals of grasses and forbs interacted differently with the carbon amendments (Figure A.3-2 and A.3-3). No significant differences were found between the biomass nutrient concentrations of our experiment and the DLG values (Table 3-2). Differences in Cl concentrations are neglected since the reference value is derived from only one sample in the DLG table. The pH values at the experimental site remained constant with 5.8 averaged over depth (0-30 cm) and treatments in both years.

Table 3-2: Comparison of the recommended nutrient supply of a dairy cow with a mean performance of 30 kg milk per day and a daily intake of 20 kg biomass DM (German Society of nutritional physiology) with the nutrient contents of the biomass from the treatment plots in the field experiment. For a further comparison, long term mean values from German grasslands (German Agricultural Society fodder quality table, 1936-2014) are displayed. Numbers in brackets give the relative increase in % compared to the recommended intake values.

	P [g kg DM^{-1}]	K [g kg DM^{-1}]	Ca [g kg DM^{-1}]	Mg [g kg DM^{-1}]	S [g kg DM^{-1}]	Fe [mg kg^{-1} DM]	Na [g kg DM^{-1}]	Mn [mg kg^{-1} DM]	Cu [mg kg^{-1} DM]	Zn [mg kg^{-1} DM]	Cl [g kg DM^{-1}]
Recommended intake	3.55	10.15	5.75	1.6	1-2	50	1.4	50	8-10	50	3.35
Control mean	3.3±0.7	16.4±4.5 (+61%)	8.8±2.1 (+53%)	3.1±0.7 (+94%)	1.8±0.3	165.7±105.6 (+235%)	0.4±0.2	110.0±51.2 (+120%)	8.0±2.7	44.1±11.5	7.8±2.1 (+132%)
Feedstock mean	3.0±0.6	16.7±4.2 (+64%)	9.1±2.0 (+58%)	2.9±0.6 (+81%)	1.8±0.2	179.3±152.2 (+259%)	0.3±0.1	145.7±84.9 (+191%)	7.7±2.3	47.1±6.6	7.6±2.0 (+134%)
Hydrochar mean	3.0±0.6	17.7±4.9 (+74%)	9.2±1.6 (+60%)	3.0±0.4 (+94%)	1.7±0.3	187.6±98.8 (+275%)	0.2±0.1	100.5±52.8 (+101%)	7.5±2.2	47.5±6.4	7.4±1.8 (+121%)
Biochar mean	3.1±0.6	18.5±4.4 (+82%)	8.9±1.5 (+55%)	2.9±0.5 +81%)	1.7±0.3	182.4±130.8 (+265%)	0.2±0.1	94.4±42.7 (+89%)	7.7±2.4	43.8±7.7	7.4±1.7 (+121%)
Minimal requirement	0.5-1	3	0.6-1	0.5	n/s	n/s	0.3	n/s	8	20	n/s
Grass biomass (German Agricultural Society table)	2.9±0.7	23.4±9.3	5.5±2.4	2.0±0.8	-	-	0.5±0.6	78.9±29.3	7.2±1.9	38.3±11.7	4

3.4 Discussion

3.4.1 Plant functional group effects

The higher nutrient concentrations generally observed in the forbs, compared to grasses, throughout all treatments (Figure 3-2 and 3-3) may possibly be explained by an improved nutrient retention capability of forbs. This is common for nutrient-poor environments (Berendse *et al.*, 1992; Aerts, 1999), as defined by biomass N-concentrations < 1.5 % (Maynard *et al.*, 1976; Whitehead, 2000; Güsewell, 2004), a threshold which was barely exceeded by the biomass harvested in our experiment, and a N : P ratio <14 (Koerselman and Meuleman, 1996; Ellenberg and Leuschner, 2010) which, with an average of 4.9, has been clearly occurred. Moreover, the nutrient needs and uptakes of forbs e.g. for Ca and Mg are generally up to five times higher than that of grasses (Bergmann, 1992).

Potassium, Mn and Cl concentrations were higher in the grass biomass, when compared to forbs. For K, the uptake from soil by plants seems to be a function of root morphology, especially root length and surface area in the top soil (Schenk and Barber, 1980; Mengel and Steffens, 1985). Thus, a reason for significantly higher K concentrations in the grass biomass could be a higher fine and fibrous root density in the upmost layer of the soil, compared to forbs (Kutschera *et al.*, 1982; Sun *et al.*, 1997; Kutschera *et al.*, 2009). Grasses have been found to contain more Mn than legumes in other experiments, and Mn uptake by plants can vary substantially according to plant species and site (Garmo *et al.*, 1986; Bergmann, 1992; Lindström *et al.*, 2013). Nevertheless, in general, site variations, with varying soil properties such as pH values found to have a greater impact on Mn uptake than plant species and plant functional groups (Hemingway, 1962; Wagner and Kaupenjohann, 2014). In our experiment, effects of soil pH on the release of Mn seem unlikely since there were no treatment effects on soil pH over the experimental period.

To our knowledge, there are no previous reports on higher Cl uptake by grasses when compared to forbs. Differences in Cl concentration among the plant groups, grasses and forbs, may also be explained by differences in the abundance of fine and main roots as well as root depth. Significant effects of biochar amendment on biomass composition have been

reported elsewhere (van de Voorde *et al.*, 2014) with legumes benefiting most from an improved availability of P, K and higher pH values due to biochar amendment, whereas the abundance of forbs was not affected.

3.4.2 Dilution effects and nutrient use efficiency

Total harvested biomass increased from 2012 to 2013 by an average 113 g m^{-2}, although fertilization was higher in 2012. This may be explained by an earlier start of the growth period in 2013 and/or a cumulative fertilization effect (Figure A.3-1). Increased biomass growth together with lower biomass N concentrations in 2013, but constant nutrient removal by the plants, points to a dilution effect, also described as a Piper-Steenbjerg effect (Wikström, 1994). The same effect could possibly also apply to Mg with a constant nutrient removal but improved plant growth and hence lower nutrient concentrations, indicating that Mg availability triggered plant growth. Low Mg concentrations of the grass biomass (0.23 %, half the concentration of the forbs) together with an improved grass biomass growth in 2013 underline this assumption especially for grasses (Figures 3-1, 3-2 and 3-3).

A decreasing nutrient concentration, accompanied by lower nutrient removals and increasing biomass from 2012 to 2013, was found for P, K, S, Cu and Cl, pointing to a suboptimal nutrient supply, especially in 2013. Still, these nutrients were not growth limiting, and the higher biomass in 2013 was accompanied by a better nutrient use efficiency in this year. A positive linear relationship of concentrations, biomass growth and nutrient removal was only found for Fe, indicating a sufficient nutrient supply. Iron concentrations in the biomass were in line with the average Fe contents of plants (50-200 mg kg^{-1}), although the total iron content may not serve as the best criterion for the Fe status of plant biomass (Bergmann, 1992). Ca and Mn concentrations were similar in 2012 and 2013, accompanied by constant nutrient removal and higher nutrient use efficiency due to increased biomass growth for Ca and constant nutrient use efficiency and an increased removal in the case of Mn. This indicates that for these elements, the dilution effect does not apply, but neither was optimum supply attained. Sodium concentrations and removals were constant over both years, indicating that these elements were not limiting.

3.4.3 Treatment effects

3.4.3.1 Macronutrients: Nitrogen, Phosphorus, Potassium, Sulphur, Magnesium and Calcium

In general, removal and concentration of N in the biomass was not influenced by any carbon amendment, compared to the control plots, indicating that either the carbon amendments had no N-limiting effect, as was reported from other studies with *Miscanthus* straw (Eiland *et al.*, 2001) or sugar beet/wheat straw hydrochar (Gajić and Koch, 2012; Bargmann *et al.*, 2014b), or more N that could be immobilized was added with the slurry. Nevertheless, we observed a grass biomass growth reduction in the hydrochar amended plots in 2011, the year of application, which was likely caused by initial N-immobilization or phytotoxic effects (Bargmann *et al.*, 2013; Busch *et al.*, 2013). However, as the results presented here show, this short term effect was outbalanced in the following years. Higher biomass N concentrations due to biochar+N-fertilizer application to soil, as found in incubation experiments (Chan *et al.*, 2008; Van Zwieten *et al.*, 2010a; Schimmelpfennig *et al.*, 2014), were not observed, likely because the biochar was not directly mixed into the soil matrix due to top dressing. Moreover, biochars´ beneficial role in soil nitrogen cycling might not be the decisive factor in soils with a naturally high nitrifier activity as in the grassland used here (Kammann *et al.*, 1998; DeLuca *et al.*, 2006).

Potassium concentrations were increased in all carbon amended plots, and correlated with the initial K concentrations of the materials, indicating a fertilization effect. Removal of K was only higher in hydrochar and biochar amended plots, compared to the control (Figure A.3-2). The difference was made up mostly by the higher share of forbs in these plots, confirming the promotion of forb over grass biomass growth by the carbon amendments (Figure 3-1). Potassium was found to be easily leached from biochar by others as well, serving as fertilizer to plants (Gaskin *et al.*, 2010; Silber *et al.*, 2010; Yao *et al.*, 2010; Angst and Sohi, 2013; Wagner and Kaupenjohann, 2014). Likewise, K from hydrochar is reportedly plant available (Gajić and Koch, 2012) and water soluble (Wagner and Kaupenjohann, 2014). A long-term K fertilization effect due to carbon amendment has been described as unlikely (Angst and Sohi, 2013), but has also been found in a natural

grass system before (van de Voorde *et al.*, 2014). Besides the direct fertilization effect, the sorption of K from slurry by negatively charged functional groups on the biochar/hydrochar surface could also be a reason for improved K availability in our experiment (Sevilla and Fuertes, 2009; Mukherjee *et al.*, 2011).

Both Ca and Mg contents were concentrated by the hydrothermal carbonization and pyrolysis process, compared to the feedstock material, nevertheless the nutrient amounts applied with all carbon amendments were quite low but were highest with hydrochar amendment (7.8 g m^{-2}) (Table 3-1). Similar Ca and Mg concentrations of the biomass from carbon amended plots, combined with a higher nutrient removal are likely due to the overall higher share of forbs in the carbon amended plots in the total biomass of the two years, compared to the control (Figures 3-1, A.3-2). The higher Ca and Mg removal in the hydrochar plots, compared to the control plots, indicate that Ca and Mg from hydrochar were easily available to plants, especially to forbs (Figure A.3-2). The influence of hydrochar on nutrient uptake or concentrations in plants is often masked by a reduced plant growth due to N-limitation or toxic effects (Gajić and Koch, 2012; Busch *et al.*, 2013; Jandl *et al.*, 2013; Wagner and Kaupenjohann, 2014) Growth reduction by N-limitation was not observed in our experiment, nevertheless N supply was rather low and a possible fertilizer effect of Ca and Mg may have been masked by suboptimal N supply.

3.4.3.2 Micronutrients: Iron, copper, zinc, manganese, sodium and chloride

Iron, Cu Zn and Cl concentrations in plant biomass were not influenced by any of the carbon amendments, compared to the control. Concentrations of Fe in the biomass suggest sufficient Fe supply in both years and all treatment plots. Surprisingly, the Fe concentrations of the carbon amendments were not reflected in the biomass from the corresponding plots (Table 3-1, Figure 3-3). This indicates that the carbon amendments were either not degraded enough to release the Fe bound in the material or that if Fe became soluble that it was taken up during the first year after application or bound in organo-mineral complexes. Bioavailability of Fe from plant residues was reported by others to be highest within the first year of amendment, even more if decomposition of the residues was enhanced by earthworms, fungi or composting (Palviainen *et al.*, 2004;

Maqueda *et al.*, 2011; Bityutskii *et al.*, 2012). The Cu results indicate that, although additional Cu was introduced with all carbon amendments, the applied surplus was apparently not available to plants. Elevated leaf concentrations of Cu were so far only reported from biochar produced from Cu-contaminated wood waste with a concentration of 22.1 g kg^{-1} (a concentration a thousand fold higher than in biochar from non-contaminated biomass), indicating that Cu from biochar itself may become available to plants at some stage (Lucchini *et al.*, 2014). Presumably, in our experiment, Cu is bound to the organic carbon fraction of the soil, in a form being not readily plant available (Sims, 1986; Beesley *et al.*, 2010).

Zinc accumulated in the carbon amendments due to the carbonization processes. Nevertheless, Zn did not accumulate in the biomass grown on carbon supplemented plots, indicating that it was not plant available. Hydrochar and biochar did not influence plant biomass Mn concentrations or uptake, although substantial amounts of Mn were added with both amendments to the soil, especially with biochar (Table 3-1). In contrast, feedstock amendment significantly increased Mn concentrations and uptake of the plants. However, Mn toxicity was not observed and has only been reported for plant Mn concentrations > 1000 mg kg^{-1}, though this depends on the plant species, and soil pH (Bergmann, 1992). It seems likely, that the provision of easily decomposable organic matter such as *Miscanthus* straw caused Mn complexes in the soil to change from less soluble forms to more plant-available forms, likely by participating in redox reactions, dissolving Mn oxides (Stone and Morgan, 1984; Shuman, 1988). It is assumed, as on-going bioturbation will foster degradation and merging of hydrochar and biochar with the soil matrix, either Mn bound in these materials will become plant available and/or hydrochar and biochar may participate in soil redox reactions, as found by Graber *et al.* (2014).

Higher Na concentrations and uptake by biomass from control plots compared to all other plots was remarkable, since Na added with the carbon amendments was in very small amounts. Considering the additional K, Ca and Mg applied with the carbon amendments, an ion antagonism in the feedstock, hydrochar and biochar plots could likely explain these differences, leading to a reduced uptake of Na. Nevertheless, the range of Na concentrations in the harvested biomass neither indicates Na deficiency nor excess.

Generally, Na deficiency is rarely reported from grassland and likewise, concentrations found here are well below critical values for excess supply (Bergmann, 1992).
Chlorine was neither added in considerable amounts with the carbon amendments, nor did the carbon amendments influence the soil´s Cl availability by complexation with e.g. Na. Thus, the effect of carbon amendments on Cl availability was negligible in the grassland under study.

3.4.4 Fodder quality

The fodder quality of the plant biomass from all plots under study with respect to the micro- and macronutrients meet (P, S, Cu and Zn) or even exceed the recommended intake for dairy cows, especially in terms of the base cations K, Ca and Mg as well as Fe, Mn and Cl (Table 3-2), without any significant positive nor negative effects of the carbon amendments. The concentration of K in the biomass of all treatments exceeded the recommended intake by 70 % on average, indicating that K supply for animal nutrition was more than sufficient, independent of carbon amendment. Excessive K intake with biomass feed can disturb Mg resorption of ruminants, leading to grass tetany in the worst case (Terörde, 1997). Nevertheless, an extra supply of Mg is only essential if K concentrations increase to 35 g kg^{-1} dry biomass or more (Kessler, 2001), which have not been reached in our experiment by far. Magnesium concentrations in our biomass were up to 94 % higher compared to the recommended intake, independent of carbon amendment, accompanied by an increase in Ca contents of up to 60 %. High Ca concentrations in biomass can reduce fodder quality if Mg, P or Vitamin D supplies are insufficient (Terörde, 1997). Commonly, a K/(Ca+Mg) ratio $\leq$ 2.2 is regarded as an indicator for low nutrient antagonism and an adequate nutritive balance (Reid and Horvath, 1980). This threshold has been met by all of our biomass with an average ratio of 1.35. In this context, high Mg and Ca concentrations as found here, can rather be considered positive. Furthermore, the Ca : P ratio of biomass can serve as indicator for an optimal uptake of Ca by ruminants. Best Ca uptake rates by ruminants are reported for biomass with a Ca : P ratio of 2 (Terörde, 1997). The mean Ca : P ratio of our biomass was with 2.9 rather high and was highest in biomass from hydrochar amended plots. Nevertheless, if requirements concerning P supply are met, the

ability of ruminants to adapt to fluctuations in Ca supply is high (Flachowsky *et al.*, 2001; McDonald, 2002). Thus, rather high Ca contents as observed here are not considered harmful for animal nutrition. Moreover, hydrochar and biochar application together with an additional P-source such as slurry may be an option to increase the fodder quality of biomass grown on such char amended soil (Sarkhot *et al.*, 2013). Iron contents in biomass from grazing land often exceed the requirements for Fe intake of ruminants, as also found here. Tolerance levels for iron are reportedly as high as 500 mg kg^{-1}, which was not reached by the biomass in any of the treatments, with a mean Fe concentration of 178.8 mg kg^{-1} (Table 3-2) (Flachowsky *et al.*, 2001).

3.5 Conclusion

Our results indicate that uncarbonized and carbonized *Miscanthus* × *giganteus* materials had neither positive nor adverse effects on biomass production in the second and third year after application. The main limitation to plant growth at the experimental site in all treatment plots was probably N, possibly masking other nutrient effects. Interactions of the carbon amendments with slurry did not occur, neither improving nor worsening the nutrient use efficiency of slurry amendment. Though total biomass growth was not affected by the carbon amendments, biochar led to a shift from grasses to forbs, leading to a total yield enriched in most nutrients. Both hydrochar and biochar led to increased K concentrations in the biomass over the two years, with a positive correlation to the initial K contents of the carbon amendments. Likewise, K removal by biomass was improved in hydro- and biochar plots, accompanied by a decreased removal of Na, indicating an ion antagonism. This leads to the assumption that, even two years after application, hydrochar and biochar exhibited a K fertilization effect. Additionally, Ca removal by biomass, especially forbs, was increased by all carbon amendments, indicating, together with Mg in hydrochar plots, plant available Ca and Mg components in the carbon amendments.

Heavy metals (Fe, Cu and Zn) were concentrated in hydrochar and biochar due to the carbonization processes, but did not accumulate in plant biomass accordingly. In terms of Mn, feedstock application led to an increased Mn concentration and removal of the plant biomass, indicating participation of the feedstock material in redox reactions in soil. Both

in terms of plant and animal health, application of carbon amendments did not harbour major risks. Recommended intakes have been met or exceeded for all nutrients except Na.,

3.6 Acknowledgments

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3.7 Appendix

Table A. 3-1: List of single grass, forb and legume species in the grassland under study

Grasses	Forbs	Legumes
Arrhenaterium elatius	*Achillea millefolia*	*Lathyrus pratensis*
Alopecurus pratensis	*Ajuga reptans*	*Lotus corniculatus*
Agrostis capillaris	*Anthriscus sylvestris*	*Medicago lupulina*
Anthoxanthum odoratum	*Bellis perennis*	*Trifolium pratensis*
Avena pubescens	*Campanula rotundifolia*	*Trifolium repens*
Dactylis glomerata	*Centaurea jacea*	*Vicia cracca*
Deschampsia cespitosa	*Cerastium holosteoides*	*Vicia sepium*
Festuca pratensis	*Cirsium oleraceum*	*Lathyrus pratensis*
Festuca rubra	*Crepis biennis*	*Lotus corniculatus*
Holcus lanatus	*Filipendula ulmaria*	*Medicago lupulina*
Lolium perenne	*Galium mollugo*	*Trifolium pratensis*
Luzula campestris	*Galium verum*	*Trifolium repens*
Phleum pratensis	*Geranium pratensis*	*Vicia cracca*
Poa pratensis	*Glechoma hederacea*	*Vicia sepium*
Poa trivialis	*Leontodon autumnalis*	
Trisetum flavescens	*Leucanthemum vulgare*	
	Lysimachia nummularia	
	Myosotis arvensis	
	Plantago lanceolata	
	Ranunculus acris	
	Ranunculus repens	
	Rumex acetosa	
	Sanguisorba officinalis	
	Saxifrage granulata	
	Sensecio jacobae	
	Stellaria graminea	
	Taraxacum officinalis	
	Veronica chamaedris	

Table A. 3-2: Detection limit, range limit and uncertainty of measurement of the X-ray fluorescence measurement process

Element	**P [%]**	**K [%]**	**S [%]**	**Ca [%]**	**Mg [%]**	**Na [%]**	**Cl [%]**	**Fe [mg kg^{-1}]**	**Mn [mg kg^{-1}]**	**Cu [mg kg^{-1}]**	**Zn [mg kg^{-1}]**
Detection limit	0.1	0.6	0.05	0.1	0.07	0.02	0.1	100	40	4	15
Range limit	0.44	3.2	0.3	1.7	0.44	0.26	0.9	1300	310	20	180
Uncertainty of measurement	1.7	1.3	1.4	1.6	1.8	4.5	1.4	2.8	1.7	5.6	2.3

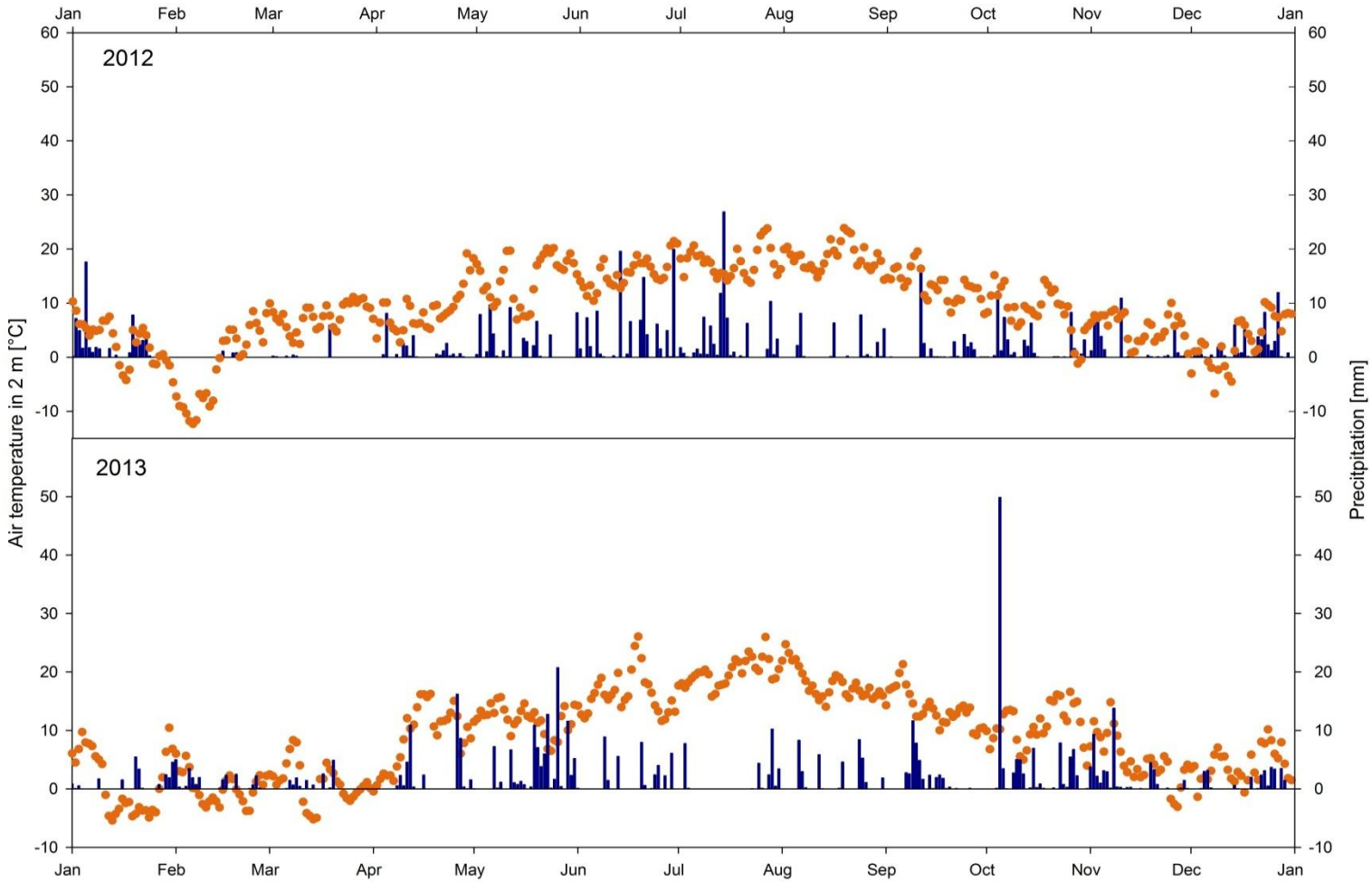

Figure A. 3-1: Mean air temperature in 2 m [°C] and precipitation [mm] of the experimental site in the years 2012 and 2013.

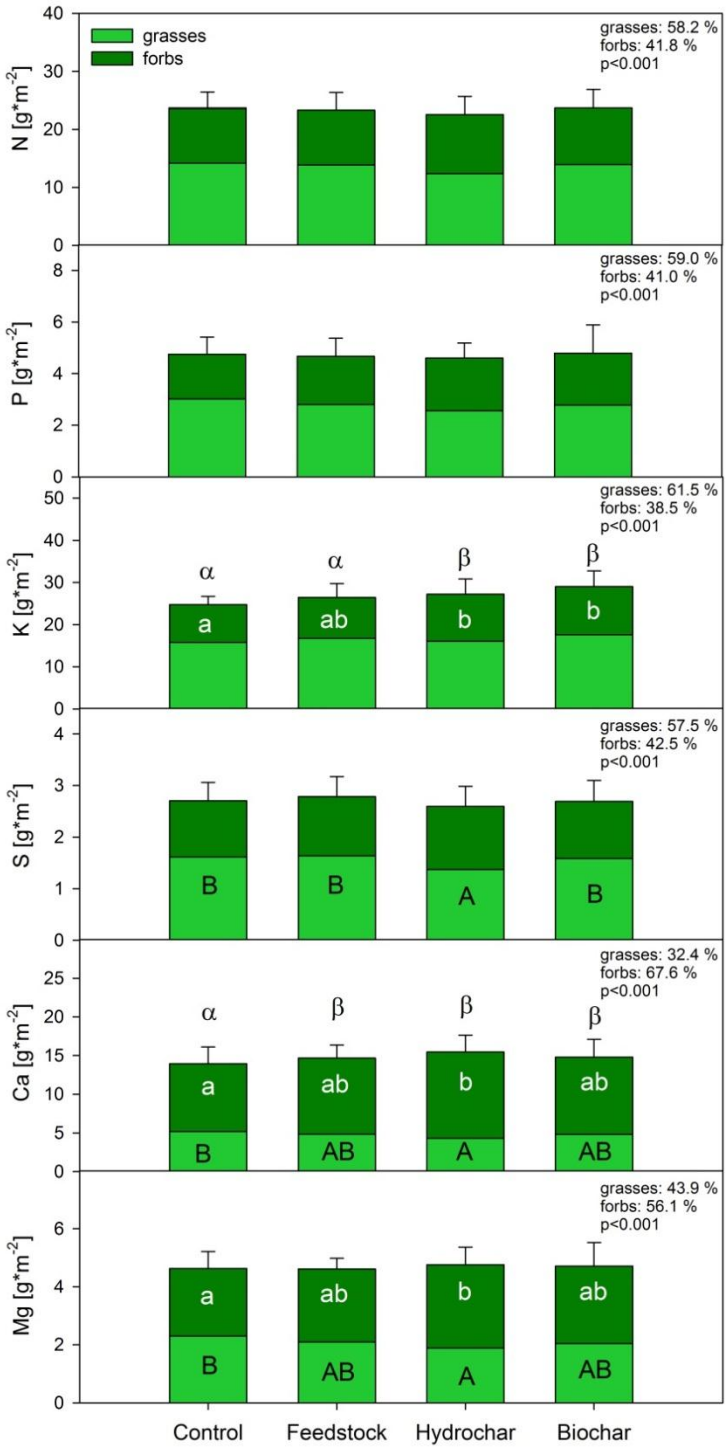

Figure A. 3-2: Mean macronutrient removals [g m^{-2}] of the two years 2012 and 2013, shown for the different treatment plots with standard deviation (n=64). Arabic letters mark significant differences in nutrient removal by grasses and forbs, respectively. Greek letters mark significant differences of the overall biomass removal (grasses+forbs).

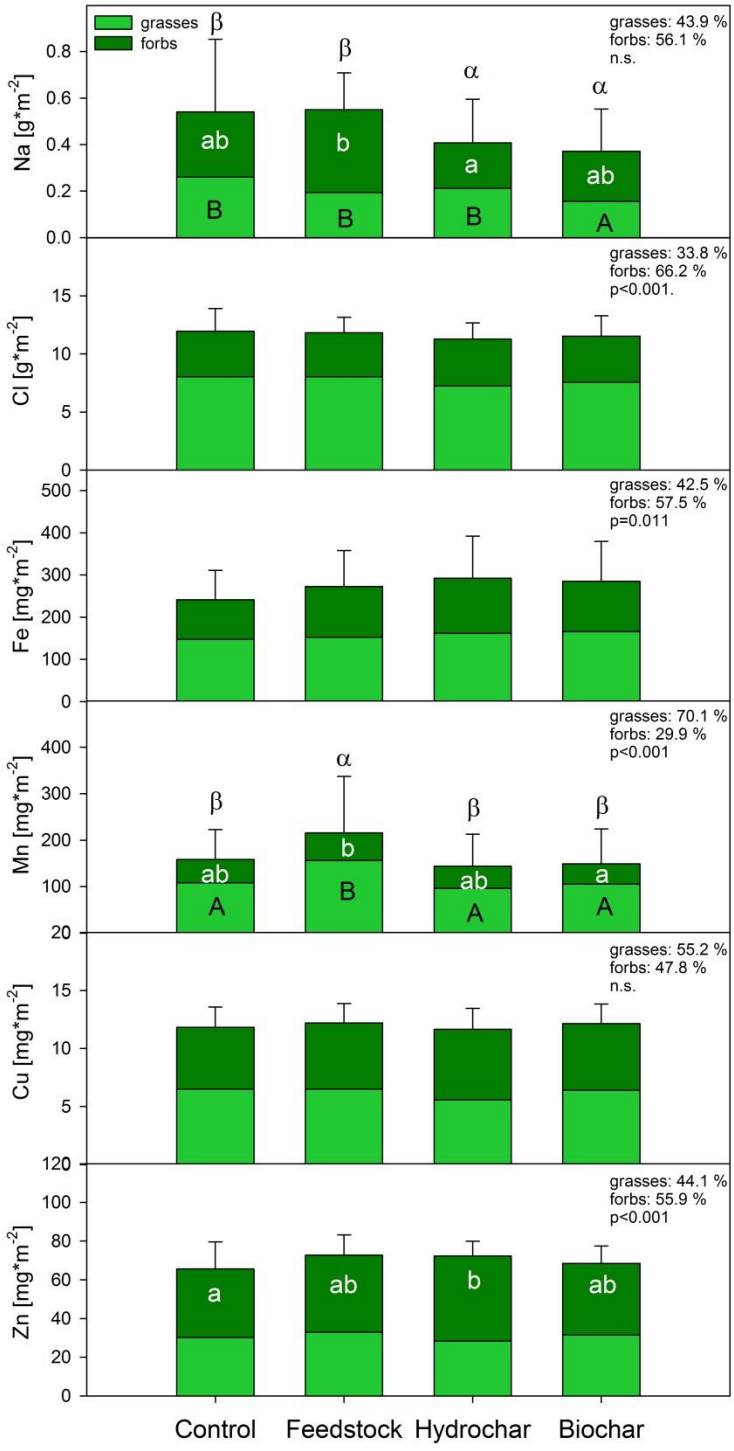

Figure A. 3-3: Mean micronutrient removals [g m^{-2}] of the two years 2012 and 2013, shown for the different treatment plots with standard deviation (n=64). Arabic letters mark significant differences in nutrient removal by grasses and forbs, respectively. Greek letters mark significant differences of the overall biomass removal (grasses+forbs).

4 Microbial community shifts 2.6 years after top dressing of *Miscanthus* biochar, hydrochar and feedstock on a temperate grassland site

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Abstract

Within the last decades, considerable knowledge has been gained on the impacts of carbonaceous soil additives such as hydrochar (or HTC) and biochar (or pyrochar) on plant growth and various soil properties. However, still little is known about the effects of hydrochar and biochar on soil microorganisms, especially from field studies. Microorganisms are closely linked to nutrient dynamics in soil and therefore are tightly linked to soil fertility. As a consequence, possible changes in the microbial community structure due to HTC/biochar soil application may lead to considerable changes in soil nutrient dynamics.

To gain insights in HTC/biochar associated long-term effects on microorganisms, soil samples were taken from a grassland field study 2.6 years (31 months) after its initiation (April 2011), where *Miscanthus* × *giganteus* feedstock, HTC and biochar mixed with pig slurry had been applied as top-dressing in a randomized block design, next to a slurry-only control (n=4, 16 plots). The samples were analyzed for microbial activity and biomass by substrate induced respiration (SIR). Bacterial and fungal fractions in soil microbial biomass (SMB) were determined using the inhibitors Streptomycin and Cycloheximide respectively.

Total SMB, microbial activity and fungal biomass were significantly higher in biochar-amended soil compared to feedstock and control treatments. The percentage of bacterial biomass was higher in the feedstock and HTC amended soil, as compared to the control. Additionally, HTC exhibited a significantly higher percentage of fungal biomass compared to the feedstock treatment, indicating a microbial community shift.

While the uncarbonized feedstock material depleted both total SMB and especially fungi, HTC and biochar did not trigger any adverse long-term effects on SMB. Rather, the observed biochar-induced stimulation of SMB may improve soil aggregation and increase the soil organic carbon content in the long term.

Keywords: biochar; hydrochar; field study; *Miscanthus* × *giganteus*; microorganisms; fungi; bacteria; microbial community shift

4.1 Introduction

Since the discovery of Terra Preta soils and the identification of charcoal as an essential component therein, many experiments on the production and application of carbonized plant material to soil have been realized. Among a variety of production processes, pyrolysis of dry plant material at 400-600°C proved to yield a low-toxic, highly porous material similar to charcoal or stone coal, suitable for soil amendment and carbon sequestration (biochar) (Schimmelpfennig and Glaser, 2012; Singh *et al.*, 2012). On the other hand, a wet, high pressure pyrolysis process, hydrothermal carbonization, suitable for the usage of wet feedstock material such biological waste (Kruse *et al.*, 2013), may generate a material suitable as peat substitute (Titirici *et al.*, 2007; Libra *et al.*, 2011). However, the effects of biochar and hydrochar (HTC) on soil microbial biomass (SMB) are still poorly understood, particularly beyond initial, short-term lab-study effects. In the short-term (< one year), biochar reportedly induced large changes in SMB composition and activity, with beneficial effects on soil and/or plant productivity (Anderson *et al.*, 2011; Kolton *et al.*, 2011; Lehmann *et al.*, 2011). These positive effects may result from biochar-induced changes in pH-value, generation of carbon-nutrient agglomerates in soil (Castaldi *et al.*, 2011), sorption of toxic substances such as heavy metals or provision of an additional C-source by biochar (Drenovsky *et al.*, 2004). Additionally, biochar was found to provide a habitat for mycorrhizal fungi, particularly by deliberate inoculation in the laboratory compared to the field (Saito, 1990; Saito and Marumoto, 2002; Quilliam *et al.*, 2013; Hammer *et al.*, 2014). However, a decrease of microbial biomass following the soil application of biochar has also been observed, resulting in lower soil C and N turnover rates (Dempster *et al.*, 2012).

HTC was found to have positive effects on growth, root colonization and spore germination of mycorrhizal fungi (Rillig *et al.*, 2010; Salem *et al.*, 2013a) as well as on the activity and abundance of SMB (Bargmann *et al.*, 2014a). Negative effects of HTC on mycorrhiza have also been reported, pointing to the occurrence of toxic compounds, mostly present in the water soluble carbon fraction (George *et al.*, 2012), whereas collembola ingested and digested unwashed HTC without being negatively affected and may even gain nutritional benefits from it (Salem *et al.*, 2013b). Yeast-derived HTC

promoted fungi, while glucose-derived HTC was mostly taken up by bacteria, indicating the importance of HTC feedstock material for any effects on microorganisms (Steinbeiss *et al.*, 2009). Besides the feedstock material, the effect of biochar and HTC on SMB depends on the production process conditions, the time following the addition to soil and the soil management and fertility (Salem *et al.*, 2013a; Muhammad *et al.*, 2014).

Considering the wide influence of SMB on the soil nutrient cycling and thus on plant growth, it is important to identify the effects of biochar/HTC on microorganisms more clearly. Yet, knowledge of the long-term effects of biochar/HTC on SMB is not sufficient to predict possible changes in an adequate way.

The economic feasibility and ecological sustainability of future large-scale carbon sequestration projects will *inter alia* depend on the availability of fast growing plants for biochar/HTC production. The *Miscanthus* × *giganteus* hybrid, a perennial C4 plant, attracted attention as a fast growing biomass plant for cultivation in temperate soil (Clifton-Brown *et al.*, 2004; Heaton *et al.*, 2008). Besides its high biomass production, the high lignin and the naturally enriched ^{13}C content (Clifton-Brown *et al.*, 2004; Pyter *et al.*, 2009; Sang and Zhu, 2011) make it an attractive biochar/HTC feedstock. Moreover, positive effects of *Miscanthus* × *giganteus* biochar on SMB have already been reported by other authors (Luo *et al.*, 2013). Thus, our aim was to evaluate the effects of field application of *Miscanthus* × *giganteus* straw, hydrochar and biochar on SMB abundance and composition after a period of 2.6 years. Based on the available studies so far, the hypothesis was that biochar would increase the abundance of both fungi and bacteria by providing a suitable habitat due to its porous nature, that HTC would promote fungal growth due to its low pH and that feedstock and HTC would support the growth of all microorganisms due to provision of a rather labile C-source.

4.2 Material and methods

4.2.1 Experimental setup

Soil samples were taken in October 2013 from an experimental grassland site of the Justus-Liebig-University Giessen, located in Linden, Germany (50°31'58.2"N 8°41'07.0"E). The

soil, a Haplic Stagnosol (WRB, 2006), had a texture of 25 % sand, 28 % clay, 47 % silt, a soil organic content (SOC) of 3.6 % and a pH of 5.8-6.0. The biochar field experiment had been installed in April 2011. A top-dressing of uncarbonized *Miscanthus* straw (feedstock, 16t ha^{-1}), hydrothermally carbonized *Miscanthus* straw (HTC, 14.5t ha^{-1}) and pyrolyzed *Miscanthus* straw (biochar, 9.3 t ha^{-1}) had been applied randomly in a quadruplicate block design, increasing the SOC content of the upper soil layer (calculated for a depth of 10 cm, bulk density = 1 g cm^{-3}) by about 16 ± 4 %. HTC material was produced by keeping *Miscanthus* straw in a water vapor atmosphere for 2 hours at a temperature of 200±3°C under pressure of 1.6 MPa (Revatec, Geeste, Germany, formerly Hydrocarb GmbH, Ohmes, Germany). Biochar was produced by pyrolyzing dry *Miscanthus* straw at 550-600°C in a continuous flow reactor (Pyreg GmbH, Bingen). The plots were fertilized with liquid pig slurry twice every year since installation of the experiment (Schimmelpfennig *et al.*, 2014). Two soil samples (volume of 231.3 cm^3) were taken from the upper soil layer (depth of 5cm) of each plot. The samples were sieved through a 2 mm mesh to separate soil and roots and stored at 4 °C for further use. Substrate induced respiration (SIR) experiments with inhibitors were carried out between November 15 and December 13, 2013. The SIR measurements without inhibitors were performed from the 17th till the 27th of March 2014.

4.2.2 Soil measurements

For carbon (C) and nitrogen (N) content determination, subsamples were oven dried at 105°C, milled and analyzed by a CNH Macro Elemental Analyzer (Hanau, Germany). For determination of the water-holding capacity (WHC), three subsamples of each treatment were weighed into a PVC-tube (closed at the lower side with mesh and filter paper) and placed into a water tank for 24 hours, to ensure that the soil mixtures were soaked with water. Thereafter the samples were taken out of the water tank, left to drain on a colander for 24 hours and reweighed. Soil water content was determined by placing another subsample in the oven for 24 hours at 105°C. The WHC for each treatment was calculated by the difference in mass between the saturated and the dry samples in relation to the dry weight, using equation 4-1:

$$WHC = \frac{(\text{soil}_{wet} - \text{soil}_{dry})}{\text{soil}_{dry}} \quad (4\text{-}1)$$

soil_{wet} water soaked, drained soil [g]
soil_{dry} dry soil [g]
WHC water holding capacity in g H_2O/g soil

4.2.3 Basal and substrate induced respiration

The basal and substrate induced respiration of microorganisms was determined following the DIN ISO (17155:2011-06 2011) guideline, based on the work of Anderson and Domsch (1973); Anderson and Domsch (1986); Anderson and Domsch (2010). Briefly, 50 g (dry weight) of field fresh soil mixture was put into a glass jar (200 ml) in three (control soil) or four repetitions (feedstock, hydrochar and biochar amended soil) and adjusted to 50 % of the WHC. The open jars were pre-incubated for three days in a dark climate chamber (20 °C, 50 % relative air humidity) to prevent overestimation of CO_2 fluxes due to soil disturbance and to trigger germination of potentially available seedlings. After pre-incubation, the jars were connected to a multiplexer coupled to an automated gas analyzer (Licor 8100A GmbH, Bad Homburg, Germany). CO_2 emissions from every jar were measured for 120 s during basal respiration, with one data point per second (Figure A. 4-1 a). In between the measurement of two jars, the analyzer was flushed for 30 s with ambient air. One measurement cycle (38 min in total starting and ending with an empty jar) was repeated consistently for the whole basal respiration period (in total 70 – 100 hours). To prevent desiccation of the soil mixtures, the jars were vented with water-saturated air from a humidifier in between the measurements. Because of total available multiplexer connections only 15 samples could be analyzed at one time. Therefore only for the control treatment three instead of four samples were analyzed. The soil CO_2 effluxes, based on the soil dry weight, were automatically calculated by regression analysis, with both exponential and linear fits.

For the determination of the microbial biomass by the substrate-induced respiration (SIR), 500 mg of a mixture of glucose, $(NH_4)_2SO_4$ and KH_2PO_4 in the ratio 6:1:0.1 was added to each sample jar. Additionally, 250 mg Talcum, used as the carrier substance for the

inhibitors, was added to the jars for SIR measurements to ensure similar conditions as during inhibitory measurements. Each of the jars was mixed thoroughly after addition of the glucose mixtures. For SIR measurements, the data logging time was reduced to 90 s per jar (Figure A. 4-1 b).

The experiment is based on the assumption that non-inhibited microorganisms are activated by the addition of glucose due to maximum cell respiration. Thus, the period between the first respiration peak caused by mixing of the soil and the second peak where proliferation of the microorganisms starts, can be interpreted as "plateau period" where nearly all microorganisms are active but still in the same abundance as before substrate addition. Consequently, the CO_2 respiration rates during the plateau period are suitable for the calculation of the soil microbial biomass (SMB) (Figure A. 4-2).

4.2.4 Determination of fungal and bacterial contribution to respiration

To determine the fungal and bacterial contribution to the bulk microbial respiration during the plateau period, we used 1) Streptomycin for the inhibition of bacteria (SIRINs) and 2) Cycloheximide for the inhibition of fungi (SIRINc) and 3) both inhibitors to suppress both fungal and bacterial growth (SIRINsc). Neither other microorganisms such as cyanobacteria, archaea, ciliates or amoeba could be captured by this method, nor the possible side effects such as promotion or die-off of non-targeted organisms (Badalucco *et al.*, 1994).

