Biochar additions to soils: effects on soil microorganisms and carbon stability

ir. Nele Ameloot

Promotors: Prof. dr. ir. Stefaan De Neve
Prof. dr. ir. Steven Sleutel
Department of Soil Management
Ghent University, Faculty of Bioscience Engineering, Ghent, Belgium.

Dean: Prof. dr. ir. Guido Van Huylenbroeck

Rector: Prof. dr. Anne De Paepe

Thesis submitted in fulfillment of the requirements for
the degree of Doctor (PhD) in Applied Biological Sciences: Land and Forest Management

Dutch translation of the title:

Biochar toedieningen aan de bodem: effecten op micro-organismen en koolstofstabiliteit

Cover illustration:

Airdried soil sampled from biochar field experiments (Bram Vervisch)

Citation

Ameloot, N. (2013). Biochar additions to soil: effects on soil microorganisms and carbon stability. Ph.D. thesis, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium, pp 179.

ISBN: 978-90-5989-664-2

Dankwoord

Stefaan en Steven, zonder jullie sterke ideeën en talloze verbeteringen zou deze thesis nooit geworden zijn wat ze nu is. Bedankt voor jullie steun en vertrouwen.

De leden van de leescommissie dr. Greet Ruysschaert, Prof. dr. Pascal Boeckx en dr. Costanza Zavalloni, bedankt voor jullie grondige lezing van en bijzonder constructieve bijdrage aan deze thesis! Ook de secretaris Prof. dr. Frederik Ronsse en voorzitster Prof. dr. Lieve Gheysen, bedankt dat jullie deze taken waargenomen hebben.

Mijn thesisstudenten Bram en Yvonne, jullie hebben beiden een mooie thesis gemaakt en ik ben dan ook zeer dankbaar dat ik jullie werk heb mogen opnemen in verschillende hoofdstukken van dit doctoraat.

In de onderzoeksgroep heb ik me steeds thuis gevoeld, dit is mede te danken aan de interessante onderzoeksthema's en de aanwezige expertise, maar vooral aan de fijne persoonlijke contacten. Ik denk daarbij aan de (ex-)collega's Mesfin, Jeroen, Peter, Yeevan, Laura, Liesbeth, David, Bram, Shamim, Kader, Azita en Sara. Ook Tina, Maarten, Sophie, Trees, Anne-Mie en Mathieu wil ik graag bedanken voor de vele analyses en de leuke gesprekken. Luc, merci voor je jeugdige enthousiasme en voor je uitgebreide kennis over zakken grond in de kelder. Alle collega's van de vakgroep 'bodembeheer', bedankt voor de aangename lunch- en koffiepauzemomenten, waar politieke en maatschappelijke thema's vlotjes overgingen in de laatste nieuwtjes uit de wondere wereld der baby's en BV's. Wat deze laatste twee werelden betreft, bleek het gedragstechnisch soms moeilijk te zijn om beide te onderscheiden.

Verder wil ik ook een aantal mensen van buiten onze onderzoeksgroep, met wie het fijn samenwerken was, danken voor hun waardevolle bijdrage aan mijn thesis. Ik denk dan in het bijzonder aan dr. Ellen Graber en dr. Frank Verheijen voor de nuttige aanvullingen van Hoofdstuk 1 en de vruchtbare samenwerking voor de organisatie van de biochar-sessie op het

Eurosoil-congres in Bari; aan Prof. dr. K.C. Das voor het genereus ter beschikking stellen van de biochar voor Hoofdstuk 2 en 5; aan Prof. dr. Frederik Ronsse, Dane Dickinson en Güray Yildiz van de vakgroep Bio-systeem techniek voor hun hulp tijdens het produceren van verschillende types biochar; aan Prof. dr. Peter Leinweber en zijn onderzoeksgroep voor de py-FIMS analyses van de biochars; aan Victoria Nelissen en dr. Greet Ruysschaert voor het gebruik van een aantal biochars uit Hoofdstuk 3; en aan de onderzoekers in Udine en Lancaster voor het bemonsteren op hun velden en de wetenschappelijke input. Ook Kristof De Beuf van de FIRE consulting dienst van de UGent wil ik graag bedanken voor zijn statistisch advies.

Familie en schoonfamilie, ik heb toch maar mooi het geluk gehad om jullie rond mij te mogen hebben. *'k Zien mè mien gat in de Diksmudse (en Kortriekse) butter gevol'n*. Bedankt!

Vrienden, meerdere keren trok ik reeds de conclusie: 'Jullie vriendschap is geen illusie'! Weet dat doorheen de wat melige klank van mijn woorden een oprechte drang bestaat om te zeggen dat ik jullie graag heb, bedankt!

Moeke, Pa en Tine, merci om er al een leven lang voor me te zijn. Jullie vormen een onvoorwaardelijke bron van liefde en ondersteuning. Ik kan jullie daarvoor nooit genoeg erkentelijk zijn.

Steven, Stella en het kleintje dat op komst is, eerst waren we een stelletje en nu zijn we een heus gezinnetje. De vele intense momenten met jullie maken me tot wie ik ben.

Table of contents

List of abbreviations

AEC	Anion Exchange Capacity
AMF	Arbuscular Mycorrhiza Fungi
βB	β-glucosidase activity
B:F ratio	Bacteria:Fungi ratio
C, H, N, O, P, S	Carbon, Hydrogen, Nitrogen, Oxygen, Phosphorus, Sulfur
CDA	Canonical Discriminant Analysis
C_0	Easily-mineralizable C (Equation 4-1)
CEC	Cation Exchange Capacity
CH_4	Methane
CO_2	Carbon dioxide
DA	Dehydrogenase Activity
DN	Dissolved Nitrogen
DOC	Dissolved Organic Carbon
GHG	Greenhouse Gas
ICP-MS	Inductively Coupled Plasma – Mass Spectrometry
k_f	Mineralization rate of the fast degradable C pool (Equation 4-1)
k_s	Mineralization rate of the slow C pool (Equation 4-1)
LTCB	Laboratory of thermo-chemical conversion of biomass
MBC	Microbial Biomass Carbon
MB	Microbial Biomass
N_2O	Nitrous oxide
NH_4^+	Ammonium
NO_3^-	Nitrate