The fungi-associated respiration (AFR) was calculated by equations 4-2 and 4-3:

$$PFR = \frac{(R_s - R_{sc})}{\left((R_s - R_{sc}) + (R_c - R_{sc}) + R_{sc}\right) * 100^{-1}} \quad (4\text{-}2)$$

$$AFR = R_{total} * PFR \quad (4\text{-}3)$$

Where PFR is the portion of fungal respiration in µg CO_2 g^{-1} soil, R_s is the respiration during the plateau period from SIRINs with inhibited bacteria, R_c is the respiration during the plateau period from SIRINc with inhibited fungi, R_{sc} the respiration of plateau period from SIRINsc with inhibited bacteria and fungi, R_{total} the respiration of the plateau period

from the SIR measurement without inhibitors and AFR the amount of fungal respiration under SIR conditions.

Bacterial respiration (ABR) was calculated using the equations 4-4 and 4-5, with R_s, R_c, R_{sc} and R_{total} being the same as in equation (4-2) and (4-3):

$$PBR = \frac{(R_c - R_{sc})}{((R_s - R_{sc}) + (R_c - R_{sc}) + R_{sc}) * 100^{-1}} \quad (4\text{-}4)$$

$$ABR = R_{total} * PBR \quad (4\text{-}5)$$

where PBR is the portion of bacterial respiration in µg CO_2 g^{-1} soil and ABR the amount of bacterial respiration from SIR measurements.

4.2.5 Determination of soil microbial biomass

Soil microbial biomass (SMB) was calculated from the CO_2 respiration rates during the plateau periods of the SIR and SIRIN measurements following equation 4-6, as given by DIN ISO (17155:2011-06 2011):

$$SMB = Cmic * SIR = 20.6 * R_{SIR} + 0.37 \quad (4\text{-}6)$$

where R_{SIR} is the substrate induced respiration in CO_2 g^{-1} soil h^{-1} and $Cmic * SIR$ is the microbial biomass in µg SMB g^{-1} dry soil mix. The constants 20.6 and 0.37 were given by DIN ISO (17155:2011-06 2011).

4.2.6 Statistics and calculations

The measured unit µmol CO_2 kg^{-1} s^{-1} was converted to µg CO_2 g^{-1} soil h^{-1} using a molar mass of CO_2 of 44.01 g mol^{-1}. Calculations were performed with Microsoft Excel 2007; statistics were carried out using SigmaPlot 11.0. All recorded flux data were checked for its R^2 value. Only data with an $R^2 > 0.9$ were considered for statistical analysis. Basal respiration rates were relatively constant, and therefore were described by the mean value and standard deviation calculated from all fluxes per jar during the basal respiration

measurement period. Bacterial and fungal contributions to respiration were calculated based on fluxes during the plateau periods of the SIR and SIRIN measurements. Subsequently, the respective microbial biomass was calculated based on the mean ± standard deviation of the respiration rates during the plateau period for each jar, resulting in 12 (control: 9) single values for each treatment. Treatment differences of normally distributed data were tested by a one way ANOVA followed by Holm-Sidak Post-hoc tests (SMB and WHC, $p < 0.05$). If the data lacked normal distribution, significant differences were determined by Kruskal-Wallis tests, followed by Dunn´s post-hoc tests (basal respiration rates, SIR/SIRIN plateau respiration rates, C and N contents and C/N ratio, $p<0.05$). Correlations of fungal and bacterial biomass with C/N ratios were determined by Spearman Rank Orders. For an evaluation of the microbial efficiency, we determined the CO_2 output per unit biomass (qCO_2), defined by the ratio of CO_2 respiration during basal respiration and unit of microbial biomass (ng CO_2 μg^{-1} SMB h^{-1}); lower values indicate a higher efficiency.

4.3 Results

4.3.1 Soil characteristics

The mean WHC of the four treatments was 1.3 g H_2O g $soil^{-1}$, with a significantly lower WHC in HTC soil compared to feedstock (Figure A.4-3). The C-contents of the soil mixtures did not differ according to the treatments; feedstock amended soil exhibited significantly higher N-contents compared to HTC and biochar-soil mixtures, leading to a lower C/N ratio compared to HTC (Table 4-1).

4.3.2 Basal respiration and respiration during plateau periods

The basal respiration was increased by all carbon amendments compared to the control ($p < 0.05$). Biochar showed the highest basal respiration, followed by feedstock and HTC. Basal respiration values ranged between 3-14 µg CO_2 g^{-1} h^{-1}. Biochar led to a significant

increase of 34 % in basal respiration (10.25 µg CO_2 g^{-1} h^{-1}) compared to the control soil (6.7 µg CO_2 g^{-1} h^{-1}) (Figure 4-1).

The plateau period following glucose and inhibitor addition could be identified clearly throughout all experiments, with the earliest start after 1.8 hours and the earliest end, due to the start of proliferation, after 6.8 hours (Figure A.4-2). The CO_2 fluxes during the plateau periods were much higher than basal respiration measurements, and different among the treatments. The qCO_2 of the feedstock was significantly higher compared to the control and biochar (4.7 ± 0.23 vs 2.53 ± 0.89 and 2.49 ± 0.85) (Table 4-2).

Table 4-1: Some characteristics of the pure carbon amendments and the soil mixtures, measured before SIR and SIRIN analyses (means ±SD). Letters indicate significant differences between the treatments (ANOVA on Ranks with Dunn´s Post-hoc test, p<0.05).

	pH (H_2O)	C-content [%]	N-content [%]	C/N ratio
soil	5.8	3.5 ± 0.01	0.33 ± 0.01	10.6
feedstock	6.8	47.94 ± 0.41	0.12 ± 0.02	399.5
HTC	5.2	50.47 ± 1.04	0.19 ± 0.02	265.6
biochar	10.1	60.8 ± 14.54	0.4 ± 0.09	152.0
Samples, collected from the plots in the field, for SIR and SIRIN				
control	5.74±0.39^{a}	3.9 ± 1.51^{a}	0.32 ± 0.12^{ab}	12.16 ± 0.73^{ab}
$soil_{feedstock}$	5.67±0.31^{a}	4.38 ± 0.6^{a}	0.43 ± 0.12^{a}	10.78 ± 2.55^{b}
$soil_{HTC}$	5.79±0.19^{a}	3.98 ± 0.96^{a}	0.31 ± 0.08^{b}	12.72 ± 0.4^{a}
$soil_{biochar}$	5.80±0.28^{a}	3.83 ± 1.08^{a}	0.30 ± 0.08^{b}	12.83 ± 0.85^{ab}

Table 4-2: Basal and substrate induced respiration rates, soil microbial biomass (SMB) and qCO_2 value [ng CO_2 μg^{-1} SMB h^{-1}] of the different soil mixtures (Mean ± standard deviation). Letters depict significant differences between the treatments ($p<0.05$). The qCO_2 value gives the respiration of every ng microbial biomass per hour during basal respiration. Note different mass unit in qCO_2.

soil	basal respiration [µg CO_2 $g^{-1}h^{-1}$]	respiration during plateau period (SIR) [µg CO_2 $g^{-1}h^{-1}$]	SMB (SIR) [µg g_{soil}^{-1}]	qCO_2 during basal respiration [ng CO_2 μg^{-1} SMB h^{-1}]
control	6.70 ± 1.30^{d}	85.93 ± 16.78^{bc}	1770.64 ± 345.75^{b}	2.53 ± 0.89^{b}
feedstock	9.03 ± 1.46^{b}	69.00 ± 24.8^{c}	1425.06 ± 510.79^{b}	4.7 ± 0.23^{a}
HTC	8.09 ± 1.65^{c}	93.72 ± 38.15^{b}	1931.05 ± 785.91^{b}	3.14 ± 0.18^{ab}
biochar	10.25 ± 1.80^{a}	136.26 ± 45.32^{a}	2807.34 ± 933.61^{a}	2.49 ± 0.85^{b}

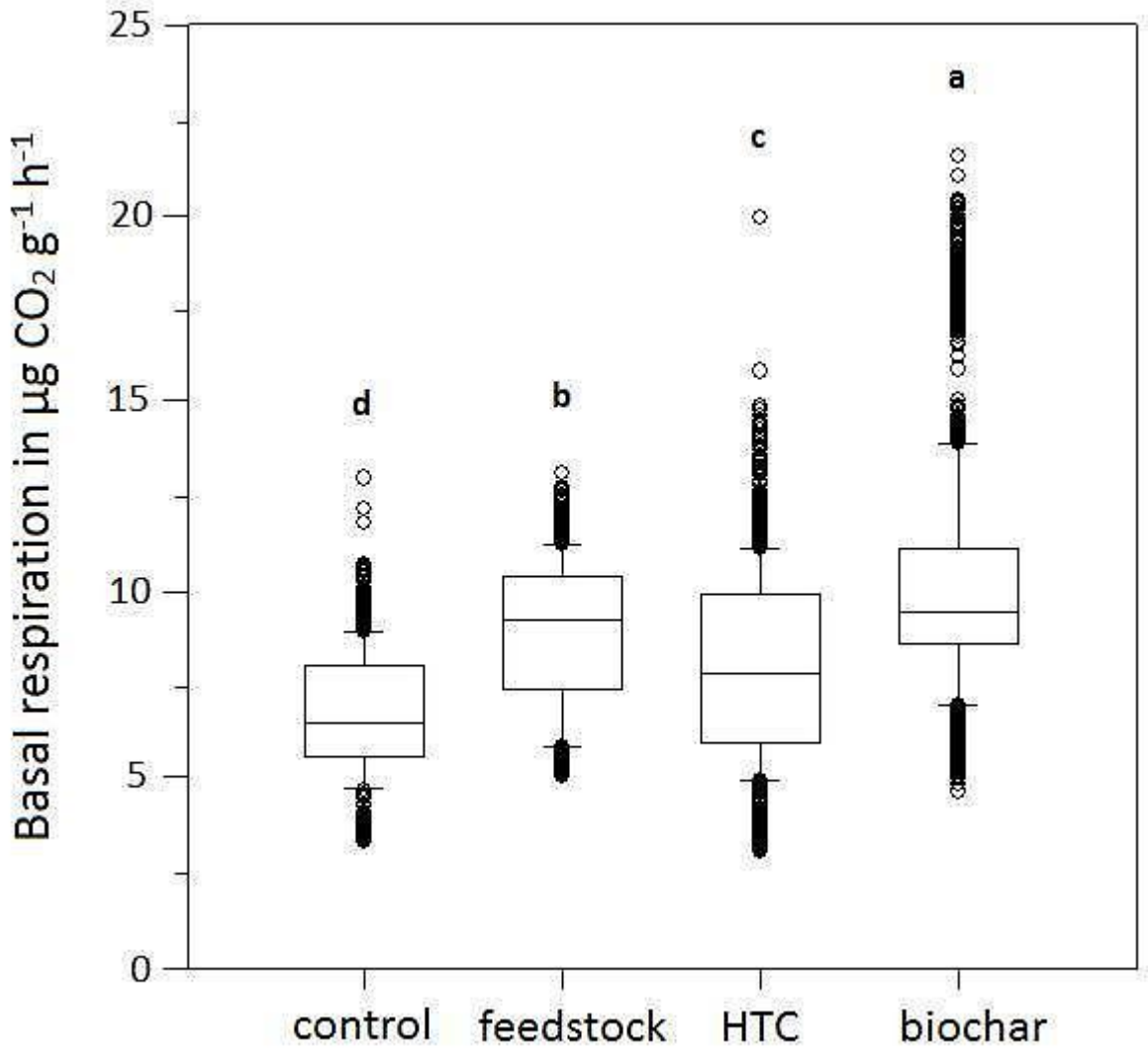

Figure 4-1: Basal respiration box plots of all basal respiration data from SIR and SIRIN, letters depict significant differences of the Medians, as tested with the Dunn`s Method ($p<0.05$).

4.3.3 Contribution of bacteria and fungi to total respiration

The contribution of bacteria (PBR) and fungi (PFR) to total respiration, as calculated by equations (4-2) and (4-4), revealed small but significant differences among the different treatments. The bacterial contribution to total soil respiration was significantly higher in feedstock and HTC amended soil, compared to the control. Total respiration of fungi (AFR) and bacteria (ABR) as calculated with equation (4-3) and (4-5) revealed a significant

higher fungal and fungal+bacterial respiration in the biochar treatments compared to all other treatments. Feedstock treatments exhibited significantly lower fungal respiration rates compared to HTC and biochar (Figure A.4-4). We found a significant positive, however weak correlation of fungi with C/N ratios ($r = 0.374$, $p < 0.025$).

4.3.4 Microbial biomass

Significant treatment differences regarding absolute microbial biomass amounts were found only for fungi, with biochar exhibiting highest amounts of fungal biomass (mean 908.08 ± 278.84 µg g^{-1}), compared to all other treatments (mean 550.39 ± 224.92 µg g^{-1}). Bacterial biomass was low throughout all treatments (mean 153.42 ± 96.40 µg g^{-1}), while the microbial biomass of other microorganisms was very variable with a mean of 1190.26 ± 428.24 µg g^{-1} (Figure 4-2). The sum of the absolute values of fungal and bacterial biomass revealed no significant treatment differences. Nevertheless when the other, non-inhibited microbial biomass was included, the highest absolute SMB values occurred in the biochar treatment (2807.34 ± 933.61 µg g^{-1}) (Figure 4-3 a).

The fungal fraction in the SMB was significantly different between feedstock (25 %) and HTC (40 %). The percentage of fungi in the biochar and control treatments ranged between 35 and 29 % (Figure 4-2 b). The fraction of bacterial biomass in SMB was increased by all carbon amendments compared to the control with significantly higher fractions in feedstock and HTC treatments (13 %) compared to the control (3 %) (Figure 4-2 d). The fraction of other microorganisms in total microbial biomass was the lowest in HTC, followed by biochar, then feedstock and the highest in the control treatment. However, only the fractions in feedstock and biochar were significantly lower compared to the control treatment (Figure 4-2 f). Combining the relative amounts of bacterial and fungal biomass revealed a significantly higher fungal plus bacterial biomass in the HTC treatments, compared to feedstock and control, which was caused by the higher relative bacterial biomass compared to the control and the higher fraction of fungal biomass especially compared to feedstock (Figure 4-3 b).

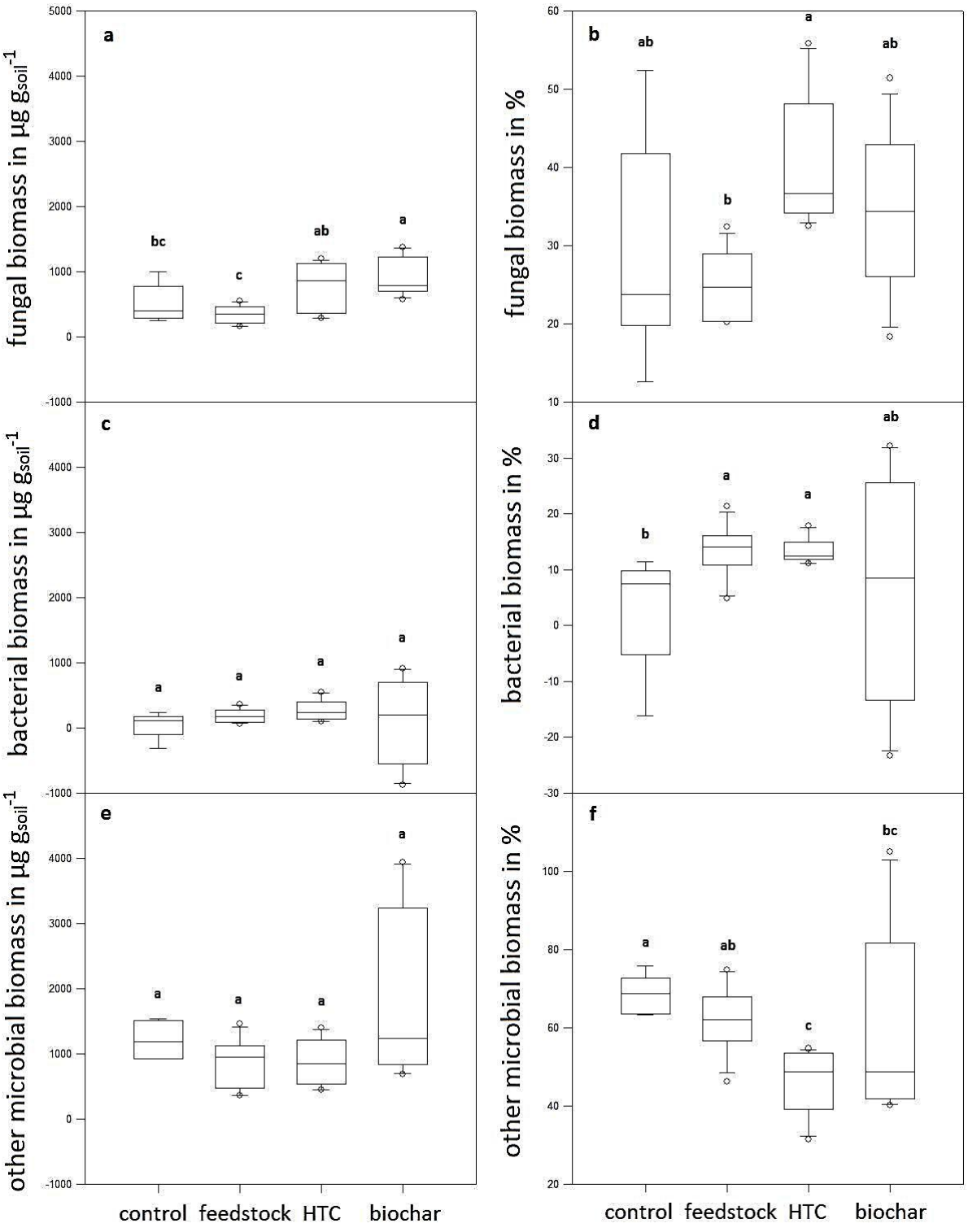

Figure 4-2: Absolute (left) and relative (right) shares of microbial biomass for fungi (a, b), bacteria (c, d) and other microbes (e, f)

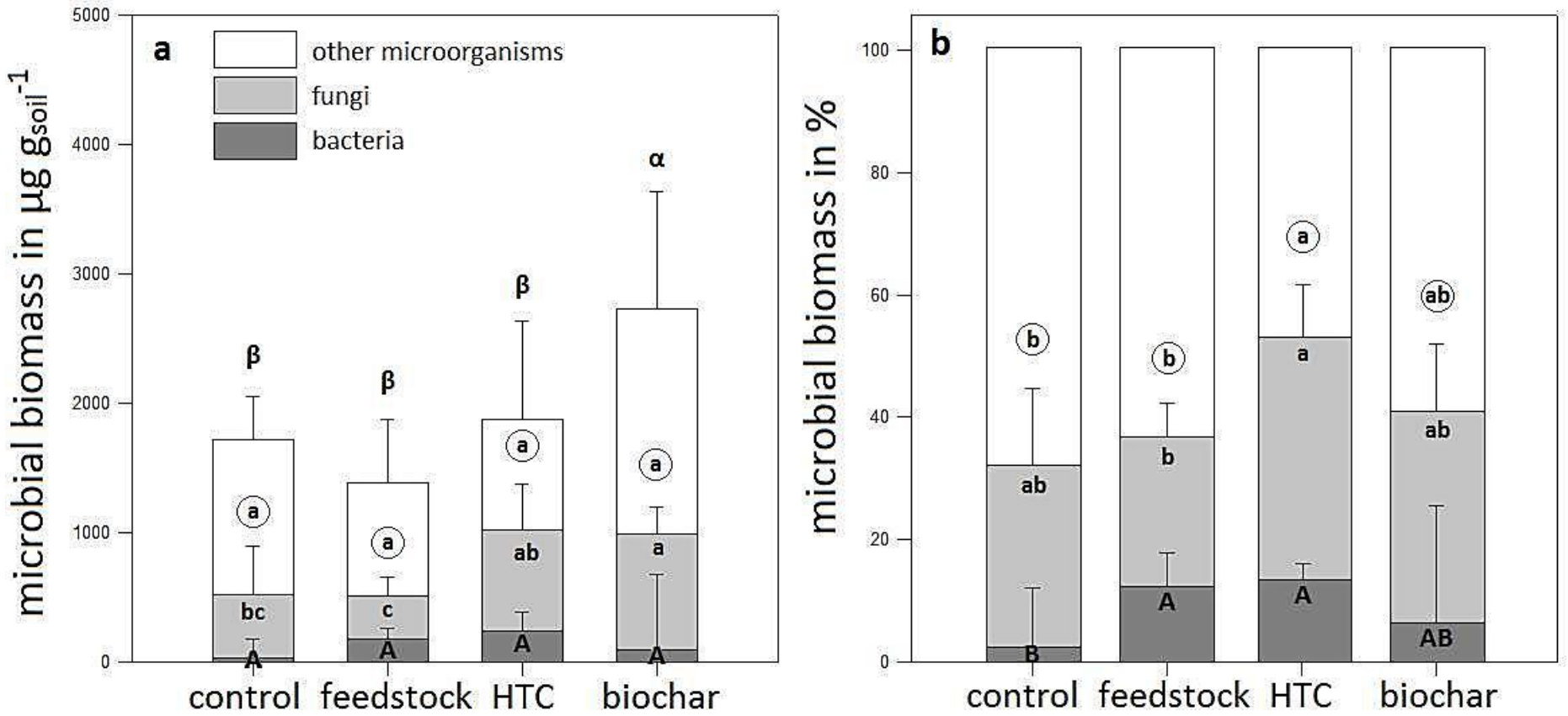

Figure 4-3: Shares of fungal and bacterial biomass in total biomass in absolute (a) and relative terms (b). Small letters depict significance of fungal fractions, capital letters of bacteria. Circled letters depict the significance of the summarized fungal and bacterial respiration and Greek letters the significance of the total microbial biomass.

4.4 Discussion

4.4.1 Experimental setup

It was assumed that by sieving the soil samples <2 mm most of the multi-cellular animals such as ants and nematodes had been removed and that the measured respiration rates therefore could be related to the target organisms. The assumption that glucose addition led to a maximum activation of microorganisms was based on the work of Lin and Brookes (1999). In this experiment, the focus was on the abundance of fungi and bacteria. However, the use of Streptomycin and Cycloheximide could very well have triggered some secondary effects, such as the inhibition of non-target microorganisms, or provision of supplementary C- and N-sources by increasing the amount of dead microbial biomass in the soil (Badalucco *et al.*, 1994). The activity of not-inhibited microbial biomass was estimated based on the differences in bacterial plus fungal respiration compared to total respiration.

4.4.2 Microbial biomass and activity

The SMB determined in this experiment is in line with results from other studies on the abundance of microorganisms in the surface soil layer of temperate, extensively cultivated grassland (Lavahun *et al.*, 1996; Jangid *et al.*, 2011). The percentages of bacteria and fungi in total SMB are with 3-13 % and 25-40 % rather low compared to other studies. The presence of the carbon amendments as well as differences in soil properties (pH, carbon content) and soil management, as well as the above mentioned methodological constraints may all be underlying causes to these inconsistencies (Anderson and Domsch, 1975; Lin and Brookes, 1999).

4.4.2.1 Feedstock

Absolute fungal biomass in the feedstock treatment was significantly lower compared to the biochar and HTC treatments, while biomass from other microbes was reduced compared to the control. However, bacterial biomass was not different from the other treatments. This resulted in the lowest overall SMB of 1425.06 µg g^{-1}. This, combined with high basal respiration rates of the feedstock treatment, likely indicates the presence of fewer but more active fungi and other non-inhibited microbes. Feedstock treatments exhibited a high WHC and N-content, compared to the other treatments. This could have triggered microbial activity (Lin and Brookes, 1999; Peacock *et al.*, 2001) (Figure A.4-3, Table 4-1). Furthermore, the low substrate induced respiration rates combined with a significantly higher qCO_2 value suggest that in the feedstock treatment a higher percentage of microorganisms was active (not dormant) before substrate addition. This indicates a lower microbial efficiency.

These results are in contrast to other medium- and long term studies (1-18 years) on the effect of straw application on soil microbial biomass, which resulted in an overall increase in microbial biomass (Powlson *et al.*, 1987; Ocio *et al.*, 1991). On the other hand, the observed increased soil N content and the low C/N values in feedstock treatments at the end of the experiment were correlated with the low fungal biomass. This is in agreement with other studies, indicating an advanced degradation of *Miscanthus* feedstock material and the release of microbially immobilized N (Neely *et al.*, 1991; Hobara *et al.*, 2014). One explanation could be that a certain fungal community specialized on lignin decomposition, first displaced other microbes and died after this C-substrate was exploited (Hatakka, 1994; Hammel, 1997). As a consequence, our measurements might have detected a reduced surviving microbial biomass, slowly rebuilding to its former abundance and composition as the carbon amendment effect of *Miscanthus* straw had subsided (Drenovsky *et al.*, 2004). The significant relative increase in bacterial biomass however indicates that bacteria could better handle the conditions caused by *Miscanthus* straw in the soil. Other studies reported that bacteria benefitted from dissolved organic carbon (DOC) more than fungi (Marschner *et al.*, 2003; Cleveland *et al.*, 2007). DOC or available carbon provided by *Miscanthus*

straw might therefore explain the microbial community shift as compared to the control soil.

4.4.2.2 HTC

SMB in the HTC amended soil was similar to the feedstock and control treatment, however it was lower compared to biochar amended soil. The basal respiration rates of the HTC amendment indicated a slightly higher microbial activity than in the control soil, nevertheless microbial efficiency, as indicated by the qCO_2 value, was not affected (Table 4-2).

The absolute amounts of SMB revealed a higher fungal biomass in HTC compared to the feedstock treatment. This suggests that degradation of HTC was not as progressed as that of feedstock material, leaving sufficient material for fungal activity. Both increased relative bacterial and fungal fractions in total SMB point to a microbial community shift due to the addition of HTC material. It seems likely that HTC triggered a very diverse, specific microbial community, which specialized on the degradation of particular de-polymerized and re-synthesized carbon compounds that originated from the production process of HTC (Waldrop and Firestone, 2004; Diakite *et al.*, 2013). As a result of the production process HTC has, compared to feedstock, an additional nutrient supply, such as phosphorus, as well as a proportional higher amount of ligneous components. This could have triggered fungal growth or the microbial community shift (Allison *et al.*, 2007; Funke and Ziegler, 2011; Funke *et al.*, 2013a). The C/N ratio is considered to be an indicator for microbial biomass, with high C/N ratios pointing to a higher abundance of fungi (Eiland *et al.*, 2001), which is consistent with our results. Other studies found that HTC supported spore germination and root colonization of mycorrhizal fungi (Rillig *et al.*, 2010). It is likely that in the current study, the leftovers of the mycorrhizal biomass, resulting from the root removal at the start of the experiment, were detected. More detailed studies aiming to identify microbial species associated with the plant-soil-HTC matrix are needed.

4.4.2.3 Biochar

Biochar amended soils exhibited a significantly higher SMB compared to all other treatments. The total microbial biomass in the biochar soil consisted of around 34.5 % fungi, leading to a significantly higher amount of fungal biomass compared to the control and feedstock treatments (Figure 4-3 a). The effectivity of the microorganisms in the biochar soils was similar to the control, as indicated by the qCO_2 ratios, suggesting a similar amount of available nutrients per mass unit of microbial biomass and consequently a higher amount of available nutrients in the biochar soil. A strong stimulation of microbial activity as indicated by high SIR values, together with qCO_2 values similar to the control, indicates that most of the microorganisms in the biochar and control treatments were inactive, which is common for soils receiving low or irregular fertilizer input, because of low nutrient availability to micro-organisms most of the time (Jenkinson and Ladd, 1981). In biochar amended soils, the significantly higher SMB indicated a stimulation of the microorganisms, likely caused by an improved nutrient retention from slurry by biochar (Taghizadeh-Toosi *et al.*, 2011a). Nevertheless, the nutrients bound to biochar could have become depleted during the long time period between slurry addition and collection of the soil samples – around 6 months –, inducing a low basal respiration and microbial activity. pH induced changes of the microbial biomass might have occurred directly after initiation of the experiment, but were not observed at the time of sampling (Table 4-1) (Baath and Anderson, 2003).

A higher abundance and activity of microorganisms in biochar amended soils compared to unamended control soil was found by others, whereby the nutrient availability of the background soil seemed to be the most important influencing factor (Kolb *et al.*, 2009; Jin, 2010; Gomez *et al.*, 2014; Muhammad *et al.*, 2014). Also, stimulation of fungi following biochar amendment has been reported elsewhere (Sun *et al.*, 2013). More specifically, biochar amendment increased the inoculation of soil with mycorrhiza, most likely by improving the habitat conditions of extraradical hyphae of mycorrhiza over saprophytic fungi, as the saprophytic fungi are not able to use biochar as a substrate (Saito and Marumoto, 2002; Quilliam *et al.*, 2012), or pH effects (Hu *et al.*, 2014). However, in our experiment, we assumedly removed a large amount of mycorrhiza with the roots in the

beginning of the experiment and fertilization with slurry may have prevented nutrient competition in our soils, as indicated by a high amount of other microorganisms in the biochar soil. Further research on the interactions of biochar and microorganisms, providing deeper insights in the different stages of fungal development on a species level following biochar amendment to soil is clearly necessary.

4.4.2.4 Comparison of treatments

The addition of *Miscanthus* feedstock to soil did not improve the conditions for microorganisms in soil. Also, HTC amended soil exhibited similar absolute microbial biomass amounts to the feedstock and control treatments. Therefore HTC seems to be an unsuitable compromise between pure *Miscanthus* straw and biochar if the aim is to increase microbial biomass. However, bacteria responded with a significant relative increase of total biomass to feedstock and HTC amendment, as compared to the control, but not to biochar amendment (Figure 4-3 b). Possibly, the effect of biochar on bacteria was masked by the dominance of a certain fungi species, inhibiting bacteria e.g. by the production of antibiotics (Boer *et al.*, 2005). Nonetheless, biochar increased the total abundance of microorganisms in soil which could be of great influence on microbial activity and nutrient turnover rates, e.g. of C and N, regarding fresh litter inputs (Vanveen *et al.*, 1984; Vanveen *et al.*, 1985). Priming experiments, focusing on the fate of labile carbon materials added to aged biochar/HTC soils, are needed in greater number to elucidate the feedback mechanisms of biochar/HTC induced soil microbial community changes on soil properties, with consequences for nutrient management and plant growth.

4.5 Conclusion

This experiment indicated that the addition of *Miscanthus* × *giganteus* material to grassland soil had long term effects on soil microbial biomass, but that these effects varied widely. The addition of pure *Miscanthus* straw did not increase SMB as would be expected due to the addition of a labile carbon source. Instead, SMB in feedstock amended soil 2.6 years after application was even lower than SMB in the control soil.

Other than expected, HTC did not cause a higher abundance of fungi as, coherently, changes in the soil pH have not been observed. On the contrary, a microbial community shift in feedstock and HTC amended soil was observed, leading to an increased fraction of bacteria in total biomass, compared to the control.

Biochar, as hypothesized, increased all microbial community groups in equal proportions, leading to a significantly higher overall microbial biomass. Our results provide indications that a biocompatible way of soil application of HTC or biochar is possible and that thereby SOC can most likely be permanently increased. However, changes in the composition of the soil microbial community following carbon amendment should be investigated in more detail, with the focus on different fertilization and soil management regimes.

4.6 Acknowledgments

We thank Gerhard Mayer, Nicol Strasilla and Christian Eckhardt for providing technical assistance with the experimental setup. This study was financed by the Hessian Agency for Environment and Geology, Germany.

4.7 Appendix

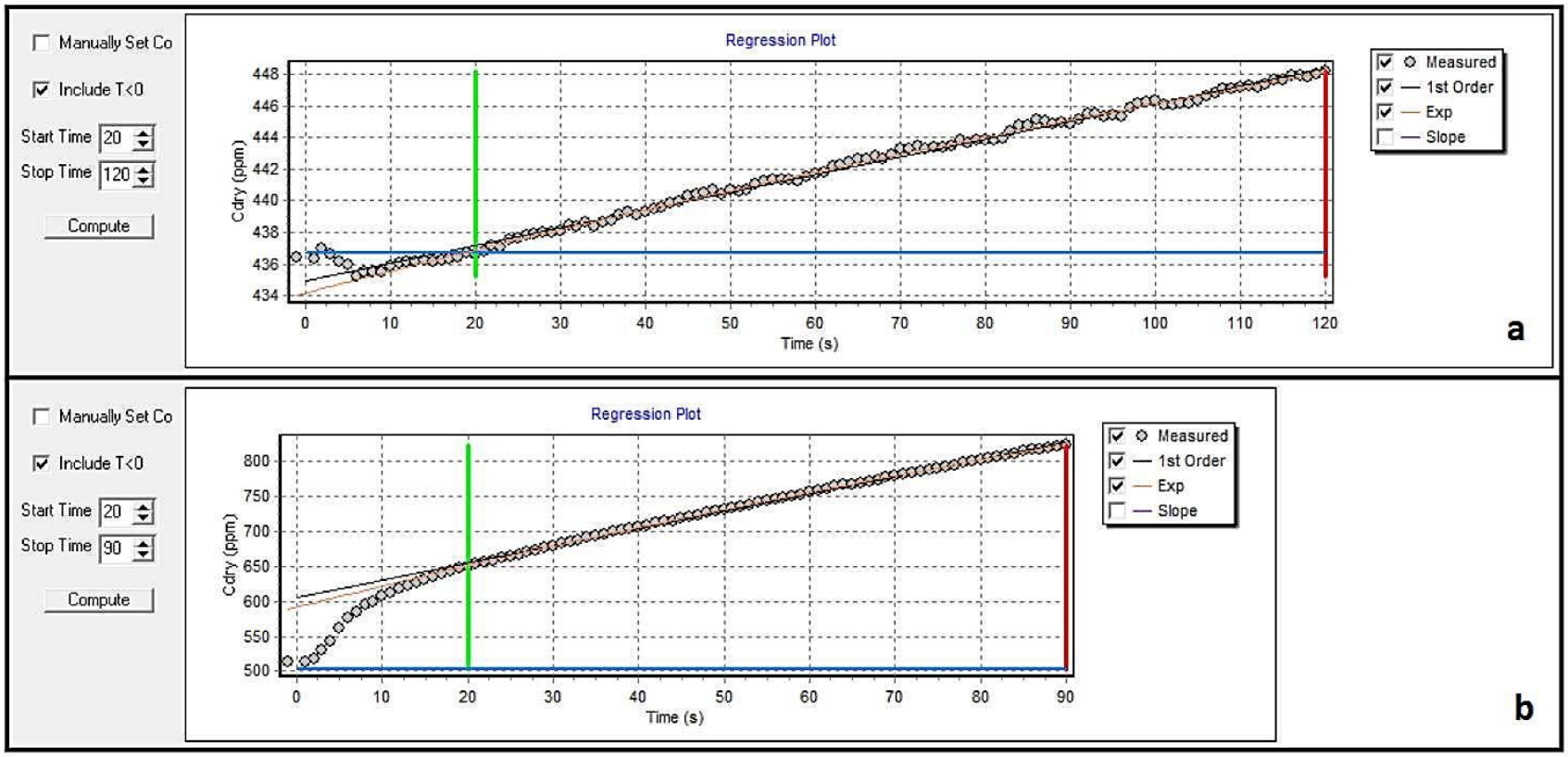

Figure A. 4-1: Measurement result of a single jar with control soil during basal respiration of SIR (a) and during plateau period of SIR (b), given by

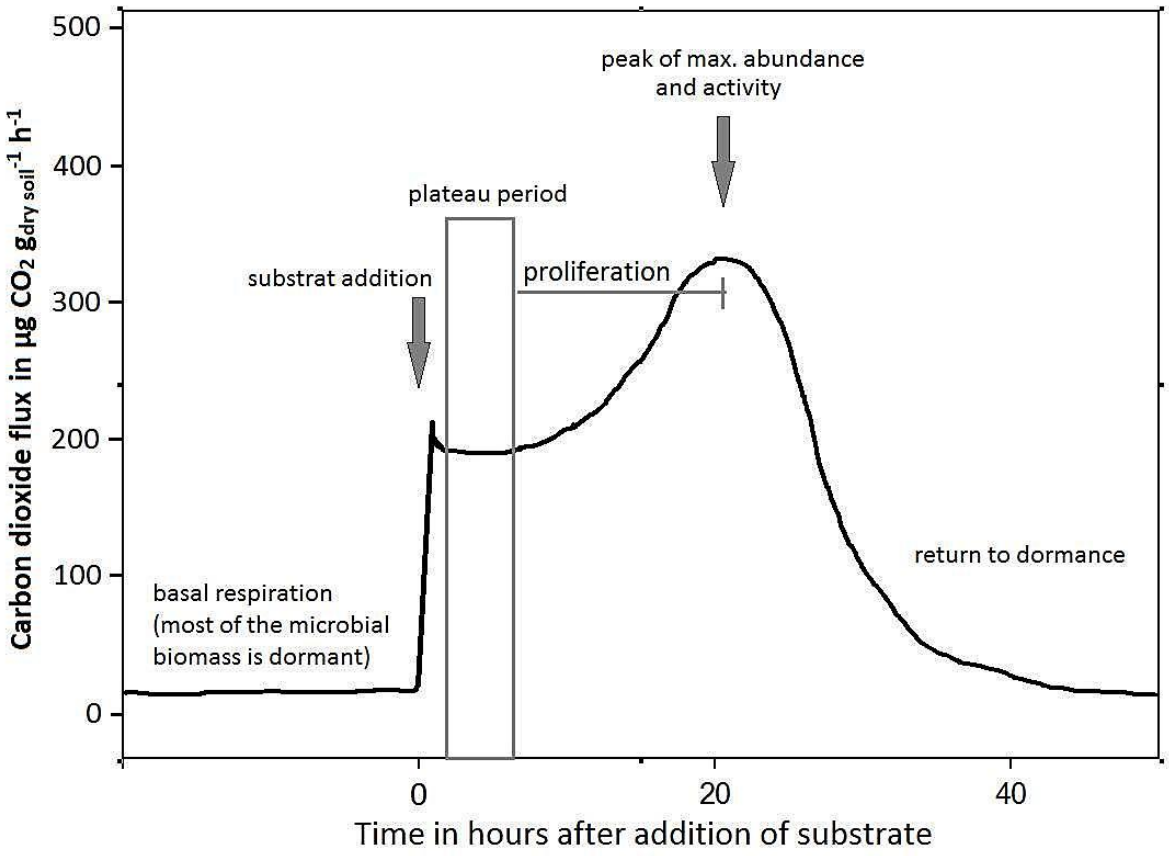

Figure A. 4-2: General scheme of SIR, based on the findings of Anderson and Domsch (1973) and DIN ISO (17155:2011-06 2011).

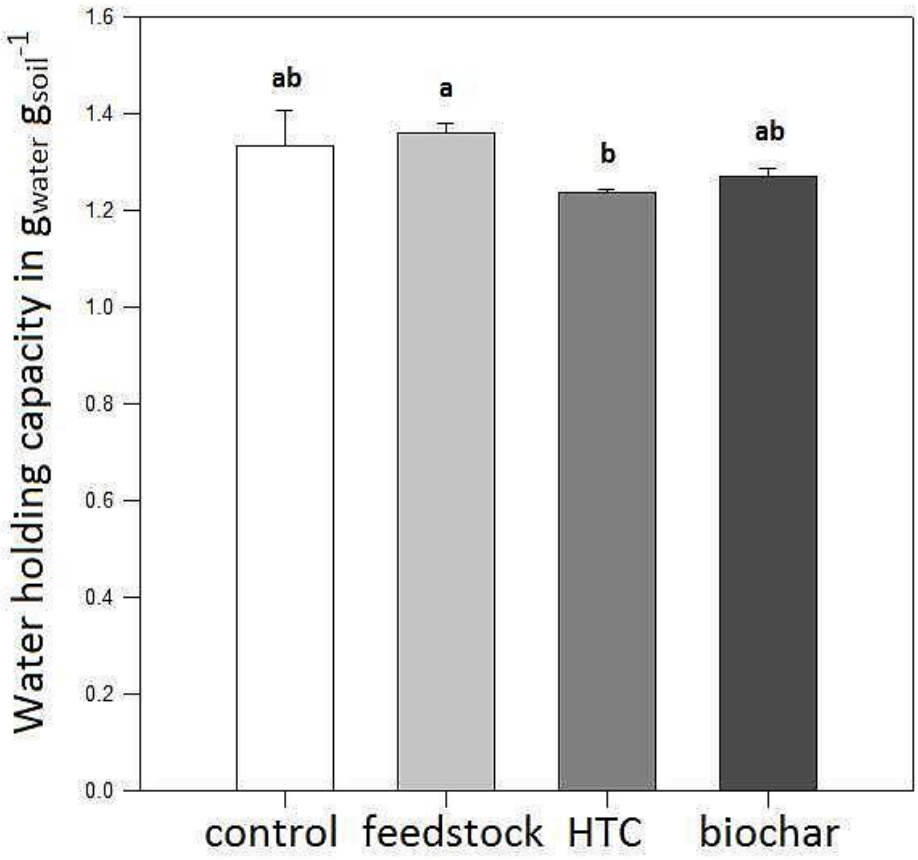

Figure A. 4-3: Water holding capacity of the different soil mixtures, as tested by a one-way ANOVA, followed by a Holm-Sidak Post-hoc test (mean ± SD). Letters depict significant differences between the treatments.

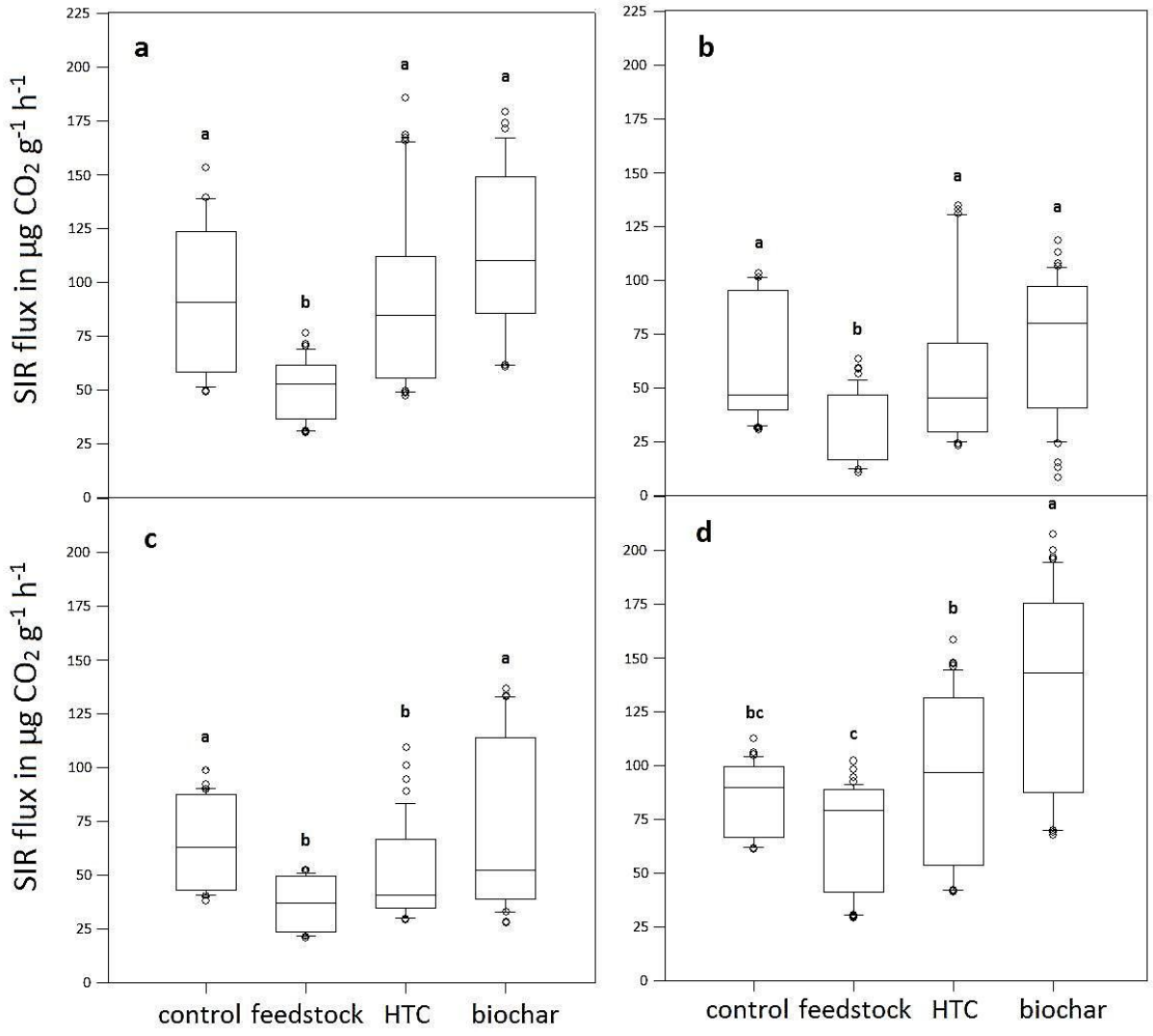

Figure A. 4-4: Comparison of the respiration rates according to the four treatments during the plateau period of SIRINs (equivalent to Rs, a), SIRINc (equivalent to Rc, b), SIRINsc (equivalent to Rsc, c) and SIR (equivalent to Rtotal, d) measurements, based on median tests followed by a Dunn´s post-hoc test ($p<0.05$). Different letters depict significant differences.

5 How to break it - degradation of *Miscanthus x giganteus* feedstock, hydrochar and biochar under the influence of simulated extreme events

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Abstract

Little is known about the degradation performance and environmental impacts of carbon amendments such as hydrochar and biochar in soil under the influence of forced weathering. Thus, we assessed the degradation and the greenhouse gas (GHG) balance of *Miscanthus x giganteus* biochar (from pyrolysis), hydrochar (from steam and water hydrothermal carbonization, HTCs and HTCw) and the uncarbonized feedstock material in two different soils, a sandy and a loamy soil, under the influence of simulated wet-dry and freeze-thaw cycles and simulated plowing, and glucose additions. Hydrochar, biochar and feedstock were mixed with a sandy and a loamy soil at a rate of 1.96 wt% and incubated at 30°C (except during freeze-thaw events) over the period of 389 days. Degradation kinetics were quantified by source partitioning of the headspace ^{13}C-CO_2 and the application of an isotope two component mixing model. Additionally, microbial biomass and composition was quantified and characterized at the end of the experimental period by chloroform fumigation extraction and phospholipid fatty acid analysis.

The molecular composition and structural properties of the carbon amendments obtained by elementary analysis and NMR spectroscopy proved to be suitable indicators for the degradation rate which followed the sequence feedstock > HTCs > HTCw > biochar ≥ control over the experimental duration. The cumulative N_2O emissions were associated/in line with the cumulative CO_2 emissions, and hence by the structural properties of the amendments. Only the addition of glucose led to slightly altered degradation, triggering temporary co-mineralization of the otherwise recalcitrant materials HTCw and biochar.

Our results show that only biochar remained stable over a period of forced weathering under high temperatures (30°C) with expected high biological activity in both soils. Moreover biochar exerted a negative priming effect, especially in sandy soil. The contribution of alternating weather conditions and possibly co-metabolism to the degradation of carbon amendments in soil was likely underestimated so far.

Keywords: biochar, hydrochar, degradation, greenhouse gases, SOC priming, *Miscanthus × giganteus*

5.1 Introduction

A range of application possibilities of carbonized biomass as well as hydrochar/biochar carbonization techniques such as hydrothermal carbonization or slow and fast pyrolysis have been developed and improved during the last decades (Meyer *et al.*, 2011). The different production processes, their potential feedstock supply as well as their resulting products may all have their specific application fields. Hydrothermal carbonization (HTCw) for example is well suited to process wet feedstock material, being energy sufficient, and yielding a material similar to brown coal or peat, i.e. a possible peat substitute (Mumme *et al.*, 2011; Cao *et al.*, 2013). An optimized HTC production process has resulted in another, even more energy conserving process when water vapor is used instead of liquid water, lately known as vapo-thermal or steam carbonization (HTCs) (Funke *et al.*, 2013b). Pyrolysis is better-suited for drier feedstocks; pyrolysis for energy and charcoal generation has been practiced likely since thousands of years (Antal Jr and Grønli, 2003). The idea to use charcoal-like remains of pyrolysis for soil C sequestration and possibly soil improvement (biochar) dates back to the discovery of anthropogenic dark earths as well as natural black soils (Chang, 1996; Reina *et al.*, 1998; Ponomarenko and Anderson, 2001; Nurul Islam *et al.*, 2005; Marris, 2006).

Theoretically, hydrochar (or HTC) and biochar (or pyrochar) should enhance the net total organic carbon (TOC) content of soils, and, in spite of short-term and transient increases in hydrochar/biochar degradation, contribute to the long-term stable carbon pool of soils (Titirici *et al.*, 2007; Stavi and Lal, 2013). Nevertheless, a reliable link of the stability of carbon amendments in soil to positive or negative feedbacks on ecosystem processes is still not known. A better predictability of the degradation potential of C amendments in soils (including indirect effects via greenhouse gas emissions) as related to their material properties would facilitate the classification and usability of specific carbon amendments for carbon capture and soil storage, and the calculation of carbon offset potentials (Gaunt and Lehmann, 2008; Whitman and Lehmann, 2009; Harvey *et al.*, 2012; Stavi, 2013).

The stability of carbon amendments in soil often depends on structural properties of the material such as cellulosic, ligneous or aromatic compounds, elemental composition and presence of functional groups (Sollins *et al.*, 1996; Spokas, 2010; Bai *et al.*, 2014).

Generally, carbonization of plant material has been found to increase the half-life when incubated with soil for about 1.6 or 3.4 years on average for hydrochar and 6.75 or 24.6-34.2 years for biochar, compared to the respective uncarbonized reference material (Steinbeiss *et al.*, 2009; Qayyum *et al.*, 2012; Bai *et al.*, 2013). As a consequence, the different carbonization processes, especially in view to hydrothermal carbonization and pyrolysis may be suitable to distinguish the properties and recalcitrance of carbon amendments against degradation (Lehmann *et al.*, 2009; Spokas, 2010; Schimmelpfennig and Glaser, 2012; Singh *et al.*, 2012). On the other hand, soil properties such as carbon and nitrogen content (Bai *et al.*, 2013) or ecosystem properties such as the presence of vegetation (root exudate supply), the abundance of soil microorganisms and most of all temperature and soil moisture are equally crucial factors determining the speed of carbon amendment degradation in soil (Kögel-Knabner *et al.*, 2008; Schmidt *et al.*, 2011).

Studies on the effects of carbon amendments on the GHG balance of soils are rather ambiguous and both positive and negative effects have been reported (Van Zwieten *et al.*, 2009; Kammann *et al.*, 2012; Mukherjee and Lal, 2013; Dicke *et al.*, 2014).

Carbon dioxide (CO_2) emissions from carbon-supplemented soils without plants can either originate from soil organic carbon (SOC) and/or amended C from the amendments. Neither a fast degradation of the carbon amendments nor an additional mineralization of SOC (positive priming) following their application is desirable. Many studies report CO_2 emission peaks after biochar and particularly hydrochar application to soil, which has been related to a labile C pool e.g. volatile compounds or carbonates, functional groups or a biochar/hydrochar induced pH shift, providing suitable conditions for microorganisms (Zimmerman, 2010; Jones *et al.*, 2011; Keith *et al.*, 2011; Wang *et al.*, 2011; Eibisch *et al.*, 2013; Farrell *et al.*, 2013; Malghani *et al.*, 2013). Subsequent biochar mineralization was reported to level out to very low rates, indicating that biochar is a rather recalcitrant, stable C-pool (Kuzyakov *et al.*, 2014), while hydrochar continued to mineralize (Kammann *et al.*, 2012; Malghani *et al.*, 2013; Bamminger *et al.*, 2014a). However, biochars´ recalcitrant behavior may be reduced by the addition of labile carbon substrates, such as glucose or straw, indicating its susceptibility to co-metabolization (Hamer *et al.*, 2004; Kuzyakov *et al.*, 2009). Biochar was found to prime SOC positively and/or negatively, depending on soil properties such as texture and SOC content, SOC structure and microbial community

composition or the biochar feedstock (Zimmerman, 2010; Keith *et al.*, 2011; Dempster *et al.*, 2012; Cely *et al.*, 2014). Concerning non-CO_2 GHG emissions, hydrochar and uncarbonized feedstock often led to higher soil N_2O emissions compared to unamended controls in incubations and field studies (Kammann *et al.*, 2012; Schimmelpfennig *et al.*, 2014). However, decreased N_2O emissions from hydrochar-amended soils have also been reported (Malghani *et al.*, 2013). Methane oxidation was typically increased by both C-amendments, yet if the soil was flooded or fertilized, the methane sink temporarily turned into a source (Kammann *et al.*, 2012; Schimmelpfennig *et al.*, 2014).

Cayuela *et al.* (2014) showed by a meta-analysis that biochar soil amendment reduced N_2O emissions on average by 54 %. Furthermore, biochar decreased methane emissions from soils by reducing the abundance of methanogenic archaea (Dong *et al.*, 2013) or by improving habitat conditions for methanotrophic bacteria, likely by an increase of the soil pH (Shen *et al.*, 2014), soil aeration (Van Zwieten *et al.*, 2009), the retention of CH_4 in biochar pores (Feng *et al.*, 2012) or provision of potassium, stimulating the methanotrophic bacteria population (Barbosa de Sousa *et al.*, 2014). However, increased CH_4 emissions were also reported, possibly due to biochar-delivered carbon compounds available to microorganisms and interactions with slurry during fertilization (Zhang *et al.*, 2010). Overall, the mechanisms and processes of biochar involvement in GHG emissions from soil are considerably influenced by soil type (SOC-content, texture, pH and nutrient status), management (fertilization), regional climate and the type of biochar (feedstock and production process conditions, C/N ratio etc.), which makes it difficult to estimate interactions and impacts (Mukherjee and Lal, 2013; Cayuela *et al.*, 2014).

Still little is known about the stability of C amendments and associated GHG fluxes in particular under weather events such as frost-thaw cycles, drought or heavy precipitation, the frequencies of which are likely to increase (Field, 2012; Reichstein, 2012). Such weather events can disrupt soil aggregates and promote soil erosion, leading to carbon and nutrient losses (Martınez-Casasnovas *et al.*, 2002; Müller *et al.*, 2003; Fry *et al.*, 2014). However, biochar and potentially hydrochar, if sufficiently stable may alleviate the impacts of some extreme weather events e.g. by improving the soils´ water holding capacity, soil aggregate formation or the recovery rate of microorganisms after drought events, depending on biochar application rate, feedstock and soil type (Liu *et al.*, 2012;

Herath *et al.*, 2013; Mukherjee and Lal, 2013; Liang *et al.*, 2014). Thus, the objectives of this study were to investigate (1) the influence of carbon amendments from a carbonization gradient on the GHG budget of two soils with different texture and SOC content, (2) whether the degradation of carbon amendments and N_2O emissions can be linked to their structural properties and (3) whether the intrinsic recalcitrance of biochar can be markedly altered by forced weathering and glucose addition, leading to priming effects, and (4) to which degree soil microorganisms are influenced by the different carbon amendments.

5.2 Material and methods

5.2.1 Material

The top layer (0-10 cm) of two contrasting temperate soils were sampled for the incubations, a grassland soil (Haplic Stagnosol, WRB (2006)) with a texture of 25 % sand, 47 % silt and 28 % clay, total organic carbon content (TOC) of 2.23 %, 0.2 % nitrogen (N) and a pH of 6.88 ± 0.02 and an agricultural Cambisol (HLUG, 2014) with a texture of 85 % sand, 10 % silt and 5 % clay, TOC of 0.63 %, 0.04% N and a pH of 5.52 ± 0.05. The field-fresh soil was sieved through a 10 mm sieve, to removee large roots, rhizomes and stones.

All carbon amendments (hereafter all termed "char" or "carbon amendment") were produced from *Miscanthus* × *giganteus* chaff, either used untreated (= feedstock), hydrothermally carbonized (steam and water atmospheres, termed hydrochar steam = HTCs and hydrochar water = HTCw in the following), or pyrolyzed (biochar). The field grown, senescent *Miscanthus* was harvested in winter 2009 when all aboveground plant material had receded. Hydrothermal carbonization (steam) was carried out by keeping the feedstock in a water vapor atmosphere for 2 hours at a temperature of 200 ± 3 °C under a pressure of 1.6 MPa (Revatec, Geeste, Germany, at that time Hydrocarb GmbH, Ohmes, Germany). For water-based HTC, the Miscanthus straw was processed at 240 °C and 3.1-3.8 MPa for 8 h in distilled water using an 18.75 L Parr series 4555 pressure reactor (Parr Instruments, Moline, IL, USA). The reactor contents were heated by an external heater operated by a Parr controller. The heating rate was set to 2 K min^{-1}. The reactor content

was not stirred. After the process, the heater was switched off and the reactor was cooled over night to about 50°C. Subsequently, the HTC slurry was removed from the reactor, filtered by a wired mesh and the resulting solids were dried for 48 h at 60 °C.

Biochar was produced using a pyrolysis unit with a continuous flow reactor at 550-600 °C and a mean residence time of the material in the reactor of 15 min (Pyreg GmbH, Bingen, Germany). All materials were ground to < 10 mm before use (SM 300, Retsch GmbH, Haan, Germany).

5.2.2 Methods

5.2.2.1 Experimental setup

The experiment was set up at the end of May 2012 and kept until mid-August 2013 (441 days, see timeline, Figure A.5-1). The carbon amendments were mixed with sandy or loamy soil at the rate of 1.96 wt % (equivalent to 29.2 or 19.6 t amendment per hectare, as calculated for an application depth of 10 cm for sandy or loamy soil, respectively), into glass incubation jars (1100 ml, Weck®, Germany) and kept inside a drying closet at 30°C to stimulate microbial activity (n = 7 per treatment). Four replicates were used for weekly measurements of the GHG fluxes of CO_2, N_2O and CH_4. The other three replicates were used to perform elementary analysis and to measure pH values in the beginning and the end of the experiment, and for substrate-induced respiration (SIR) measurement after eight months. The water content of the samples was set to 60 % of the maximum water holding capacity (WHC) and adjusted weekly (Figure A.5-1), whereby the WHC of each mixture was previously determined as described by Schimmelpfennig *et al.* (2014).

The soil mixtures were fertilized with dissolved NH_4NO_3 five times during the experimental period, equivalent to 50 µg nitrogen (N) per g soil mix a time, to stimulate microbial activity and trigger N_2O emissions (Figure A.5-1).

We forced the degradation of the carbon amendments by the simulation of several "weather events" in the experiment, which are termed "sections" in the following. The first four months (days 0-131) of the incubation are considered a period of labile char-carbon degradation with a constant WHC of 60 % and no further disturbances (Section 1). On day

132, we increased the water content of the soil mixtures to 100 % WHC, with a subsequent drying phase decreasing the WHC to 50 % (Section 2, days 132-232). From day 233 onward, several freeze/thaw cycles were performed, where the jars were repeatedly put to either 4°C or -20° C over several days and taken out to thaw and to perform gas flux measurements (Section 3, days 232-302). On day 303 a plowing event was simulated by stirring the soil mixtures with a plastic spoon (Section 4, days 303-353). After 12 months of incubation (t_1 in the following), a priming experiment was carried out by adding 80 mg glucose powder g^{-1} soil mix, as a labile carbon source to each jar (Section 5, days 354-399).

5.2.2.2 Analytical procedures

Soil pH (H_2O) was measured using a pH meter (ratio 2.5:1 soil/water) (InoLab; WTW, Weilheim, Germany). Elementary analyses including carbon (C), nitrogen (N), sulfur (S) and hydrogen (H) of the raw materials and the soil mixtures were conducted using an elementary analyzer (CHNS Macro/VarioMax Elemental Analyzer, Elementar Analysensysteme GmbH, Hanau, Germany). The Oxygen content (in %) was determined as difference between 100 and the sum of the other measured elements plus ash, see Table 5-1.

Table 5-1: Elemental composition of the carbon amendments, means ± SD (n=3). Oxygen values were determined by difference (100-the sum of the other elements and ash). All values refer to the dry weight. Letters depict significant treatment differences (One way ANOVA, Holm-Sidak Post-hoc test P<0.05)

Material	C[%]	N[%]	S[%]	H[%]	Ash[%]	O[%]	H/C atomic ratio	O/C atomic ratio	C/N mass ratio
Feedstock	**47.94**±0.41^{A}	**0.12**±0.02^{A}	**0.19**±0.00^{A}	**5.71**±0.06^{D}	**1.57**±0.67^{A}	**44.48**±0.73^{D}	**1.42**±0.01^{D}	**0.70**±0.01^{D}	**412.14**±93.15^{B}
HTCs	**50.47**±1.04^{B}	**0.19**±0.02^{B}	**0.22**±0.01^{AB}	**5.19**±0.11^{C}	**3.56**±0.60^{B}	**40.38**±1.28^{C}	**1.22**±0.01^{C}	**0.40**±0.01^{B}	**263.93**±20.31^{A}
HTCw	**66.35**±0.28^{C}	**0.24**±0.01^{C}	**0.25**±0.04^{B}	**4.40**±0.09^{B}	**2.05**±0.06^{A}	**26.71**±0.26^{B}	**0.79**±0.01^{B}	**0.46**±0.02^{C}	**274.88**±11.52^{A}
Biochar	**75.65**±1.09^{D}	**0.43**±0.04^{D}	**0.19**±0.01^{A}	**0.79**±0.04^{A}	**18.45**±0.24^{C}	**4.48**±1.30^{A}	**0.12**±0.00^{A}	**0.04**±0.01^{A}	**175.86**±15.93^{A}

Structural analysis of all carbon amendments was carried out by high-resolution ^{13}C solid-state NMR spectroscopy (cross polarization-magic angle spinning) on a Unity INOVA 400 spectrometer (Varian Inc. USA). The spectra were Fourier transformed, base lines were corrected and integrated with the spectrometer's software package VNMRJ 2.2D. For quantification of selected structural elements, all spectra were divided in four sections and their signal intensities were determined (normalized to a total intensity of 100). Section boundaries were 0-50 ppm (aliphatic C), 50-60 ppm (-O-CH_3 = lignin), 60-110 ppm (cellulose), 110-220 ppm (aromatic C).

GHG fluxes were determined on a weekly basis following the method described by Hutchinson and Livingston (1993) adapted to jar measurements (Kammann *et al.*, 2009). Briefly, after jar closure, 50 ml gas samples were taken with syringes three times in equal time steps over 0.5 – 2 hours (depending on the incubation temperature). Gas samples were analyzed within 24 hours on a gas chromatograph equipped with a flame ionization detector (for CH_4) and an electron capture detector (for N_2O and CO_2; set-up according to Loftfield *et al.* (1997). The GHG fluxes were calculated by linear regression, considering the ideal gas law as well as average air pressure and temperature during the sampling period (Kammann *et al.*, 2009).

The overall GHG budget of the soil mixtures was expressed as CO_2 equivalents (CO_2eq), calculated from the CO_2, N_2O and CH_4 fluxes using the respective 100-year global warming potentials (GWPs) as given in the IPCC 4AR (GWP N_2O = 298, GWP CH_4 = 25) (IPCC, 2007).

At the end of Section 1, prior to fertilization (day 115) and subsequent glucose addition (day 368, Section 5), gas samples for the analysis of the isotopic composition of CO_2 (Microgas IRMS, Isoprime Ltd., Wytenshaw, UK) were taken with syringes and transferred into evacuated 12 ml vials (Labco, UK) and measured against the IAEA standards CH6, 305 and 310, Vienna, Austria.

The $^{13}C/^{12}C$ ratios of the soil and the carbon amendments were determined using an elementary analyzer coupled to an isotope ratio mass spectrometer (EA-IRMS, Eurovector, Thermo Fisher Delta V Advantage, Thermo Fisher Scientific GmbH, Dreieich, Germany).

5.2.2.3 Carbon budget and isotope mass balance calculation

The TOC contents of the soil mixtures at t_1 (prior to the priming experiment, days 0-365) and t_2 (after the priming experiment, days 366-399) were determined by subtracting the cumulated CO_2-C losses from the initial C-amounts within the soil mixtures. The relative carbon losses of the soil mixtures and their respective shares (SOC or char-C) were calculated using the isotopic signature of the headspace CO_2 sampled at day 115 for the period prior to the priming experiment (t_0-t_1), and the signature of the gas sampled after glucose addition (day 368) for the period during the priming experiment (period t_1-t_2), compared to a control (Hamer *et al.*, 2004). The loss of char-C was estimated by determining the relative C-share in CO_2 using equation 5-1:

$$^{13}CO_2(\%) = \frac{(\delta mix - \partial soil)}{(\delta char - \partial soil)} * 100 \qquad (5\text{-}1)$$

where $^{13}CO_2$ is the percentage of char-C in the respired CO_2, δmix is the isotopic ratio of the soil mix (soil+char (+glucose)), δsoil is the isotopic ratio of the pure soil (+glucose) and δchar the isotopic ratio of the specific char (Amelung *et al.*, 2008; Luo *et al.*, 2011). The isotope ratios of the soil mixtures were obtained from $^{13}CO_2$ gas measurements on day 115 (end of Section 1) and day 368 (start of Section 5) respectively, with source partitioning using Keeling plots. The calculation is based on the following assumptions: 1. the integrated ^{13}C signature for the soil-plus-glucose CO_2 would be identical for the control and the char-amended soils. Possible interactions of glucose and char mineralization were not taken into consideration. 2. The respective signatures of CO_2-C originating from the amendment or soil are similar to that of their sources. 3. The calculated ratio of the measurement in September can be extrapolated to all CO_2 measurement time points in the period before glucose addition (t_0-t_1) and the ratio in the beginning of the priming experiment applies to all subsequent measurements of the priming section (t_1-t_2). From the calculated cumulative char-C loss, the daily char-C loss was determined according to the five Sections, and the influence of glucose addition on char-C mineralization was quantified.

The mean residence time (MRT = $k^{-1}/365$) and half-life ($k^{-1}/365$) of the carbon amendments were estimated using a single exponential decay model (Lehmann *et al.*, 2009), since our data did not comply with the requirements for a double exponential model such as constant experimental conditions and an experimental duration of several years (Kuzyakov *et al.*, 2009).

5.2.2.4 Extractable organic carbon, microbial biomass and community composition

At the end of the experiment (t_3), the soil mixtures were analyzed for their extractable organic carbon (EOC) and soil microbial biomass carbon (C_{mic}) by the chloroform-fumigation-extraction (CFE) method (Vance *et al.*, 1987). In brief, 10 g of the chloroform fumigated soil subsample (24 h at 22 °C) were extracted with 40 ml 0.5 M K_2SO_4 on a horizontal shaker for 30 min at 250 rpm and centrifuged for 30 min at 4400 g. Another 10 g subsample remained non-fumigated but was extracted similar to the fumigated sample. C and N in supernatants of fumigated and non-fumigated samples were measured on a Multi N/C 2100S TOC/TN-analyzer (Analytik Jena, Jena, Germany). C_{mic} was calculated by subtracting the extractable C values of the non-fumigated from that of the fumigated samples. For calculation of C_{mic}, the conversion factor k_{EC} 0.45 (Joergensen, 1996) was used. EOC was calculated from C concentration in supernatants of the non-fumigated samples.

Phospholipid fatty acids (PLFA) of the soil mixtures were extracted following the method of Frostegård *et al.* (1993). A Bligh and Dyer solution (chloroform, methanol, citrate buffer, pH=4, 1:2:0.8;v/v/v) was used to extract glycol-, neutral- and phospholipid fatty acids and the fractions were separated by silica acid columns (0.5 g silicic acid, 3 ml: Varian Medical Systems, Palo Alto, California). PLFAs were classified according to Frostegård and Bååth (1996), Zelles (1999) and Kaiser *et al.* (2010), whereby the branched fatty acids i15:0, a15:0, i16:0 and i17:0 were summed as Gram-positive, the cy17:0 and cy19:0 as Gram-negative bacteria, the biomarker 16:1ω7 served as additional fatty acid for identification of total bacteria and 18:2ω6,9c for fungal PLFA. Total PLFA ($PLFA_{mic}$) is the sum of bacterial and fungal PLFA.

For a correlation of the microbial parameters with CO_2 fluxes, we extrapolated and cumulated the fluxes from the day 399, the time point when the glucose effect had been leveled out, to the end of the experiment (day 441, t_3).

5.2.3 Statistics and calculations

Statistics were carried out using SigmaPlot 11.0 (Systat Software Inc., San José, California, USA), IBM SPSS Statistics Version 20 (IBM Corporation, Armonk, New York, USA) and Xact 8.03 (SciLab, Saint Yrieix, France). The cumulated CO_2, N_2O, CH_4 and CO_2eq fluxes were integrated over the experiment duration using linear interpolation between measurement dates. Differences in CO_2eq over the incubation period were determined by a Repeated Measurements ANOVA and Post-hoc tests (Tukey HSD), factoring in the different time intervals between the gas measurements. Overall treatment effects, char-C losses in g per day within the five time sections, and differences between the single sections within one treatment were tested by one-way ANOVAs followed by LSD or Holm-Sidak post-hoc tests, or by ANOVA on Ranks followed by SNK or Tukey HSD post-hoc tests. Differences in the char-C degradation rates according to soil or treatments were determined by one-way ANOVAs on Ranks followed by Tukey HSD Post-hoc tests.

CO_2 and N_2O flux sums, percent SOC, char-C and TOC (SOC+char-C, excluding glucose-C) losses, C_{mic}, EOC, pH, WHC and MRT were tested for significant effects of the influencing factors "treatment" (C additive form) and "soil type" by two-way ANOVAs followed by Holm-Sidak post-hoc tests (CI=95 %). CH_4 flux sums were tested by a two-way ANOVA on ranks due to lack of normal distribution or heterogeneity of variances, followed by a Tukey HSD post-hoc test (CI=95 %; Table 5-2). Correlations of the cumulated CO_2 and N_2O-N fluxes were tested by Spearman Rank Order Correlation, since the data were not normally distributed. Correlations between microbial parameters and pH and CO_2 (t_2-t_3) fluxes were performed using Pearson Correlation. Correlations of the ratio of initial TOC [g] / remaining TOC [g] (g C_{in}/g C_{rem}) and the characterization parameters H/C-O/C ratio, cellulose and aromatics content of the char samples were calculated using Michaelis-Menten equations.

Table 5-2: Statistical results of the two-way ANOVA with the factors soil, treatment and soil x treatment interaction (CI = 95%); MRT, mean residence time; WHC, water holding capacity.

Parameter tested	soil		treatment		soil x treatment		displayed in
	F	***p***	***F***	***p***	***F***	***p***	
Cumulated CO_2 fluxes [g CO_2 kg^{-1} soil mix]	8.28	**0.007**	510.23	**<0.001**	17.08	**<0.001**	Fig. 5-1
Cumulated N_2O fluxes [mg N kg^{-1} soil mix]	2.77	0.106	75.57	**<0.001**	2.36	0.076	Fig. 5-1
Cumulated CH_4 fluxes [µg CH_4 kg^{-1} soil mix	445.10	**<0.001**	24.80	**<0.001**	19.23	**<0.001**	Fig. 5-1
SOC loss [% of initial]	866.04	**<0.001**	93.77	**<0.001**	40.40	**<0.001**	Fig. 5-5,-6,-7
Char-C loss [%of initial]	0.56	0.459	761.86	**<0.001**	53.43	**<0.001**	Fig. 5-5
TOC loss [%of initial]	2.23	0.146	469.14	**<0.001**	16.61	**<0.001**	Fig. 5-5
C_{mic} [µg C g^{-1} dw]	85.06	**<0.001**	6.68	**<0.001**	6.23	**<0.001**	Fig. 5-8
EOC [µg C g^{-1} dw]	364.56	**<0.001**	45.54	**<0.001**	25.14	**<0.001**	Fig. 5-8
$PLFA_{mic}$ [nmol g^{-1}]	940.56	**<0.001**	16.26	**<0.001**	23.88	**<0.001**	Fig. 5-9
Gram positive bacteria [nmol g^{-1}]	1356.21	**<0.001**	5.17	**0.003**	5.98	**0.001**	Fig. 5-9
Gram negative bacteria [nmol g^{-1}]	1029.93	**<0.001**	12.24	**<0.001**	8.05	**<0.001**	Fig. 5-9
Fungi [nmol g^{-1}]	12.37	**0.001**	10.37	**<0.001**	39.44	**<0.001**	Fig. 5-9
MRT [years]	8.45	**0.008**	583.36	**<0.001**	72.03	**<0.001**	Table 5-5
WHC_{max} [%]	452.60	**<0.001**	18.50	**<0.001**	3.64	**0.022**	Fig. A.5-2
pH at initiation of experiment	2050.83	**<0.001**	57.35	**<0.001**	8.34	**<0.001**	Table A.5-1
pH at the end of the experiment	7163.15	**<0.001**	116.43	**<0.001**	77.70	**<0.001**	Table A.5-1

5.3 Results

5.3.1 pH and WHC

Feedstock initially increased the pH value of pure sandy soil (pH 5.52) by half a unit, as did biochar in both, the sandy and loamy soil (Table A.5-1). At the end of the experiment, pH values had decreased significantly compared to initial pH values, especially in the loamy soil (on average from 5.33 to 4.62). While the pH values of the sandy control soil remained similar to initial values, all carbon amended soils exhibited a pH drop of 0.5 units. Initial treatment differences in loamy soil, had been leveled out during the experimental period.

The WHC_{max} was increased by all carbon amendments by 20 % compared to the control in both soils, with the exception of biochar in the loamy soil (Figure A.5-2).

5.3.2 CO_2, N_2O and CH_4 fluxes

The cumulated CO_2 emissions (days 0-399, t_0-t_2) ranged from 3.68 to 32.78 g CO_2 kg^{-1} soil mix (Figure 5-1 b; for CO_2, N_2O and CH_4 flux rates as measured see Figures A.5-3 - A.5-8). Emissions from the feedstock treatment were higher in the sandy soil (32.78 g CO_2 kg^{-1} soil mix), compared to the loamy soil (25.50 g CO_2 kg^{-1} soil mix, $p < 0.001$) (Figure 5-1 a-c). Carbon dioxide emissions from the control/HTCs/HTCw treatments were not different between the two soil types. Emissions from the biochar treatments were 3.68 and 6.46 g kg^{-1} soil and lower in sandy compared to loamy soil, respectively ($p < 0.05$). The cumulative degradation pattern was similar in both soils and followed the carbonization degree of the material (feedstock > HTCs > HTCw > biochar ≥ control). Cumulative N_2O emissions ranged between 0.95-14.55 mg N_2O-N kg^{-1} soil mix. Emissions from the feedstock treatment were higher from loamy compared to sandy soil ($p < 0.05$), whereas no soil-treatment interactions were found for the other treatments. Nitrous oxide fluxes from feedstock/HTCs/HTCw treatments were higher than those from the control and biochar treatments in both soils ($p < 0.001$), with highest fluxes from feedstock amended loamy

soil (Figure 5-1 d-f). Emissions from the biochar treatments were not different compared to emissions from the control in both soils.

Cumulated methane fluxes from the sandy soil were negligible and emissions ranged from -0.02 to 72.10 CH_4 µg kg^{-1} soil mix. Methane fluxes in the loamy soil were dominated by methane oxidation ranging from -71.99 to -302.99 µg CH_4 kg^{-1}. Here, soil methane oxidation capacity was improved by all carbon amendments, inversely to their carbonization degree, with highest methane oxidation rates in feedstock-amended soil (Figure 5-1 g-i).

The cumulated N_2O emissions correlated significantly with the cumulated CO_2 emissions in both soils ($r = 0.832$ in sandy soil, $r = 0.901$ in loamy soil, $p < 0.01$) (Figure 5-2).

5.3.3 Shares of the single GHGs in total CO_2-equivalent emissions

Carbon dioxide made up for 85.6 % and 87.5 % and N_2O for 14.4 % and 12.5 % of the total CO_2eq in the sandy and loamy soil, respectively, with large treatment differences. Methane fluxes corresponded to 0.03 % (sandy soil) and 0.16 % (loamy soil) of total CO_2eq, with only the feedstock treatment in loamy soil leading to a higher share in CO_2eq compared to the control ($p < 0.05$, Figure A.5-9, Table A.5-2).

The N_2O emissions during the simulated weather events were very variable. In particular, water logging in Section 2 led to N_2O emission peaks (Figure A.5-5 and A.5-6) which contributed disproportionally strongly to the CO_2eq sum during the wetting-drying cycle.

As a consequence, rather the CO_2eq fluxes than the sole CO_2 fluxes during the single Sections were used for an overall evaluation of the effects of the carbon amendments under simulated weather conditions on the GHG budget of the soils.

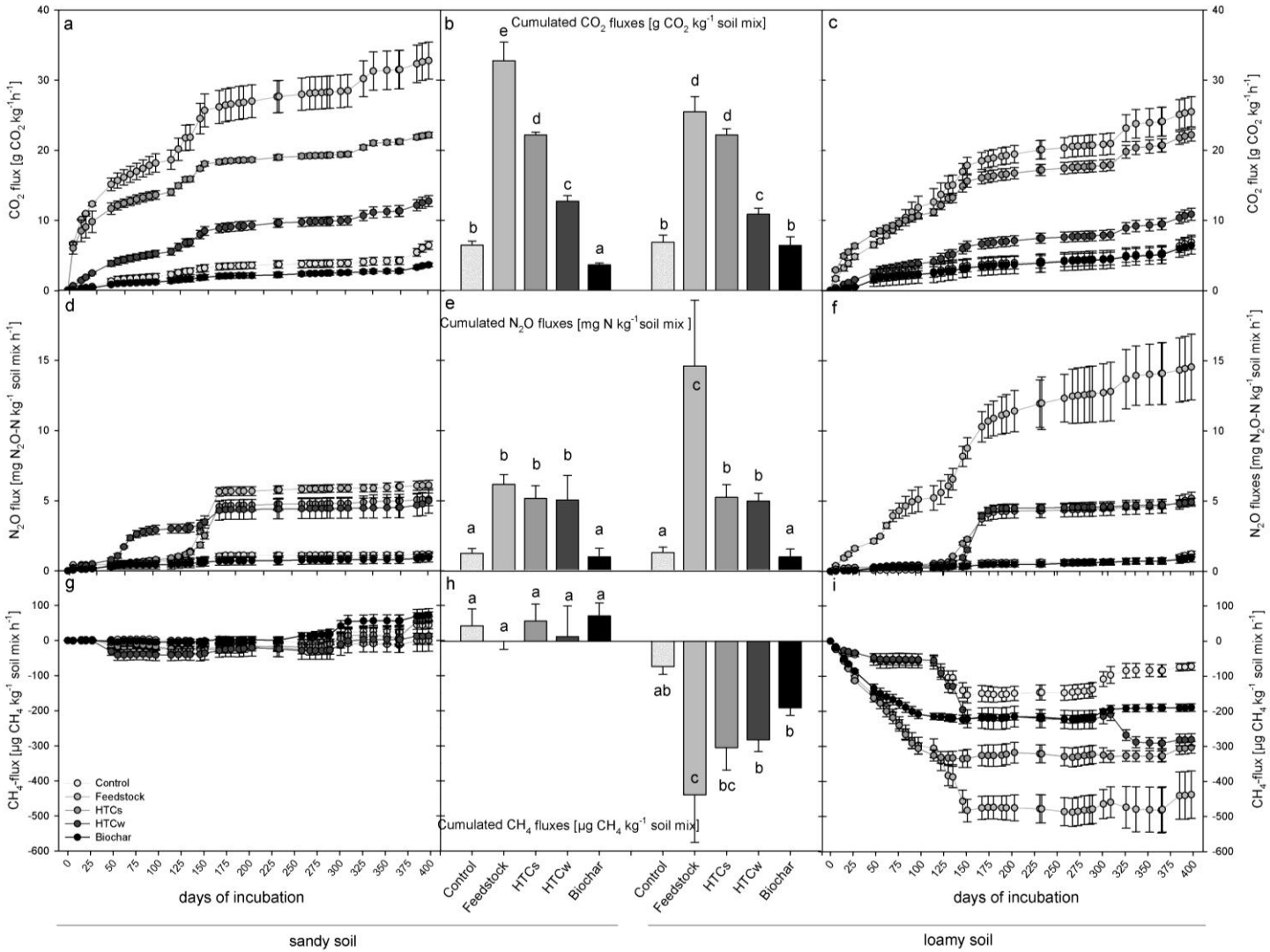

Figure 5-1: Cumulated mean CO_2, N_2O and CH_4 fluxes over time (left, a, d, g; and right, c, f, i) and total GHG flux sums at the end of the experiment (middle, b, e, h). Error bars indicate standard deviation (n=4); letters mark significant differences between treatments (one way ANOVA and Holm-Sidak post-hoc test (CO_2, N_2O), or ANOVA on Ranks and Tukey post-hoc test (CH_4), CI = 95%).

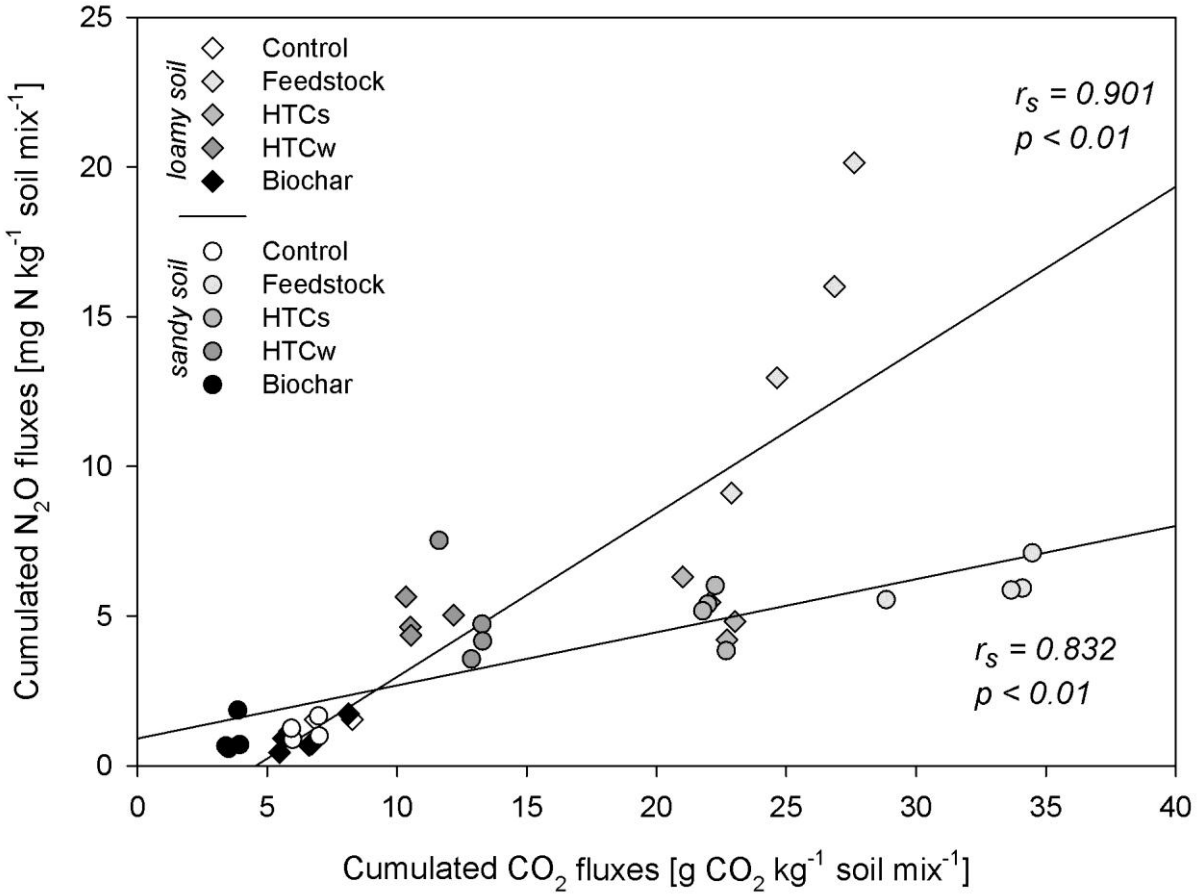

Figure 5-2: Pearson correlation of cumulated CO_2 and N_2O fluxes [g kg^{-1} soil mix].

5.3.4 GHG (CO_2eq) emissions during simulated weather and priming events

Most of the CO_2eq (mean 58.4 % in sandy soil and 54.3 % in loamy soil) were lost during the period of labile carbon degradation (Section 1) in the first four months, enhanced by two fertilization events (Figures 5-3 and 5-4). The WHC increase to 100 % and subsequent drying (Section 2) led to a renewed emission outburst in both soils, amounting to 20.4 and 19.1 % of the total cumulated CO_2eq emissions (with the dry-up period contributing less than the wetting). Thereafter, neither the third, fourth and fifth fertilization event, nor the freeze-thaw cycles (Section 3) led to larger GHG emission outbursts, contributing only 2.4 and 3.8 % to the total cumulated CO_2eq emissions in sandy and loamy soil, respectively. Plowing (Section 4) and glucose addition (priming, Section 5) stimulated emissions from all treatments in both soils, contributing 10.4/11.9 % (Section 4) and 8.6/11.0 % (Section 5) to total CO_2eq emissions in the sandy/loamy soils, respectively.

Total CO_2eq emissions from both control soils were similar in Sections 1, 2 and 4 (labile carbon degradation, wet-dry and plowing). During Section 3 (freeze-thaw), the loamy control soil showed larger emissions than the sandy soil, while it was vice versa during Section 5 (priming) (Figures 5-3 and 5-4; Table 5-3). The non- or low-carbonized carbon amendments led to significantly higher GHG emission sums in most of the Sections (1, 2 and 4: feedstock > HTCs > HTCw), compared to the control and biochar treatments. During Section 3 (freeze-thaw), the sandy control soil emitted the lowest CO_2eq sum, followed by biochar/HTCs/HTCw while feedstock treatments exhibited the highest emissions. In the loamy soil, during freeze-thaw events, emissions from the HTCw treatment were as low as from the control, followed by biochar < HTCs/feedstock treatments. Subsequent to glucose addition (Section 5, priming), in sandy soil, the control and HTCw treatments showed the highest CO_2eq emissions, followed by the feedstock and HTCs/biochar treatments. In loamy soil, the CO_2eq emissions in Section 5 were not different between treatments (Figures 5-3 and 5-4, Table 5-3).