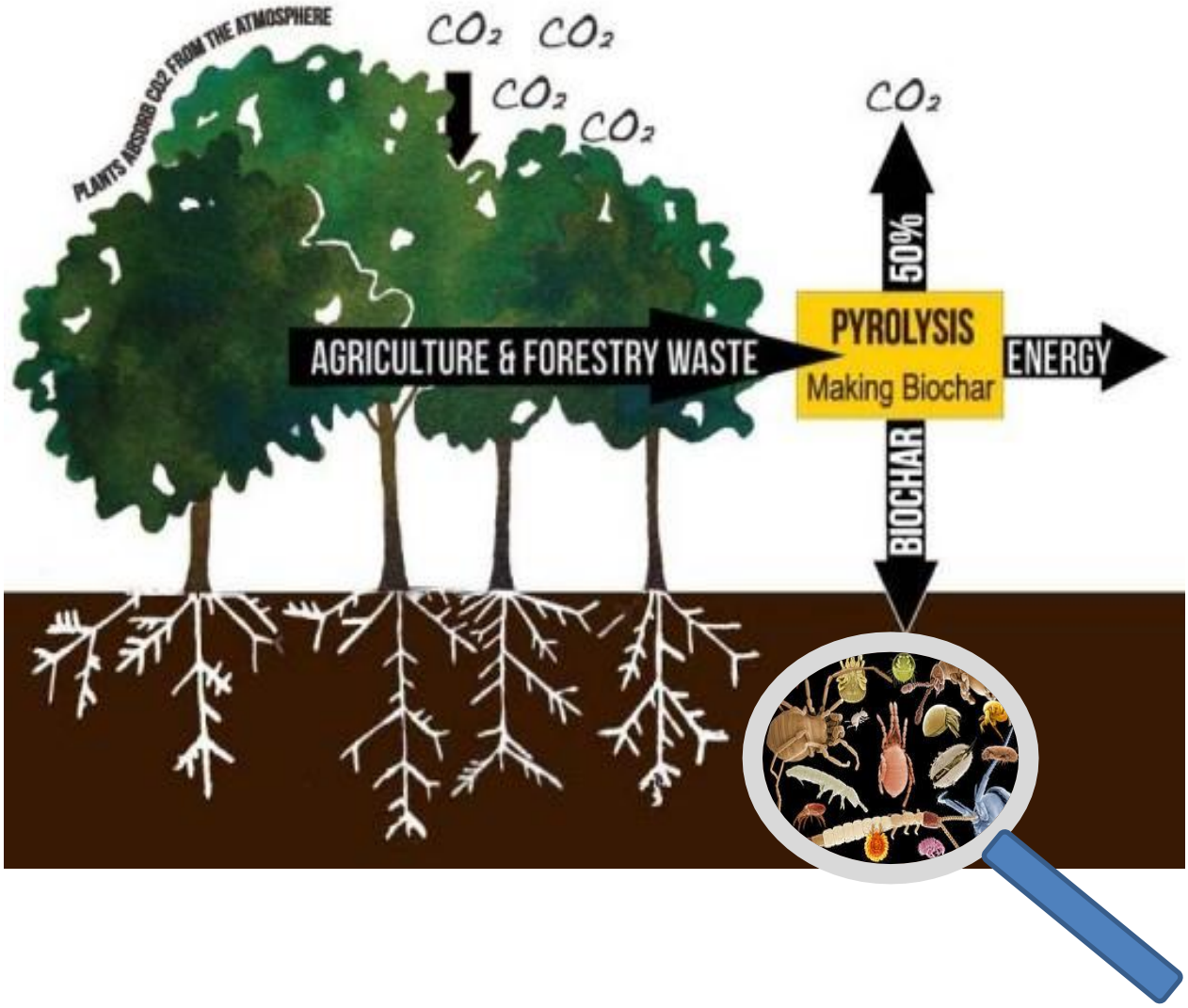
OM	Organic Matter
PAH	Polyaromatic hydrocarbon
PC	Principal Component
PCA	Principal Component Analysis
PLFA	Phospholipid Fatty Acid
POM	Particulate Organic Matter
Py-FIMS	Pyrolysis-Field Ionization Mass Spectroscopy
SA	Surface Area
SOC	Soil Organic Carbon
SOM	Soil Organic Matter
TII	Total Ion Intensity
TOC	Total Organic Carbon
TPF	Triphenylformazan
TPV	Total Pore Volume
TTC	Tetrazolium chloride
SCDFC	Standardized Canonical Discriminant Function Coefficients
Syngas	Synthetic Gas
VM	Volatile Matter
WFPS	Water-Filled Pore Space
WHC	Water Holding Capacity
WL	Weight Loss
WRB	World Reference Base

List of tables

List of figures

Chapter 1:

Biochar stability and soil microorganisms



Compiled from:

Ameloot, N., Graber, E., Verheijen, F., De Neve, S., 2013. Interactions between biochar stability and soil organisms: Review and research needs. European Journal of Soil Science, 64, 379-390.

1.1 Context and problem statement

Increasing levels of greenhouse gases (GHGs), such as carbon dioxide (CO_2), nitrous oxide (N_2O) and methane (CH_4) are confronting the world with one of its most important ecological challenges in history; being climate change (IPCC, 2007). Burning of fossil and biomass fuels as well as the decomposition of organic matter and deforestation are contributing to these increasing GHG emissions. Due to climate change the world is already facing increasing temperatures, extreme weather events, and desertification. In order to combat these problems we can for example build dam constructions to protect ourselves against increasing sea levels. However, acting pre-emptive by reducing greenhouse gas emissions, seems to hold more potential.

GHG emissions, and more specifically CO_2 emissions, can be reduced by planting trees and sequestering carbon (C) into the living biomass. Additionally, well thought management of grasslands, croplands, peatlands and forests and the reduction of the global livestock are other climate change mitigating solutions. The soil as well can be an important C sink; more than 80% of the terrestrial organic C is found in the soil. However, (parts of) the soil organic carbon (SOC) can mineralize at the time scale of several decades, whereby CO_2 is again released in the atmosphere. It is thus clear that maintaining the soil C sink is essential in combating climate change.

The production and insertion into the soil of a stable biomass-derived C product has been proposed as one possible solution for reducing net GHG emissions from agroecosystems. By the carbonization of biomass during a pyrogenic process, a recalcitrant carbon-rich by-product, biochar is formed. Because of its chemically recalcitrant nature, biochar incorporation promises sequestering of organic carbon (OC) into soils. This is supported by the discovery in the 19th century by Charles Hartt of anthropogenic *Terra Preta* soils in the Amazonian Region. Application of large amounts of charcoal, manure and animal bones by pre-Colombian farmers to weathered Amazonian soils was the basis for the creation of these *Terra Preta* soils (Glaser et al., 2002; Glaser, 2007; Lehmann, 2007). A higher microbial diversity in comparison to adjacent soils has been found (Kim et al., 2007; O'Neill et al., 2009) next to greater crop productivity, nutrient contents, and higher contents of organic carbon and charcoal compared to adjacent non-amended soils. The total C storage in these soils may be as high as 250 t C ha^{-1} m^{-1} compared to typical values of 100 t C ha^{-1} m^{-1} in

adjacent soils derived from similar parent material (Glaser et al., 2002). This discovery has inspired scientists to create '*Terra Preta nova'* soils by the addition of biochar, i.e. charred biomass produced by pyrolysis, with the intent to improve soil functions whilst mitigating climate change through sequestration of OC (Figure 1-1). Ample research has shown that biochar application can moreover increase soil productivity, by improving both chemical and physical soil properties (Glaser et al., 2002; Lehmann, 2007; Jeffery et al., 2011).

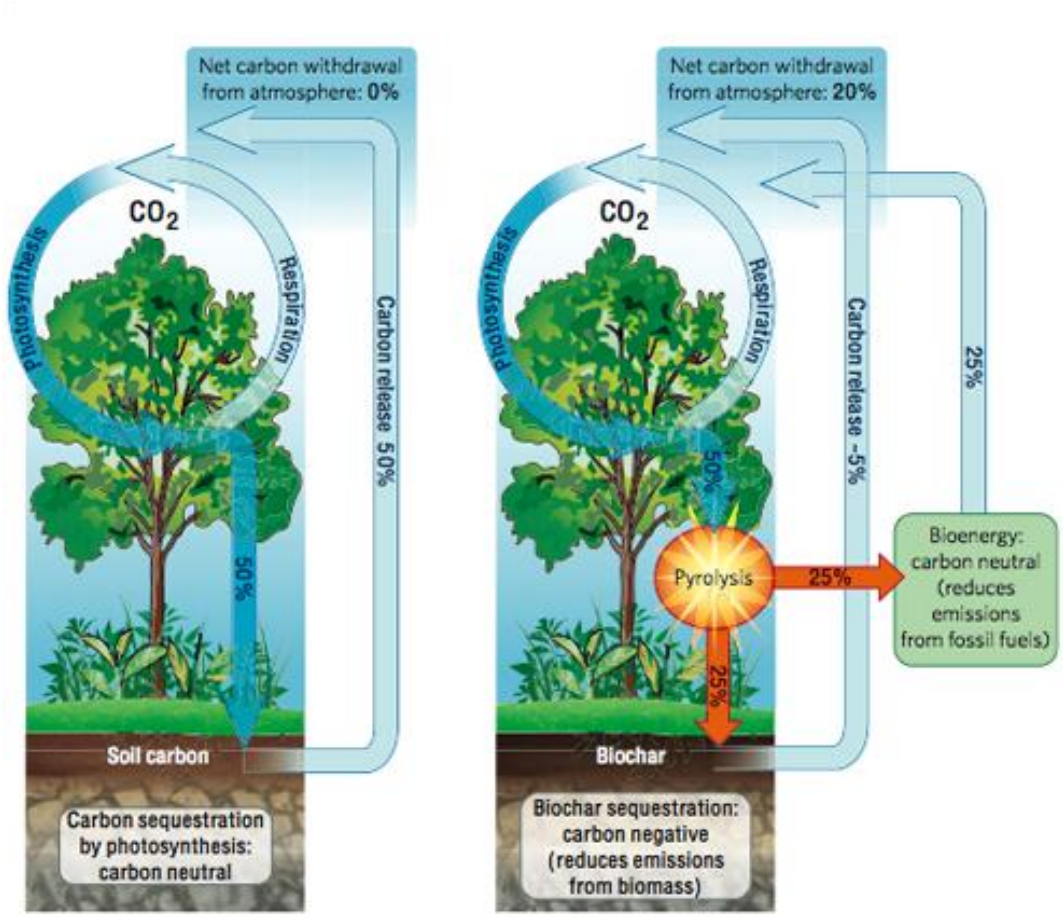


Figure 1-1 Biochar provides a net-sink of CO_2 from the atmosphere (Lehmann, 2007)

The activities of soil biota are critical to the wellbeing of human societies because their activities underpin the delivery of major ecosystem services, such as decomposition of organic matter and consequential nutrient cycling, N fixation, improvement of soil structure and water filtration, soil restoration after pollution, etc. All these key ecosystem functions would not exist, if there would be no living soil biota (bacteria, fungi, microfauna like protozoa and nematodes, mesofauna like microarthropods and enchitraeids, and macrofauna

like earthworms and millipeds). This would have tremendous impacts on food production and the functioning of natural ecosystems. Some processes like decomposition and nutrient cycling can be provided by a broad range of soil organisms, while other more specific ecosystem services, like N fixation, plant associations with mycorrhiza and improvements of soil structure and aeration through earthworm activity, are only provided by a narrow range of soil organisms. Therefore maintaining a high level of soil biodiversity is the key to provide all these important ecosystem functions.

When biochar is applied to soils, it is likely that soil organisms are influenced. Biochar, with its porous nature and reactive surface, will change the microbial habitat, in this way microorganisms will be indirectly influenced by the presence of biochar in the soil. Additionally, some biochar compounds may be used as a food source by soil organisms. This interaction between biochar and soil organisms will therefore be of importance for biochar stability against biological degradation and consequently for its C sequestration potential. Moreover, some studies showed that biochar addition to soils may influence native soil organic matter (SOM) mineralization (Zimmerman, 2010). There is thus an immerging need to quantify the stability of biochar in the soil and the role of soil organisms.

1.2 Biochar production

In the scientific literature, the terms biochar, black carbon, charcoal and char are often used interchangeably without clear distinction. Black carbon often represents a continuum of naturally carbonized products, ranging from slightly charred biomass to highly condensed refractory soot, while charcoal, char or biochar mostly refers to human-made carbonized products. For this thesis, a wide scope of research concerning charcoal/biochar and black carbon additions to soils and their biological effects were considered.

Charcoal can be present in soils as a result of natural or human-induced fires (Schmidt et al., 1999; Skjemstad et al., 2002). In recent years, special attention has been paid to biochar, i.e. charred biomass that is produced from a wide range of feedstocks (biomass) and through a wide range of pyrolysis conditions, with the intent to improve soil functions while sequestering carbon. Pyrolysis is the combustion of biomass in the absence of oxygen (O_2) or in O_2-limited conditions. Biochar can be produced by simple combustion techniques, as is often the case in developing countries, however, such techniques are not energy efficient, are

often very polluting and result in low temperature slow pyrolysis chars with relatively heterogeneous properties.

Biochar production can by optimized by working under controlled conditions as found in industrial pyrolysis processes. During this carbonization process large amounts of hydrogen, oxygen and volatile compounds of the original biomass evaporate into a synthetic gas (syngas), which can be condensed into a combustible bio-oil. Simultaneously, a carbon-rich by-product biochar is produced.

Slow pyrolysis is the thermal carbonization of biomass at slow heating rates at low to medium temperatures (450 to 650°C). During this process biochar, bio-oil and syngas are produced in proportions which are strongly dependent of the original feedstock and the pyrolysis temperature. High temperatures result in lower biochar yields, in favor of bio-oil and syngas yields (Demirbas, 2001). Slow pyrolysis including steam gasification, liberates additional proportions of syngas. The resulting biochar is relatively stable, which makes it attractive as a product able to sequester C into soils.

Fast pyrolysis (Figure 1-2) is characterized by rapid biomass heating at moderate temperatures (350 – 500 °C) and leads to a greater proportion of bio-oil and less biochar (Sohi et al., 2009). Gasification of biomass is performed under higher temperatures (800 °C) during longer vapor residence times and some oxygen is allowed in the atmosphere. During this process 85% of the biomass is converted to syngas and only 10% to biochar (Sohi et al., 2010). Depending on the pyrolysis temperature, duration and the source material, different types of biochar are produced of which the quality can differ enormously (Gundale and DeLuca, 2006).