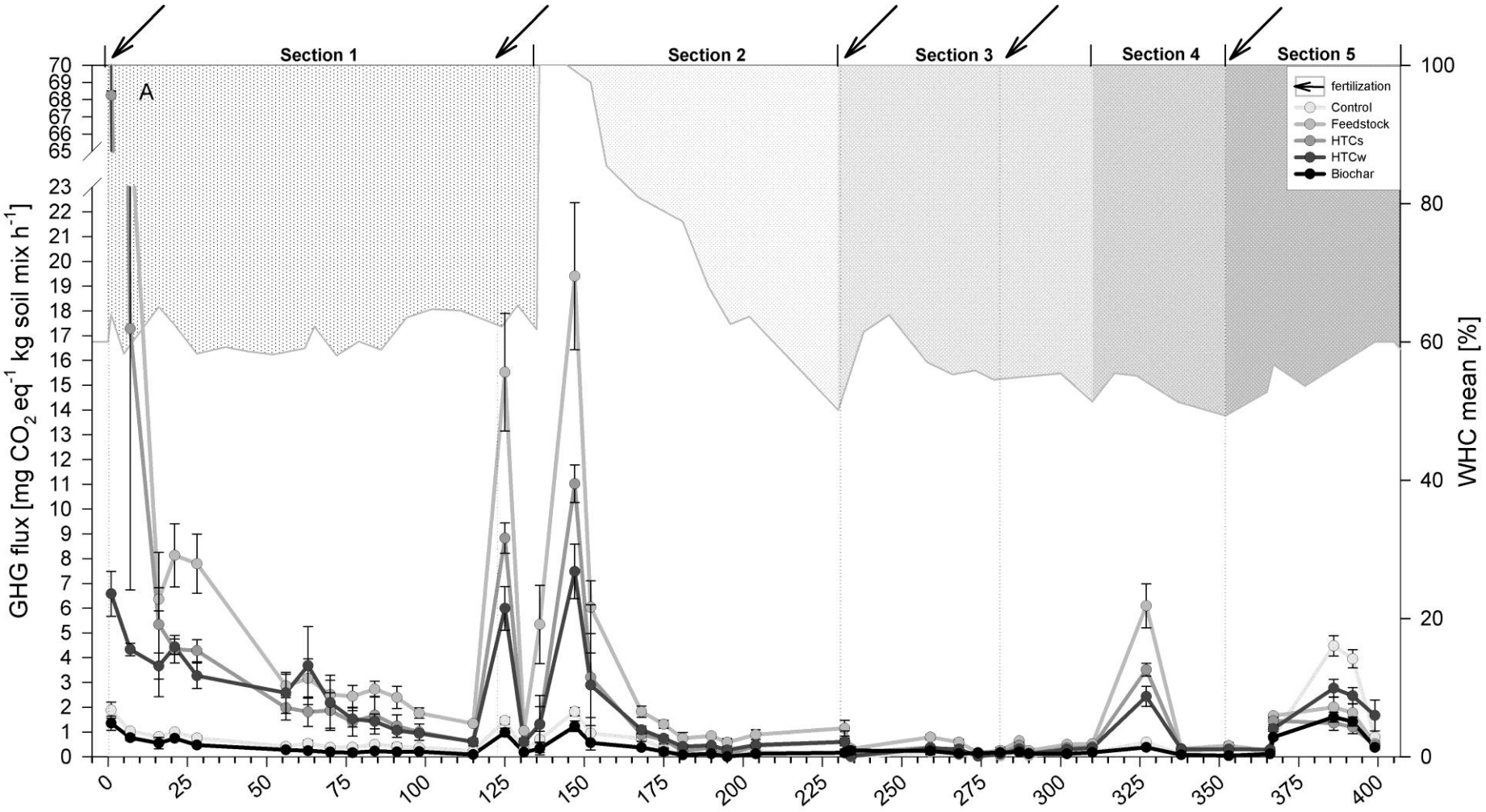

Figure 5-3: Total CO_2eq fluxes [g CO_2 kg-1 soil mix, left Y-axis] of the five treatments over the incubation period with mean and standard deviation. The white area marks the WHC [in %, right Y-axis]; differently patterned areas mark the five Sections of the simulated weather events and disturbances. A: sandy soil

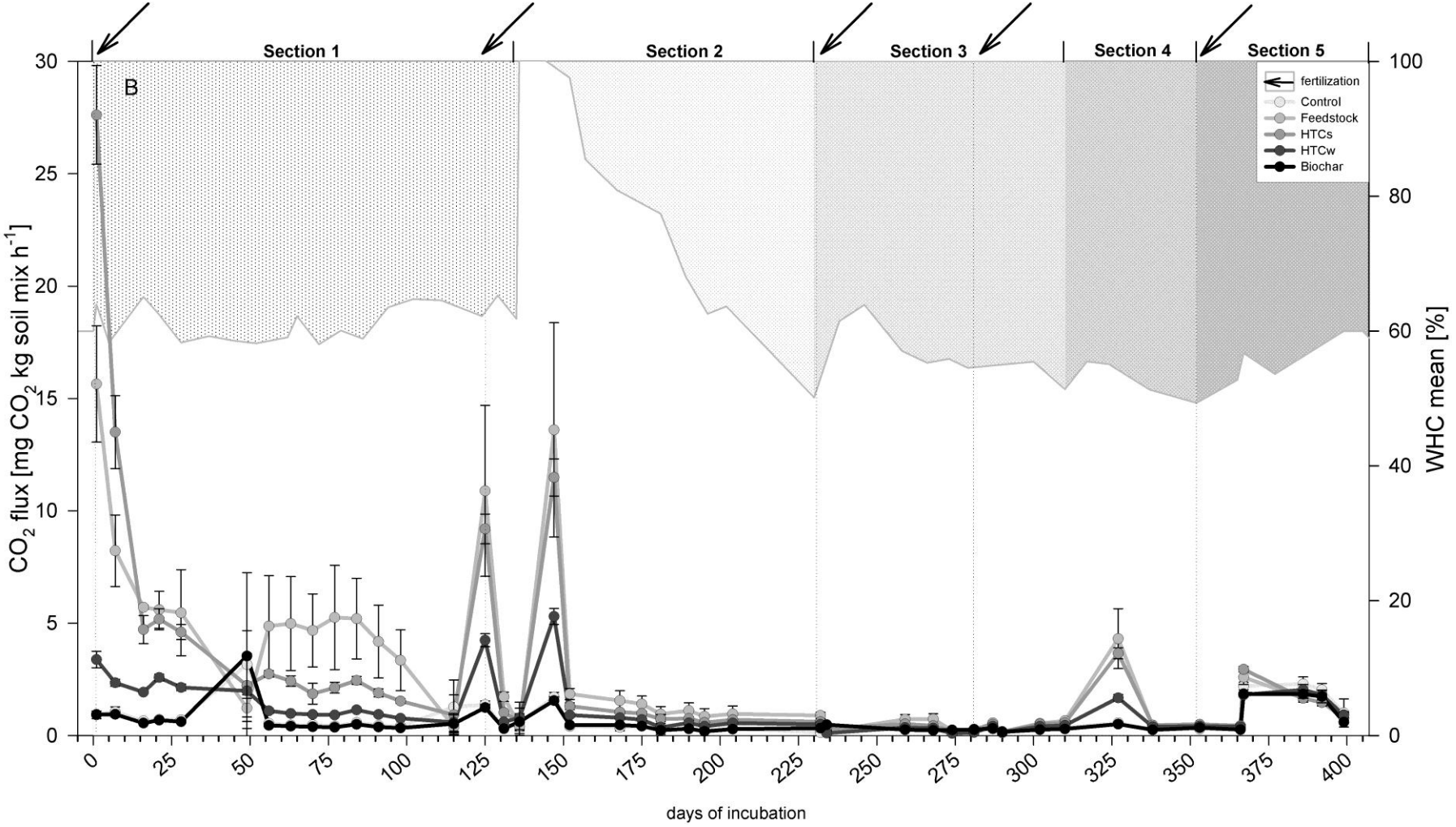

Figure 5-4: Total CO_2eq fluxes [g CO_2 kg-1 soil mix, left Y-axis] of the five treatments over the incubation period with mean and standard deviation. The white area marks the WHC [in %, right Y-axis]; differently patterned areas mark the five Sections of the simulated weather events and disturbances. B:

The carbon amendments interacted differently with the two soils during the five sections (Table 5-3). None of the carbon amendments showed a distinctive degradation pattern according to the soil type except biochar, with higher CO_2eq emissions from the loamy compared to the sandy soil throughout all sections, except Section 1 (Table 5-3).

5.3.5 Char-C losses during the simulated weather and priming sections

The fractions of char-C and SOC in total CO_2 emissions were different for the period before and during the priming experiment, revealing an altered degradation behavior (Table A.5-3). During Section 1 (days 0-131), the ratio of char-C/SOC in total emissions followed the carbonization degree with highest ratios for feedstock and HTCs, followed by HTCw and biochar. Glucose addition in Section 5 altered the source partitions of SOC- and char-C in CO_2 emissions, increasing the fractions of HTCw- and biochar-C and decreasing the fractions of feedstock and HTCs in total CO_2 emissions.

The mean daily char-C loss in the five sections was lowest in Section 3 (freeze-thaw) ($p < 0.05$), compared to all other sections, with no significant differences among the other Sections. Testing the losses of the single treatments for each soil showed very different daily C losses of the carbon amendments (Table 5-4). Mean daily C losses were highest in the initial phase of the experiment (Section 1, labile carbon) for feedstock and HTCs in both soils. Losses of HTCw were slightly elevated in the beginning (Section 1), decreased in the following Sections and were triggered again in Section 5 (priming) by glucose addition similar in the two soils. Consequently, initial and final HTCw degradation rates (Sections 1 and 5) were higher than during the simulated weather events (Sections 2-4: wet-dry, freeze-thaw and plowing). Biochar degradation in both sandy and loamy soil was slightly increased in the beginning, compared to Sections 2- 4, and was increased by glucose addition, resulting in highest mean char-C losses during Section 5 (Table 5-4). Expressed as percentages of the total char-C loss, 1.3, 3.3, 16.0 and 52.1 % and 3.2, 5.3, 16.4 and 25.1 % were lost during priming from the feedstock, HTCs, HTCw and biochar treatments in sandy and loamy soil.

Table 5-3: Mean cumulated GHG (CO_2eq) fluxes, means (n=4) ± SD during the different Sections [g CO_2eq kg^{-1} soil mix]. Letters indicate significant treatment differences in every section across both soil types (One-way ANOVA and Holm-Sidak Post-hoc tests, CI=95 %).

Sections	Sandy soil					Loamy soil				
	Ctrl	Fstck	HTC_s	HTC_w	BC	Ctrl	Fstck	HTC_s	HTC_w	BC
Section 1 (labile carbon)	**2.71**±0.55^{A}	**22.38**±1.7^{E}	**15.98**±0.6^{D}	**8.28**±0.43^{C}	**1.72**±0.48^{A}	**2.77**±0.51^{A}	**15.45**±1.4^{D}	**13.16**±0.3^{C}	**5.04**±0.66^{B}	**2.70**±1.24^{A}
Section 2 (wet-dry)	**1.24**±0.09^{B}	**8.23**±0.80^{E}	**4.10**±0.13^{D}	**3.49**±0.30^{D}	**0.74**±0.13^{A}	**1.05**±0.19^{B}	**5.15**±1.44^{D}	**4.05**±0.21^{D}	**2.42**±0.17^{C}	**1.12**±0.09^{B}
Section 3 (freeze-thaw)	**0.16**±0.01^{A}	**0.82**±0.09^{D}	**0.42**±0.04^{B}	**0.34**±0.03^{B}	**0.32**±0.08^{B}	**0.39**±0.06^{B}	**0.76**±0.20^{D}	**0.64**±0.01^{D}	**0.42**±0.02^{B}	**0.50**±0.01^{C}
Section 4 (plowing)	**0.49**±0.02^{B}	**4.02**±0.57^{E}	**2.41**±0.11^{D}	**1.78**±0.24^{C}	**0.36**±0.07^{A}	**0.68**±0.07^{B}	**3.10**±0.80^{D}	**2.70**±0.15^{D}	**1.45**±0.11^{C}	**0.62**±0.03^{B}
Section 5 (priming)	**2.26**±0.2^{D}	**1.44**±0.17^{B}	**1.08**±0.17^{A}	**1.73**±0.18^{C}	**0.96**±0.06^{A}	**1.73**±0.14^{C}	**1.57**±0.11^{C}	**1.68**±0.07^{C}	**1.55**±0.13^{C}	**1.43**±0.09^{C}

Table 5-4: Mean daily char-C-loss [mg d^{-1}] during the five sections, means ± SD. Letters indicate significant differences of one treatment across the five sections (One-way ANOVA and Holm-Sidak Post-hoc tests (1) or Anova on ranks and Tukey Post-hoc test (2) CI=95%)

	Sandy soil				Loamy soil			
Sections	Feedstock[1)]	HTC_s[1)]	HTC_w[2)]	BC[1)]	Feedstock[2)]	HTC_s[1)]	HTC_w[1)]	BC[2)]
Section 1 (labile carbon)	39.56 ± 3.28^{C}	26.48 ± 0.63^{D}	5.86 ± 0.52^{B}	0.60 ± 0.14^{B}	14.67 ± 1.4^{B}	18.11 ± 0.90^{C}	4.42 ± 0.67^{B}	2.33 ± 1.13^{B}
Section 2 (wet-dry)	13.14 ± 1.72^{B}	6.84 ± 0.23^{C}	3.16 ± 0.15^{AB}	0.31 ± 0.06^{A}	6.58 ± 1.83^{AB}	7.23 ± 0.37^{ABC}	2.75 ± 0.19^{AB}	1.21 ± 0.10^{A}
Section 3 (freeze-thaw)	1.98 ± 1.35^{A}	6.84 ± 0.23^{C}	0.50 ± 0.05^{A}	0.20 ± 0.06^{A}	1.39 ± 0.36^{A}	1.65 ± 0.03^{A}	0.69 ± 0.04^{A}	0.77 ± 0.02^{A}
Section 4 (plowing)	6.56 ± 6.66^{AB}	7.47 ± 0.53^{C}	2.85 ± 0.35^{AB}	0.22 ± 0.03^{A}	7.85 ± 2.02^{AB}	9.56 ± 0.55^{B}	3.27 ± 0.24^{AB}	$1.33\ 1 \pm 0.07^{AB}$
Section 5 (priming)	0.72 ± 1.45^{A}	3.93 ± 0.52^{B}	5.63 ± 0.77^{B}	3.33 ± 0.17^{C}	2.64 ± 0.18^{A}	5.24 ± 0.22^{AB}	5.05 ± 0.42^{B}	4.25 ± 0.27^{C}

5.3.6 Carbon balance and degradation rate

The relative remaining carbon contents (SOC, "char"-carbon and TOC) of the soil mixtures after 13 months of incubation (t_2) are given as percent of the initial C (=100 %, t_0) in Figure 5-5. The relative SOC losses from sandy soil were higher throughout all treatments. In sandy soil, SOC loss of the feedstock and HTCs treatments was similar to that of the control, whereas HTCw increased SOC losses. In loamy soil, SOC loss of the feedstock treatment was higher compared to the control soil, whereas no significant SOC loss was observed for the other treatments, except for biochar. Biochar amendment led to significantly lower SOC losses in both soils, compared to the respective controls (Figure 5-5 A).

Char-C loss followed the carbonization degree in sandy soil: feedstock > HTCs > HTCw > biochar. Also in loamy soil, feedstock/HTCs degradation was higher than that of HTCw/biochar. Feedstock degradation was higher in sandy soil compared to loamy soil, whereas it was vice versa for biochar with higher degradation rates in loamy than sandy soil (Figure 5-5 B, Figures 5-6 and 5-7). Comparing initial and end char-C amounts of the single treatments revealed that all carbon amendments except biochar lost significant amounts of initial char-C over the incubation period in both soils. Practically all C was lost from feedstock in sandy soil (97.7 ± 4.7 %), whereas it was more stable in loamy soil (43.3 ± 9.0 % C loss) (Table 5-2: significant soil × treatment interactions). Degradation of HTCs and HTCw was not different between soil types with mean C loss of 42.9 % from HTCs and 10.6 % from HTCw. Biochar-C loss was insignificant in both soils with 98.5 and 96.3 % of the initial carbon remaining at the end of the experiment in sandy and loamy soil, respectively.

TOC (SOC+char-C) loss from the treatments control, feedstock, HTCs and HTCw was higher in sandy soil, compared to loamy soil, whereas TOC loss from biochar treatments was low and not soil dependent. TOC loss was highest from the feedstock treatments in both soils, followed by HTCs>control>HTCw>biochar treatments in sandy soil and by HTCs/HTCw/control>biochar in loamy soil (Figure 5-5 C).

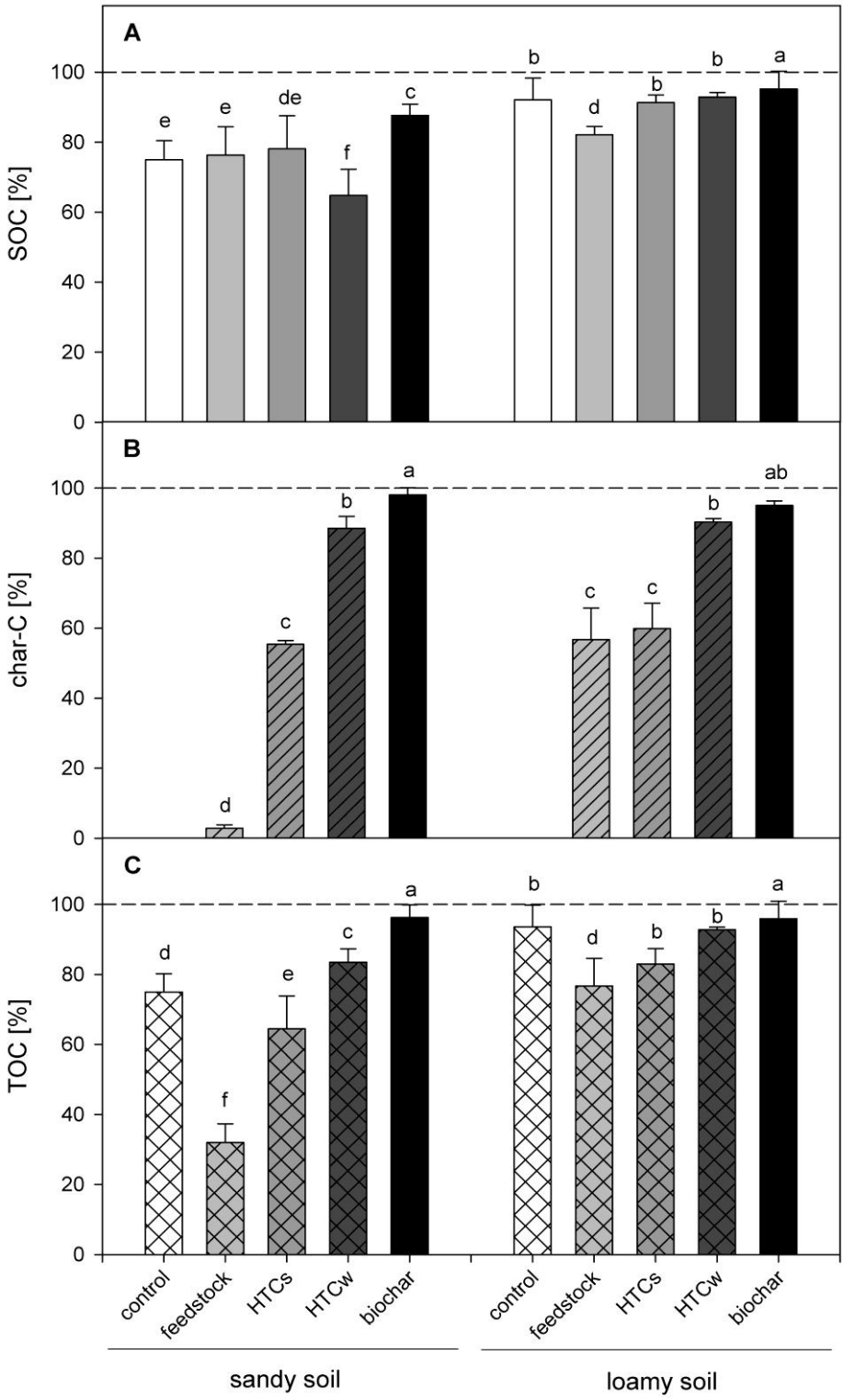

Figure 5-5: Percental SOC, char-C and TOC-remains (A, B and C) at the end of the incubation period (n=4), means ± SD. Letters depict significant treatment differences, as determined by one way ANOVAs and Holm-Sidak or LSD Post-hoc tests or an ANOVA on Ranks with a SNK Post-hoc test (CI = 95%).

Total carbon buildup by carbon amendments was more pronounced in the sandy soil. There was a negative carbon buildup due to feedstock amendment and a significant linear increase in carbon buildup depending on the carbonization degree of the substrates, with C gains of initial carbon for HTCs, HTCw and biochar, respectively. In loamy soil, the C buildup was less pronounced (Table 5-5).

All structural properties (H-C/O-C ratio, cellulose and aromatics content) were in line with the ratio of initial TOC / remaining TOC (C_{in}/C_{rem}), independent of the soil type ($p<0.001$). In sandy soil, the correlations of C_{in}/C_{rem} were highest for cellulose and H/C ratios (r^2_{adj} = 0.992 and 0.993, respectively); in loamy soil, best fits were obtained with O/C ratios (r^2_{adj} = 0.972) (Figures A.5-10 and A.5-11).

5.3.7 MRT and half life

The single exponential decay model fitted the data with R^2 values ranging from 0.92 to 0.95. Both MRT and half-life of the carbon amendments depended linearly on their carbonization degree and on soil type, with the highest MRT of around 90 years for biochar in sandy soil (Table 5-5). In loamy soil, biochar MRT and half-life was significantly lower than in sandy soil (27 years), nevertheless it was still significantly higher than the MRT and half-life of all other carbon amendments (2-12 years). MRT and half-life of feedstock and HTCw was significantly higher in loamy soil compared to sandy soil (2 vs 0.3 years and 12 vs 10 years, respectively), whereas MRT and half-life of HTCs material was similar in both soils (≈ 2 years).

Table 5-5: Remains of initial C, total C buildup at the end of the experiment (t2) in %, mean residence time (MRT) and half-life [years] of the carbon amendments in the two different soils (mean ± SD). Letters depict significant treatment differences across both soil type (ANOVA on Ranks, followed by a SNK-post-hoc-test, CI=95%).

Soil	Sandy soil				Loamy soil			
Treatment	Feedstock	HTCs	HTCw	Biochar	Feedstock	HTCs	HTCw	Biochar
Remains of initial char-C [%]	2.35± 4.69^{A}	55.24 ± 3.07^{B}	88.56 ± 0.85^{C}	98.09 ± 0.07^{C}	56.72 ± 9.04^{B}	59.05 ± 7.28^{B}	90.32 ± 0.95^{C}	94.94 ± 1.30^{C}
TOC-buildup [%]	-22.71±24.48^{A}	111.75±20.75^{D}	215.67±12.62^{E}	303.79±14.88^{F}	2.99±8.26^{A}	31.07±5.92^{B}	59.47±1.14^{C}	58.18±14.3^{C}
MRT [years]	0.29 ± 0.12^{A}	2.23 ± 0.20^{B}	10.72 ± 0.91^{C}	90.67 ± 4.64^{F}	1.97 ± 0.58^{B}	2.34 ± 0.57^{B}	12.44 ± 1.10^{D}	27.31 ± 6.79^{E}
Half-life [years]	0.20 ± 0.08^{A}	1.54 ± 0.14^{B}	7.43 ± 0.63^{C}	62.84 ± 3.22^{F}	1.37 ± 0.40^{B}	1.62 ± 0.39^{B}	8.62 ± 0.76^{D}	18.93 ± 4.71^{E}

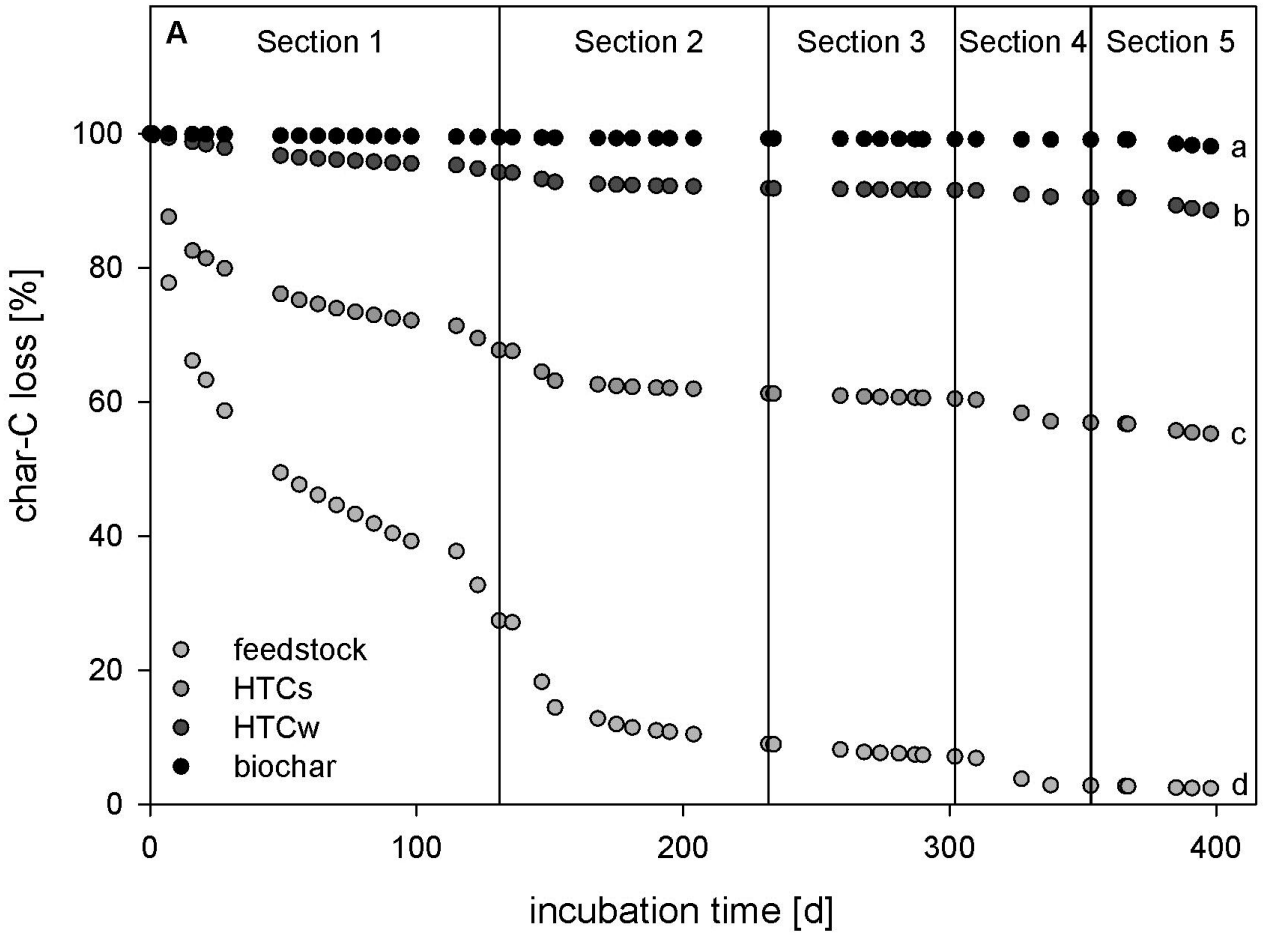

Figure 5-6: Percental char-C-loss over the incubation period of 389 days, separated according to the different sections. Letters depict significant differences among the treatments (Kruskal-Wallis-ANOVA on Ranks and Tukey test, CI=95%). A=sandy soil

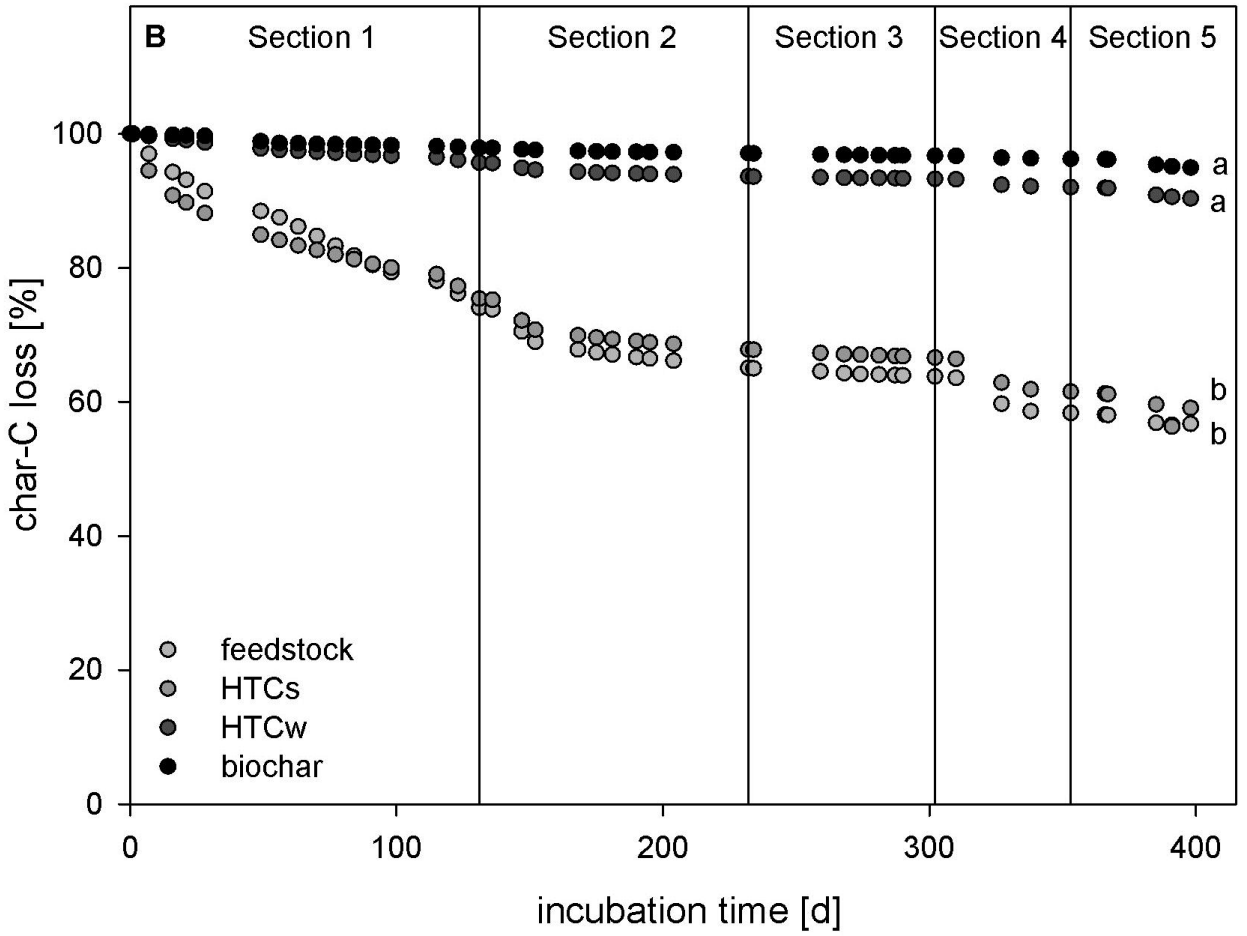

Figure 5-7: Percental char-C-loss over the incubation period of 389 days, separated according to the different sections. Letters depict significant differences among the treatments (Kruskal-Wallis-ANOVA on Ranks and Tukey test, CI=95%). B=loamy soil.

5.3.8 C_{mic} and EOC with appending CO_2 emissions

Both mean C_{mic} and EOC were higher in loamy soil (C_{mic} = 266.8 µg g^{-1} dw, EOC = 200.2 µg g^{-1} dw) compared to sandy soil (C_{mic} = 149.9 µg g^{-1} dw, EOC = 119.8 µg g^{-1} dw, $p < 0.001$), throughout all treatments except HTCw with similar values in both soils. HTCs led to highest EOC and C_{mic} amounts in loamy soil, whereas HTCw exhibited highest C_{mic} and EOC in sandy soil (Figure 5-8 A, B). Both C_{mic} and EOC correlated positively with the cumulated CO_2 flux (t_2-t_3) ($r = 0.681$, $p < 0.001$ and $r = 0.815$, $p < 0.001$) and final pH values ($r = 0.67$, $p < 0.001$ and $r = 0.68$ $p < 0.001$). The CO_2 flux (t_2-t_3) was different for the factors soil and treatment with significant interactions for all treatments except the control treatment, where fluxes were similar in sandy and loamy soil. All other treatments exhibited higher fluxes in the loamy soil. The CO_2 flux patterns were slightly altered compared to the experimental period before glucose addition, with a shift from feedstock mineralization towards HTCs and/or HTCw mineralization; fluxes from biochar treatment were reduced to levels similar to the time period before glucose addition, in both soils (Figure 5-8 C).

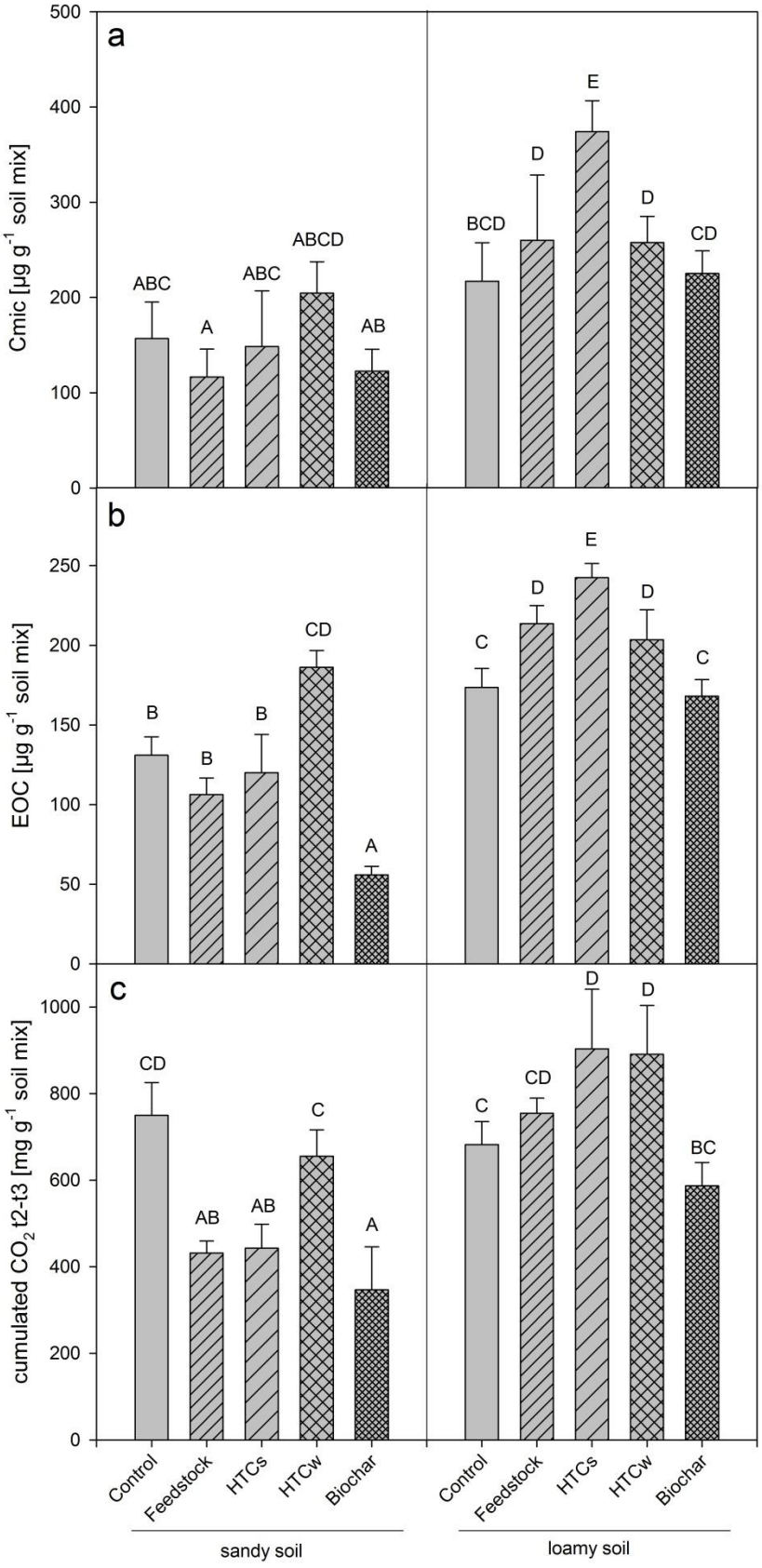

Figure 5-8: Mean and standard deviation of Cmic, EOC [µg g^{-1} soil mix] and cumulated CO_2 fluxes [mg g^{-1} soil mix] at the final period of the experiment in the two different soils. Letters depict significant treatment differences (Two way ANOVA and Holm-Sidak post-hoc test, CI = 95%).

5.3.9 PLFAs

All bacterial (gram-positive and gram-negative) and fungal PLFAs were more abundant in loamy soil (total mean 22.4 nmol g^{-1} soil mix dw, HTCs > control/feedstock/HTCw/biochar), compared to sandy soil (7.7 nmol g^{-1} soil mix dw, no differences between treatments) (Figure 5-9). The carbon amendments had significant effects on most PLFA groups, with significant interactions regarding the soil type. In the loamy soil, HTCs caused a significantly larger abundance of gram-positive bacteria compared to all other treatments. The abundance of gram-negative bacteria followed the sequence control/biochar/HTCs < HTCw < feedstock in sandy soil and HTCs > control/feedstock/HTCw/biochar in loamy soil. Also, the fungal biomass exhibited treatment differences in the sequence HTCs ≤ biochar ≤ feedstock/HTCw < control in the sandy soil and control/biochar < feedstock/HTCw < HTCs in the loamy soil.

In the loamy soil, fungi, gram-positive and gram-negative bacteria correlated well with EOC contents ($r = 0.778$, $p < 0.001$; $r = 0.710$, $p < 0.001$; $r = 0.827$, $p < 0.001$). In sandy soil, only gram-positive bacteria correlated weekly with EOC ($r = 0.535$, $p < 0.05$). Single and total PLFA groups correlated with the cumulated CO_2 flux (t_2-t_3) ($r = 0.725$, $p < 0.001$).

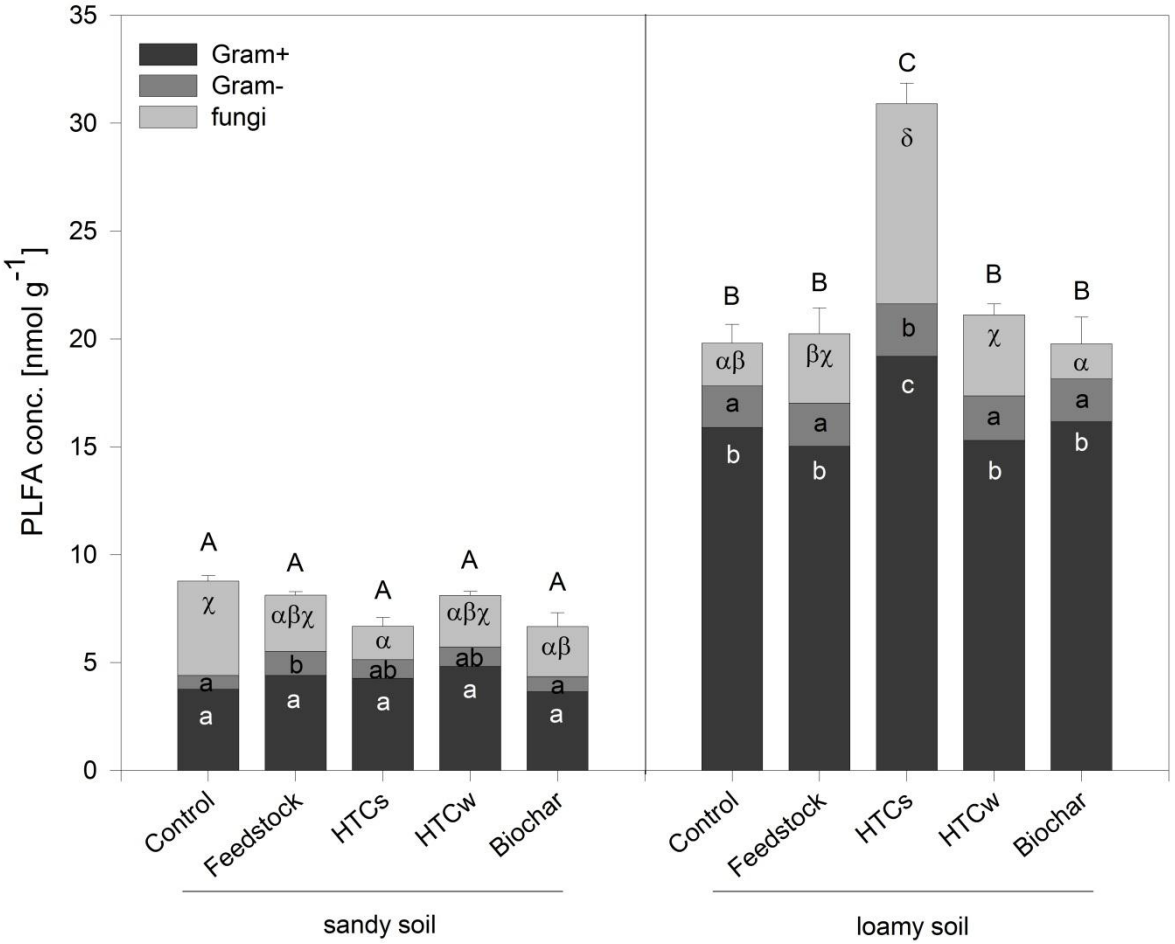

Figure 5-9: Total mean PLFA concentration [nmol g^{-1}] ± SD and their $gram^{+}$, $gram^{-}$ and fungal shares given in grey shades. The white lowercase letters indicate significant treatment differences in $gram^{+}$ bacteria concentrations, black lowercase letters mark treatment differences in gram- bacteria concentrations, Greek letters show differences between fungal PLFA concentrations and uppercase letters give differences in total PLFA concentrations.

5.4 Discussion

5.4.1 C-degradation

Recent results suggest that laboratory incubation studies under constant conditions may underestimate the degradability of carbonized plant material compared to alternating conditions including weather changes or nutrient/labile carbon supply (Ventura *et al.*, 2014). Hence, in this study, a series of alternating conditions including nitrogen and labile-C addition were applied to accelerate degradation under high soil temperatures (except freeze-thaw cycles). The results suggest that especially C from feedstock and steam-carbonized HTCs was readily available to microorganisms, as indicated by high amounts of cellulose and large H/C-O/C ratios (Siu and Reese, 1953; Eibisch *et al.*, 2013). Water-carbonized HTCw was less degradable than HTCs and feedstock, but still significantly more degradable than biochar. Interestingly, HTCw triggered the degradation of native SOC rather than being mineralized itself, which was also found by others, possibly due to supply of extra nutrients to microorganisms for enhanced SOC degradation (Steinbeiss *et al.*, 2009; Bamminger *et al.*, 2014a). Nevertheless, the relative TOC loss was reduced by HTCw in sandy soil and was similar to the control in loamy soil, indicating, together with the relative C buildup at the end of the experiment, its suitability for carbon accumulation especially in sandy soils (Table 5-5, Figure 5-4). Biochar reduced the SOC- and TOC-losses from both soils, though the reduction was more distinct in sandy soil, pointing to a negative priming effect. Negative priming following biochar amendment to soil has been observed before, mostly as medium- to long-term effect (5-16 months) (Jones *et al.*, 2011; Zimmerman *et al.*, 2011; Maestrini *et al.*, 2014), whereas in the short-term (18-90 days), biochar amendment often led to positive priming of SOC (Zimmerman *et al.*, 2011; Maestrini *et al.*, 2014). Nevertheless, in our experiment, biochar did neither induce positive priming of SOC in the short- or the long term, independent of the soil type, nor was mineralized in significant amounts itself, which was also found by Cross and Sohi (2011). Consequently, the TOC contents in the sandy and loamy soils after biochar application

remained significantly increased compared to all other carbon amendments, regardless of the alternating experimental conditions applied to foster decomposition.