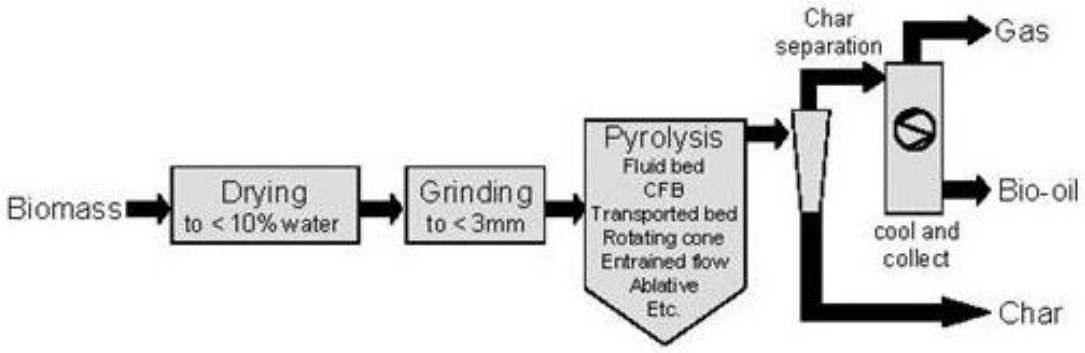


Figure 1-2 Fast pyrolysis of biochar (Sohi et al., 2009)

1.3 Biochar properties

1.3.1 Physical biochar properties

Biomass material or feedstock and the pyrolysis conditions (including treatments of the biomass and the biochar before and after pyrolysis, respectively) determine the physical properties of the biochar. The pore volume and the specific internal surface of the biochar are the most important physical parameters, influencing soil processes, including biological soil parameters. The pyrolysis conditions affect the formation of cracks in the biochar, volatile compounds, and arrangement of the C crystals. The most important parameters are the pyrolysis temperature, the pressure, the residence time, the dimensions of the pyrolysis oven, the pre-treatments (drying and chemical activation) and the type of inert gas, used to realize the anaerobic conditions (N_2, He) (Downie et al., 2009).

The molecular structure determines directly the porosity and specific internal surface of the biochar. The structure of biochar can be considered as amorfe, however crystalline areas are found (Qadeer et al., 1994). These crystalline particles are aromatic plates, which are linked to each other at random places. The non-crystalline biochar matrix consists of aromatic-alifatic organic compounds and inorganic compounds (mineral ash) (Emmerich et al., 1987). Into the biochar matrix cracks and pores, originating from the biomass are found (Brown et al., 2006; Downie et al., 2009). Pyrolysis results in alterations of the biomass macro- and microstructure, with progressive homogenization of wood cell walls and the disappearance of the middle lamella. The increase in porosity of biochar may be attributed to water molecules released by dehydroxylation (Chan et al., 2008), rendering the biochar structure porous and increasing the internal surface area (Downie et al., 2009; Ascough et al., 2010; Van Zwieten et al., 2010). Specific surface area varies from a few $m^2\ g^{-1}$ to more than 400 $m^2\ g^{-1}$ biochar, depending on biomass and pyrolysis conditions (Glaser et al., 2002; Hilscher et al., 2009).

The internal surface area is typically measured by the BET-N_2 sorption technique. There is a strong relationship between the pyrolysis temperature and the BET surface area (Figure 1-3). BET surface area increases till an optimal temperature of around 700°C is reached, whereafter it drops. The surface area of the biochar determines the adsorption capacity of the biochar, and impacts the retention of water, nutrients and organic compounds by biochar, when added to the soil (Brown et al., 2006).

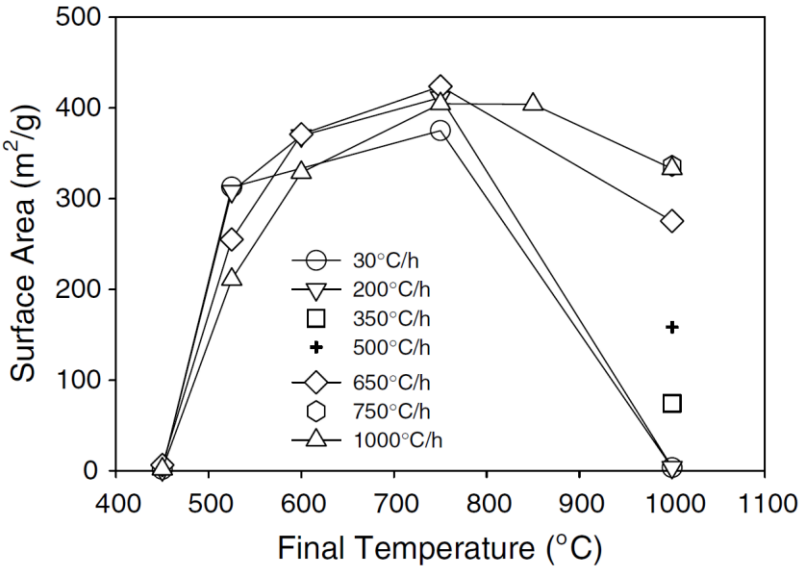


Figure 1-3 Relationship between pyrolysis temperature and BET surface area (Brown et al., 2006)

The biochar porosity can be divided into three pore classes: a) micropores (<2 nm), b) mesopores (between 2 and 50 nm) and c) macropores (>50 nm) (Downie et al., 2009). Macropores are relevant for the aeration, and the water holding capacity of the soil amended with biochar, while they also provide a habitat for soil microorganisms. When added to the soils the higher biochar porosity compared to the soil porosity, may induce a reduction of soil density.

1.3.2 Chemical biochar properties

pH and cation exchange capacity (CEC) of biochar are inseparably linked to each other. The CEC of biochar is defined as the amount of exchangeable cations linked to its surface. The higher the pH of biochar, the higher its CEC (Glaser et al., 2002). CEC and pH are to a large extent influenced by the biochar feedstock. K, Na, Mg, and P in the biomass may form O-groups at the surface of the biochar, which increases the CEC (Gaskin et al., 2008). With increasing pyrolysis temperature, the CEC and the pH increases, with a temperature optimum of 450-550 °C (Lehmann, 2007). Due to hydrolysis of Ca, K and Mg salts in the feedstock the biochar's pH increases and this increase is closely related to the ash content of biochar. Since

the majority of mineral compounds will remain in the biochar during pyrolysis, the ash content is highly dependent from the type of feedstock, used for biochar production. High pH in the biochar can be due to the presence of ash, basic functional groups, carbonates or oxides (e.g. CaO) or through a negative charge on its surface. Due to abiotic and biotic oxidation of biochar in the soil the pH of the biochar may decrease (Cheng et al., 2006).

A second important chemical characteristic of biochar is the O:C (oxygen to carbon) ratio (Spokas, 2010). The higher this ratio, the higher the amount of hydroxyl, carboxyl and carbonyl compounds in the biochar. During pyrolysis a great amount of the H (hydrogen) and O (oxygen) is lost as bio-oil or bio-gas, while the C content is accumulating into the biochar and increases with increasing pyrolysis temperatures. Cellulose, lignin and to a lesser extend hemicellulose compounds in the biomass decompose during pyrolysis and volatile compounds are formed by the breakdown or rearrangement of original biomass compounds (Spokas et al., 2011) and are then released (devolatilization). According to Ronsse et al. (2013) slow pyrolysis subjects the original feedstock to a series of devolatilization reactions, while progressively leaving behind an increasingly condensed biochar matrix. In this way, lower amounts of volatile compounds are retained in the biochar matrix at higher pyrolysis temperatures. Additionally, some initially devolatilized compounds may recondense in the aromatic biochar matrix (Imam and Capareda, 2012; Kloss et al., 2012). According to Graber et al. (2010), volatile compounds consist of a wide array of relatively small molecules, including *n*-alkanoic acids, hydroxy and acetoxy acids, benzoic acids, diols, triols, and phenols.