Biochar was less degraded in sandy soil, compared to loamy soil. The low initial SOC content of the sandy soil could imply that C-saturation was not given at the beginning of the experiment. Thus, a small but considerable carbon storage capacity, emanating from the clay content (5 %) could have facilitated biochar stabilization by the formation of clay-biochar complexes (Baiamonte *et al.*, 2014). Additionally, the increase of the soils´ WHC due to biochar amendment compared to the control could have facilitated the formation of soil aggregates in the biochar amended sandy soil (Piccolo *et al.*, 1996; Glaser *et al.*, 2002; Dugan *et al.*, 2010), even though such effects are reported to occur only in the long term and with biochar application rates > 20t/ha (Borchard *et al.*, 2014a; Tammeorg *et al.*, 2014). Moreover, these properties could apply to all carbon amendments in sandy soil and may thus not fully explain the stabilization of organic material by the biochar in sandy soil. Hence, sorption of (dissolved) organic material onto the biochar surface or encapsulation into biochar pores, limiting the substrate available to microorganisms, may better explain the negative priming effects observed here (Kasozi *et al.*, 2010; Zimmerman *et al.*, 2011; Barnes *et al.*, 2014; Lu *et al.*, 2014). Chemical as well as structural properties of carbon(ized) substrates such as H/C-O/C ratios and/or the amount of aromatic carbon compounds have long been identified as indicators for recalcitrance against degradation (Krull *et al.*, 2009; Lee *et al.*, 2010; Nguyen *et al.*, 2010; Spokas, 2010; Schimmelpfennig and Glaser, 2012; Singh *et al.*, 2012), together with the ash content (Bai *et al.*, 2013). This indication was confirmed by our experiment for the continuum of all four different carbon amendments, as the ratios of initial/remaining TOC correlated well with all structural properties (Figures A.5-10 and A.5-11). However, as the identical carbon amendments, namely feedstock and biochar, interacted differently with the two soils, it seems obvious that soil characteristics such as diverse microbial populations in the different soils influenced the C degradation patterns as well (Nguyen *et al.*, 2014). Feedstock material was less degraded in loamy soil, most likely due to aggregate formation, rendering it more recalcitrant against microbial decomposition compared to sandy soil (Zhang *et al.*, 2014b).

Besides abiotic factors, negative priming of SOC observed with biochar was also attributed to biotic factors such as pH induced changes of the microbial population or inhibition of

microbial metabolism by potentially toxic compounds released from biochar (Cross and Sohi, 2011; Zimmerman *et al.*, 2011; Jones *et al.*, 2012). Inhibitory effects e.g. by toxic components seem unlikely here, since the biochar did not contain toxic substances when analyzed (unpublished results). Furthermore, substrate induced respiration measurements using subsamples of this experiment 6 months after initiation showed that microbial biomass was increased but not decreased by all carbon amendments, compared to the control. Microbial biomass followed the reverse degradation order in the sandy soil (feedstock >HTCs > HTCw > biochar > control) and the order HTCs > feedstock > biochar > HTCw > control in the loamy soil (Eckhardt, 2013). Nevertheless, negative priming caused by a decrease of the microbial and enzymatic activity has been found by others, possibly due to sorption of quorum sensing molecules onto biochar, making them less available to soil microorganisms (Chintala *et al.*, 2014).

The slightly acidic pH of the sandy soil was increased significantly by biochar amendment at the start of the experiment and thus could have improved the milieu for microbial metabolism (Farrell *et al.*, 2013); however, the observed decrease in carbon mineralization here counteracts this assumption.

5.4.2 Kinetics, carbon loss and gain

The amount of C lost from the different carbon amendments during the experiment (Table 5) is slightly higher than data reported from other authors. Bai *et al.* (2013) used the same chars (except HTCw), mixed them with a range of soils and reported C-losses of 32-37 % from feedstock (here: 43-98 %), 27-30 % C-loss from HTCs (here: 41-45 %) and 0-3% C-loss from biochar (here: 2-5 %) over a period of 200 days. The differences are easily explained by a longer experimental duration, a higher incubation temperature and the alternating, diverse degradation-promoting conditions that were applied in this study. Nguyen *et al.* (2014) incubated a biochar produced at 450 °C from switchgrass together with five different soils at 25 °C and 60 % WHC. Their reported carbon losses in the range of 2.1-4.1 % are in line with the results obtained here.

Fang *et al.* (2014) incubated mixtures of woody (*Eucalyptus salinga* Sm.) biochar produced at 550 °C, with a range of soils over 12 months at 20-40 °C and reported biochar-

C losses of 0.30-0.42 % and 0.97-1.16 % at 20 and 40 °C respectively. Here, lower values may be explained by the difference in feedstock, since woody biochars are considered more stable than biochar from more brittle plant material such as leaves, due to a higher amount of fused aromatic C-structures and lower ash (SiO_2) and mineral contents (Kloss *et al.*, 2012; Singh *et al.*, 2012; Zhao *et al.*, 2013a).

The calculated MRT and half-lives are, especially in view to biochar in loamy soil, rather short compared to the MRT of 550 °C biochar reported in other studies (90-10^{14} years) (Keith *et al.*, 2011; Gajić and Koch, 2012). This might be attributable to the simulated weather events plus nitrogen and glucose additions during the experiment, since the fluctuations in the C-degradation rates following these events were mostly very high and consisted of several degradation boosts. Thus, the results suggest that, the influence of soil type and climatic conditions on the degradation of carbon amendments might have been underestimated. Nonetheless, our general result, the prolongation of the half-life of uncarbonized *Miscanthus* × *giganteus* straw by hydrothermal carbonization and pyrolysis are in good agreement with the results of Bai *et al.* (2013) and (Qayyum *et al.*, 2012), although none of these studies used a continuum of four carbonization types or simulated weather events, fertilization and priming. Thus, the results confirm the suitability of hydrochar (especially HTCw) and more so biochar to increase the soils´ TOC content and to sequester carbon even under exposure to degradation-promoting conditions.

5.4.3 N_2O emissions in relation to the carbonization grade

All carbon amendments except biochar significantly increased the cumulative N_2O emissions over the course of the incubation study compared to the control. Biochar reduced the N_2O emissions by 26.5 and 8.5 % in sandy and loamy soil compared to the control however the reductions were not significant. Biochar application frequently reduced soil N_2O emissions particularly after N fertilization (Van Zwieten *et al.*, 2010b; Kammann *et al.*, 2012) or during freeze-thaw cycles (Kettunen and Saarnio, 2013), and even in the presence of N_2O-producing earthworms (Augustenborg *et al.*, 2012). The largest, almost dramatic N_2O emission increase was observed with the straw-like uncarbonized *Miscanthus* feedstock amended to loamy soil, while the increase was lower in the sandy

soil (although highest among all carbon amendments). The same effect – increased N_2O emissions with uncarbonized compared to pyrolyzed wheat straw – was observed by Cheng *et al.* (2012) in a field study on Chernozem soil. Wolf *et al.* (2010) observed that grazing, i.e. the removal of grassy litter, reduced the N_2O emissions of Mongolian steppe significantly, and that grassy litter decomposition may be an underestimated source of N_2O formation in natural or semi-natural grasslands. The latter, as well as our findings, are in line with earlier observations in the grassland field site (where the loamy soil for the incubation had been taken): Schimmelpfennig *et al.* (2014) observed significantly increased N_2O emissions in the second year after top-dressing of *Miscanthus* straw; no increase was found when the same amount of straw-C was applied as biochar. Rather, N_2O emissions tended to be reduced during a freeze-thaw period (Schimmelpfennig *et al.*, 2014). The reduction in N_2O emissions by (woody) biochar amendment is a common finding confirmed by meta-analysis (Cayuela *et al.*, 2014; Van Zwieten *et al.*, 2015).

For hydrochar, less information is available. While Malghani *et al.* (2013) observed N_2O emission reductions following hydrochar application to non-fertilized soils, Kammann *et al.* (2012) reported that soil N_2O emissions were greatly stimulated in an incubation study after mineral-N fertilizer had been added to loamy soil amended with two different hydrochars. In the above-mentioned grassland field study, Schimmelpfennig *et al.* (2014) found no changes in the N_2O emissions due to hydrochar application. However, this study indicates that hydrochar amendments still stimulated N_2O emissions significantly compared to the unamended control, but that the increase was reduced (more so with increasing HTC carbonization grade) and retarded in time and/or dependent on N-fertilization compared to the uncarbonized feedstock.

In our study, differences in the water/oxygen content of the soils were likely eliminated by adjusting the WHC (Case *et al.*, 2012). However, continuous O_2 consumption during the mineralization of the more labile carbon compounds may have reduced the redox potential of the soil mixtures, triggering the use of NO_3^- as electron donor for denitrification (Miller *et al.*, 2008). Especially in the SOC richer loamy soil, a higher amount of anaerobic microsites may have, together with the higher pH, promoted denitrifying bacteria (Firestone, 1982; Herold *et al.*, 2012). Moreover, fungal co-denitrification often dominates N_2O emissions in temperate grassland soil (Laughlin and Stevens, 2002). A larger fungal

biomass fraction was indeed confirmed for the loamy-soil HTCs and HTCw treatments at the end of the incubation study ($p<0.001$), whereas the fungi in the feedstock treatment were only slightly increased. Here, the microbial community composition likely had changed over the course of the experimental period. The easily accessible feedstock C may have been depleted so that the fungal biomass was reduced again when finally measured. Summarizing the evidence obtained here and earlier (Schimmelpfennig *et al.*, 2014), we have evidence that N_2O production by denitrifying fungi may have been responsible for the higher N_2O emissions with uncarbonized or weaker carbonized materials (Laughlin and Stevens, 2002; Oehl *et al.*, 2010), and that this effect was more pronounced in the loamy than sandy soil. Additionally, biochar but not hydrochar application offers the possibility to reduce N_2O emissions from soils compared to the application of decomposable straw materials. The most interesting open question is if the combination of labile straw material with biochar would result in lower-than-straw-alone N_2O emissions.

5.4.4 Predicting N_2O emissions: correlation of CO_2 and N_2O

Our results revealed that a high amount of cumulative CO_2 emissions (i.e. mineralizable carbon) resulted in high cumulative N_2O emissions. Vice versa, the lack of an additional organic carbon source (control) as well as a carbon amendment with little mineralizable carbon (biochar) resulted in low N_2O emissions. This behavior only became evident because of the carbonization gradient used in our experiment and due to the varied incubation conditions over 13 months. Other char-application studies where CO_2 and N_2O emissions were monitored simultaneously did not report a correlation, but they did not cumulate the fluxes for comparison (Van Zwieten *et al.*, 2010b). As to hydrochar, such a relation was found earlier, with high mean CO_2/N_2O fluxes from beet/bark hydrochar applied to a loamy soil, assumedly due to stimulation of microbial activity (Kammann *et al.*, 2012). Our results suggest that using the CO_2 emissions after litter amendment for predicting cumulative N_2O emissions may be a promising approach that deserves further study.

5.4.5 Methane fluxes

The methane fluxes in the experiment were negligible in the sandy soil, whereas methane oxidation was improved by all carbon amendments in loamy soil, inversely to their carbonization degree (strongest with feedstock, lowest by biochar). Karhu *et al.* (2011) also observed a doubling of the CH_4 uptake in grassland soil when biochar was plowed into the top soil; the authors attributed this to improved soil aeration. This can largely be excluded for this study since the WHC was adjusted to equal values and the most labile substrates, where more O_2 must have been consumed by decomposition, showed the strongest stimulation in CH_4 oxidation. The capacity of soil to take up methane is generally a function of land use, with tilled agricultural soil exhibiting lower oxidation potentials than no-tilled, temperate soils (Mosier and Delgado, 1997). The methane oxidation capacity of soils was found to correlate with the nitrogen status of the soil, in a way that high amounts of available N limited the activity of methanotrophs in non-wetland soils (Chan and Parkin, 2001; Aronson and Helliker, 2010). Correspondingly, an effective sorption and/or immobilization of NH_4^+ or NO_3^- by biochar and/or HTC could have kept methane oxidation intact, whereas freely available NH_4^+ or NO_3^- may have inhibited methane oxidation in the control treatments (Bédard and Knowles, 1989; Dunfield and Knowles, 1995; Schimmelpfennig *et al.*, 2014). Additionally, thermally desorbable organic compounds on the biochar surface (e.g. alkanes, ketones, aromatics) have been identified as an underlying cause of improved methane oxidation of soil amended with biochar (Borchard *et al.*, 2014b). Also an increase in the abundance and activity of methane-producing saprophytic fungi, degrading especially the more labile materials feedstock and HTC might have stimulated methanotrophic bacteria, leading to an improved methane oxidation of the soil mixtures (Lenhart *et al.*, 2012). More mechanistic studies are necessary to identify the causes for the observed increase in the CH_4 consumption following carbon amendment.

5.4.6 Simulated weather events

Initial CO_2 outbursts following the admixture of biochar, hydrochar and feedstock material such as straw to soil have been widely reported (Zimmerman, 2010; Qayyum *et al.*, 2012;

Eibisch *et al.*, 2013; Fang *et al.*, 2014), and have been confirmed by our experiment for feedstock and both hydrochars. In contrast to most reported studies, CO_2 emissions from biochar amended soil in our experiment were never higher than emissions from the control soil. Rather, they were mostly lower than the control, especially in the sandy soil (except during freeze-thaw events). This might be, on the one hand, due to the different properties (low temperature biochar ≤ 375°C) (Bruun *et al.*, 2008), or smaller particle sizes of the biochar used in other studies (Jones *et al.*, 2011). On the other hand, our biochar has been stored 12 months in a closed but not air-tight box before use, thus some production prone, easily degradable volatile compounds might have outgassed over the storage time.

By directed N-fertilization, we aimed at lowering the C/N ratio of the soil to facilitate C degradation and trigger CO_2 and/or N_2O emissions (Chantigny *et al.*, 1999). This occurred for the first two fertilization events and led to a CO_2 emissions peak in the feedstock and HTC treatments, indicating the presence of labile, biologically available carbon compounds. None of the other fertilization events triggered further peak emissions from any of the treatments, suggesting that most of the labile compounds had been degraded by then. Only the disturbance of the soil structure by simulation of plowing led to renewed C and N degradation of feedstock and hydrochar in both soils, assumedly due to destruction of soil aggregates, improving the accessibility of carbon compounds, and provision of oxygen (West and Marland, 2002). Similar results have been reported from an experiment with two hydrochars and four biochars mixed with a Luvisol, where simulated plowing led to high CO_2 and N_2O emissions from the hydrochar soil mixtures, compared to the control (Kammann *et al.*, 2012).

Water logging of N-rich soil promotes denitrifying enzyme gene expression in bacteria using NO_3^- as electron acceptor. The increase of the WHC to 100 %, simulating heavy rainfall, led to an immediate short-term increase of CO_2 and N_2O emissions from all treatments, though emissions from biochar treatments were still lower than or equal to the control soil. Kammann *et al.*, (2012) found higher N_2O emissions from biochar amended soil in nearly water-logged soils (80 % WHC) after N-fertilization only after long-term (1.5 years) incubation of the soil at high (70 % WHC) water regimes.

Freeze-thaw cycles had unexpectedly a rather small though stimulating effect on the GHG emissions. This might be explained by the experimental setup, i.e. the disturbed soil

structure and removal of vegetation, since the die-off of fine roots and disruption of soil aggregates largely contributed to CO_2 and N_2O emissions during freeze-thaw events (Logsdail and Webber, 1959). Freeze-thaw cycles led to significantly higher CO_2 emissions from biochar amended soil compared to the control soil, indicating some small effects of freeze-thaw events on biochar degradation. This can be explained by the disruption of small biochar-soil aggregates, generating particles susceptible to microbial attack (Eastman, 2011), In another experiment, biochar significantly reduced both ammonium and nitrate leaching and N_2O emissions from vegetated soil cores subjected to freeze-thaw events, assumedly due to N-retention (Kettunen and Saarnio, 2013). The same tendency was observed in data from a grassland field experiment, indicating that this interaction requires further attention (Schimmelpfennig *et al.*, 2014).

5.4.7 Priming experiment

Glucose addition led to positive priming of char-C, indicating that char-C was susceptible to co-metabolism during glucose mineralization, especially since N was not a limiting factor (Kuzyakov *et al.*, 2000). The relative fraction of positive priming (although low in absolute terms) was most prominent for the higher-temperature chars such as HTCw and biochar, especially for biochar in sandy soil, where the biochar may have been less protected within soil aggregates compared to loamy soil (Hamer *et al.*, 2004). Glucose addition increased mean biochar mineralization by the factor 6.5, compared to the average daily flux of all other sections (Table 5-4), as was found also by (Kuzyakov *et al.*, 2009), incubating Haplic Luvisol with ryegrass char. This could indicate that possibly similar biochar compounds may generally be prone to co-metabolization.

Glucose addition had little priming effects on the degradation of feedstock and HTCs, likely due to the fact that these materials had been largely mineralized already (Table 5-4). Thus, labile-C priming (e.g. root exudates) and freeze-thaw cycles deserve further study with regard to their impact on long-term biochar stability.

5.4.8 Microbial biomass and composition

Microbial biomass and composition were analyzed at the end of the experimental period, and thus correspond to the end of the proliferation period following glucose amendment (Stotzky, 1965). Therefore, the results reflect the microbial population and related microbial data at the experimental period when the glucose effect had leveled off. EOC, C_{mic} and all $PLFA_{mic}$ groups were higher in the loamy soil compared to sandy soil. This is likely due to the loamy texture, supporting aggregate formation and habitats for microorganisms (Monreal and Kodama, 1997). Additionally, disturbance of the soil structure and aggregates during glucose admixture likely triggered the availability of aggregate protected SOC and EOC to microorganisms (Rovira and Greacen, 1957). The considerable drop of the pH of the soil mixtures, especially in the loamy soil (about one unit) confirms the higher biological activity in loamy soil, leaving protons as metabolite from oxidation.

The increase in the microbial biomass and activity following EOC provision in soil, especially for fungi is well known (Burford and Bremner, 1975). Interestingly, only HTCs increased the total microbial biomass in loamy soil significantly, coinciding with high EOC values in this treatment (Figure 5-6 a, b). An increase in C_{mic} due to hydrochar amendment (maize silage, application rate 4-8 g kg^{-1}) compared to the arable control soil was also found by Bamminger *et al.* (2014a), even in the same magnitude (doubling of C_{mic} compared to the control). Concentrations of nutrients plus depolymerization of carbon structures during the hydrothermal production process, increasing the availability of both nutrients and carbon to microorganisms are likely the reasons for the preference of microorganisms for HTCs, as compared to all other materials including the uncarbonized feedstock (Funke *et al.*, 2013a; Funke *et al.*, 2013b).

C_{mic} in the HTCw treatment in sandy soil was higher compared to the feedstock treatment, most likely driven by the enhanced EOC concentration in this treatment compared to the other treatments. Additionally, glucose amendment may have induced a microbial community change or triggered the activity/enzyme production of a certain microbial group that was able to degrade HTCw (Allison, 2005). In consistence with the cumulative CO_2 emissions, the low C_{mic} values in feedstock and HTCs amended sandy soil imply that

most of the material had indeed been degraded in the preceding experimental period (Figures 5-6 and 5-7). C_{mic}, PLFA and especially EOC in biochar amended sandy soil were low, leading consequently to low CO_2 emissions (Figure 5-6 C). A lower fungal and total biomass in the biochar- compared to the control soil indicates on the one hand higher persistence of microbial biomass in the control soil after glucose addition, on the other hand that biochar decreased the availability of organic material to microorganisms, possibly by sorption mechanisms or blocking of pores, also inducing the negative priming effect found earlier in the experiment. Biochar amendment to loamy soil did not cause differences to the control soil; if EOC sorption occurred it may not have caused effects due to much higher background C values which may be the reason for the low response in this soil even after various degradation promoting treatments.

5.5 Conclusion

The addition of four carbon amendments along a carbonization gradient had largely different effects on the overall GHG budget of the two soils under study. On average, CO_2 accounted for 86.5 % of the cumulative GHG equivalents, highlighting the importance of the stability of carbon amendments in soil with respect to GHG mitigation and C sequestration. Highest degradation rates were found for feedstock, significantly declining along the carbonization gradient down to nearly zero (biochar). Nitrous oxide emissions mainly followed these degradation patterns, reinforcing the CO_2 impact of the single treatments. The influence of the carbon amendments on CH_4 fluxes was restricted to loamy soil, where the intrinsic methane uptake was improved by all treatments, which was interesting but did not contribute much to the overall GHG budget.

Among the simulated weather events employed to "break" the C amendments, freeze-thaw events led to the lowest GHG emissions of all treatments, while the periods of initial carbon loss, wet-dry cycles, plowing and glucose addition led to diverse treatment effects. Most "char-C" was lost from feedstock and HTCs during the initial period of labile carbon degradation, leveling off gradually during the subsequent experimental sections. Only plowing renewed stimulated mineralization, especially that of HTCs material in both soils. Consequently, further carbon loss following glucose addition was mostly insignificant. C-

losses from HTCw and biochar treatments were lower throughout. Biochar-C loss was only triggered by glucose addition, indicating that biochar was degraded mainly by co-mineralization. As a consequence, carbon was lost in significant amounts from all carbon amendments except biochar over the experimental period in both soils. HTCw and feedstock additionally led to a positive priming of SOC in sandy or loamy soil, respectively, whereas biochar rather induced negative priming of SOC, especially in sandy soil. Overall, only biochar and to some extent also HTCw increased the TOC content of both soils significantly over the whole experimental period. Thus, depending on the site specific soil properties, HTCw and more so biochar may aid in medium- to long-term carbon build-up, providing a basis for the accounting of carbon credits, while the less carbonized materials seem unsuitable.

Even under the degradation-promoting incubation conditions applied here, the MRT and half-life of the C materials could clearly be linked to their carbonization grade. Thereby, the structural properties such as cellulose content or the H/C-O/C ratios were suitable predictors for the carbon losses during the experimental "breaking" period over one year in both soils. Since the cumulative N_2O emissions were well predictable from the cumulative CO_2 emissions, the estimation of the overall environmental impact of the carbon amendments might simply be tied to their structural properties. We conclude that among the variety of the material under study, only biochar remained stable over a period of forced degradation and moreover stabilized soil organic matter noticeably in both sandy and loamy soil, likely by sorption of dissolved organic carbon compounds, leading to a negative priming effect.

Since incubations only allow first insights, verification of the degradation behavior of carbon amendments under the influence of "extreme" weather events in field experiments, including vegetation and its root-C delivery as well as its below-ground micro-biome are necessary.

5.6 Acknowledgments

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5.7 Appendix

Table A. 5-1: pH values of the soil-substrate mixes, at initiation and end of the experiment (n=3 ± SD). Letters indicate significant treatment differences at the different time points (ANOVA+Holm-Sidak post-hoc test (CI =95%)

Treatment / Soil	Control	Feedstock	HTCs	HTCw	Biochar
Sandy t0	5.52 ± 0.05^{A}	5.98 ± 0.09^{C}	5.77 ± 0.07^{B}	5.64 ± 0.03^{AB}	6.19 ± 0.12^{D}
Sandy t3	5.55 ± 0.02^{C}	5.34 ± 0.01^{B}	5.38 ± 0.04^{B}	5.09 ± 0.02^{A}	5.53 ± 0.02^{C}
Loamy t0	6.88 ± 0.02^{A}	6.98 ± 0.09^{A}	7.02 ± 0.07^{A}	6.88 ± 0.07^{A}	7.34 ± 0.07^{B}
Loamy t3	4.64 ± 0.03^{A}	4.65 ± 0.02A^{A}	4.61 ± 0.04^{A}	4.63 ± 0.01^{A}	4.71 ± 0.01^{A}

Table A. 5-2: Greenhouse gas emissions (CO_2, N_2O, and CH4) over the incubation period, expressed in GHG equivalents with their respective shares in percent

Soil type	Treatment	GHG eq [g CO_2 kg^{-1} soil mix]	% CO_2	% N_2O	% CH_4
Sandy soil	Control	7.0 ± 0.7	93.5	6.4	0.0
	Feedstock	35.6 ± 2.9	92.0	8.0	0.0
	HTCs	24.6 ± 0.4	97.5	2.5	0.0
	HTCw	15.1 ± 0.4	84.5	15.5	0.0
	Biochar	4.1 ± 0.5	60.6	39.36	0.1
Loamy soil	Control	7.5 ± 1.1	92.3	7.8	-0.1
	Feedstock	32.3 ± 4.3	79.0	21.1	-0.1
	HTCs	24.6 ± 0.5	90.2	9.9	-0.1
	HTCw	13.2 ± 0.9	82.7	17.5	-0.2
	Biochar	6.9 ± 1.5	93.8	6.4	-0.2

Table A. 5-3: Shares of char-C in total CO_2 fluxes [%] at the end of Section 1 (21st of September 2012) and the Start of Section 5 (31st of May 2013), as determined by Keeling Plots and an isotope mixing model.

Soil type	Treatment	Char-C in total CO_2 [%]	
		End of Section 1 (21.09.2012)	Start of Section 5 (31.05.2013)
Sandy soil	Feedstock	87.3 ± 2.0	≈ 27
	HTCs	80.4 ± 5.1	≈ 61
	HTCw	41.3 ± 6.7	≈ 63
	Biochar	17.9 ± 27.8	≈ 40
Loamy soil	Feedstock	47.2 ± 9.6	≈ 27
	HTCs	66.2 ± 7.8	≈ 52
	HTCw	42.0 ± 9.2	≈ 56
	Biochar	40.0 ± 27.2	≈ 51

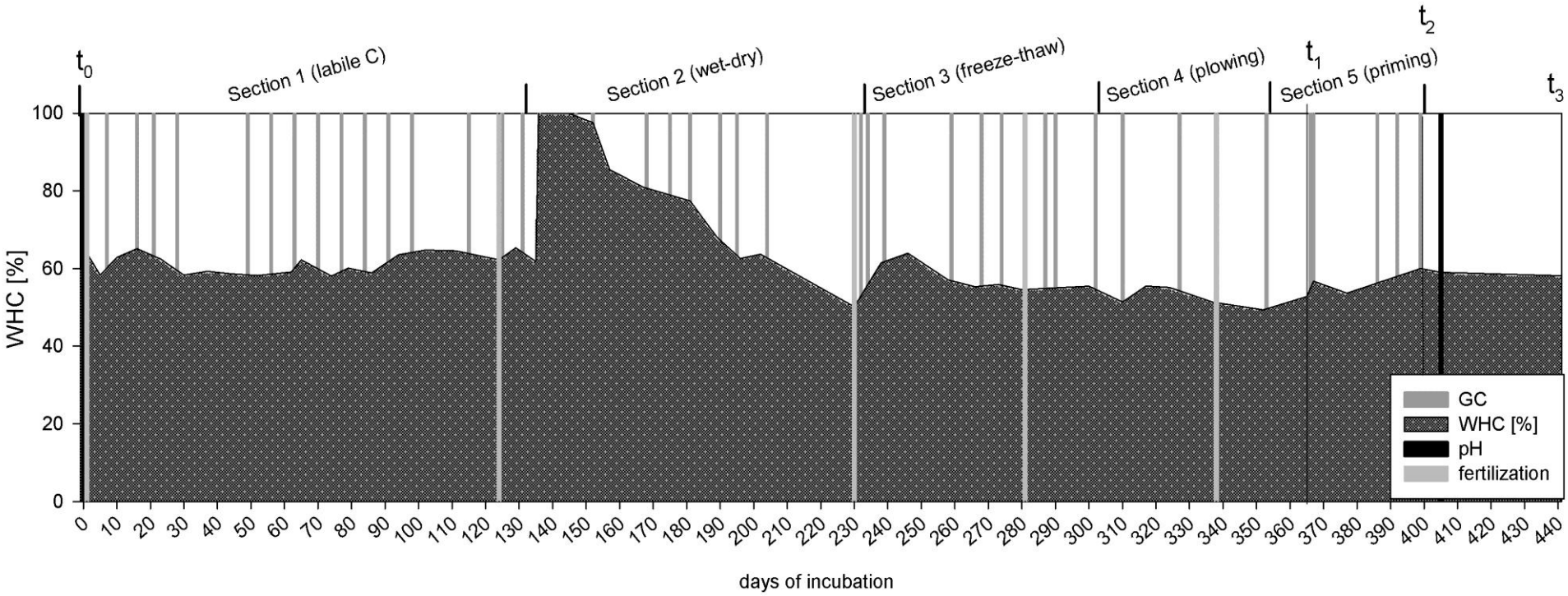

Figure A. 5-1: Timeline over the experimental period, marking the five different Sections, time points of fertilization pH analyses.

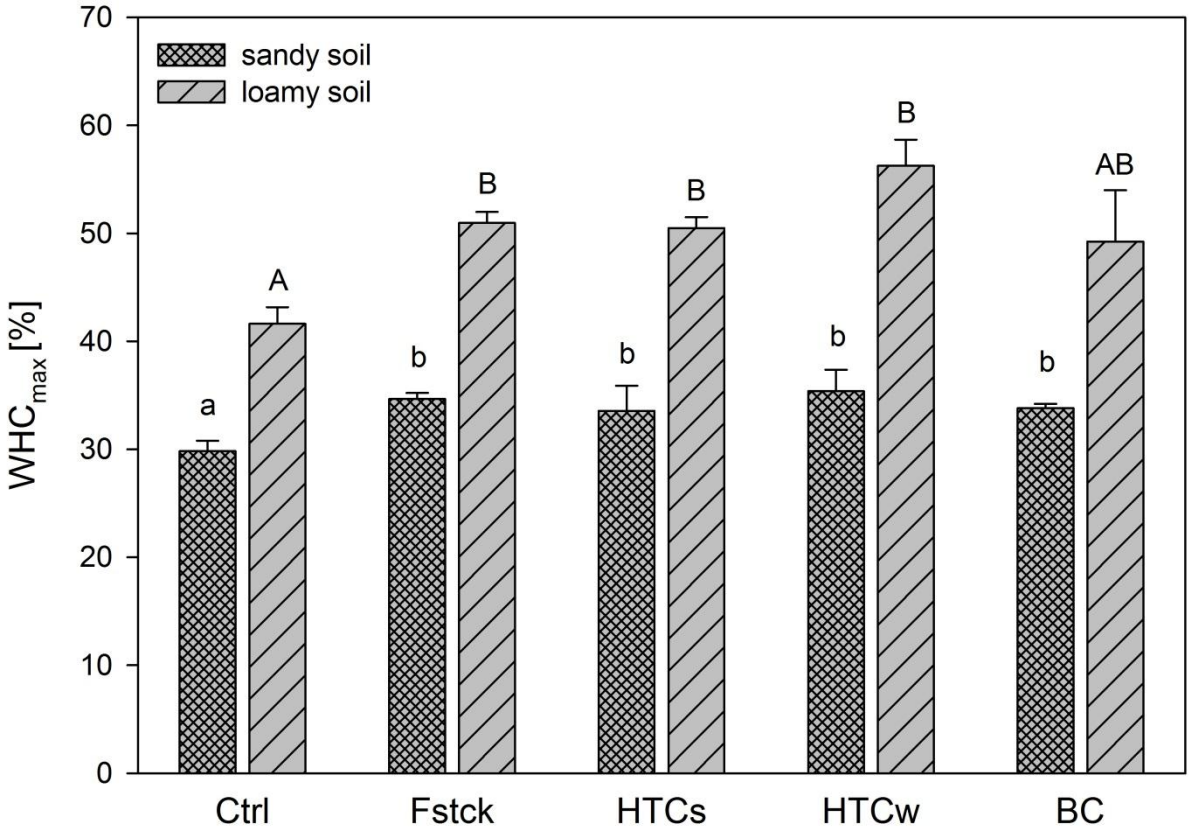

Figure A. 5-2: Initial maximum water holding capacity [%] of the soil-substrate mixtures. Letters depict significant differences between treatments in sandy (small letters) and loamy (big letters) soil (two-way ANOVA with Holm-Sidak Post-hoc test CI = 95%).

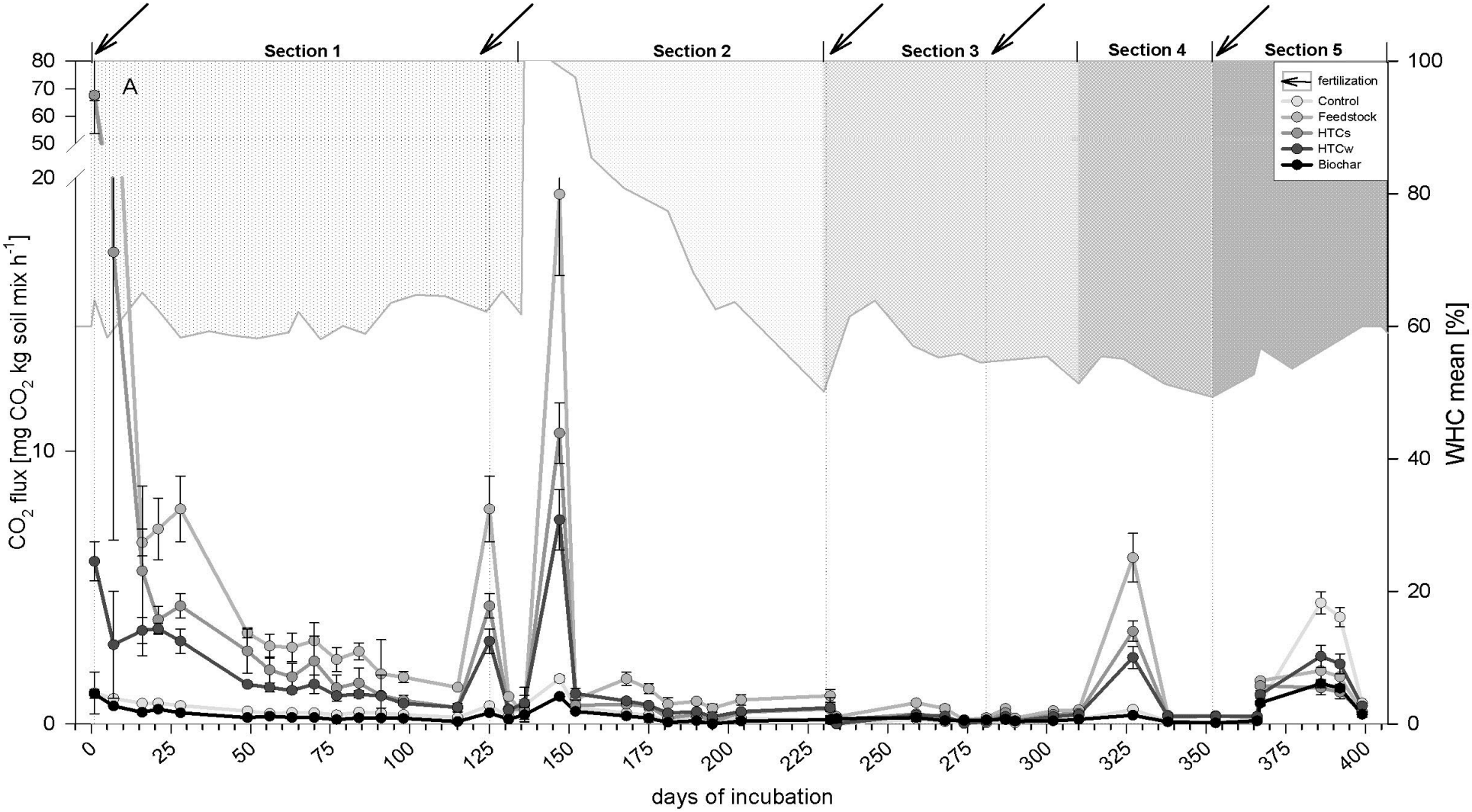

Figure A. 5-3: CO_2 flux rates [mg CO_2 kg soil mix h^{-1}] over the incubation period A=sandy soil

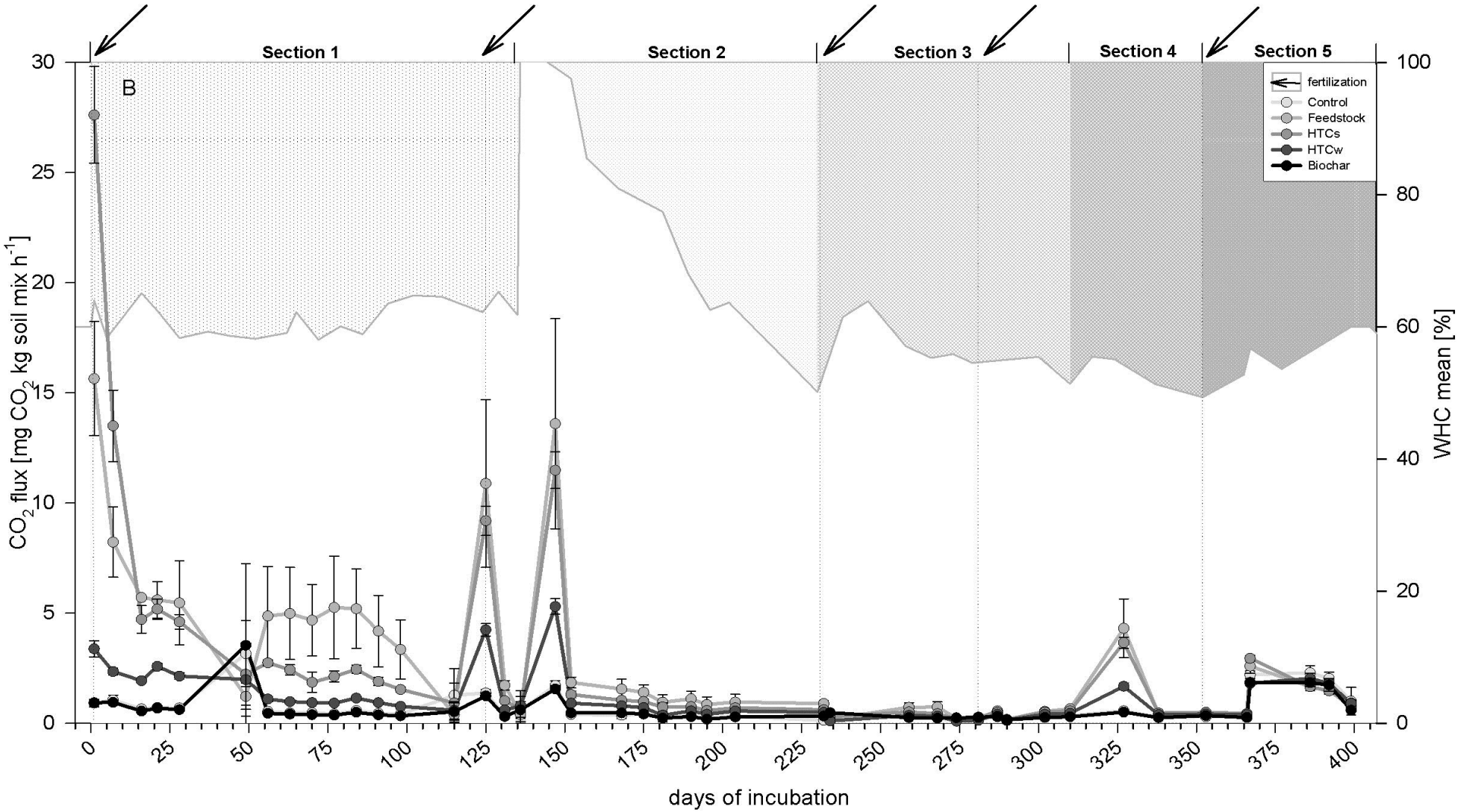

Figure A. 5-4: CO_2 flux rates [mg CO_2 kg soil mix h^{-1}] over the incubation period. B= loamy soil

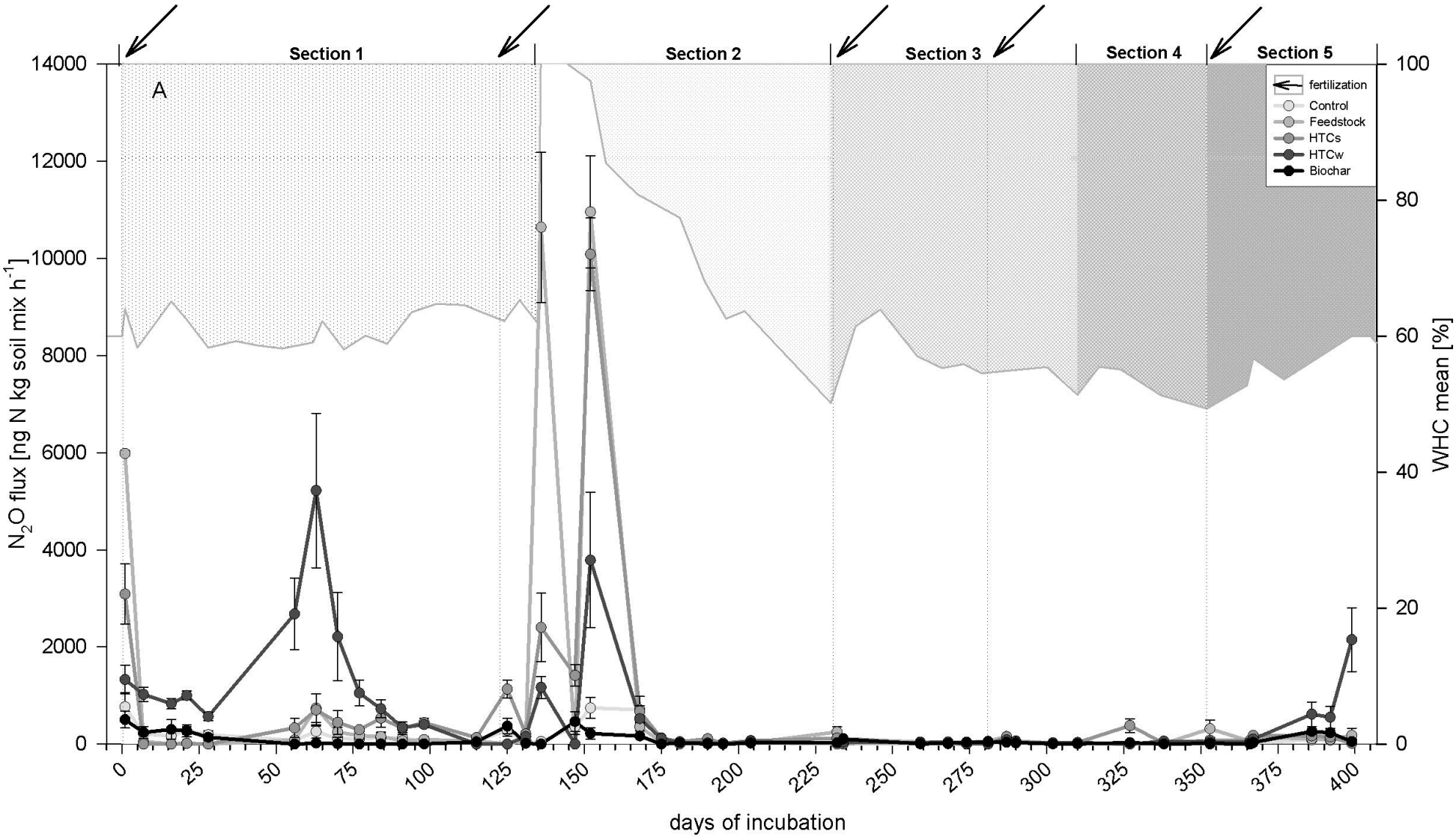

Figure A. 5-5: N_2O flux rates [µg N_2O kg soil mix h^{-1}] over the incubation period. A=sandy soil