1.4 Effect of biochar on soil organisms

1.4.1 Biochar as a food source for soil organisms

During the carbonization of biomass, a shift occurs in the chemical composition towards molecules that are more resistant to microbial decomposition such as highly condensed aromatic structures. Despite the recalcitrant nature of biochar, several studies have reported increased soil respiration rates when biochar was added to soils (Pietikainen et al., 2000; Baldock and Smernik, 2002; Bruun et al., 2008; Das et al., 2008; Zimmerman, 2010; Qayyum et al., 2011; Zavalloni et al., 2011). Lower oak biochar mineralization rates

(approximately 10 mg C g^{-1} char, estimated from Figure 1 of Zimmerman (2010)) were reported in sterilized incubation than when inoculated with microorganisms (mineralization rates of approximately 20 mg C g^{-1} char), emphasizing the importance of soil microorganisms for biochar degradation (Zimmerman, 2010). In many cases, C mineralization after biochar addition shows an initial flush, after which CO_2 evolution continues at much lower rates, similar to the biphasic mineralization rates observed after addition of non-pyrolyzed organic materials to soils. Das et al. (2008) observed this phenomenon in soils amended by biochar made from poultry litter, and attributed it to the presence of labile compounds in the poultry litter biochar. Biphasic biochar mineralization patterns suggest that apart from the initial flush attributed to the establishment of soil incubations and wetting of soils (Clein and Schimel, 1994; Fierer and Schimel, 2003), labile or volatile components of biochar are rapidly degraded, followed by slow to negligible degradation of the condensed aromatic ring structures (Smith et al., 2010; Cross and Sohi, 2011). After mineralization of the labile biochar-C pool in the short-term, mineralization rates in biochar-amended soils drop dramatically and are nearly equal to rates in treatments without biochar. The time lag is highly dependent on the biochar type, biochar application rate, and soil characteristics. The initial stage of fast mineralization has been reported to last between 6 (Smith et al., 2010) to 60 days (Kuzyakov et al., 2009; Steinbeiss et al., 2009), during which 2 to 20% of the biochar-C can be mineralized. For example, after an initial fast biochar decomposition rate of 2% biochar-C (assessed by ^{14}C isotopic labelling of rye grass biochars) during two months, the respiration rate slowed 10-fold (Kuzyakov et al., 2009). Increased enzyme activities of β-glucosidase and β-N-acetylglucosaminidase, indicative of microbial activity, were observed for a period of 7 days following the addition of 2% (w w^{-1}) fast pyrolysis biochar having a 40% labile organic fraction (Bailey et al., 2011).

The direct incorporation of OC (organic carbon) from biochar into the microbial biomass is often measured by the fumigation-extraction procedure in combination with biochar-C labelling (Vance et al., 1987; Bruun et al., 2008; Kuzyakov et al., 2009). Incorporation rates of ryegrass biochar into microbial biomass after 624 days amounted to 1.5 and 2.6% of biochar-C (Kuzyakov et al., 2009). This shows the low availability of biochar as a food source for microorganisms after a long incubation time. Yet, several fungal saprophytes are able to use biochar as a food source, depending on the production of a range of extra-cellular enzymes that break down various biochar components. This ability is attributed to several Basidiomycetes, such as the white-rot fungi *Nematoloma frowardii* and

Clitocybula duseni (Hofrichter et al., 1999), and several Ascomycetes, like *Tricoderma sp.* and *Penicillium sp.* (Laborda et al., 1999). The production of enzymes such as manganese peroxidase, fenoloxidase, laccases and lignine peroxidases and reactive phenoxy and peroxyradicals are essential for the degradation of aromatic structures in biochar (Hofrichter et al., 1999; Hockaday, 2006; Atkinson et al., 2010). Several wood-decaying fungi even have the capacity to use biochars (Baldock and Smernik, 2002; Hamer et al., 2004; Wengel et al., 2006) and coal (Hofrichter et al., 1997) as their sole C source. Also, strains of *mycobacterium* sp. and the fungus *Beauveria sulfurescens* were found to be able to hydroxylate aromatic biochar compounds (Novak et al., 2010). It is clear that biochar is not biologically inert and that some degree of biochar microbial decomposition is likely to occur. However, biochar might be stabilized by the secretion of mycorrhizal mycelium and polysaccharides, which has been found to be involved in the formation of stabile organic matter (OM) aggregates and in the physical protection of SOM, but this hypothesis still needs confirmation in the case of biochar (Rillig and Mummey, 2006).

As an important commentary, it should be noted that apparent biochar mineralization rates are not only biotically mediated, but may be influenced by some abiotic processes, including dissolution of carbonates and chemisorption. Carbonates formed during pyrolysis may be abiotically dissolved when the biochar is added to the soil (Bruun et al., 2008; Spokas et al., 2009; Jones et al., 2011). Bruun et al. (2008) reported higher initial biochar-C respiration rates in soil with biochars with a higher carbonate content compared to the rates from soil with biochars with a low carbonate content. Moreover, soil carbonates themselves may dissolve as a result of biochar-borne organic acids, resulting in an over-estimation of respired C (Tamir et al., 2011). Apparent respiration rates in biochar-amended soils may also be either reduced or increased by abiotic chemisorption processes. On the one hand, CO_2 can be chemisorbed at biochar surfaces, reducing apparent respiration rates (Thies and Rillig, 2009). Alternatively, CO_2 may be released abiotically from biochar during chemisorption of oxygen (O_2) (Puri et al., 1958; Hshieh and Richards, 1989; Cheng et al., 2006; Zimmerman, 2010). These abiotic oxidation mechanisms impede the interpretation of biochar mineralization rates.

Clearly, more research about the biological degradability of biochars is needed, particularly considering the increasing variety of feedstocks and pyrolysis conditions for biochar production, in combination with the full variety of soil conditions.

1.4.2 Indirect effect of biochar on soil organisms

Soil microorganisms are indirectly influenced by the addition of biochar in various ways. The porous nature of biochar may stimulate soil microorganisms by providing favorable microsites for microorganisms, as well as shelter against predatory soil fauna and desiccation (Warnock et al., 2007). Additionally, biochar provides a suitable porous habitat for the propagation of microorganisms into the soil solution (Pietikainen et al., 2000).

Also, the adsorption of toxic organic compounds and labile native SOM compounds at biochar surfaces may either promote or reduce (respectively) the suitability of biochar as a favourable habitat (Wardle et al., 1998; Pietikainen et al., 2000; Thies and Rillig, 2009). Absorption of toxic compounds, such as pesticides, on the biochar surface has been reported (Loganathan et al., 2009; Qiu et al., 2009; Graber et al., 2011a; Graber et al., 2011b) and may reduce the availability of these detrimental substances, which may cause a promotion of soil organisms. Labile native SOM compounds may be stabilized in the presence of biochar, due to adsorption on the biochar surface (Cross and Sohi, 2011; LeCroy et al., 2013) or due to physical trapping in biochar micropores, that are too small for soil organisms (Major et al., 2010). These stabilized compounds are not available anymore as a substrate for soil organisms, which may affect soil microorganisms negatively. For some compounds increased absorption may be linked to the cation exchange capacity (CEC) of the biochar surface (Silber et al., 2010). Sometimes biochar additions can increase the CEC of soils substantially, from 10% to more than 100% of the original CEC of the soil (without biochar), depending on the soil type, biochar type and experimental circumstances (Glaser et al., 2002; Chan et al., 2008; Steiner et al., 2008; Van Zwieten et al., 2010). It has been suggested that biochar CEC increases over time in soil due to oxidation of edges of the aromatic backbone of charcoal, yielding carboxylic groups (Glaser et al., 2002). Adsorption of anions, such as nitrate (NO_3^-) may also occur at biochars with high anion exchange capacity (AEC).