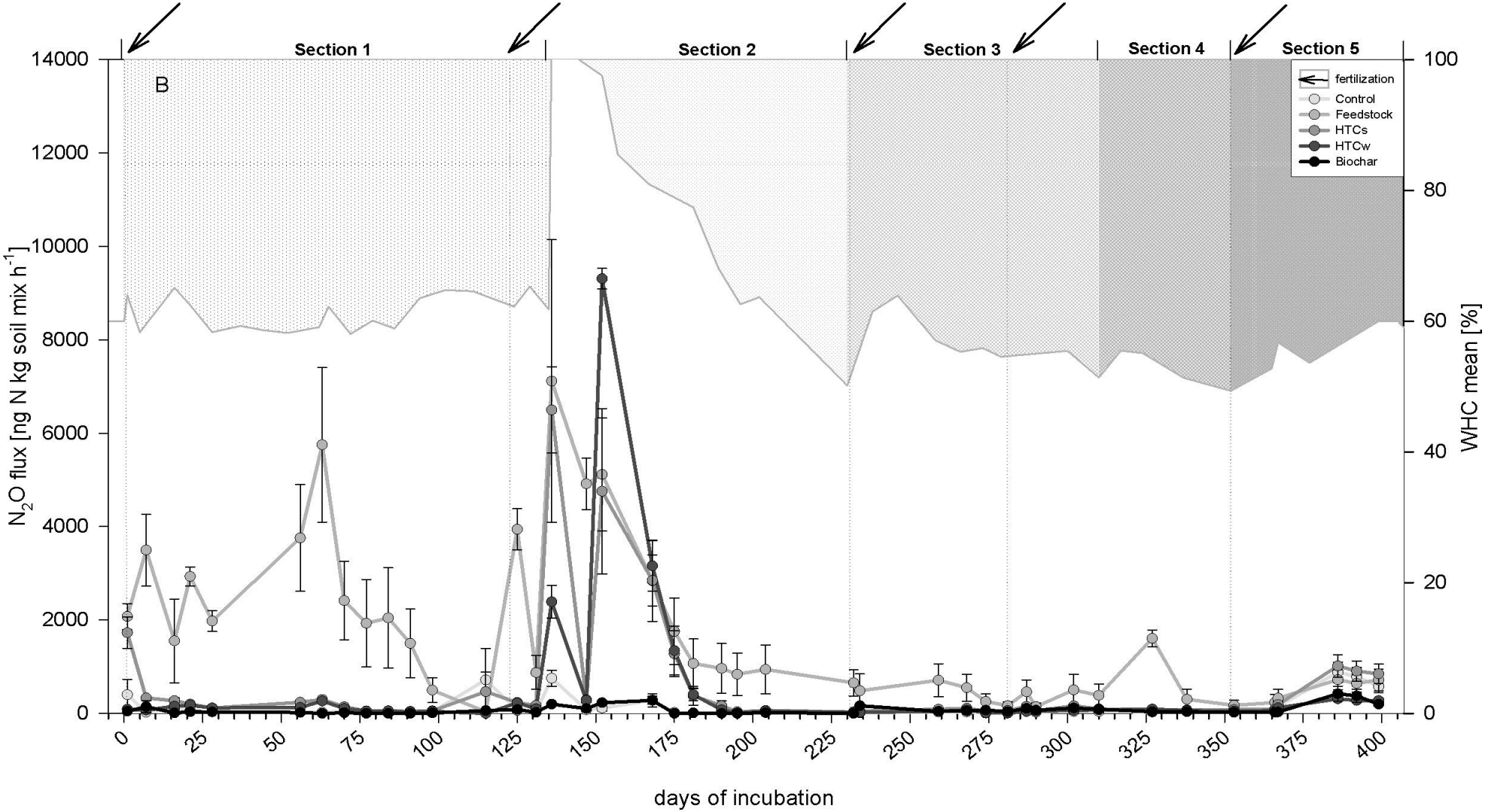

Figure A. 5-6: N_2O flux rates [µg N_2O kg soil mix h^{-1}] over the incubation period. B= loamy soil

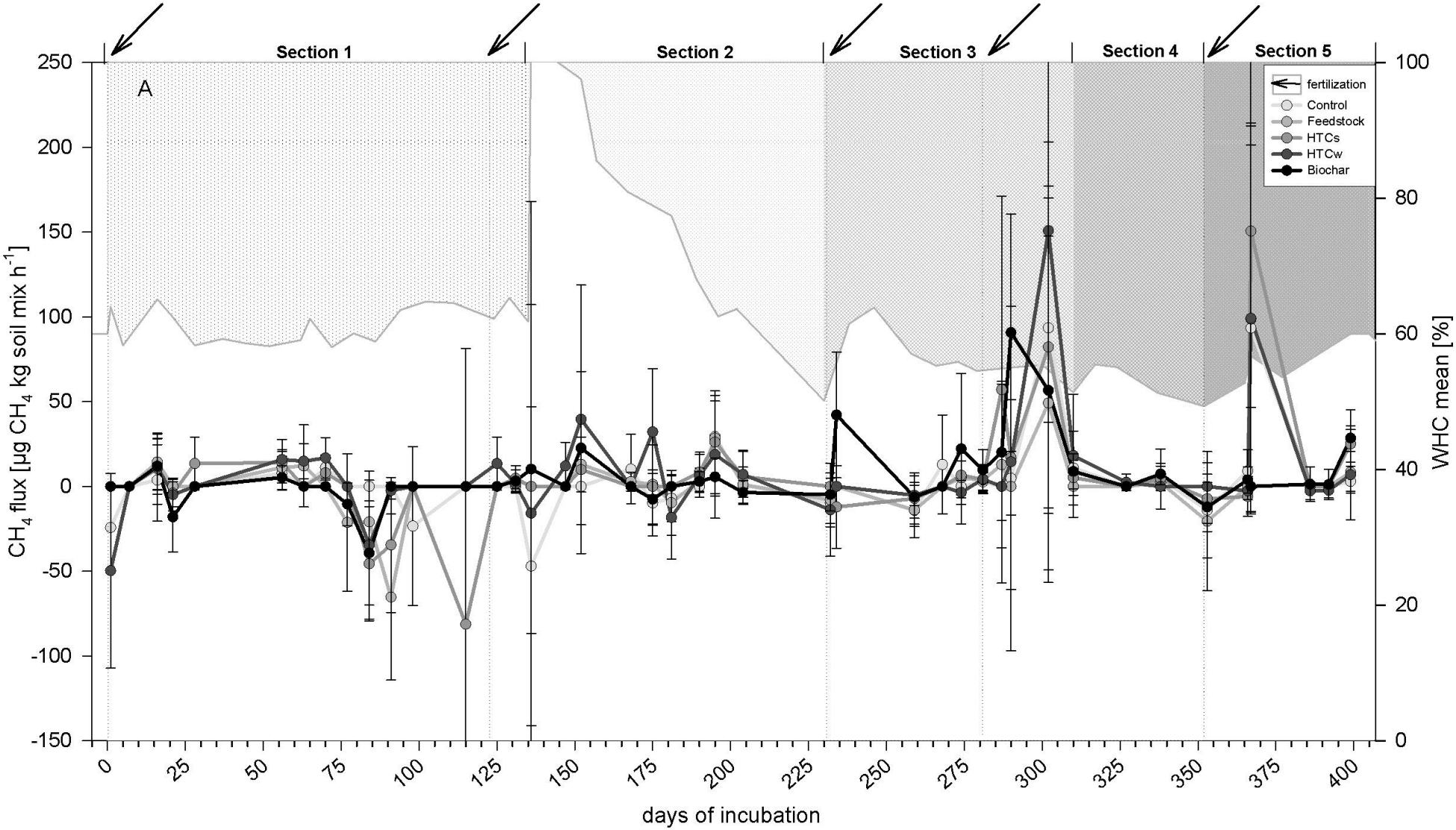

Figure A. 5-7: CH_4 flux rates [µg CH_4 kg soil mix h^{-1}] over the incubation period. A=sandy soil

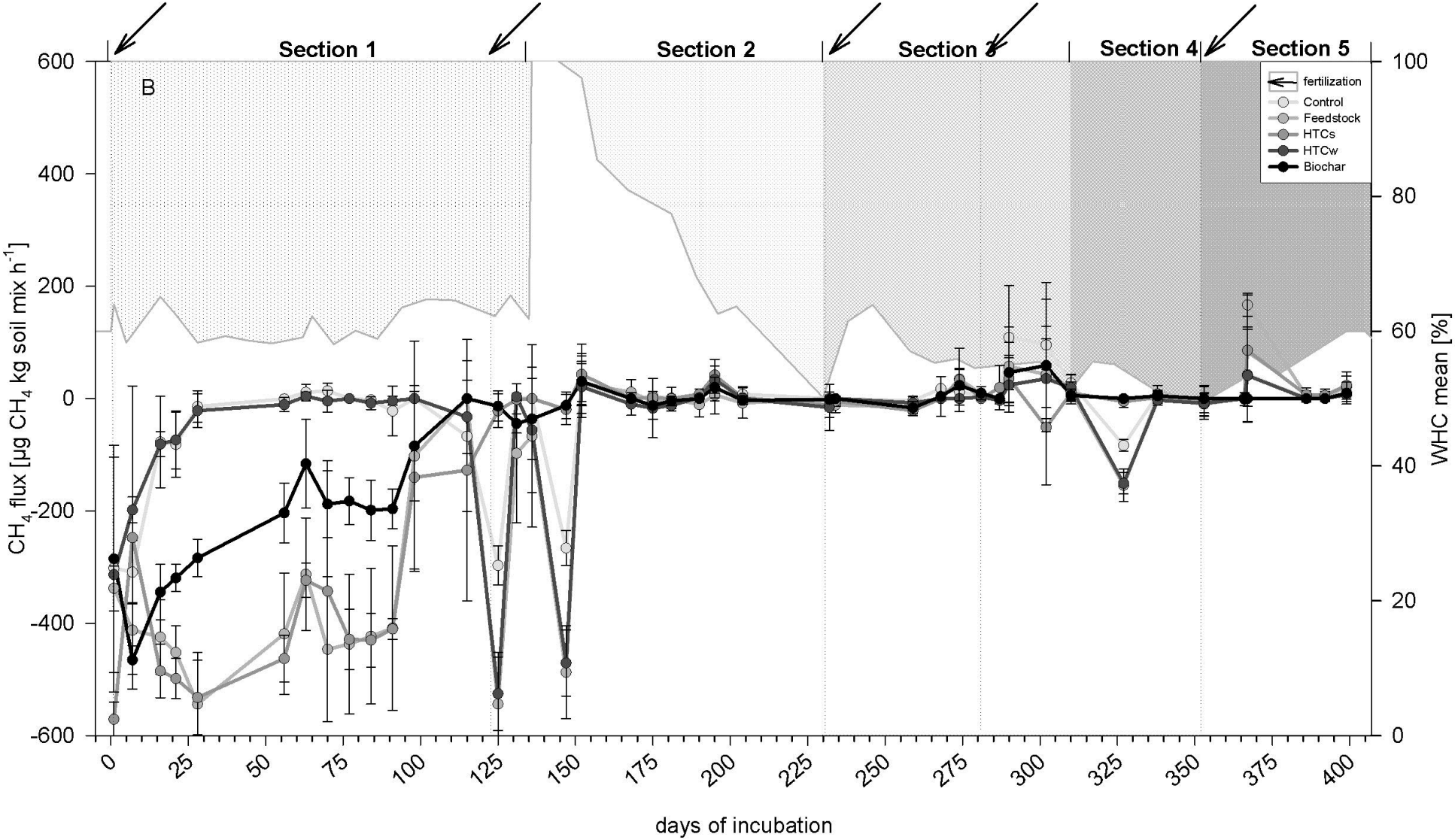

Figure A. 5-8: CH_4 flux rates [µg CH_4 kg soil mix h^{-1}] over the incubation period. B= loamy soil

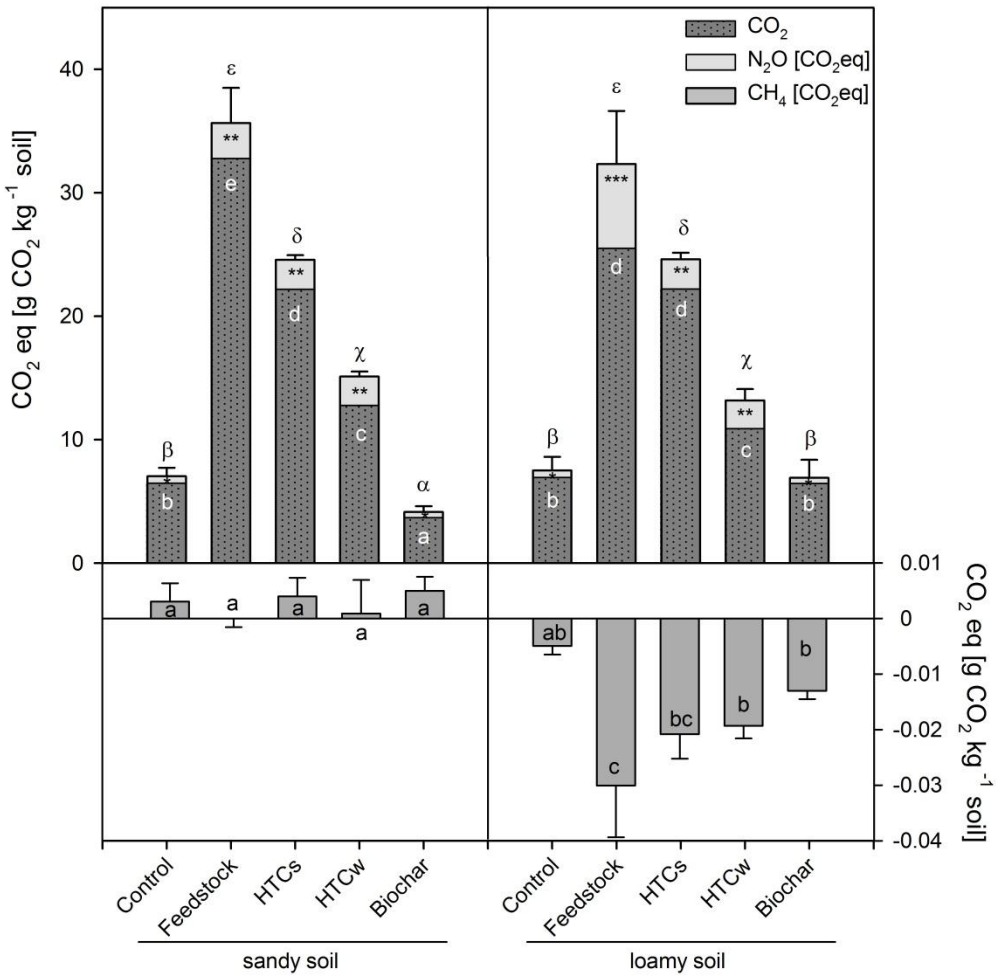

Figure A. 5-9: Shares of the single GHGs in total CO_2eq fluxes [g CO_2 kg^{-1} soil mix] of the two soils. Latin letters and asterisks mark differences according to the single GHGs (one way ANOVA followed by Tukey HSD post-hoc tests, CI = 95%), Greek letters mark significant differences of the total cumulated CO_2eq emissions. Note different scales.

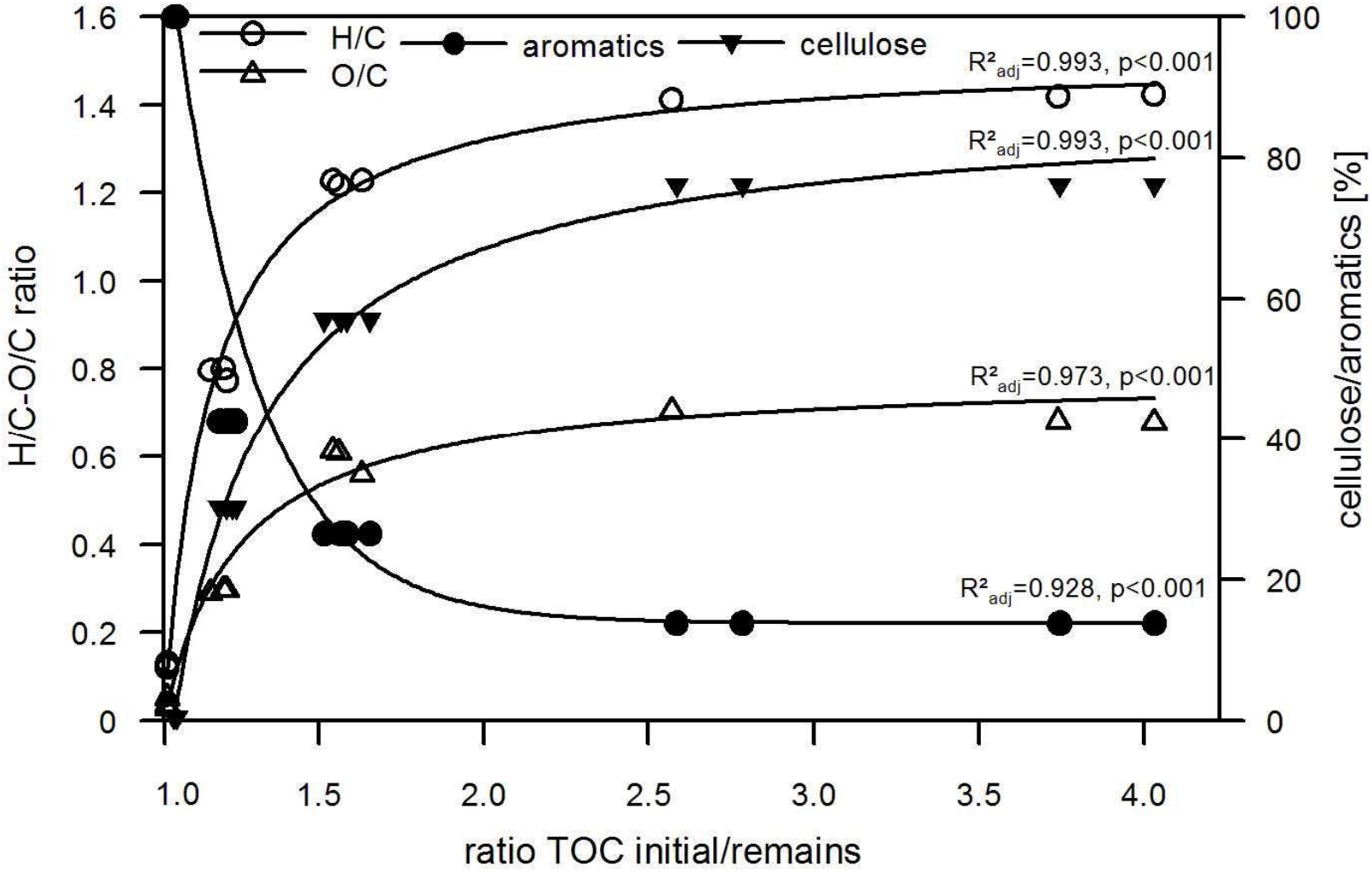

Figure A. 5-10: Non-linear regressions of the elemental composition and structural properties of the different carbon amendments with C initial/C remaining ratios. Sandy soil.

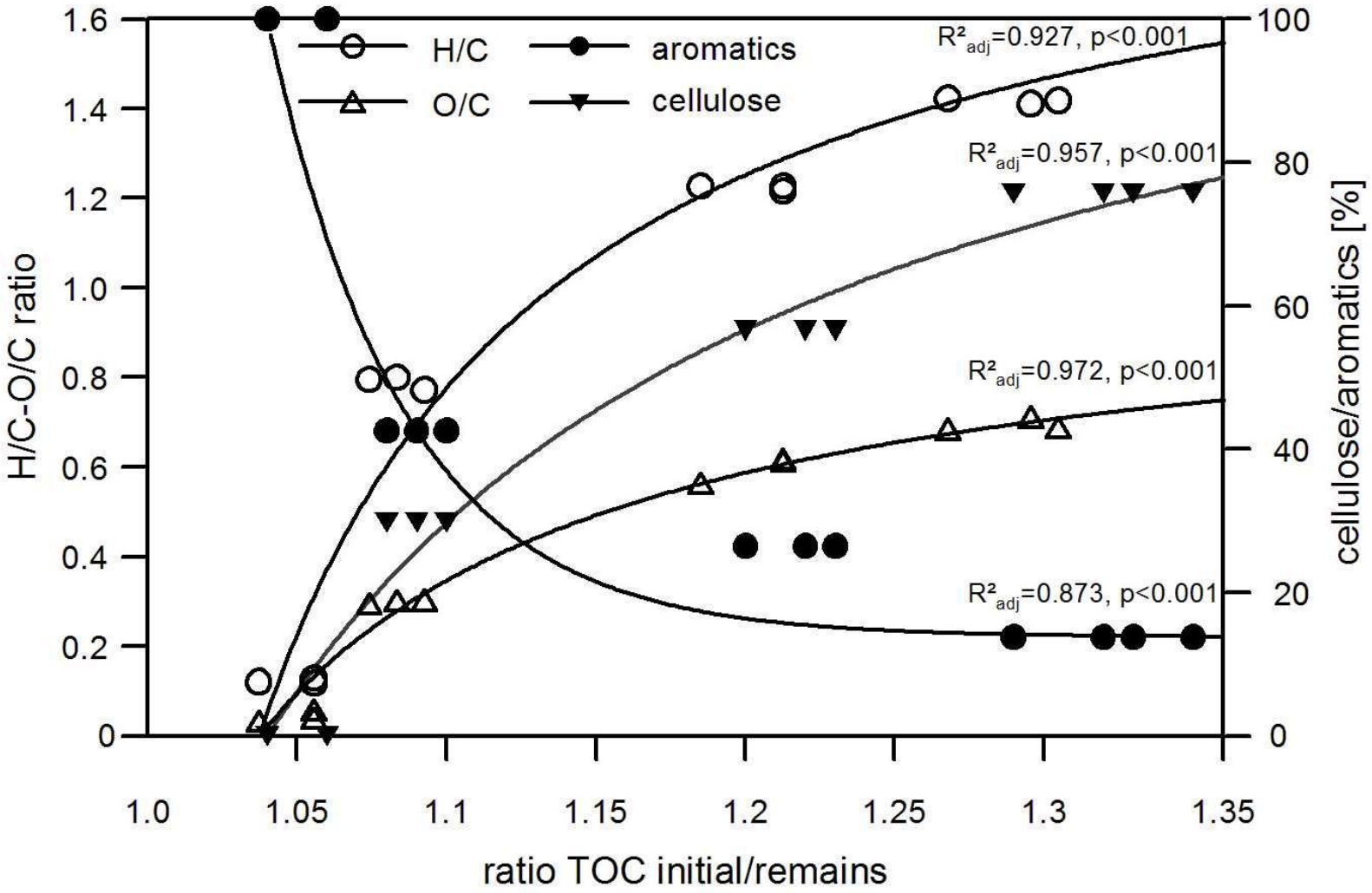

Figure A. 5-11: Non-linear regressions of the elemental composition and structural properties of the different carbon amendments with C initial/C remaining ratios. Loamy soil.

6 References

ABEL S., PETERS A., TRINKS S., SCHONSKY H., FACKLAM M. AND WESSOLEK G. (2013). Impact of biochar and hydrochar addition on water retention and water repellency of sandy soil. *Geoderma*, **202**, 183-191.

AERTS R. (1999). Interspecific competition in natural plant communities: mechanisms, trade-offs and plant-soil feedbacks. *Journal of Experimental Botany*, **50**, 29-37.

AGUILAR-CHÁVEZ Á., DÍAZ-ROJAS M., CÁRDENAS-AQUINO M. D. R., DENDOOVEN L. AND LUNA-GUIDO M. (2012). Greenhouse gas emissions from a wastewater sludge-amended soil cultivated with wheat (*Triticum spp.* L.) as affected by different application rates of charcoal. *Soil Biology and Biochemistry*, **52**, 90-95.

ALLEN A., JARVIS S. AND HEADON D. (1996). Nitrous oxide emissions from soils due to inputs of nitrogen from excreta return by livestock on grazed grassland in the UK. *Soil Biology and Biochemistry*, **28**, 597-607.

ALLISON S. D. (2005). Cheaters, diffusion and nutrients constrain decomposition by microbial enzymes in spatially structured environments. *Ecology Letters*, **8**, 626-635.

ALLISON V., CONDRON L., PELTZER D., RICHARDSON S. AND TURNER B. (2007). Changes in enzyme activities and soil microbial community composition along carbon and nutrient gradients at the Franz Josef chronosequence, New Zealand. *Soil Biology and Biochemistry*, **39**, 1770-1781.

ALVUM-TOLL K., KARLSSON T. AND STRÖM H. (2011). Biochar as soil amendment: a comparison between plant materials for biochar production from three regions in Kenya, p. 74. Uppsala, Sweden: Swedish University of Agricultural Sciences, Department of soil and Environment.

AMARAL J. A., REN T. AND KNOWLES R. (1998). Atmospheric methane consumption by forest soils and extracted bacteria at different pH values. *Applied and environmental microbiology*, **64**, 2397-2402.

AMBERGER A., VILSMEIER K. AND GUTSER R. (1982). Stickstofffraktionen verschiedener Güllen und deren Wirkung im Pflanzenversuch. *Zeitschrift für Pflanzenernährung und Bodenkunde*, **145**, 325-336.

AMELOOT N., DE NEVE S., JEGAJEEVAGAN K., YILDIZ G., BUCHAN D., FUNKUIN Y. N., PRINS W., BOUCKAERT L. AND SLEUTEL S. (2013a). Short-term CO_2 and N_2O emissions and microbial properties of biochar amended sandy loam soils. *Soil Biology and Biochemistry*, **57**, 401-410.

AMELOOT N., GRABER E. R., VERHEIJEN F. G. A. AND DE NEVE S. (2013b). Interactions between biochar stability and soil organisms: review and research needs. *European Journal of Soil Science*, **64**, 379-390.

AMELUNG W., BRODOWSKI S., SANDHAGE-HOFMANN A. AND BOL R. (2008). Combining biomarker with stable isotope analyses for assessing the transformation and turnover of soil organic matter. *Advances in Agronomy*, **100**, 155-250.

AMON B., KRYVORUCHKO V., AMON T. AND ZECHMEISTER-BOLTENSTERN S. (2006). Methane, nitrous oxide and ammonia emissions during storage and after application of dairy cattle slurry and influence of slurry treatment. *Agriculture, Ecosystems and Environment*, **112**, 153-162.

ANDERSON C. R., CONDRON L. M., CLOUGH T. J., FIERS M., STEWART A., HILL R. A. AND SHERLOCK R. R. (2011). Biochar induced soil microbial community change:

implications for biogeochemical cycling of carbon, nitrogen and phosphorus. *Pedobiologia*, **54**, 309-320.

ANDERSON J. AND DOMSCH K. (1973). Quantification of bacterial and fungal contributions to soil respiration. *Archiv Fur Mikrobiologie*, **93**, 113-127.

ANDERSON J. AND DOMSCH K. (1975). Measurement of bacterial and fungal contributions to respiration of selected agricultural and forest soils. *Canadian Journal of Microbiology*, **21**, 314-322.

ANDERSON T. H. AND DOMSCH K. (1986). Carbon assimilation and microbial activity in soil. *Zeitschrift für Pflanzenernährung und Bodenkunde*, **149**, 457-468.

ANDERSON T. H. AND DOMSCH K. (2010). Soil microbial biomass: the eco-physiological approach. *Soil Biology and Biochemistry*, **42**, 2039-2043.

ANGIN D. AND SENSOZ S. (2014). Effect of pyrolysis temperature on chemical and surface properties of biochar of rapeseed (*Brassica napus* L.). *International Journal of Phytoremediation*, **16**, 684-693.

ANGST T. E. AND SOHI S. P. (2013). Establishing release dynamics for plant nutrients from biochar. *GCB Bioenergy*, **5**, 221-226.

ANGST T. E., SIX J., REAY D. S. AND SOHI S. P. (2014). Impact of pine chip biochar on trace greenhouse gas emissions and soil nutrient dynamics in an annual ryegrass system in California. *Agriculture Ecosystems and Environment*, **191**, 17-26.

ANTAL JR M. J. AND GRØNLI M. (2003). The Art, Science, and Technology of charcoal production. *Industrial and Engineering Chemistry Research*, **42**, 1619-1640.

ARONSON E. AND HELLIKER B. (2010). Methane flux in non-wetland soils in response to nitrogen addition: a meta-analysis. *Ecology*, **91**, 3242-3251.

ATKINSON C. J., FITZGERALD J. D. AND HIPPS N. A. (2010). Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant and soil*, **337**, 1-18.

AUGUSTENBORG C. A., HEPP S., KAMMANN C., HAGAN D., SCHMIDT O. AND MÜLLER C. (2012). Biochar and earthworm effects on soil nitrous oxide and carbon dioxide emissions. *Journal of environmental quality*, **41**, 1203-1209.

BAATH E. AND ANDERSON T. H. (2003). Comparison of soil fungal/bacterial ratios in a pH gradient using physiological and PLFA-based techniques. *Soil Biology and Biochemistry*, **35**, 955-963.

BADALUCCO L., POMARE F., GREGO S., LANDI L. AND NANNIPIERI P. (1994). Activity and degradation of Streptomycin and Cycloheximide in soil. *Biology and Fertility of Soils*, **18**, 334-340.

BAI M., WILSKE B., BUEGGER F., ESPERSCHÜTZ J., KAMMANN C. I., ECKHARDT C., KOESTLER M., KRAFT P., BACH M. AND FREDE H.-G. (2013). Degradation kinetics of biochar from pyrolysis and hydrothermal carbonization in temperate soils. *Plant and soil*, **372**, 375-387.

BAI M., WILSKE B., BUEGGER F., BRUUN E. W., BACH M., FREDE H.-G. AND BREUER L. (2014). Biodegradation measurements confirm the predictive value of the O:C-ratio for biochar recalcitrance. *Journal of Plant Nutrition and Soil Science*, **177**, 633-637.

BAIAMONTE G., DE PASQUALE C., MARSALA V., CIMÒ G., ALONZO G., CRESCIMANNO G. AND CONTE P. (2014). Structure alteration of a sandy-clay soil by biochar amendments. *Journal of Soils and Sediments*, 1-9.

BALL P. R. AND KEENEY D. (1981). Nitrogen losses from urine-affected areas of a New Zealand pasture under contrasting seasonal conditions, pp. 342-344.

BAMMINGER C., MARSCHNER B. AND JÜSCHKE E. (2014a). An incubation study on the stability and biological effects of pyrogenic and hydrothermal biochar in two soils. *European Journal of Soil Science*, **65**, 72-82.

BAMMINGER C., ZAISER N., ZINSSER P., LAMERS M., KAMMANN C. AND MARHAN S. (2014b). Effects of biochar, earthworms, and litter addition on soil microbial activity and abundance in a temperate agricultural soil. *Biology and Fertility of Soils*, **50**, 1189-1200.

BARBOSA DE SOUSA A. M., SOARES SANTOS R. R. AND GEHRING C. (2014). Charcoal in Amazonian paddy soil-Nutrient availability, rice growth and methane emissions. *Journal of Plant Nutrition and Soil Science*, **177**, 39-47.

BARGMANN I., GREEF J. M. AND KÜCKE M. (2012). Keimung, Wachstum und Nährstoffdynamik in Boden und Pflanze nach dem Einbringen von Biokohlen aus hydrothermaler Carbonierung (Hydrochar) und Pyrolyse (Biochar). *Kommissionen IV und VI der Deutschen Bodenkundlichen Gesellschaft Gesellschaft für Pflanzenbauwissenschaften e. V.*, 60.

BARGMANN I., RILLIG M. C., BUSS W., KRUSE A. AND KUECKE M. (2013). Hydrochar and biochar effects on germination of spring barley. *Journal of Agronomy and Crop Science*, **199**, 360-373.

BARGMANN I., MARTENS R., RILLIG M. C., KRUSE A. AND KUECKE M. (2014a). Hydrochar amendment promotes microbial immobilization of mineral nitrogen. *Journal of Plant Nutrition and Soil Science*, **177**, 59-67.

BARGMANN I., RILLIG M. C., KRUSE A., GREEF J.-M. AND KÜCKE M. (2014b). Initial and subsequent effects of hydrochar amendment on germination and nitrogen uptake of spring barley. *Journal of Plant Nutrition and Soil Science*, **177**, 68-74.

BARGMANN I., RILLIG M. C., KRUSE A., GREEF J.-M. AND KUECKE M. (2014c). Effects of hydrochar application on the dynamics of soluble nitrogen in soils and on plant availability. *Journal of Plant Nutrition and Soil Science*, **177**, 48-58.

BARNES R. T., GALLAGHER M. E., MASIELLO C. A., LIU Z. AND DUGAN B. (2014). Biochar-induced changes in soil hydraulic conductivity and dissolved nutrient fluxes constrained by laboratory experiments. *PLoS ONE*, **9**, e108340.

BARONTI S., VACCARI F. P., MIGLIETTA F., CALZOLARI C., LUGATO E., ORLANDINI S., PINI R., ZULIAN C. AND GENESIO L. (2014). Impact of biochar application on plant water relations in *Vitis vinifera* (L.). *European Journal of Agronomy*, **53**, 38-44.

BEAUCHAMP E., TREVORS J. AND PAUL J. (1989). Carbon sources for bacterial denitrification. *Advances in Soil Science*, **10**, 113-142.

BECK D. A., JOHNSON G. R. AND SPOLEK G. A. (2011). Amending greenroof soil with biochar to affect runoff water quantity and quality. *Environmental Pollution*, **159**, 2111-2118.

BECKER R., DORGERLOH U., HELMIS M., MUMME J., DIAKITÉ M. AND NEHLS I. (2012). Hydrothermally carbonized plant materials: patterns of volatile organic compounds detected by gas chromatography. *Bioresource Technology*, **130**, 621-628.

BÉDARD C. AND KNOWLES R. (1989). Physiology, biochemistry, and specific inhibitors of CH_4, NH_4^+, and CO oxidation by methanotrophs and nitrifiers. *Microbiological reviews*, **53**, 68-84.

BEESLEY L., MORENO-JIMÉNEZ E. AND GOMEZ-EYLES J. L. (2010). Effects of biochar and greenwaste compost amendments on mobility, bioavailability and toxicity of inorganic and organic contaminants in a multi-element polluted soil. *Environmental Pollution*, **158**, 2282-2287.

BELL M. AND WORRALL F. (2011). Charcoal addition to soils in NE England: A carbon sink with environmental co-benefits? *Science of the Total Environment*, **409**, 1704-1714.

BERENDSE F., ELBERSE W. T. AND GEERTS R. (1992). Competition and nitrogen loss from plants in grassland ecosystems. *Ecology*, **73**, 46-53.

BERGE N. D., KAMMANN C., RO K. AND LIBRA J. (2013). Environmental Applications of Hydrothermal Carbonization Technology: Biochar Production, Carbon Sequestration, and Waste Conversion. In: TITIRICI M. M. (ed.) *Sustainable Carbon Materials from Hydrothermal Processes*, pp. 295-340. Chichester, UK: John Wiley & Sons, Ltd.

BERGMANN W. (1992). Nutritional disorders of plants *Development visual and analytical diagnosis. Jena, Germany: Gustav Fischer*, p. 741. Jena: Gustav Fischer Verlag Jena.

BIEDERMAN L. A. AND HARPOLE W. S. (2013). Biochar and its effects on plant productivity and nutrient cycling: a meta-analysis. *Global Change Biology Bioenergy*, **5**, 202-214.

BISCHOFF W.-A., SCHWARZ A. AND PFENNING J. (2012). Pyrolyse-und HTC-Kohlen als Bodenverbesserer auf sandigen Böden im Gemüsebau. *Kommissionen IV und VI der Deutschen Bodenkundlichen Gesellschaft Gesellschaft für Pflanzenbauwissenschaften e. V.*, 56.

BITYUTSKII N. P., KAIDUN P. I. AND YAKKONEN K. L. (2012). The earthworm (*Aporrectodea caliginosa*) primes the release of mobile and available micronutrients in soil. *Pedobiologia*, **55**, 93-99.

BLACKWELL P., RIETHMULLER G. AND COLLINS M. (2009). Biochar application to soil. In: LEHMANN J. AND JOSEPH S. (eds.) *Biochar for Environmental Management: Science and Technology*, pp. 207-226. London, UK: Earthscan.

BOER W. D., FOLMAN L. B., SUMMERBELL R. C. AND BODDY L. (2005). Living in a fungal world: impact of fungi on soil bacterial niche development. *FEMS Microbiology Reviews*, **29**, 795-811.

BOL R., MOERING J., KUZYAKOV Y. AND AMELUNG W. (2003). Quantification of priming and CO_2 respiration sources following slurry-C incorporation into two grassland soils with different C content. *Rapid Communications in Mass Spectrometry*, **17**, 2585-2590.

BORCHARD N., PROST K., KAUTZ T., MOELLER A. AND SIEMENS J. (2012). Sorption of copper (II) and sulphate to different biochars before and after composting with farmyard manure. *European Journal of Soil Science*.

BORCHARD N., SIEMENS J., LADD B., MOELLER A. AND AMELUNG W. (2014a). Application of biochars to sandy and silty soil failed to increase maize yield under common agricultural practice. *Soil and Tillage Research*, **144**, 184-194.

BORCHARD N., SPOKAS K., PROST K. AND SIEMENS J. (2014b). Greenhouse gas production in mixtures of soil with composted and noncomposted biochars is governed by char-associated organic compounds. *Journal of Environmental Quality*, **43**, 971-979.

BREWER C. E., CHUANG V. J., MASIELLO C. A., GONNERMANN H., GAO X., DUGAN B., DRIVER L. E., PANZACCHI P., ZYGOURAKIS K. AND DAVIES C. A. (2014). New approaches to measuring biochar density and porosity. *Biomass and Bioenergy*, **66**, 176-185.

BRUUN E. W., MULLER-STOVER D., AMBUS P. AND HAUGGAARD-NIELSEN H. (2011). Application of biochar to soil and N_2O emissions: potential effects of blending fast-

pyrolysis biochar with anaerobically digested slurry. *European Journal of Soil Science*, **62**, 581-589.

BRUUN S., JENSEN E. S. AND JENSEN L. S. (2008). Microbial mineralization and assimilation of black carbon: Dependency on degree of thermal alteration. *Organic Geochemistry*, **39**, 839-845.

BRUUN S., CLAUSON-KAAS S., BOBULSKA L. AND THOMSEN I. K. (2014). Carbon dioxide emissions from biochar in soil: role of clay, microorganisms and carbonates. *European Journal of Soil Science*, **65**, 52-59.

BUDAI A., WANG L., GRONLI M., STRAND L. T., ANTAL M. J., JR., ABIVEN S., DIEGUEZ-ALONSO A., ANCA-COUCE A. AND RASSE D. P. (2014). Surface properties and chemical composition of corncob and miscanthus biochars: Effects of production temperature and method. *Journal of Agricultural and Food Chemistry*, **62**, 3791-3799.

BURFORD J. AND BREMNER J. (1975). Relationships between the denitrification capacities of soils and total, water-soluble and readily decomposable soil organic matter. *Soil Biology and Biochemistry*, **7**, 389-394.

BUSCH D., KAMMANN C., GRÜNHAGE L. AND MÜLLER C. (2012). Simple biotoxicity tests for evaluation of carbonaceous soil additives: Establishment and reproducibility of four test procedures. *Journal of Environmental Quality*, **41**, 1023-1032.

BUSCH D., STARK A., KAMMANN C. I. AND GLASER B. (2013). Genotoxic and phytotoxic risk assessment of fresh and treated hydrochar from hydrothermal carbonization compared to biochar from pyrolysis. *Ecotoxicology and Environmental Safety*, **97**, 59-66.

BUSS W. AND MASEK O. (2014). Mobile organic compounds in biochar - A potential source of contamination - Phytotoxic effects on cress seed (*Lepidium sativum*) germination. *Journal of Environmental Management*, **137**, 111-119.

CAO X., RO K. S., LIBRA J. A., KAMMANN C. I., LIMA I., BERGE N., LI L., LI Y., CHEN N. AND YANG J. (2013). Effects of biomass types and carbonization conditions on the chemical characteristics of hydrochars. *Journal of Agricultural and Food Chemistry*, **61**, 9401-9411.

CAO X., ZHONG L., PENG X., SUN S., LI S., LIU S. AND SUN R. (2014). Comparative study of the pyrolysis of lignocellulose and its major components: Characterization and overall distribution of their biochars and volatiles. *Bioresource Technology*, **155**, 21-27.

CASE S. D. C., MCNAMARA N. P., REAY D. S. AND WHITAKER J. (2012). The effect of biochar addition on N_2O and CO_2 emissions from a sandy loam soil - The role of soil aeration. *Soil Biology and Biochemistry*, **51**, 125-134.

CASTALDI S., RIONDINO M., BARONTI S., ESPOSITO F., MARZAIOLI R., RUTIGLIANO F., VACCARI F. AND MIGLIETTA F. (2011). Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. *Chemosphere*, **85**, 1464-1471.

CASTRO M. S., MELLILO J. M., STEUDLER P. A. AND CHAPMAN J. W. (1994). Soil moisture as a predictor of methane uptake by temperate forest soils. *Canadian Journal of Forest Research*, **24**, 1805-1810.

CASTRO M. S., STEUDLER P. A., MELLILO J. M., ABER J. D. AND BOWDEN R. D. (1995). Factors controlling atmospheric methane consumption by temperate forest soils. *Global Biogeochemical Cycles*, **9**, 1-10.

CAYUELA M. L., OENEMA O., KUIKMAN P. J., BAKKER R. R. AND VAN GROENIGEN J. W. (2010). Bioenergy by-products as soil amendments? Implications for carbon

sequestration and greenhouse gas emissions. *Global Change Biology Bioenergy*, **2**, 201-213.

CAYUELA M. L., SÁNCHEZ-MONEDERO M. A., ROIG A., HANLEY K., ENDERS A. AND LEHMANN J. (2013). Biochar and denitrification in soils: when, how much and why does biochar reduce N_2O emissions? *Scientific Reports*, **3**.

CAYUELA M. L., VAN ZWIETEN L., SINGH B. P., JEFFERY S., ROIG A. AND SÁNCHEZ-MONEDERO M. A. (2014). Biochar's role in mitigating soil nitrous oxide emissions: A review and meta-analysis. *Agriculture, Ecosystems and Environment*, **191**, 5-16.

CELY P., TARQUIS A., PAZ-FERREIRO J., MÉNDEZ A. AND GASCÓ G. (2014). Factors driving the carbon mineralization priming effect in a sandy loam soil amended with different types of biochar. *Solid Earth*, **5**, 585-594.

CHADWICK D. AND PAIN B. (1997). Methane fluxes following slurry applications to grassland soils: laboratory experiments. *Agriculture, Ecosystems and Environment*, **63**, 51-60.

CHAN A. AND PARKIN T. (2001). Methane oxidation and production activity in soils from natural and agricultural ecosystems. *Journal of Environmental Quality*, **30**, 1896-1903.

CHAN K., VAN ZWIETEN L., MESZAROS I., DOWNIE A. AND JOSEPH S. (2008). Agronomic values of greenwaste biochar as a soil amendment. *Soil Research*, **45**, 629-634.

CHAN K. Y. AND XU Z. (2009). Biochar: nutrient properties and their enhancement. In: LEHMANN J. AND JOSEPH S. (eds.) *Biochar for Environmental Management: Science and Technology*, pp. 67-84. London, UK: Earthscan.

CHANG Y.-M. (1996). On pyrolysis of waste tire: degradation rate and product yields. *Resources, Conservation and Recycling*, **17**, 125-139.

CHANTIGNY M. H., ANGERS D. A., PRÉVOST D., SIMARD R. R. AND CHALIFOUR F.-P. (1999). Dynamics of soluble organic C and C mineralization in cultivated soils with varying N fertilization. *Soil Biology and Biochemistry*, **31**, 543-550.

CHANTIGNY M. H., ROCHETTE P. AND ANGERS D. A. (2001). Short-term C and N dynamics in a soil amended with pig slurry and barley straw: A field experiment. *Canadian Journal of Soil Science*, **81**, 131-137.

CHAPIN III F. S., CHAPIN M. C., MATSON P. A. AND VITOUSEK P. (2011). Principles of terrestrial ecosystem ecology. Springer, Heidelberg: Springer Science and Business Media.

CHEN C., PHILLIPS I., CONDRON L., GOLORAN J., XU Z. AND CHAN K. (2012). Impacts of greenwaste biochar on ammonia volatilisation from bauxite processing residue sand. *Plant and Soil*, 1-12.

CHENG Y., CAI Z.-C., CHANG S., WANG J. AND ZHANG J.-B. (2012). Wheat straw and its biochar have contrasting effects on inorganic N retention and N_2O production in a cultivated Black Chernozem. *Biology and Fertility of Soils*, **48**, 941-946.

CHIA C. H., SINGH B. P., JOSEPH S., GRABER E. R. AND MUNROE P. (2014). Characterization of an enriched biochar. *Journal of Analytical and Applied Pyrolysis*, **108**, 26-34.

CHINTALA R., SCHUMACHER T. E., KUMAR S., MALO D. D., RICE J. A., BLEAKLEY B., CHILOM G., CLAY D. E., JULSON J. L., PAPIERNIK S. K. AND GU Z. R. (2014). Molecular characterization of biochars and their influence on microbiological properties of soil. *Journal of Hazardous Materials*, **279**, 244-56.

CLAYTON H., MCTAGGART I., PARKER J., SWAN L. AND SMITH K. (1997). Nitrous oxide emissions from fertilised grassland: A 2-year study of the effects of N fertiliser form and environmental conditions. *Biology and Fertility of Soils*, **25**, 252-260.

CLEMENS J. AND WULF S. (2005). Reduktion der Ammoniakausgasung aus Kofermentationssubstraten und Gülle während der Lagerung und Ausbringung durch interne Versaurung mit in NRW anfallenden organischen Kohlenstofffraktionen. *Forschungsvorhaben im Auftrag des Ministeriums für Umwelt und Naturschutz, Landwirtschaft und Verbraucherschutz des Landes Nordrhein-Westfalen, Bonn, Germany*, 41.

CLEVELAND C. C., NEMERGUT D. R., SCHMIDT S. K. AND TOWNSEND A. R. (2007). Increases in soil respiration following labile carbon additions linked to rapid shifts in soil microbial community composition. *Biogeochemistry*, **82**, 229-240.

CLIFTON-BROWN J. C., STAMPFL P. F. AND JONES M. B. (2004). Miscanthus biomass production for energy in Europe and its potential contribution to decreasing fossil fuel carbon emissions. *Global Change Biology*, **10**, 509-518.

CLOUGH T. J., BERTRAM J. E., RAY J., CONDRON L. M., O'CALLAGHAN M., SHERLOCK R. R. AND WELLS N. (2010). Unweathered wood biochar impact on nitrous oxide emissions from a bovine-urine-amended pasture soil. *Soil Science Society of America Journal*, **74**, 852-860.

CLOUGH T. J. AND CONDRON L. M. (2010). Biochar and the nitrogen cycle: Introduction. *Journal of environmental quality*, **39**, 1218-1223.

CLOUGH T. J., CONDRON L. M., KAMMANN C. AND MÜLLER C. (2013). A review of biochar and soil nitrogen dynamics. *Agronomy*, **3**, 275-293.

CONTE P., HANKE U. M., MARSALA V., CIMO G., ALONZO G. AND GLASER B. (2014). Mechanisms of water interaction with pore systems of hydrochar and pyrochar from poplar forestry waste. *Journal of Agricultural and Food Chemistry*, **62**, 4917-4923.

CORNELISSEN G., RUTHERFORD D., ARP H. P. H., DOERSCH P., KELLY C. N. AND ROSTAD C. (2013). Sorption of pure N2O to biochars and other organic and inorganic materials under anhydrous conditions. *Environmental Science and Technology*.

CRANE-DROESCH A., ABIVEN S., JEFFERY S. AND TORN M. S. (2013). Heterogeneous global crop yield response to biochar: a meta-regression analysis. *Environmental Research Letters*, **8**.

CROSS A. AND SOHI S. P. (2011). The priming potential of biochar products in relation to labile carbon contents and soil organic matter status. *Soil Biology and Biochemistry*, **43**, 2127-2134.

CZEPIEL P. M., CRILL P. M. AND HARRISS R. C. (1995). Environmental factors influencing the variability of methane oxidation in temperate zone soils. *Journal of Geophysical Research*, **100**, 9359-9364.

DELUCA T., MACKENZIE M., GUNDALE M. AND HOLBEN W. (2006). Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa pine forests. *Soil Science Society of America Journal*, **70**, 448-453.

DEMPSTER D., GLEESON D., SOLAIMAN Z., JONES D. AND MURPHY D. (2012). Decreased soil microbial biomass and nitrogen mineralisation with Eucalyptus biochar addition to a coarse textured soil. *Plant and Soil*, **354**, 311-324.

DIAKITE M., PAUL A., JAEGER C., PIELERT J. AND MUMME J. (2013). Chemical and morphological changes in hydrochars derived from microcrystalline cellulose and investigated by chromatographic, spectroscopic and adsorption techniques. *Bioresource Technology*, **150**, 98-105.

DIAZ-ROJAS M., AGUILAR-CHAVEZ A., DEL ROSARIO CARDENAS-AQUINO M., MANUEL RUIZ-VALDIVIEZO V., HERNANDEZ-VALDEZ E., LUNA-GUIDO M., OLALDE-PORTUGAL V. AND DENDOOVEN L. (2014). Effects of wastewater sludge, urea and

charcoal on greenhouse gas emissions in pots planted with wheat. *Applied Soil Ecology*, **73**, 19-25.

DICKE C., LANZA G., MUMME J., ELLERBROCK R. AND KERN J. (2014). Effect of hydrothermally carbonized char application on trace gas emissions from two sandy soil horizons. *Journal of Environmental Quality*, **43**, 1790-1798.

DING Y., LIU Y. X., WU W. X., SHI D. Z., YANG M. AND ZHONG Z. K. (2010). Evaluation of biochar effects on nitrogen retention and leaching in multi-layered soil columns. *Water, Air and Soil Pollution*, **213**, 47-55.

DOMENE X., MATTANA S., HANLEY K., ENDERS A. AND LEHMANN J. (2014). Medium-term effects of corn biochar addition on soil biota activities and functions in a temperate soil cropped to corn. *Soil Biology and Biochemistry*, **72**, 152-162.

DONG D., YANG M., WANG C., WANG H., LI Y., LUO J. AND WU W. (2013). Responses of methane emissions and rice yield to applications of biochar and straw in a paddy field. *Journal of Soils and Sediments*, **13**, 1450-1460.

DOWNIE A. E., VAN ZWIETEN L., SMERNIK R. J., MORRIS S. AND MUNROE P. R. (2011). *Terra Preta Australis*: Reassessing the carbon storage capacity of temperate soils. *Agriculture, Ecosystems and Environment*, **140**, 137-147.

DOYDORA S. A., CABRERA M. L., DAS K. C., GASKIN J. W., SONON L. S. AND MILLER W. P. (2011). Release of nitrogen and phosphorus from poultry litter amended with acidified biochar. *International Journal of Environmental Research and Public Health*, **8**, 1491-1502.

DRENOVSKY R., VO D., GRAHAM K. AND SCOW K. (2004). Soil water content and organic carbon availability are major determinants of soil microbial community composition. *Microbial Ecology*, **48**, 424-430.

DU Z., WANG Y., HUANG J., LU N., LIU X., LOU Y. AND ZHANG Q. (2014). Consecutive biochar application alters soil enzyme activities in the winter wheat-growing season. *Soil Science*, **179**, 75-83.

DUGAN E., VERHOEF A., ROBINSON S. AND SOHI S. (2010). Bio-char from sawdust, maize stover and charcoal: Impact on water holding capacities (WHC) of three soils from Ghana. Proceedings of the 19th World Congress of Soil Science, Symposium, pp. 9-12.

DUNFIELD P. AND KNOWLES R. (1995). Kinetics of inhibition of methane oxidation by nitrate, nitrite, and ammonium in a Humisol. *Applied and Environmental Microbiology*, **61**, 3129-3135.

EASTMAN C. M. (2011). *Soil physical characteristics of an Aeric Ochraqualf amended with Biochar* Masters Thesis, The Ohio State University.

ECKHARDT C. (2013). *Der Einfluss von Biokohle auf die mikrobielle Aktivität im Boden* Diploma Thesis, Justus Liebig University Giessen.

ECKMEIER E., GERLACH R., GEHRT E. AND SCHMIDT M. W. (2007). Pedogenesis of Chernozems in central Europe—a review. *Geoderma*, **139**, 288-299.

EIBISCH N., HELFRICH M., DON A., MIKUTTA R., KRUSE A., ELLERBROCK R. AND FLESSA H. (2013). Properties and degradability of hydrothermal carbonization products. *Journal of Environmental Quality*, **42**, 1565-1573.

EIBISCH N., SCHROLL R., FUSS R., MIKUTTA R., HELFRICH M. AND FLESSA H. (2015). Pyrochars and hydrochars differently alter the sorption of the herbicide isoproturon in an agricultural soil. *Chemosphere*, **119**, 155-62.

EILAND F., KLAMER M., LIND A.-M., LETH M. AND BÅÅTH E. (2001). Influence of initial C/N ratio on chemical and microbial composition during long term composting of straw. *Microbial Ecology*, **41**, 272-280.

ELAIGWU S. E., ROCHER V., KYRIAKOU G. AND GREENWAY G. M. (2014). Removal of Pb^{2+} and Cd^{2+} from aqueous solution using chars from pyrolysis and microwave-assisted hydrothermal carbonization of *Prosopis africana* shell. *Journal of Industrial and Engineering Chemistry*, **20**, 3467-3473.

ELLENBERG H. AND LEUSCHNER C. (2010). Vegetation Mitteleuropas mit den Alpen, p. 1333. Stuttgart: Ulmer UTB.

FANG Y., SINGH B., SINGH B. AND KRULL E. (2014). Biochar carbon stability in four contrasting soils. *European Journal of Soil Science*, **65**, 60-71.

FARRELL M., KUHN T. K., MACDONALD L. M., MADDERN T. M., MURPHY D. V., HALL P. A., SINGH B. P., BAUMANN K., KRULL E. S. AND BALDOCK J. A. (2013). Microbial utilisation of biochar-derived carbon. *Science of the Total Environment*, **465**, 288-297.

FELBER R., LEIFELD J., HORAK J. AND NEFTEL A. (2014). Nitrous oxide emission reduction with greenwaste biochar: comparison of laboratory and field experiments. *European Journal of Soil Science*, **65**, 128-138.

FENG Y., XU Y., YU Y., XIE Z. AND LIN X. (2012). Mechanisms of biochar decreasing methane emission from Chinese paddy soils. *Soil Biology and Biochemistry*, **46**, 80-88.

FIELD C. B. (2012). Managing the risks of extreme events and disasters to advance climate change adaptation: Special report of the intergovernmental panel on climate change: Cambridge University Press.

FIRESTONE M. (1982). Biological denitrification. *Nitrogen in agricultural soils*, 289-326.

FLACHOWSKY G., JEROCH H., KIRCHGESSNER M., PALLAUF J., PFEFFER E., SCHULZ E. AND STAUDACHER W. (2001). Empfehlungen zur Energie- und Nährstoffversorgung der Milchkühe und Aufzuchtrinder *Energie- und Nährstoffbedarf landwirtschaftlicher Nutztiere*. Frankfurt am Main: DLG-Verlags-GmbH.

FLESSA H., WILD U., KLEMISCH M. AND PFADENHAUER J. (1998). Nitrous oxide and methane fluxes from organic soils under agriculture. *European Journal of Soil Science*, **49**, 327-335.

FOCHT D., STOLZY L. AND MEEK B. (1979). Sequential reduction of nitrate and nitrous oxide under field conditions as brought about by organic amendments and irrigation management. *Soil Biology and Biochemistry*, **11**, 37-46.

FROSTEGÅRD Å., BÅÅTH E. AND TUNLID A. (1993). Shifts in the structure of soil microbial communities in limed forests as revealed by phospholipid fatty acid analysis. *Soil Biology and Biochemistry*, **25**, 723-730.

FROSTEGÅRD Å. AND BÅÅTH E. (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. *Biology and Fertility of Soils*, **22**, 59-65.

FRY E. L., MANNING P. AND POWER S. A. (2014). Ecosystem functions are resistant to extreme changes to rainfall regimes in a mesotrophic grassland. *Plant and soil*, **381**, 351-365.

FUNKE A. AND ZIEGLER F. (2011). Heat of reaction measurements for hydrothermal carbonization of biomass. *Bioresource Technology*, **102**, 7595-7598.

FUNKE A., MUMME J., KOON M. AND DIAKITE M. (2013a). Cascaded production of biogas and hydrochar from wheat straw: Energetic potential and recovery of carbon and plant nutrients. *Biomass and Bioenergy*, **58**, 229-237.

FUNKE A., REEBS F. AND KRUSE A. (2013b). Experimental comparison of hydrothermal and vapothermal carbonization. *Fuel Processing Technology*, **115**, 261-269.

GAJIĆ A. AND KOCH H.-J. (2012). Sugar beet (L.) growth reduction caused by hydrochar is related to nitrogen supply. *Journal of Environmental Quality*, **41**, 1067-1075.

GAO Y., WANG X., WANG J., LI X., CHENG J., YANG H. AND CHEN H. (2013). Effect of residence time on chemical and structural properties of hydrochar obtained by hydrothermal carbonization of water hyacinth. *Energy*, **58**, 376-383.

GARMO T. H., FROSLIE A. AND HOIE R. (1986). Levels of copper, molybdenum, sulphur, zinc, selenium, iron and manganese in native pasture plants from a mountain area in southern Norway. *Acta Agriculturae Scandinavica*, **36**, 147-161.

GASKIN J. W., SPEIR R. A., HARRIS K., DAS K., LEE R. D., MORRIS L. A. AND FISHER D. S. (2010). Effect of peanut hull and pine chip biochar on soil nutrients, corn nutrient status, and yield. *Agronomy Journal*, **102**, 623-633.

GAUNT J. L. AND LEHMANN J. (2008). Energy balance and emissions associated with biochar sequestration and pyrolysis bioenergy production. *Environmental Science and Technology*, **42**, 4152-4158.

GEORGE C., WAGNER M., KUECKE M. AND RILLIG M. C. (2012). Divergent consequences of hydrochar in the plant-soil system: Arbuscular mycorrhiza, nodulation, plant growth and soil aggregation effects. *Applied Soil Ecology*, **59**, 68-72.

GLASER B., LEHMANN J. AND ZECH W. (2002). Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal–a review. *Biology and Fertility of Soils*, **35**, 219-230.

GLASER B., PARR M., BRAUN C. AND KOPOLO G. (2009). Biochar is carbon negative. *Nature Geoscience*, **2**, 2.

GOMEZ J. D., DENEF K., STEWART C. E., ZHENG J. AND COTRUFO M. F. (2014). Biochar addition rate influences soil microbial abundance and activity in temperate soils. *European Journal of Soil Science*, **65**, 28-39.

GRABER E. R., TSECHANSKY L., LEW B. AND COHEN E. (2014). Reducing capacity of water extracts of biochars and their solubilization of soil Mn and Fe. *European Journal of Soil Science*, **65**, 162-172.

GRONAUER A. (1993). Einflußfaktoren auf die Ammoniakfreisetzung aus Flüssigmist als Grundlage verfahrenstechnischer Verbesserungen: Bayer. Staatsministerium f. Ernährung, Landwirtschaft u. Forsten, Ref. Landmaschinenwesen u. Energiewirtschaft.

GUEERENA D., LEHMANN J., HANLEY K., ENDERS A., HYLAND C. AND RIHA S. (2013). Nitrogen dynamics following field application of biochar in a temperate North American maize-based production system. *Plant and Soil*, **365**, 239-254.

GULLEDGE J., DOYLE A. P. AND SCHIMEL J. P. (1997). Different NH_4^+-inhibition patterns of soil CH_4 consumption: A result of distinct CH_4-oxidizer populations across sites? *Soil Biology and Biochemistry*, **29**, 13-21.

GURWICK N. P., MOORE L. A., KELLY C. AND ELIAS P. (2013). A systematic review of biochar research, with a focus on its stability in situ and its promise as a climate mitigation strategy. *PLoS One*, **8**, e75932.

GÜSEWELL S. (2004). N: P ratios in terrestrial plants: variation and functional significance. *New Phytologist*, **164**, 243-266.

HAENI C., KUPPER T., JOCHER M., NEFTEL A. AND SINTERMANN J. (2012). Amendment of biochar to slurry: A possibility to mitigate ammonia emissions? Proceedings of the International Symposium on Emissions of Gas and Dust from Livestock, pp. 10-13.

HALE S. E., HANLEY K., LEHMANN J., ZIMMERMAN A. R. AND CORNELISSEN G. (2011). Effects of chemical, biological, and physical aging as well as soil addition on the

sorption of pyrene to activated carbon and biochar. *Environmental Science and Technology*, **45**, 10445-10453.

HAMER U., MARSCHNER B., BRODOWSKI S. AND AMELUNG W. (2004). Interactive priming of black carbon and glucose mineralisation. *Organic Geochemistry*, **35**, 823-830.

HAMMEL K. (1997). Fungal degradation of lignin. In: CADISCH G. AND GILLER K. E. (eds.) *Driven by nature: plant litter quality and decomposition*, pp. 33-45. Wallingford, UK: CAB International.

HAMMER E. C., BALOGH-BRUNSTAD Z., JAKOBSEN I., OLSSON P. A., STIPP S. L. AND RILLIG M. C. (2014). A mycorrhizal fungus grows on biochar and captures phosphorus from its surfaces. *Soil Biology and Biochemistry*, **77**, 252-260.

HAMMOND J., SHACKLEY S., PRENDERGAST-MILLER M., COOK J., BUCKINGHAM S. AND PAPPA V. A. (2013). Biochar field testing in the UK: outcomes and implications for use. *Carbon Management*, **4**, 159-170.

HARTER J., KRAUSE H.-M., SCHUETTLER S., RUSER R., FROMME M., SCHOLTEN T., KAPPLER A. AND BEHRENS S. (2014). Linking N_2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community. *Isme Journal*, **8**, 660-674.

HARVEY O. R., KUO L.-J., ZIMMERMAN A. R., LOUCHOUARN P., AMONETTE J. E. AND HERBERT B. E. (2012). An index-based approach to assessing recalcitrance and soil carbon sequestration potential of engineered black carbons (biochars). *Environmental Science and Technology*, **46**, 1415-1421.

HATAKKA A. (1994). Lignin-modifying enzymes from selected white-rot fungi: production and role from in lignin degradation. *FEMS Microbiology Reviews*, **13**, 125-135.

HE C., GIANNIS A. AND WANG J.-Y. (2013). Conversion of sewage sludge to clean solid fuel using hydrothermal carbonization: Hydrochar fuel characteristics and combustion behavior. *Applied Energy*, **111**, 257-266.

HEATON E. A., DOHLEMAN F. G. AND LONG S. P. (2008). Meeting US biofuel goals with less land: the potential of *Miscanthus*. *Global Change Biology*, **14**, 2000-2014.

HEMINGWAY R. (1962). Copper, molybdenum, manganese and iron contents of herbage as influenced by fertilizer treatments over a three-year period. *Grass and Forage Science*, **17**, 182-187.

HERATH H., CAMPS-ARBESTAIN M. AND HEDLEY M. (2013). Effect of biochar on soil physical properties in two contrasting soils: an Alfisol and an Andisol. *Geoderma*, **209**, 188-197.

HEROLD M. B., BAGGS E. M. AND DANIELL T. J. (2012). Fungal and bacterial denitrification are differently affected by long-term pH amendment and cultivation of arable soil. *Soil Biology and Biochemistry*, **54**, 25-35.

HILSCHER A. AND KNICKER H. (2011). Degradation of grass-derived pyrogenic organic material, transport of the residues within a soil column and distribution in soil organic matter fractions during a 28month microcosm experiment. *Organic Geochemistry*, **42**, 42-54.

HLUG (2014). BodenViewer Hessen: Hessian Agency for Geology and the Environment.

HOBARA S., OSONO T., HIROSE D., NORO K., HIROTA M. AND BENNER R. (2014). The roles of microorganisms in litter decomposition and soil formation. *Biogeochemistry*, **118**, 471-486.

HOSSAIN M. K., STREZOV V., CHAN K. Y., ZIOLKOWSKI A. AND NELSON P. F. (2011). Influence of pyrolysis temperature on production and nutrient properties of wastewater sludge biochar. *Journal of Environmental Management*, **92**, 223-228.

HOUBEN D., EVRARD L. AND SONNET P. (2013). Mobility, bioavailability and pH-dependent leaching of cadmium, zinc and lead in a contaminated soil amended with biochar. *Chemosphere*, **92**, 1450-1457.

HOUGHTON J. T., MEIRA FILHO L. G., GRIGGS D. J. AND MASKELL K. (1997). Stabilization of atmospheric greenhouse gases: physical, biological and socio-economic implications: Intergovernmental Panel on Climate Change.

HRAPOVIC L. AND ROWE R. (2002). Intrinsic degradation of volatile fatty acids in laboratory-compacted clayey soil. *Journal of contaminant hydrology*, **58**, 221-242.

HU J., WU F., WU S., LAM C. L., LIN X. AND WONG M. H. (2014). Biochar and *Glomus caledonium* influence Cd accumulation of Upland Kangkong (*Ipomoea aquatica* Forsk.) intercropped with Alfred Stonecrop (*Sedum alfredii* Hance). *Scientific Reports*, **4**.

HUA L., WU W., LIU Y., MCBRIDE M. B. AND CHEN Y. (2009). Reduction of nitrogen loss and Cu and Zn mobility during sludge composting with bamboo charcoal amendment. *Environmental Science and Pollution Research*, **16**, 1-9.

HUTCHINSON G. AND LIVINGSTON G. (1993). Use of chamber systems to measure trace gas fluxes. In: ROLSTON D. E., DUXBURY J. M., HARPER L. A. AND MOSIER A. (eds.) *Agricultural ecosystem effects on trace gases and global climate change*, pp. 63-78: Amer Society of Agronomy.

HÜTSCH B. W. (2001). Methane oxidation in non-flooded soils as affected by crop production—invited paper. *European Journal of Agronomy*, **14**, 237-260.

IBI (2014). Standardized Product Definition and Product Testing Guidelines for Biochar that is used in soil, p. 60. Westerville: International Biochar Initiative.

IPCC (2007). Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. In: SOLOMON S., D. QIN, M. MANNING, Z. CHEN, M. MARQUIS, K.B. AVERYT, AND MILLER M. T. A. H. L. (eds.), p. 996. Cambridge, NY.

IPCC (2013). Climate Change 2013: The Physical Science Basis. Contribution of Working Group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. In: STOCKER T. F., D. QIN, G.-K. PLATTNER, M. TIGNOR, S.K. ALLEN, J. BOSCHUNG, A. NAUELS, Y. XIA, V. BEX AND P.M. MIDGLEY (ed.), 5 edn, p. 1535. Cambridge, United Kingdom and New York, NY, USA.

IPPOLITO J., NOVAK J., BUSSCHER W., AHMEDNA M., REHRAH D. AND WATTS D. (2012). Switchgrass biochar affects two Aridisols. *Journal of Environmental Quality*, **41**, 1123-1130.

JAAFAR N. M., CLODE P. L. AND ABBOTT L. K. (2014). Microscopy observations of habitable space in biochar for colonization by fungal hyphae from soil. *Journal of Integrative Agriculture*, **13**, 483-490.

JÄGER H.-J., SCHMIDT S. W., KAMMANN C., GRÜNHAGE L., MÜLLER C. AND HANEWALD K. (2003). The University of Giessen free-air carbon dioxide enrichment study: description of the experimental site and of a new enrichment system. *Journal of Applied Botany*, **77**, 117-127.

JANDL G., ECKHARDT K.-U., BARGMANN I., KÜCKE M., GREEF J.-M., KNICKER H. AND LEINWEBER P. (2013). Hydrothermal carbonization of biomass residues: mass spectrometric characterization for ecological effects in the soil–plant system. *Journal of Environmental Quality*, **42**, 199-207.

JANGID K., WILLIAMS M. A., FRANZLUEBBERS A. J., SCHMIDT T. M., COLEMAN D. C. AND WHITMAN W. B. (2011). Land-use history has a stronger impact on soil microbial

community composition than aboveground vegetation and soil properties. *Soil Biology and Biochemistry*, **43**, 2184-2193.

JANZE S. (2006). *Auswirkungen von erhöhtem CO_2 auf die Vegetation eines Grünlandes* PhD thesis, Justus Liebig University Giessen.

JEFFERY S., VERHEIJEN F., VAN DER VELDE M. AND BASTOS A. (2011). A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. *Agriculture, Ecosystems and Environment*, **144**, 175-187.

JENKINSON D. AND LADD J. (1981). Microbial biomass in soil: measurement and turnover. *Soil Biochemistry*, Vol. 5. New York, USA, Marcel Dekker.

JIN H. (2010). *Characterization of microbial life colonizing biochar and biochar-amended soils* PhD thesis. Cornell University.

JOERGENSEN R. G. (1996). The fumigation-extraction method to estimate soil microbial biomass: Calibration of the k_{EC} value. *Soil Biology and Biochemistry*, **28**, 25-31.

JONES A., JONES D.L. AND CROSS P. (2014). The carbon footprint of UK sheep production: current knowledge and opportunities for reduction in temperate zones. *The Journal of Agricultural Science*, **152**, 288-308.

JONES D.L., MURPHY D., KHALID M., AHMAD W., EDWARDS-JONES G. AND DELUCA T. (2011). Short-term biochar-induced increase in soil CO_2 release is both biotically and abiotically mediated. *Soil Biology and Biochemistry*, **43**, 1723-1731.

JONES D. L., ROUSK J., EDWARDS-JONES G., DELUCA T. H. AND MURPHY D. V. (2012). Biochar-mediated changes in soil quality and plant growth in a three year field trial. *Soil Biology and Biochemistry*, **45**, 113-124.

JONES D. L. AND QUILLIAM R. S. (2014). Metal contaminated biochar and wood ash negatively affect plant growth and soil quality after land application. *Journal of Hazardous Materials*, **276**, 362-370.

JOSEPH S., GRABER E.R., CHIA C., MUNROE P., DONNE S., THOMAS T., NIELSEN S., MARJO C., RUTLIDGE H., PAN G.X., LI L., TAYLOR P., RAWAL A. AND HOOK J (2013). Shifting paradigms: development of high-efficiency biochar fertilizers based on nano-structures and soluble components. *Carbon Management*, 4, 323-343.

KAISER C., FRANK A., WILD B., KORANDA M. AND RICHTER A. (2010). Negligible contribution from roots to soil-borne phospholipid fatty acid fungal biomarkers 18: 2ω6, 9 and 18: 1ω9. *Soil Biology and Biochemistry*, **42**, 1650-1652.

KALDERIS D., KOTTI M. S., MENDEZ A. AND GASCO G. (2014). Characterization of hydrochars produced by hydrothermal carbonization of rice husk. *Solid Earth*, **5**, 477-483.

KAMMANN C., GRÜNHAGE L., MÜLLER C., JACOBI S. AND JÄGER H.-J. (1998). Seasonal variability and mitigation options for N_2O emissions from differently managed grasslands. *Environmental Pollution*, **102**, 179-186.

KAMMANN C., MÜLLER C., GRÜNHAGE L. AND JÄGER H.-J. (2008). Elevated CO_2 stimulates N_2O emissions in permanent grassland. *Soil Biology and Biochemistry*, **40**, 2194-2205.

KAMMANN C., HEPP S., LENHART K. AND MUELLLER C. (2009). Stimulation of methane consumption by endogenous CH_4 production in aerobic grassland soil. *Soil Biology and Biochemistry*, **41**, 622-629.

KAMMANN C., RATERING S., ECKHARD C. AND MUELLER C. (2012). Biochar and hydrochar effects on greenhouse gas (carbon dioxide, nitrous oxide, and methane) fluxes from soils. *Journal of Environmental Quality*, **41**, 1052-1066.

KAMMANN C., MESSERSCHMIDT N., MÜLLER C., STEFFENS D., SCHMIDT H.-P. AND KOYRO H.-W. (2013). Significant plant growth stimulation by composted vs. untreated

biochar. Proceedings of the 2nd Nordic Biochar Symposium, Helsinki, 14.-15. February.

KAMMANN C. AND GRABER E. (2015). Biochar effects on plant ecophysiology. In: LEHMANN J., JOSEPH, S. (ed.) *Biochar: Science, Technology and Implementation, 2nd Edition*, pp. 389-418. London, UK: Earthscan.

KARHU K., MATTILA T., BERGSTRÖM I. AND REGINA K. (2011). Biochar addition to agricultural soil increased CH_4 uptake and water holding capacity–Results from a short-term pilot field study. *Agriculture, Ecosystems and Environment*, **140**, 309-313.

KASOZI G. N., ZIMMERMAN A. R., NKEDI-KIZZA P. AND GAO B. (2010). Catechol and humic acid sorption onto a range of laboratory-produced black carbons (biochars). *Environmental Science and Technology*, **44**, 6189-6195.

KEITH A., SINGH B. AND SINGH B. P. (2011). Interactive priming of biochar and labile organic matter mineralization in a smectite-rich soil. *Environmental Science and Technology*, **45**, 9611-9618.

KESSLER J. (2001). Mineralstoffversorgung der Milchkuh auf einen Blick *rap aktuell*, p. 4. MTL SA, Villars-sur-Glâne: Eidgenössische Forschungsanstalt für Nutztiere.

KETTUNEN R. AND SAARNIO S. (2013). Biochar can restrict N_2O emissions and the risk of nitrogen leaching from an agricultural soil during the freeze-thaw period. *Agricultural and Food Science*, **22**, 373-379.

KLOSS S., ZEHETNER F., DELLANTONIO A., HAMID R., OTTNER F., LIEDTKE V., SCHWANNINGER M., GERZABEK M. H. AND SOJA G. (2012). Characterization of slow pyrolysis biochars: effects of feedstocks and pyrolysis temperature on biochar properties. *Journal of Environmental Quality*, **41**, 990-1000.

KLOSS S., ZEHETNER F., OBURGER E., BUECKER J., KITZLER B., WENZEL W. W., WIMMER B. AND SOJA G. (2014a). Trace element concentrations in leachates and mustard plant tissue (*Sinapis alba* L.) after biochar application to temperate soils. *Science of the Total Environment*, **481**, 498-508.

KLOSS S., ZEHETNER F., WIMMER B., BUECKER J., REMPT F. AND SOJA G. (2014b). Biochar application to temperate soils: Effects on soil fertility and crop growth under greenhouse conditions. *Journal of Plant Nutrition and Soil Science*, **177**, 3-15.

KNOWLES O., ROBINSON B., CONTANGELO A. AND CLUCAS L. (2011). Biochar for the mitigation of nitrate leaching from soil amended with biosolids. *Science of the Total Environment*, **409**, 3206-3210.

KOERSELMAN W. AND MEULEMAN A. F. (1996). The vegetation N: P ratio: a new tool to detect the nature of nutrient limitation. *Journal of Applied Ecology*, **33**, 1441-1450.

KÖGEL-KNABNER I., GUGGENBERGER G., KLEBER M., KANDELER E., KALBITZ K., SCHEU S., EUSTERHUES K. AND LEINWEBER P. (2008). Organo-mineral associations in temperate soils: Integrating biology, mineralogy, and organic matter chemistry. *Journal of Plant Nutrition and Soil Science*, **171**, 61-82.

KOLB S. E., FERMANICH K. J. AND DORNBUSH M. E. (2009). Effect of charcoal quantity on microbial biomass and activity in temperate soils. *Soil Science Society of America Journal*, **73**, 1173-1181.

KOLTON M., HAREL Y. M., PASTERNAK Z., GRABER E. R., ELAD Y. AND CYTRYN E. (2011). Impact of biochar application to soil on the root-associated bacterial community structure of fully developed greenhouse pepper plants. *Applied and Environmental Microbiology*, **77**, 4924-4930.

KOTTEK M., GRIESER J., BECK C., RUDOLF B. AND RUBEL F. (2006). World map of the Köppen-Geiger climate classification updated. *Meteorologische Zeitschrift*, **15**, 259-263.

KRULL E. S., BALDOCK J. A., SKJEMSTAD J. O. AND SMERNIK R. J. (2009). Characteristics of biochar: organo-chemical properties. *Biochar for Environmental Management: Science and Technology*. *Earthscan, London*, 53-65.

KRUSE A., FUNKE A. AND TITIRICI M.-M. (2013). Hydrothermal conversion of biomass to fuels and energetic materials. *Current Opinion in Chemical Biology*, **17**, 515-521.

KUTSCHERA L., LICHTENEGGER E. AND SOBOTIK M. (1982). Wurzelatlas mitteleuropäischer Grünlandpflanzen: Gustav Fischer Verlag.

KUTSCHERA L., LICHTENEGGER E. AND SOBOTIK M. (2009). Wurzelatlas der Kulturpflanzen gemäßigter Gebiete mit Arten des Feldgemüsebaues: DLG-Verlag.

KUZYAKOV Y., FRIEDEL J. AND STAHR K. (2000). Review of mechanisms and quantification of priming effects. *Soil Biology and Biochemistry*, **32**, 1485-1498.

KUZYAKOV Y., SUBBOTINA I., CHEN H., BOGOMOLOVA I. AND XU X. (2009). Black carbon decomposition and incorporation into soil microbial biomass estimated by C-14 labeling. *Soil Biology and Biochemistry*, **41**, 210-219.

KUZYAKOV Y., BOGOMOLOVA I. AND GLASER B. (2014). Biochar stability in soil: decomposition during eight years and transformation as assessed by compound-specific ^{14}C analysis. *Soil Biology and Biochemistry*, **70**, 229-236.

LAIRD D., FLEMING P., WANG B., HORTON R. AND KARLEN D. (2010a). Biochar impact on nutrient leaching from a Midwestern agricultural soil. *Geoderma*, **158**, 436-442.

LAIRD D. A., FLEMING P., DAVIS D. D., HORTON R., WANG B. AND KARLEN D. L. (2010b). Impact of biochar amendments on the quality of a typical Midwestern agricultural soil. *Geoderma*, **158**, 443-449.

LASER H. (2007). Effects of liming and nitrogen application on the trace element concentrations of pastures in low mountain range. *Plant Soil and Environment*, **53**, 258.

LAUGHLIN R. J. AND STEVENS R. J. (2002). Evidence for fungal dominance of denitrification and codenitrification in a grassland soil. *Soil Science Society of America Journal*, **66**, 1540-1548.

LAUGHLIN R. J., STEVENS R. J., MÜLLER C. AND WATSON C. J. (2008). Evidence that fungi can oxidize NH_4^+ to NO_3^- in a grassland soil. *European Journal of Soil Science*, **59**, 285-291.

LAVAHUN M., JOERGENSEN R. AND MEYER B. (1996). Activity and biomass of soil microorganisms at different depths. *Biology and Fertility of Soils*, **23**, 38-42.

LEE J. W., KIDDER M., EVANS B. R., PAIK S., BUCHANAN III A., GARTEN C. T. AND BROWN R. C. (2010). Characterization of biochars produced from cornstovers for soil amendment. *Environmental Science and Technology*, **44**, 7970-7974.

LEHMANN J., GAUNT J. AND RONDON M. (2006). Biochar sequestration in terrestrial ecosystems–a review. *Mitigation and Adaptation Strategies for Global Change*, **11**, 395-419.

LEHMANN J. (2007). A handful of carbon. *Nature*, **447**, 143-144.

LEHMANN J., CZIMCZIK C., LAIRD D. AND SOHI S. (2009). Stability of biochar in soil. In: LEHMANN J. AND JOSEPH S. (eds.) *Biochar for Environmental Management: Science and Technology*, pp. 183-205. London, UK: Earthscan.

LEHMANN J. AND JOSEPH S. (2009). Biochar for Environmental Management: an Introduction. In: LEHMANN J. AND JOSEPH S. (eds.) *Biochar for Environmental Management: Science and Technology*, pp. 1-12. London, UK: Earthscan.

LEHMANN J., RILLIG M. C., THIES J., MASIELLO C. A., HOCKADAY W. C. AND CROWLEY D. (2011). Biochar effects on soil biota–a review. *Soil Biology and Biochemistry*, **43**, 1812-1836.

LEHMANN J., ABIVEN S., KLEBER M., PAN G., SINGH B. P., SOHI S. AND ZIMMERMAN A. (2015). Persistence of biochar in soil. In: LEHMANN J. AND JOSEPH S. (eds.) *Biochar: Science, Technology and Implementation,* pp. 233-280. London, UK: Earthscan.

LENHART K., BUNGE M., RATERING S., NEU T. R., SCHÜTTMANN I., GREULE M., KAMMANN C., SCHNELL S., MÜLLER C. AND ZORN H. (2012). Evidence for methane production by saprotrophic fungi. *Nature communications*, **3**, 1046.

LIANG C., ZHU X., FU S., MÉNDEZ A., GASCÓ G. AND PAZ-FERREIRO J. (2014). Biochar alters the resistance and resilience to drought in a tropical soil. *Environmental Research Letters*, **9**, 064013.

LIBRA J. A., RO K. S., KAMMANN C., FUNKE A., BERGE N. D., NEUBAUER Y., TITIRICI M.-M., FÜHNER C., BENS O., KERN J. AND EMMERICH K.-H. (2011). Hydrothermal carbonization of biomass residuals: A comparative review of the chemistry, processes and applications of wet and dry pyrolysis. *Biofuels*, **2**, 71-106.

LIN Q. AND BROOKES P. C. (1999). An evaluation of the substrate-induced respiration method. *Soil Biology and Biochemistry*, **31**, 1969-1983.

LIN Y., MUNROE P., JOSEPH S., KIMBER S. AND VAN ZWIETEN L. (2012). Nanoscale organo-mineral reactions of biochars in ferrosol: an investigation using microscopy. *Plant and Soil*, **357**, 369-380.

LINDSTRÖM B., FRANKOW-LINDBERG B., DAHLIN A., WIVSTAD M. AND WATSON C. (2013). Micronutrient concentrations in common and novel forage species and varieties grown on two contrasting soils. *Grass and Forage Science*, **68**, 427-436.

LIU X.-H., HAN F.-P. AND ZHANG X.-C. (2012). Effect of biochar on soil aggregates in the Loess Plateau: results from incubation experiments. *International Journal of Agriculture and Biology*, **14**, 975-979.

LIU Y. X., YANG M., WU Y. M., WANG H. L., CHEN Y. X. AND WU W. X. (2011). Reducing CH_4 and CO_2 emissions from waterlogged paddy soil with biochar. *Journal of Soils and Sediments*, **11**, 930-939.

LOFTFIELD N., FLESSA H., AUGUSTIN J. AND BEESE F. (1997). Automated gas chromatographic system for rapid analysis of the atmospheric trace gases methane, carbon dioxide, and nitrous oxide. *Journal of Environmental Quality*, **26**, 560-564.

LOGSDAIL D. AND WEBBER L. (1959). Effect of frost action on structure of Haldimand clay. *Canadian Journal of Soil Science*, **39**, 103-106.

LORENZ K. AND LAL R. (2014). Biochar application to soil for climate change mitigation by soil organic carbon sequestration. *Journal of Plant Nutrition and Soil Science*.

LU W., DING W., ZHANG J., LI Y., LUO J., BOLAN N. AND XIE Z. (2014). Biochar suppressed the decomposition of organic carbon in a cultivated sandy loam soil: A negative priming effect. *Soil Biology and Biochemistry*, **76**, 12-21.

LUCCHINI P., QUILLIAM R., DELUCA T. H., VAMERALI T. AND JONES D. L. (2014). Increased bioavailability of metals in two contrasting agricultural soils treated with waste wood-derived biochar and ash. *Environmental Science and Pollution Research*, **21**, 3230-3240.

LUO F., SONG J., XIA W., DONG M., CHEN M. AND SOUDEK P. (2014). Characterization of contaminants and evaluation of the suitability for land application of maize and sludge biochars. *Environmental Science and Pollution Research*, **21**, 8707-8717.

LUO Y., DURENKAMP M., DE NOBILI M., LIN Q. AND BROOKES P. (2011). Short term soil priming effects and the mineralisation of biochar following its incorporation to soils of different pH. *Soil Biology and Biochemistry*, **43**, 2304-2314.

LUO Y., DURENKAMP M., DE NOBILI M., LIN Q., DEVONSHIRE B. J. AND BROOKES P. C. (2013). Microbial biomass growth, following incorporation of biochars produced at 350 degrees C or 700 degrees C, in a silty-clay loam soil of high and low pH. *Soil Biology and Biochemistry*, **57**, 513-523.

MAAG M. AND VINTHER F. P. (1996). Nitrous oxide emission by nitrification and denitrification in different soil types and at different soil moisture contents and temperatures. *Applied Soil Ecology*, **4**, 5-14.

MAESTRINI B., HERRMANN A. M., NANNIPIERI P., SCHMIDT M. W. AND ABIVEN S. (2014). Ryegrass-derived pyrogenic organic matter changes organic carbon and nitrogen mineralization in a temperate forest soil. *Soil Biology and Biochemistry*, **69**, 291-301.

MAJOR J. AND HUSK B. (2010). Commercial scale agricultural biochar field trial in Québec, Canada, over two years: Effects of biochar on soil fertility, biology, crop productivity and quality, pp. 1-38. Drummondville (Québec): BlueLeaf Inc.

MALGHANI S., GLEIXNER G. AND TRUMBORE S. E. (2013). Chars produced by slow pyrolysis and hydrothermal carbonization vary in carbon sequestration potential and greenhouse gases emissions. *Soil Biology and Biochemistry*, **62**, 137-146.

MANYÀ J. J. (2012). Pyrolysis for biochar purposes: a review to establish current knowledge gaps and research needs. *Environmental Science and Technology*, **46**, 7939-7954.