The addition of biochar may induce an increase in soil pH, through the negative charge on the surface that buffers acidity in soils and the presence of mineral ashes in the biochar, which has a positive effect on soil microbial activity in acidic soils (Pietikainen et al., 2000; Birk et al., 2009; Van Zwieten et al., 2010). Several studies confirm that biochar improves the physical and chemical soil quality, including increased organic C content, Ca content, pH and consequent lower content of exchangeable Al, which make it a suitable habitat for microorganisms (Yamato et al., 2006; Chan et al., 2008; Van Zwieten et al., 2010).

One factor that should not be overlooked is the potential of biochar additions to increase soil water holding capacity (WHC) (Glaser et al., 2002; Brokhoff, 2010; Verheijen et al., 2010; Jeffery et al., 2011; Karhu et al., 2011), which may increase the suitability of biochar-amended soils as a microbial habitat, although biochar pores can become anoxic when saturated with water. In Figure 1-4 a schematic overview of possible indirect effects, influencing soil

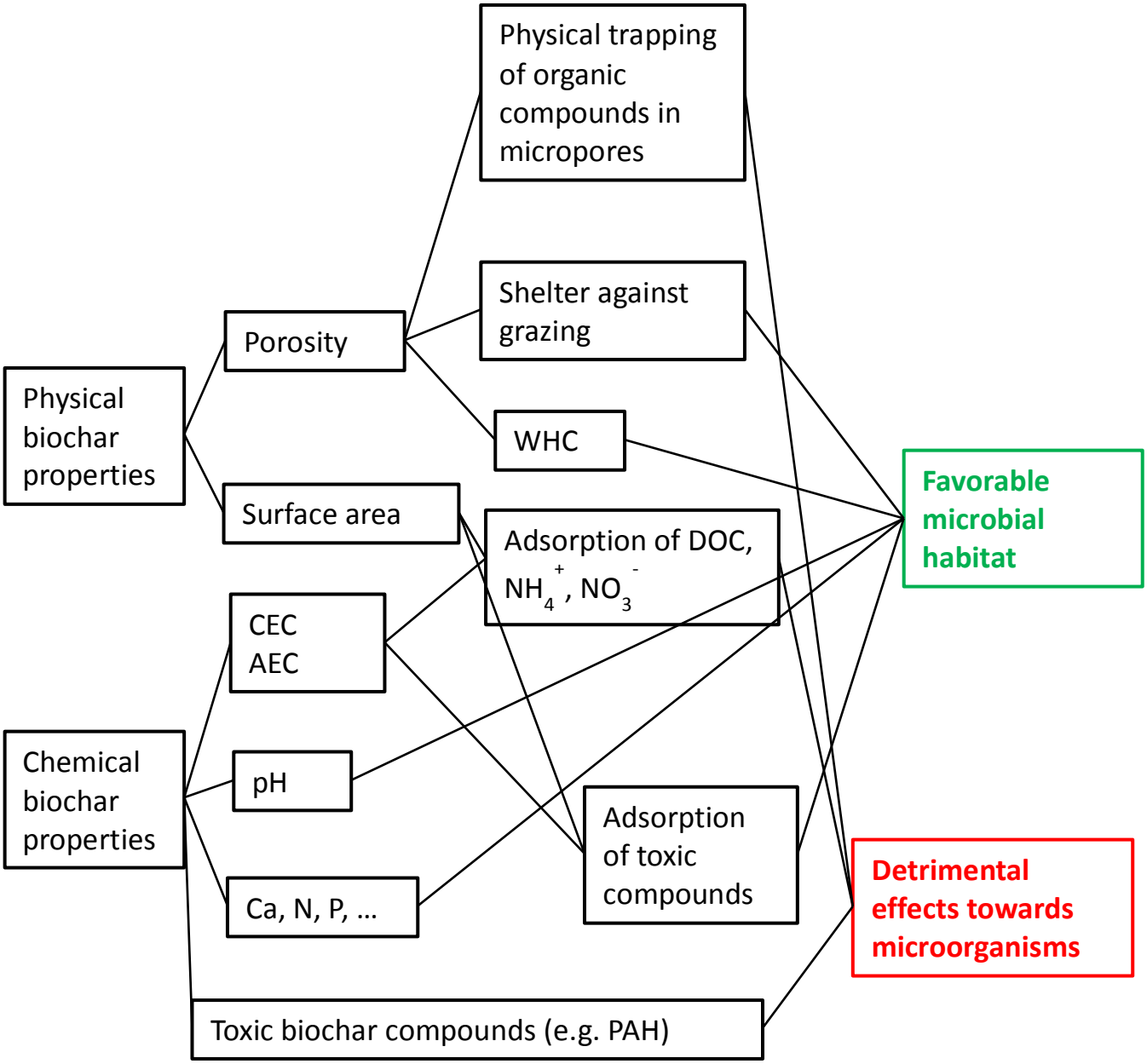


Figure 1-4 Indirect effects of biochar on soil biological properties

1.5 Factors influencing CO_2 release from biochar degradation

To investigate the importance of various factors on biochar-C mineralization (including biochar production methods, soil properties, and incubation parameters), we made a statistical

analysis of 8 studies assessing biochar-C mineralization following ^{14}C or ^{13}C isotopic labelling of the biochar. Only studies which provided enough data on C mineralization from biochar-C after the addition of biochar to soil, biochar properties and incubation parameters were considered (Bruun et al., 2008; Hilscher et al., 2009; Kuzyakov et al., 2009; Major et al., 2010; Keith et al., 2011; Luo et al., 2011; Zimmerman et al., 2011; Santos et al., 2012). Not all the variables included were scale variables and we intended to include not only linear, but all monotonic relationships between variables; therefore we used a Spearman rank correlation (IBM SPSS statistics 19.0, SPSS inc., Chicago, USA). We carried out both the Pearson correlation and the Spearmans' rank correlation: in most significant cases the Spearmans' rho values were greater than the Pearsons' correlation coefficients, indicating that the variables were monotonically related to each other, rather than being linearly correlated. The daily rate of mineralized biochar-OC was calculated as the amount of mineralized biochar-OC divided by the duration in days of each study and was corrected for incubation temperature, according to De Neve et al. (1996). Non-parametrical Spearman rank correlation analysis was performed between the percentage of daily mineralized biochar-OC corrected and pyrolysis temperature, pyrolysis residence time, native SOC content, biochar-C content, soil-biochar incubation time and biochar application rate. Another variable intended to be included into this meta-analysis was the biochar C:N ratio, an important parameter influencing microbial decomposition processes in the soil (Cleveland and Liptzin, 2007). However, not all studies reported on this parameter, so the data could not be included in the analysis. A significant positive correlation between pyrolysis temperature and the C content of the biochar was found (Table 1-1), in line with other reports (Baldock and Smernik, 2002; Spokas, 2010; Zimmerman, 2010). Significant negative correlations were found between the biochar-C mineralization rate, the pyrolysis temperature and the biochar-C content, which reflects the impact of increasing pyrolysis temperatures, and hence degree of aromaticity, on increasing biochar stability. It may also indicate that higher temperature biochars are more toxic to microbial activity, perhaps due to their highly basic surfaces, or perhaps due to specific toxic chemical formed at higher temperatures. A negative correlation between biochar-C mineralization rates and the duration of the incubation content was found, demonstrating the stability of biochar in the long term.

Table 1-1 Spearmans' rho values of the non-parametrical correlation between the daily mineralized OC from biochar-soil incubations (%biochar-C min day^{-1}), pyrolysis temperature (°C), pyrolysis retention time (min), soil organic carbon (SOC) content (%), incubation duration (days), biochar application rate (w w^{-1}) and C content of the biochars (%) (n=28). Correlations without a clear potential cause-effect relationship were omitted.

	Biochar-C content	Daily biochar mineralization
Temperature	0.67*	-0.59*
Retention time	-0.32	0.02
SOC content		-0.35
Incubation duration		-0.60*
Application rate		-0.17
Biochar-C content		-0.48*

* Correlation is significant at 0.01 level (no additional correlations were significant at the P<0.05 level)

1.5.1 Influence of biochar production and application parameters

Biochar properties and their effect on the microbial community vary significantly according to pyrolysis conditions and source material properties (Pietikainen et al., 2000; Das et al., 2008; Sohi et al., 2009; Sohi et al., 2010). Importantly, the source material from which biochar is produced has an impact on the soil microbial response to biochar amendments. The higher the lignin content of the feedstock, the higher the aromatic C content and the C:N ratio of the resulting biochar; this decreases the biochar mineralization rate. For example, higher C mineralization rates have been reported for rye grass biochars (2% – 3.5 % of C mineralized after 48 days) as compared with pine wood biochars (only 0.2% – 0.4 %) produced under the same pyrolysis conditions (Hilscher et al., 2009). Glucose-derived biochar had a more pronounced aromatic structure, in contrast to the low condensation level of yeast-derived biochar, although the biochars were produced under the same pyrolysis conditions (Steinbeiss et al., 2009). Consequently, the yeast biochar stimulated soil microorganisms more than the glucose-derived biochar, leading to higher C respiration rates (loss of 7% C versus 3% C respectively in arable soil) and to higher loss of the native SOM (priming effect).

Pyrolysis temperatures have a significant influence on biochar pH, CEC, OC content, O:C ratio, porosity and surface area, and consequently on the microbial community (Pietikainen et al., 2000; Lehmann, 2007). Indeed, increased charring temperature leads to a higher resistance to microbial decay (Baldock and Smernik, 2002; Hamer et al., 2004; Bruun et al., 2008; Spokas, 2010; Zimmerman, 2010). For example, the modelled carbon half-lives

of oak biochars ranged from 840 to $4.0x10^7$ years, for pyrolysis temperatures of 250°C to 650°C, respectively (Zimmerman, 2010). Spokas (2010) introduced the O:C ratio as an important and easy-to-measure parameter to predict biochar stability, such that biochar O:C ratios, which decrease with increasing pyrolysis temperature, were negatively correlated to biochar stability (Spokas, 2010).

Volatile matter content was found to decrease with increasing pyrolysis temperature (Spokas et al., 2011), and biochars with high labile organic matter contents (i.e. formed at relatively low pyrolysis temperatures) had higher mineralization rates when added to soils (Cross and Sohi, 2011). Additionally, with increasing pyrolysis temperature, the structural heterogeneity of biochars has been found to decrease. This feature appeared to play an important role in inhibiting the growth of several fungal species, such as *Pleurotus pulmonarius* and *Coriolus versicolor*, on biochar substrates (Ascough et al., 2010). Also, the residence time during pyrolysis has an influence on the microbial response when biochar is added to soils. Longer residence time during pyrolysis result in a more completely pyrolyzed biochars with less volatile matter, which are thus less suitable for microbial degradation (Bruun et al., 2012).

The microbial response to biochar application depends also on the particle size of the biochars. Zimmerman (2010) reported that biochar-C mineralization rates of fine biochar particles (< 0.25 mm) were about 1.5 times higher than of the coarse fraction (> 0.25 mm), even though the fine and coarse fractions had similar N_2-BET specific surface area. This indicates that internal surface accessibility may be one factor controlling the microbial decomposition of biochars. Research into how biochar particles disintegrate over time after application to soil, and how their external/internal surface areas change accordingly, will be pivotal in gaining a better understanding (quantitative as well as qualitative) of the soil microbial response to biochar application.

1.5.2 Influence of native SOM – priming and co-metabolism

Native SOM content and associated microbial community structure influence the impact of biochar application on the soil microbial community. Mineralization of organic substrates and native SOM can be enhanced or decreased by the presence of biochar in soils; this is the so-called positive or negative priming effect of biochar on organic matter (Hamer et

al., 2004; Novak et al., 2010; Cross and Sohi, 2011; Keith et al., 2011; Luo et al., 2011; Zimmerman et al., 2011). Several authors observed a higher microbial biomass in biochar-organic material mixtures than would have been expected on the basis of the components considered separately. The addition of biochar, with labile compounds that can be used as a substrate, might boost microbial activity. Through this increased microbial activity native SOM may be co-metabolized (Kuzyakov et al., 2000). The native SOM can be co-metabolized due to substrate-induced microbial growth (Kuzyakov et al., 2000) or microorganism might decompose SOM to acquire N needed for the decomposition of biochar (Blagodatskaya and Kuzyakov, 2008). Mineralization of SOM or added organic substrates is likely to be inhibited (negative priming) when biochars have a high microporosity (i.e. specific surface area greater than 200 m^2 g^{-1}). Soluble constituents from the organic matter may diffuse into the micropores of the biochar particles and are adsorbed there, where they are physically protected against further mineralization, since these pores are too small for microorganisms (Hamer et al., 2004; Hilscher et al., 2009; Cross and Sohi, 2011; Zimmerman et al., 2011). Consequently, the mineralization of native SOM may be either stimulated (Steinbeiss et al., 2009; Major et al., 2010; Luo et al., 2011; Zimmerman et al., 2011) or reduced (Spokas et al., 2009; Cross and Sohi, 2011; Zimmerman et al., 2011) by the presence of biochar.