MAQUEDA C., HERENCIA J. F., RUIZ J. C. AND HIDALGO M. F. (2011). Organic and inorganic fertilization effects on DTPA-extractable Fe, Cu, Mn and Zn, and their concentration in the edible portion of crops. *Journal of Agricultural Science*, **149**, 461-472.

MARRIS E. (2006). Putting the carbon back: Black is the new green. *Nature*, **442**, 624-626.

MARSCHNER P., KANDELER E. AND MARSCHNER B. (2003). Structure and function of the soil microbial community in a long-term fertilizer experiment. *Soil Biology and Biochemistry*, **35**, 453-461.

MARTINEZ-CASASNOVAS J., RAMOS M. AND RIBES-DASI M. (2002). Soil erosion caused by extreme rainfall events: mapping and quantification in agricultural plots from very detailed digital elevation models. *Geoderma*, **105**, 125-140.

MATZNER E. AND BORKEN W. (2008). Do freeze-thaw events enhance C and N losses from soils of different ecosystems? A review. *European Journal of Soil Science*, **59**, 274-284.

MAYNARD D., BARKER A., MINOTTI P. AND PECK N. (1976). Nitrate accumulation in vegetables. *Advances in Agronomy*, **28**, 71-118.

MCCORMACK S., VANBERGEN A., BARDGETT R., HOPKINS D. AND OSTLE N. (2012). Biochar and biological carbon cycling in temperate soils. Proceedings of the EGU General Assembly Conference Abstracts, p. 10996.

MCDONALD P. (2002). Animal nutrition. Harlow, UK: Pearson education.

MEINKEN E. (2014). Eignung von Pflanzenkohle als Torfersatzsstoff in gärtnerischen Kultursubstraten, In: MAURER-WOHLARTZ S. (ed). Proceedings of the Qualitätssicherung und Umwelteffekte von Pflanzenkohle Conference, Hannover, p. 32.

MENGEL K. AND STEFFENS D. (1985). Potassium uptake of rye-grass (*Lolium perenne*) and red clover (*Trifolium pratense*) as related to root parameters. *Biology and Fertility of Soils*, **1**, 53-58.

MEYER S., GLASER B. AND QUICKER P. (2011). Technical, economical, and climate-related aspects of biochar production technologies: a literature review. *Environmental Science and Technology*, **45**, 9473-9483.

MILLER M., ZEBARTH B., DANDIE C., BURTON D., GOYER C. AND TREVORS J. (2008). Crop residue influence on denitrification, N_2O emissions and denitrifier community abundance in soil. *Soil Biology and Biochemistry*, **40**, 2553-2562.

MIMMO T., PANZACCHI P., BARATIERI M., DAVIES C. A. AND TONON G. (2014). Effect of pyrolysis temperature on miscanthus (*Miscanthus* × *giganteus*) biochar physical, chemical and functional properties. *Biomass and Bioenergy*, **62**, 149-157.

MISSELBROOK T., SMITH K., JOHNSON R. AND PAIN B. (2002). SE—structures and environment: Slurry application techniques to reduce ammonia emissions: Results of some UK field-scale experiments. *Biosystems Engineering*, **81**, 313-321.

MÖLLER A. (2014). Biokohle aus Sicht des Bodenschutzes in Niedersachsen, In: MAURER-WOHLARTZ S. (ed). Proceedings of the Qualitätssicherung und Umwelteffekte von Pflanzenkohle Conference, Hannover, p. 32.

MONAGHAN R. AND BARRACLOUGH D. (1993). Nitrous oxide and dinitrogen emissions from urine-affected soil under controlled conditions. *Plant and Soil*, **151**, 127-138.

MONREAL C. AND KODAMA H. (1997). Influence of aggregate architecture and minerals on living habitats and soil organic matter. *Canadian Journal of Soil Science*, **77**, 367-377.

MONTANARELLA L. AND LUGATO E. (2013). The application of biochar in the EU: Challenges and opportunities. *Agronomy*, **3**, 462-473.

MOSIER A. AND DELGADO J. (1997). Methane and nitrous oxide fluxes in grasslands in western Puerto Rico. *Chemosphere*, **35**, 2059-2082.

MUHAMMAD N., DAI Z., XIAO K., MENG J., BROOKES P. C., LIU X., WANG H., WU J. AND XU J. (2014). Changes in microbial community structure due to biochars generated from different feedstocks and their relationships with soil chemical properties. *Geoderma*, **226**, 270-278.

MUKHERJEE A., ZIMMERMAN A. AND HARRIS W. (2011). Surface chemistry variations among a series of laboratory-produced biochars. *Geoderma*, **163**, 247-255.

MUKHERJEE A. AND LAL R. (2013). Biochar impacts on soil physical properties and greenhouse gas emissions. *Agronomy*, **3**, 313-339.

MUKHERJEE A. AND LAL R. (2014). The biochar dilemma. *Soil Research*, **52**, 217-230.

MÜLLER C., MARTIN M., STEVENS R., LAUGHLIN R., KAMMANN C., OTTOW J. AND JÄGER H.-J. (2002). Processes leading to N_2O emissions in grassland soil during freezing and thawing. *Soil Biology and Biochemistry*, **34**, 1325-1331.

MÜLLER C., KAMMANN C., OTTOW J. AND JÄGER H. J. (2003). Nitrous oxide emission from frozen grassland soil and during thawing periods. *Journal of Plant Nutrition and Soil Science*, **166**, 46-53.

MUMME J., ECKERVOGT L., PIELERT J., DIAKITÉ M., RUPP F. AND KERN J. (2011). Hydrothermal carbonization of anaerobically digested maize silage. *Bioresource Technology*, **102**, 9255-9260.

NEELY C. L., BEARE M. H., HARGROVE W. L. AND COLEMAN D. C. (1991). Relationships between fungal and bacterial substrate-induced respiration, biomass and plant residue decomposition. *Soil Biology and Biochemistry*, **23**, 947-954.

NELISSEN V., SAHA B. K., RUYSSCHAERT G. AND BOECKX P. (2014). Effect of different biochar and fertilizer types on N_2O and NO emissions. *Soil Biology and Biochemistry*, **70**, 244-255.

NELSON N. O., AGUDELO S. C., YUAN W. AND GAN J. (2011). Nitrogen and phosphorus availability in biochar-amended soils. *Soil Science*, **176**, 218.

NEVES E. G., PETERSEN J. B., BARTONE R. N. AND DA SILVA C. A. (2003). Historical and socio-cultural origins of Amazonian dark earth *Amazonian Dark Earths*, pp. 29-50: Springer.

NGUYEN B. T., LEHMANN J., HOCKADAY W. C., JOSEPH S. AND MASIELLO C. A. (2010). Temperature sensitivity of black carbon decomposition and oxidation. *Environmental Science and Technology*, **44**, 3324-3331.

NGUYEN B. T., KOIDE R. T., DELL C., DROHAN P., SKINNER H., ADLER P. R. AND NORD A. (2014). Turnover of soil carbon following addition of switchgrass-derived biochar to four soils. *Soil Science Society of America Journal*, **78**, 531-537.

NOVAK J. M., SPOKAS K. A., CANTRELL K. B., RO K. S., WATTS D. W., GLAZ B., BUSSCHER W. J. AND HUNT P. G. (2014). Effects of biochars and hydrochars produced from lignocellulosic and animal manure on fertility of a Mollisol and Entisol. *Soil Use and Management*, **30**, 175-181.

NURUL ISLAM M., RAFIQUL ALAM BEG M. AND ROFIQUL ISLAM M. (2005). Pyrolytic oil from fixed bed pyrolysis of municipal solid waste and its characterization. *Renewable Energy*, **30**, 413-420.

OCIO J., MARTINEZ J. AND BROOKES P. (1991). Contribution of straw-derived N to total microbial biomass N following incorporation of cereal straw to soil. *Soil Biology and Biochemistry*, **23**, 655-659.

OEHL F., LACZKO E., BOGENRIEDER A., STAHR K., BÖSCH R., VAN DER HEIJDEN M. AND SIEVERDING E. (2010). Soil type and land use intensity determine the composition of arbuscular mycorrhizal fungal communities. *Soil Biology and Biochemistry*, **42**, 724-738.

OGAWA M. AND OKIMORI Y. (2010). Pioneering works in biochar research, Japan. *Australian Journal of Soil Research*, **48**, 489-500.

OLARIETA J. R., PADRO R., MASIP G., RODRIGUEZ-OCHOA R. AND TELLO E. (2011). 'Formiguers', a historical system of soil fertilization (and biochar production?). *Agriculture Ecosystems and Environment*, **140**, 27-33.

ORAM N. J., VAN DE VOORDE T. F., OUWEHAND G.-J., BEZEMER T. M., MOMMER L., JEFFERY S. AND GROENIGEN J. W. V. (2014). Soil amendment with biochar increases the competitive ability of legumes via increased potassium availability. *Agriculture, Ecosystems and Environment*, **191**, 92-98.

PACHOLSKI A., CAI G., NIEDER R., RICHTER J., FAN X., ZHU Z. AND ROELCKE M. (2006). Calibration of a simple method for determining ammonia volatilization in the field–comparative measurements in Henan Province, China. *Nutrient Cycling in Agroecosystems*, **74**, 259-273.

PAIN B. F., PHILLIPS V. R., CLARKSON C. R. AND KLARENBEEK J. V. (1989). Loss of nitrogen through ammonia volatilisation during and following the application of pig or cattle slurry to grassland. *Journal of the Science of Food and Agriculture*, **47**, 1-12.

PALVIAINEN M., FINÉR L., KURKA A.-M., MANNERKOSKI H., PIIRAINEN S. AND STARR M. (2004). Release of potassium, calcium, iron and aluminium from Norway spruce, Scots pine and silver birch logging residues. *Plant and soil*, **259**, 123-136.

PARSHETTI G. K., HOEKMAN S. K. AND BALASUBRAMANIAN R. (2013). Chemical, structural and combustion characteristics of carbonaceous products obtained by hydrothermal carbonization of palm empty fruit bunches. *Bioresource Technology*, **135**, 683-689.

PEACOCK A. D., MULLEN M. D., RINGELBERG D. B., TYLER D. D., HEDRICK D. B., GALE P. M. AND WHITE D. C. (2001). Soil microbial community responses to dairy manure or ammonium nitrate applications. *Soil Biology and Biochemistry*, **33**, 1011-1019.

PETERSEN S. O., AMON B. AND GATTINGER A. (2005). Methane oxidation in slurry storage surface crusts. *Journal of Environmental Quality*, **34**, 455-461.

PICCOLO A., PIETRAMELLARA G. AND MBAGWU J. (1996). Effects of coal derived humic substances on water retention and structural stability of Mediterranean soils. *Soil Use and Management*, **12**, 209-213.

PIELERT J., KURZ J., GEORGE E., DOMINIK P. AND KAUPENJOHANN M. (2012). Pre-treated hydrochar as a soil conditioner? Results from a pot trial with *Zea mays*. Proceedings of the 1st international Biochar Summer School, Potsdam.

PIETIKÄINEN J., KIIKKILÄ O. AND FRITZE H. (2000). Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. *Oikos*, **89**, 231-242.

PONOMARENKO E. AND ANDERSON D. (2001). Importance of charred organic matter in Black Chernozem soils of Saskatchewan. *Canadian Journal of Soil Science*, **81**, 285-297.

POWLSON D., PROOKES P. AND CHRISTENSEN B. T. (1987). Measurement of soil microbial biomass provides an early indication of changes in total soil organic matter due to straw incorporation. *Soil Biology and Biochemistry*, **19**, 159-164.

PROMMER J., WANEK W., HOFHANSL F., TROJAN D., OFFRE P., URICH T., SCHLEPER C., SASSMANN S., KITZLER B., SOJA G. AND HOOD-NOWOTNY R. C. (2014). Biochar decelerates soil organic nitrogen cycling but stimulates soil nitrification in a temperate arable field trial. *PLoS One*, **9**.

PROST K., BORCHARD N., SIEMENS J., KAUTZ T., SEQUARIS J.-M., MOELLER A. AND AMELUNG W. (2013). Biochar affected by composting with farmyard manure. *Journal of Environmental Quality*, **42**, 164-172.

PYTER R., HEATON E., DOHLEMAN F., VOIGT T. AND LONG S. (2009). Agronomic experiences with *Miscanthus* × *giganteus* in Illinois, USA. In: MIELENZ J. R. (ed.) *Biofuels Methods and Protocols*, pp. 41-52. New York: Humana Press.

QAYYUM M. F., STEFFENS D., REISENAUER H. P. AND SCHUBERT S. (2012). Kinetics of carbon mineralization of biochars compared with wheat straw in three soils. *Journal of Environmental Quality*, **41**, 1210-1220.

QUILLIAM R. S., MARSDEN K. A., GERTLER C., ROUSK J., DELUCA T. H. AND JONES D. L. (2012). Nutrient dynamics, microbial growth and weed emergence in biochar amended soil are influenced by time since application and reapplication rate. *Agriculture Ecosystems and Environment*, **158**, 192-199.

QUILLIAM R. S., GLANVILLE H. C., WADE S. C. AND JONES D. L. (2013). Life in the 'charosphere' - Does biochar in agricultural soil provide a significant habitat for microorganisms? *Soil Biology and Biochemistry*, **65**, 287-293.

RAJKOVICH S., ENDERS A., HANLEY K., HYLAND C., ZIMMERMAN A. R. AND LEHMANN J. (2012). Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. *Biology and Fertility of Soils*, **48**, 271-284.

RAVISHANKARA A., DANIEL J. S. AND PORTMANN R. W. (2009). Nitrous oxide (N_2O): The dominant ozone-depleting substance emitted in the 21st century. *Science*, **326**, 123-125.

REDDY K. R., YARGICOGLU E. N., YUE D. AND YAGHOUBI P. (2014). Enhanced microbial methane oxidation in landfill cover soil amended with biochar. *Journal of Geotechnical and Geoenvironmental Engineering*, **140**.

REHRAH D., REDDY M. R., NOVAK J. M., BANSODE R. R., SCHIMMEL K. A., YU J., WATTS D. W. AND AHMEDNA M. (2014). Production and characterization of biochars from agricultural by-products for use in soil quality enhancement. *Journal of Analytical and Applied Pyrolysis*, **108**, 301-309.

REICHSTEIN M. (2012). Carbon management under extremes. *Carbon Management*, **3**, 113-115.

REID R. AND HORVATH D. (1980). Soil chemistry and mineral problems in farm livestock. A review. *Animal Feed Science and Technology*, **5**, 95-167.

REINA J., VELO E. AND PUIGJANER L. (1998). Thermogravimetric study of the pyrolysis of waste wood. *Thermochimica Acta*, **320**, 161-167.

REX D., SCHIMMELPFENNIG S., JANSEN-WILLEMS A., MOSER G., KAMMANN C. AND MÜLLER C. (2015). Microbial community shifts 2.6 years after top dressing of Miscanthus biochar, hydrochar and feedstock on a temperate grassland site. *Submitted to Plant and Soil for publication*.

REZA M. T., LYNAM J. G., UDDIN M. H. AND CORONELLA C. J. (2013). Hydrothermal carbonization: Fate of inorganics. *Biomass and Bioenergy*, **49**, 86-94.

RILLIG M. C., WAGNER M., SALEM M., ANTUNES P. M., GEORGE C., RAMKE H.-G., TITIRICI M.-M. AND ANTONIETTI M. (2010). Material derived from hydrothermal carbonization: Effects on plant growth and arbuscular mycorrhiza. *Applied Soil Ecology*, **45**, 238-242.

ROGOVSKA N., LAIRD D. A., RATHKE S. J. AND KARLEN D. L. (2014). Biochar impact on Midwestern Mollisols and maize nutrient availability. *Geoderma*, **230**, 340-347.

ROVIRA A. AND GREACEN E. (1957). The effect of aggregate disruption on the activity of microorganisms in the soil. *Crop and Pasture Science*, **8**, 659-673.

SAARNIO S., HEIMONEN K. AND KETTUNEN R. (2013). Biochar addition indirectly affects N_2O emissions via soil moisture and plant N uptake. *Soil Biology and Biochemistry*, **58**, 99-106.

SAHRAWAT K. AND KEENEY D. (1986). Nitrous oxide emission from soils. *Advances of Soil Science*, **4**, 103-148.

SAITO M. (1990). Charcoal as a micro-habitat for VA mycorrhizal fungi, and its practical implication. *Agriculture, Ecosystems and Environment*, **29**, 341-344.

SAITO M. AND MARUMOTO T. (2002). Inoculation with arbuscular mycorrhizal fungi: the status quo in Japan and the future prospects *Diversity and Integration in Mycorrhizas*, pp. 273-279: Springer.

SALEM M., KOHLER J., WURST S. AND RILLIG M. C. (2013a). Earthworms can modify effects of hydrochar on growth of Plantago lanceolata and performance of arbuscular mycorrhizal fungi. *Pedobiologia*, **56**, 219-224.

SALEM M., KOHLER J. AND RILLIG M. C. (2013b). Palatability of carbonized materials to Collembola. *Applied Soil Ecology*, **64**, 63-69.

SANG T. AND ZHU W. (2011). China's bioenergy potential. *Global Change Biology Bioenergy*, **3**, 79-90.

SARKHOT D., GHEZZEHEI T. AND BERHE A. (2013). Effectiveness of biochar for sorption of ammonium and phosphate from dairy effluent. *Journal of Environmental Quality*, **42**, 1545-1554.

SCHELLNHUBER H. J., CRAMER W., NAKICENOVIC N., WIGLEY T. AND YOHE G. (2006). Avoiding dangerous climate change, p. 406. New York: Cambridge University Press.

SCHENK M. AND BARBER S. (1980). Potassium and phosphorus uptake by corn genotypes grown in the field as influenced by root characteristics. *Plant and Soil*, **54**, 65-76.

SCHEUB U., PIEPLOW, H., SCHMIDT, H.-P. (2013). Terra Preta. Die schwarze Revolution aus dem Regenwald: Mit Klimagärtnern die Welt retten und gesunde Lebensmittel produzieren. München: oekom verlag

SCHIMMELPFENNIG S. AND GLASER B. (2012). One step forward toward characterization: some important material properties to distinguish biochars. *Journal of Environmental Quality*, **41**, 1001-1013.

SCHIMMELPFENNIG S., MUELLER C., GRUENHAGE L., KOCH C. AND KAMMANN C. (2014). Biochar, hydrochar and uncarbonized feedstock application to permanent grassland-Effects on greenhouse gas emissions and plant growth. *Agriculture Ecosystems and Environment*, **191**, 39-52.

SCHIMMELPFENNIG S., KAMMANN C., MOSER G., GRÜNHAGE L. AND MÜLLER C. (2015a). Changes in macro- and micronutrient contents of grasses and forbs following Miscanthus x giganteus feedstock, hydrochar and biochar application to temperate grassland. *Journal of Grass and Forage Science, accepted*.

SCHIMMELPFENNIG S., MUMME J., KAMMANN C., MARHAN S., BAMMINGER C., MOSER G., KERN J. AND MÜLLER C. (2015b). How to break it - degradation of Miscanthus x giganteus feedstock, hydrochar and biochar under the influence of simulated extreme events. *Submitted to Soil Biology and Biochemistry for publication*.

SCHMIDT H.-P., KAMMANN C., NIGGLI C., EVANGELOU M. W. H., MACKIE K. A. AND ABIVEN S. (2014). Biochar and biochar-compost as soil amendments to a vineyard soil: Influences on plant growth, nutrient uptake, plant health and grape quality. *Agriculture Ecosystems and Environment*, **191**, 117-123.

SCHMIDT M. W., TORN M. S., ABIVEN S., DITTMAR T., GUGGENBERGER G., JANSSENS I. A., KLEBER M., KÖGEL-KNABNER I., LEHMANN J. AND MANNING D. A. (2011). Persistence of soil organic matter as an ecosystem property. *Nature*, **478**, 49-56.

SCURLOCK J. AND HALL D. (1998). The global carbon sink: a grassland perspective. *Global Change Biology*, **4**, 229-233.

SEVILLA M. AND FUERTES A. B. (2009). Chemical and structural properties of carbonaceous products obtained by hydrothermal carbonization of saccharides. *Chemistry-A European Journal*, **15**, 4195-4203.

SHEN J., TANG H., LIU J., WANG C., LI Y., GE T., JONES D. L. AND WU J. (2014). Contrasting effects of straw and straw-derived biochar amendments on greenhouse gas emissions within double rice cropping systems. *Agriculture Ecosystems and Environment*, **188**, 264-274.

SHERLOCK R. R., SOMMER S. G., KHAN R. Z., WOOD C., GUERTAL E. A., FRENEY J. R., DAWSON C. O. AND CAMERON K. C. (2002). Ammonia, methane, and nitrous oxide emission from pig slurry applied to a pasture in New Zealand. *Journal of Environmental Quality*, **31**, 1491-1501.

SHINDELL D. T., FALUVEGI G., KOCH D. M., SCHMIDT G. A., UNGER N. AND BAUER S. E. (2009). Improved attribution of climate forcing to emissions. *Science*, **326**, 716-718.

SHUMAN L. (1988). Effect of organic matter on the distribution of manganese, copper, iron, and zinc in soil fractions. *Soil Science*, **146**, 192-198.

SIGUA G. C., NOVAK J. M., WATTS D. W., CANTRELL K. B., SHUMAKER P. D., SZOEGI A. A. AND JOHNSON M. G. (2014). Carbon mineralization in two ultisols amended with different sources and particle sizes of pyrolyzed biochar. *Chemosphere*, **103**, 313-321.

SILBER A., LEVKOVITCH I. AND GRABER E. (2010). pH-dependent mineral release and surface properties of cornstraw biochar: agronomic implications. *Environmental Science and Technology*, **44**, 9318-9323.

SIMS J. T. (1986). Soil pH effects on the distribution and plant availability of manganese, copper, and zinc. *Soil Science Society of America Journal*, **50**, 367-373.

SINGH B. P., HATTON B. J., SINGH B., COWIE A. L. AND KATHURIA A. (2010). Influence of biochars on nitrous oxide emission and nitrogen leaching from two contrasting soils. *Journal of. Environmental Quality*, **39**, 1224.

SINGH B. P., COWIE A. L. AND SMERNIK R. J. (2012). Biochar carbon stability in a clayey soil as a function of feedstock and pyrolysis temperature. *Environmental Science and Technology*, **46**, 11770-11778.

SINGH B. P. AND COWIE A. L. (2014). Long-term influence of biochar on native organic carbon mineralisation in a low-carbon clayey soil. *Scientific Reports*, **4**.

SINGH N., ABIVEN S., MAESTRINI B., BIRD J. A., TORN M. S. AND SCHMIDT M. W. I. (2014). Transformation and stabilization of pyrogenic organic matter in a temperate forest field experiment. *Global Change Biology*, **20**, 1629-1642.

SINGLA A. AND INUBUSHI K. (2014). Effect of biochar on CH_4 and N_2O emission from soils vegetated with paddy. *Paddy and Water Environment*, **12**, 239-243.

SIU R. AND REESE E. (1953). Decomposition of cellulose by microorganisms. *The Botanical Review*, **19**, 377-416.

SOHI S., KRULL E., LOPEZ-CAPEL E. AND BOL R. (2010). A review of biochar and its use and function in soil. *Advances in Agronomy*, **105**, 47-82.

SOINNE H., HOVI J., TAMMEORG P. AND TURTOLA E. (2014). Effect of biochar on phosphorus sorption and clay soil aggregate stability. *Geoderma*, **219**, 162-167.

SOLLINS P., HOMANN P. AND CALDWELL B. A. (1996). Stabilization and destabilization of soil organic matter: mechanisms and controls. *Geoderma*, **74**, 65-105.

SOMMER S. G., FRIIS E., BACH A. AND SCHJØRRING J. K. (1997). Ammonia volatilization from pig slurry applied with trail hoses or broadspread to winter wheat: effects of crop developmental stage, microclimate, and leaf ammonia absorption. *Journal of Environmental Quality*, **26**, 1153-1160.

SOMMER S. G. AND HUTCHINGS N. (2001). Ammonia emission from field applied manure and its reduction—invited paper. *European Journal of Agronomy*, **15**, 1-15.

SONG W. AND GUO M. (2012). Quality variations of poultry litter biochar generated at different pyrolysis temperatures. *Journal of Analytical and Applied Pyrolysis*, **94**, 138-145.

SONG X. D., XUE X. Y., CHEN D. Z., HE P. J. AND DAI X. H. (2014). Application of biochar from sewage sludge to plant cultivation: Influence of pyrolysis temperature and biochar-to-soil ratio on yield and heavy metal accumulation. *Chemosphere*, **109**, 213-220.

SØRENSEN P. (1998). Carbon mineralization, nitrogen immobilization and pH change in soil after adding volatile fatty acids. *European Journal of Soil Science*, **49**, 457-462.

SPOKAS K. A., KOSKINEN W. C., BAKER J. M. AND REICOSKY D. C. (2009). Impacts of woodchip biochar additions on greenhouse gas production and sorption/degradation of two herbicides in a Minnesota soil. *Chemosphere*, **77**, 574-581.

SPOKAS K. A. AND REICOSKY D. C. (2009). Impact of sixteen different biochars on soil greenhouse gas production. *Annals of Environmental Science*, **3**, 179-193.

SPOKAS K. A. (2010). Review of the stability of biochar in soils: predictability of O: C molar ratios. *Carbon Management*, **1**, 289-303.

SPOKAS K. A. AND BOGNER J. E. (2011). Limits and dynamics of methane oxidation in landfill cover soils. *Waste Management*, **31**, 823-832.

SPOKAS K. A., NOVAK J. M. AND VENTEREA R. T. (2012). Biochar's role as an alternative N-fertilizer: ammonia capture. *Plant and Soil*, **350**, 35-42.

SPOKAS K. A. (2013). Impact of biochar field aging on laboratory greenhouse gas production potentials. *Global Change Biology Bioenergy*, **5**, 165-176.

STAVI I. (2013). Biochar use in forestry and tree-based agro-ecosystems for increasing climate change mitigation and adaptation. *International Journal of Sustainable Development and World Ecology*, **20**, 166-181.

STAVI I. AND LAL R. (2013). Agroforestry and biochar to offset climate change: a review. *Agronomy for Sustainable Development*, **33**, 81-96.

STEINBEISS S., GLEIXNER G. AND ANTONIETTI M. (2009). Effect of biochar amendment on soil carbon balance and soil microbial activity. *Soil Biology and Biochemistry*, **41**, 1301-1310.

STEINER C., DAS K. C., MELEAR N. AND LAKLY D. (2010). Reducing nitrogen loss during poultry litter composting using biochar. *Journal of Environmental Quality*, **39**, 1236-1242.

STEWART C. E., ZHENG J., BOTTE J. AND COTRUFO M. F. (2013). Co-generated fast pyrolysis biochar mitigates green-house gas emissions and increases carbon sequestration in temperate soils. *Global Change Biology Bioenergy*, **5**, 153-164.

STONE A. T. AND MORGAN J. J. (1984). Reduction and dissolution of manganese (III) and manganese (IV) oxides by organics: 2. Survey of the reactivity of organics. *Environmental Science and Technology*, **18**, 617-624.

STOTZKY G. (1965). Microbial respiration. *Methods of Soil Analysis. Part 2. Chemical and Microbiological Properties*, 1550-1572.

SUN D., MENG J. AND CHEN W. (2013). Effects of abiotic components induced by biochar on microbial communities. *Acta Agriculturae Scandinavica Section B-Soil and Plant Science*, **63**, 633-641.

SUN G., COFFIN D. P. AND LAUENROTH W. K. (1997). Comparison of root distributions of species in North American grasslands using GIS. *Journal of Vegetation Science*, **8**, 587-596.

SUN L., LI L., CHEN Z., WANG J. AND XIONG Z. (2014a). Combined effects of nitrogen deposition and biochar application on emissions of N_2O, CO_2 and NH_3 from agricultural and forest soils. *Soil Science and Plant Nutrition*, **60**, 254-265.

SUN Y., GAO B., YAO Y., FANG J., ZHANG M., ZHOU Y., CHEN H. AND YANG L. (2014b). Effects of feedstock type, production method, and pyrolysis temperature on biochar and hydrochar properties. *Chemical Engineering Journal*, **240**, 574-578.

SUN Z., BRUUN E. W., ARTHUR E., DE JONGE L. W., MOLDRUP P., HAUGGAARD-NIELSEN H. AND ELSGAARD L. (2014c). Effect of biochar on aerobic processes, enzyme activity, and crop yields in two sandy loam soils. *Biology and Fertility of Soils*, **50**, 1087-1097.

TAGHIZADEH-TOOSI A., CLOUGH T. J., CONDRON L. M., SHERLOCK R. R., ANDERSON C. R. AND CRAIGIE R. A. (2011a). Biochar incorporation into pasture soil suppresses in situ nitrous oxide emissions from ruminant urine patches. *Journal of environmental quality*, **40**, 468-476.

TAGHIZADEH-TOOSI A., CLOUGH T. J., SHERLOCK R. R. AND CONDRON L. M. (2011b). Biochar adsorbed ammonia is bioavailable. *Plant and Soil*, 1-13.

TAGHIZADEH-TOOSI A., CLOUGH T. J., SHERLOCK R. R. AND CONDRON L. M. (2012). A wood based low-temperature biochar captures NH_3-N generated from ruminant urine-N, retaining its bioavailability. *Plant and Soil*, **353**, 73-84.

TAMMEORG P., SIMOJOKI A., MÄKELÄ P., STODDARD F. L., ALAKUKKU L. AND HELENIUS J. (2014). Biochar application to a fertile sandy clay loam in boreal conditions: effects on soil properties and yield formation of wheat, turnip rape and faba bean. *Plant and soil*, **374**, 89-107.

TERÖRDE H. (1997). *Untersuchungen zur Nähr-und Mineralstoffversorgung von Mutterkuhherden auf ausgesuchten Standorten in Mecklenburg-Vorpommern*, Freie Universität Berlin, Universitätsbibliothek.

THIES J. E. AND RILLIG M. C. (2009). Characteristics of biochar: biological properties. In: LEHMANN J. AND JOSEPH S. (eds.) *Biochar for Environmental Management: Science and Technology*, pp. 85-105. London, UK: Earthscan.

TITIRICI M.-M., THOMAS A. AND ANTONIETTI M. (2007). Back in the black: hydrothermal carbonization of plant material as an efficient chemical process to treat the CO_2 problem? *New Journal of Chemistry*, **31**, 787-789.

TITIRICI M.-M. (2013). Sustainable carbon materials from hydrothermal processes. Chichester: John Wiley & Sons, Ltd.

UCHIMIYA M. (2014). Influence of pH, ionic strength, and multidentate ligand on the interaction of Cd-II with biochars. *ACS Sustainable Chemistry and Engineering*, **2**, 2019-2027.

UMWELTBUNDESAMT (2011). Daten zur Umwelt *Umwelt und Landwirtschaft*, p. 100. Dessau-Roßlau: Umweltbundesamt.

VACCARI F. P., BARONTI S., LUGATO E., GENESIO L., CASTALDI S., FORNASIER F. AND MIGLIETTA F. (2011). Biochar as a strategy to sequester carbon and increase yield in durum wheat. *European Journal of Agronomy*, **34**, 231-238.

VAN DE VOORDE T. F. J., BEZEMER T. M., VAN GROENIGEN J. W., JEFFERY S. AND MOMMER L. (2014). Soil biochar amendment in a nature restoration area: effects on plant productivity and community composition. *Ecological Applications*, **24**, 1167-1177.

VAN WESENBEECK S., PRINS W., RONSSE F. AND ANTAL M. J., JR. (2014). Sewage sludge carbonization for biochar applications. Fate of heavy metals. *Energy and Fuels*, **28**, 5318-5326.

VAN ZWIETEN L., SINGH B., JOSEPH S., KIMBER S., COWIE A. AND CHAN K. Y. (2009). Biochar and emissions of non-CO_2 greenhouse gases from soil. *Biochar for Environmental Management: Science and Technology. Earthscan, London,* 227-249.

VAN ZWIETEN L., KIMBER S., DOWNIE A., MORRIS S., PETTY S., RUST J. AND CHAN K. (2010a). A glasshouse study on the interaction of low mineral ash biochar with nitrogen in a sandy soil. *Soil Research*, **48**, 569-576.

VAN ZWIETEN L., KIMBER S., MORRIS S., DOWNIE A., BERGER E., RUST J. AND SCHEER C. (2010b). Influence of biochars on flux of N_2O and CO_2 from Ferrosol. *Soil Research*, **48**, 555-568.

VAN ZWIETEN L., KAMMANN C., CAYUELA M. L., SINGH B., JOSEPH S., KIMBER S., S. D., CLOUGH T. J. AND SPOKAS K. A. (2015). Biochar effects on nitrous oxide and methane emissions from soil. In: LEHMANN J. AND JOSEPH S. (eds.) *Biochar: Science, Technology and Implementation*, pp. 487-518. London, UK: Earthscan.

VANCE E., BROOKES P. AND JENKINSON D. (1987). An extraction method for measuring soil microbial biomass C. *Soil Biology and Biochemistry*, **19**, 703-707.

VANDRÉ R. (1997). Ammoniakausgasung aus Gülle nach der Ausbringung. Bayreuth: Lehrstuhl für Bodenkunde und Bodengeographie der Univ. Bayreuth, 1997.

VANVEEN J. A., LADD J. N. AND FRISSEL M. J. (1984). Modeling C and N turnover through the microbial biomass in soil. *Plant and Soil*, **76**, 257-274.

VANVEEN J. A., LADD J. N. AND AMATO M. (1985). Turnover of carbon and nitrogen through the microbial biomass in a sandy loam and a clay soil incubated with [^{14}C(U)] glucose and [^{15}N]$(NH_4)_2SO_4$ under different moisture regimes. *Soil Biology and Biochemistry*, **17**, 747-756.

VELTHOF G., BRADER A. AND OENEMA O. (1996). Seasonal variations in nitrous oxide losses from managed grasslands in the Netherlands. *Plant and Soil*, **181**, 263-274.

VENTURA M., SORRENTI G., PANZACCHI P., GEORGE E. AND TONON G. (2013). Biochar reduces short-term nitrate leaching from a horizon in an apple orchard. *Journal of Environmental Quality*, **42**, 76-82.

VENTURA M., ALBERTI G., VIGER M., JENKINS J. R., GIRARDIN C., BARONTI S., ZALDEI A., TAYLOR G., RUMPEL C. AND MIGLIETTA F. (2014). Biochar mineralization and priming effect on SOM decomposition in two European short rotation coppices. *Global Change Biology Bioenergy*.

VERHOEVEN E. AND SIX J. (2014). Biochar does not mitigate field-scale N_2O emissions in a Northern California vineyard: An assessment across two years. *Agriculture Ecosystems and Environment*, **191**, 27-38.

WAGNER A. AND KAUPENJOHANN M. (2014). Suitability of biochars (pyro-and hydrochars) for metal immobilization on former sewage-field soils. *European Journal of Soil Science*, **65**, 139-148.

WALDROP M. P. AND FIRESTONE M. K. (2004). Microbial community utilization of recalcitrant and simple carbon compounds: impact of oak-woodland plant communities. *Oecologia*, **138**, 275-284.

WANG J., ZHANG M., XIONG Z., LIU P. AND PAN G. (2011). Effects of biochar addition on N_2O and CO_2 emissions from two paddy soils. *Biology and Fertility of Soils*, **47**, 887-896.

WANG J., PAN X., LIU Y., ZHANG X. AND XIONG Z. (2012). Effects of biochar amendment in two soils on greenhouse gas emissions and crop production. *Plant and Soil*, **360**, 287-298.

WARNOCK D. D., LEHMANN J., KUYPER T. W. AND RILLIG M. C. (2007). Mycorrhizal responses to biochar in soil–concepts and mechanisms. *Plant and soil*, **300**, 9-20.

WARNOCK D. D., MUMMEY D. L., MCBRIDE B., MAJOR J., LEHMANN J. AND RILLIG M. C. (2010). Influences of non-herbaceous biochar on arbuscular mycorrhizal fungal abundances in roots and soils: Results from growth-chamber and field experiments. *Applied Soil Ecology*, **46**, 450-456.

WATZINGER A., FEICHTMAIR S., KITZLER B., ZEHETNER F., KLOSS S., WIMMER B., ZECHMEISTER-BOLTENSTERN S. AND SOJA G. (2014). Soil microbial communities responded to biochar application in temperate soils and slowly metabolized C-13-labelled biochar as revealed by C-13 PLFA analyses: results from a short-term incubation and pot experiment. *European Journal of Soil Science*, **65**, 40-51.

WEST T. AND MARLAND G. (2002). Net carbon flux from agricultural ecosystems: methodology for full carbon cycle analyses. *Environmental Pollution*, **116**, 439-444.

WHITEHEAD D. C. (2000). Nutrient elements in grassland: soil-plant-animal relationships: Cabi.

WHITMAN T. AND LEHMANN J. (2009). Biochar-One way forward for soil carbon in offset mechanisms in Africa? *Environmental Science and Policy*, **12**, 1024-1027.

WHITMAN T., ENDERS A. AND LEHMANN J. (2014). Pyrogenic carbon additions to soil counteract positive priming of soil carbon mineralization by plants. *Soil Biology and Biochemistry*, **73**, 33-41.

WIEDNER K., NAISSE C., RUMPEL C., POZZI A., WIECZOREK P. AND GLASER B. (2013). Chemical modification of biomass residues during hydrothermal carbonization - What makes the difference, temperature or feedstock? *Organic Geochemistry*, **54**, 91-100.

WINSLEY P. (2007). Biochar and bioenergy production for climate change mitigation. *New Zealand Science Review*, **64**, 5-10.

WOLF B., ZHENG X., BRÜGGEMANN N., CHEN W., DANNENMANN M., HAN X., SUTTON M. A., WU H., YAO Z. AND BUTTERBACH-BAHL K. (2010). Grazing-induced reduction of natural nitrous oxide release from continental steppe. *Nature*, **464**, 881-884.

WOOLF D., AMONETTE J. E., STREET-PERROTT F. A., LEHMANN J. AND JOSEPH S. (2010). Sustainable biochar to mitigate global climate change. *Nature Communications*, **1**, 56.

WRB (2006). World reference base for soil resources. *World Soil Resources Reports*, **103**.

XIAO L., BI E., DU B., ZHAO X. AND XING C. (2014). Surface characterization of maize-straw-derived biochars and their sorption performance for MTBE and benzene. *Environmental Earth Sciences*, **71**, 5195-5205.

XIE Z., XU Y., LIU G., LIU Q., ZHU J., TU C., AMONETTE J. E., CADISCH G., YONG J. W. H. AND HU S. (2013). Impact of biochar application on nitrogen nutrition of rice, greenhouse-gas emissions and soil organic carbon dynamics in two paddy soils of China. *Plant and Soil*, **370**, 527-540.

XUE Y., GAO B., YAO Y., INYANG M., ZHANG M., ZIMMERMAN A. R. AND RO K. S. (2012). Hydrogen peroxide modification enhances the ability of biochar (hydrochar) produced from hydrothermal carbonization of peanut hull to remove aqueous heavy metals: Batch and column tests. *Chemical Engineering Journal*, **200**, 673-680.

YAN W., HOEKMAN S. K., BROCH A. AND CORONELLA C. J. (2014). Effect of Hydrothermal Carbonization Reaction Parameters on the Properties of Hydrochar and Pellets. *Environmental Progress and Sustainable Energy*, **33**, 676-680.

YANAI Y., TOYOTA K. AND OKAZAKI M. (2007). Effects of charcoal addition on N_2O emissions from soil resulting from rewetting air-dried soil in short-term laboratory experiments. *Soil Science and Plant Nutrition*, **53**, 181-188.

YAO F., ARBESTAIN M. C., VIRGEL S., BLANCO F., AROSTEGUI J., MACIÁ-AGULLÓ J. AND MACÍAS F. (2010). Simulated geochemical weathering of a mineral ash-rich biochar in a modified Soxhlet reactor. *Chemosphere*, **80**, 724-732.

YOO G. AND KANG H. (2012). Effects of Biochar Addition on Greenhouse Gas Emissions and Microbial Responses in a Short-Term Laboratory Experiment. *Journal of Environmental Quality*, **41**, 1193-1202.

ZAVALLONI C., ALBERTI G., BIASIOL S., VEDOVE G. D., FORNASIER F., LIU J. AND PERESSOTTI A. (2011). Microbial mineralization of biochar and wheat straw mixture in soil: A short-term study. *Applied Soil Ecology*, **50**, 45-51.

ZELLES L. (1999). Fatty acid patterns of phospholipids and lipopolysaccharides in the characterisation of microbial communities in soil: a review. *Biology and Fertility of Soils*, **29**, 111-129.

ZHANG A., CUI L., PAN G., LI L., HUSSAIN Q., ZHANG X., ZHENG J. AND CROWLEY D. (2010). Effect of biochar amendment on yield and methane and nitrous oxide emissions from a rice paddy from Tai Lake plain, China. *Agriculture, Ecosystems and Environment*, **139**, 469-475.

ZHANG A., LIU Y., PAN G., HUSSAIN Q., LI L., ZHENG J. AND ZHANG X. (2012). Effect of biochar amendment on maize yield and greenhouse gas emissions from a soil organic carbon poor calcareous loamy soil from Central China Plain. *Plant and soil*, **351**, 263-275.

ZHANG J.-H., LIN Q.-M. AND ZHAO X.-R. (2014a). The hydrochar characters of municipal sewage sludge under different hydrothermal temperatures and durations. *Journal of Integrative Agriculture*, **13**, 471-482.

ZHANG P., WEI T., JIA Z., HAN Q., REN X. AND LI Y. (2014b). Effects of straw incorporation on soil organic matter and soil water-stable aggregates content in semiarid regions of northwest China. *PLoS One*, **9**.

ZHANG Q.-Z., DIJKSTRA F. A., LIU X.-R., WANG Y.-D., HUANG J. AND LU N. (2014c). Effects of biochar on soil microbial biomass after four years of consecutive application in the north China plain. *PLoS One*, **9**.

ZHAO L., CAO X., MAŠEK O. AND ZIMMERMAN A. (2013a). Heterogeneity of biochar properties as a function of feedstock sources and production temperatures. *Journal of Hazardous Materials*, **256**, 1-9.

ZHAO X., YAN X., WANG S., XING G. AND ZHOU Y. (2013b). Effects of the addition of rice-straw-based biochar on leaching and retention of fertilizer N in highly fertilized cropland soils. *Soil Science and Plant Nutrition*, **59**, 771-782.

ZHENG J., STEWART C. E. AND COTRUFO M. F. (2012). Biochar and nitrogen fertilizer alters soil nitrogen dynamics and greenhouse gas fluxes from two temperate soils. *Journal of Environmental Quality*, **41**, 1361-1370.

ZIMMERMAN A. R. (2010). Abiotic and microbial oxidation of laboratory-produced black carbon (biochar). *Environmental Science and Technology*, **44**, 1295-1301.

ZIMMERMAN A. R., GAO B. AND AHN M.-Y. (2011). Positive and negative carbon mineralization priming effects among a variety of biochar-amended soils. *Soil Biology and Biochemistry*, **43**, 1169-1179.

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Erklärung

Ich erkläre hiermit, dass ich die vorgelegte Dissertation selbstständig und ohne unerlaubte fremde Hilfe und nur mit den Hilfen angefertigt habe, die ich in der Dissertation angegeben habe.
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Sonja Schimmelpfennig

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