The interaction or priming effects of biochar and organic matter may work in both directions, since the addition of easily-degradable substrates (Hamer et al., 2004; Kuzyakov et al., 2009; Luo et al., 2011) or the presence of labile SOM (Keith et al., 2011) in biochar-amended soils also increases biochar decomposition. This priming effect of added organic matter on biochar is often called *co-metabolic decomposition*. Microbial enzymes involved in the decomposition of other substrates resulted in enhanced decomposition of biochar in soils, with repeated additions of the organic material to biochar-amended soils resulting in yet greater co-metabolic biochar decomposition (Kuzyakov et al., 2009; Keith et al., 2011). This is attributed to the better adaptation of the microorganisms to decompose biochar (Hamer et al., 2004). However, the enhanced biochar decay due to the addition of easily-degradable substrate was mainly observed during the first days following the addition of organic matter (Hamer et al., 2004). Biochar decomposition in the rhizosphere may therefore be increased by the enhanced microbial activity due to the continuous addition of carbohydrates in root exudates and other rhizodeposits. At least locally in the rhizosphere, co-metabolism may be a major biochar decomposition pathway (Hamer et al., 2004; Kuzyakov et al., 2009).

1.5.3 Influence of vegetation and mineral soil properties

Vegetation properties may also influence the interaction between biochar and soil microorganisms. Compared to adjacent unamended soils where microbial activity was inhibited due to the presence of allelopathic organic compounds originating from the vegetation, there was higher microbial activity in biochar-amended soils due to adsorption of these allelopathic substances on the biochar surface (Kolb et al., 2009). In this way, the adsorption of toxic organic compounds to the biochar surface may result in increased vigor of microorganisms compared to unamended soils (Wardle et al., 2008; Kolb et al., 2009). The adsorption of various root exudates by biochar may also influence the rhizosphere microbial population.

Given that organic matter binds to clay surfaces, it is possible that biochar particles also interact with clay surfaces, which may diminish its availability as a food source for soil organisms and increase its resistance to biodegradation (Chenu and Plante, 2006). Joseph et al. (2010) hypothesized that biochar – mineral soil interactions were comparable to organic matter – mineral soil interactions. Indeed, stabilization of biochar-C was reported by Bolan et al. (2012), and was ascribed to the adsorption of organic compounds onto the clay minerals, or physical entrapment in small pores in aggregates inaccessible to microbes.

1.6 Biochar and the N cycle

During ammonification aerobic and anaerobic soil organisms metabolise organic compounds and transform them to ammonium (NH_4^+). This NH_4^+ is transformed to nitrate (NO_3^-) through nitrification by aerobic soil microorganisms. N immobilisation occurs when compounds with a high C:N ratio are degraded by soil organisms with a lower C:N ratio. These organisms need to obtain their tissue C:N ratio and will take up NH_4^+ or NO_3^- (through assimilative nitrate reduction), which will decrease the amount of mineral N in the soil. In the short-term, degradation of labile biochar compounds with a high C:N ratio may thus cause N-immobilisation (Clough et al., 2013). In this way a temporal organic N pool is established in the soil. Nelissen et al. (2012) observed increased gross N minerlization rates in the short term when biochar was added to the soil. These increased rates were attributed to the decomposition of SOM (priming) by microorganisms in order to acquire N. Gross N

mineralization rates were higher in soils amended with biochar with a larger labile C fraction, suggesting that microorganisms were activated by the presence of volatile compounds in the biochar. This N may then be immobilized in the microbial biomass (Nelissen et al., 2012). N immobilization may prevent N leaching from the soil profile, however crops might also experience N deficiency. After the labile biochar compounds are degraded this N immobilisation is likely to stop and the recalcitrant biochar matrix remains in the soil (DeLuca et al., 2009). In the long term, chemical and physical biochar characteristics will influence the N cycles in the biochar-amended soil. Adsorption of NH_4^+ onto the biochar surface with a high CEC can occur. On the one hand this can decrease nitrification rates and thus the amount of available nitrate in the soil (Major et al., 2010). However, the NH_4^+ is not irreversibly bound to biochar and can be made available again when levels are low. Increased mineral N contents in the soils are also possible when biochar with a high pH is incorporated into the soil. It is well known that soil nitrifying community is favoured by less acidic soil conditions. The addition of biochar with a high pH could in this way increase nitrification rates in soils. Nitrification rates can also increase when OC compounds with a high C:N ratio are adsorbed onto the biochar surface, reducing the bioavailability of these compounds that would otherwise cause N immobilisation in the soil.

1.7 Objectives and thesis outline

A starting hypothesis of this thesis is that biochar additions to soil impacts soil microorganisms. Directly, by microbial assimilation of biochar compounds and indirectly through changing the microbial habitat. The overall aim of this thesis is to investigate the impact of biochars differing in their chemical composition, biological stability, and duration of soil incorporation on selected soil biological parameters.

The biochemical composition and structure of biochar logically determine its stability against biological degradation in the soil. Both strongly result from variation in feedstock type and pyrolysis conditions, but specific molecular-level information on the biologically labile fractions of biochar is often limited. To investigate the relation between short-term stability of biochar and its biochemical composition (**Objective 1**), we characterized six biochar types by means of pyrolysis-field ionization mass spectroscopy (Py-FIMS). These biochars were amended to a sandy loam soil and the short-term stability was tested during a soil incubation

experiment with follow-up of soil CO_2 emission (Chapter 2). The modeled kinetic parameters of this mineralization experiment were correlated to the biochar molecular markers tentatively assigned to biochemical compound classes.

Because of its highly reactive surface area biochar has been shown to hold the capability to adsorb organic compounds. Therefore, we may expect that soil microbial measurements involving extraction after the addition of chemicals to soils, would be influenced by the presence of biochar. Morever, labile biochar compounds may be extracted during microbial soil assays. As a result, several methodological challenges may arise when determining soil biological properties in the presence of biochar. Before engaging experiments to assess the effect of biochar on microbial functioning we tested if such interference occurs and if microbial assays are applicable to biochar-amended soil (**Objective 2**). To this end several biochar types were added to two soil types immediately before analyzing soil microbial activity (enzyme activity), biomass (fumigation-extraction) and community structure (PLFA analysis) (Chapter 3).

A primary aim of this thesis was to assess short-term effects of biochar on soil microbial properties (**Objective 3**). Four different biochar types, produced from two different feedstocks and at two pyrolysis temperatures were incubated in a sandy loam soil. After 14 weeks microbial activity (enzyme activity and CO_2 emission), biomass and community structure (PLFA analysis) was determined (Chapter 4). We tested the hypothesis that the microbial activity and community structure of soils amended with biochar were determined by the type of biochar feedstock, and the pyrolysis temperature.

Multiple edaphic and environmental factors steer the microbial population and numerous interactive effects exist between most of these. One dominant obvious key soil trait controlling both the physical as well as the chemical and biological soil properties is SOM content. Expanding our analysis in chapter 4, our aim was to investigate if the native SOM content has an interactive control exerted by biochar on the soil microbial community and its activity (Chapter 5) (**Objective 4**). We added several biochar types to two adjacent soils with contrasting SOM levels and through an incubation study N mineralization rates and soil biological parameters were investigated. Additionally, SOM quality was investigated through a combined physico-chemical SOM fractionation method.

Addition of fresh biochar to soils during incubation experiments only allows to assess short-term effects of biochar onto the soil microbial community. Biologically labile biochar

compounds seem to be important as an available food source for soil microorganisms in the short-term. However, an unresolved question to date is if biochar still exerts a similar control on microbial activity when volatile biochar components are degraded. Moreover, next to loss of a labile biochar fraction, after its incorporation in the soil, biochar ages through oxidation, both biologically and abiotically mediated. By investigating soil microbial properties of established field experiments the effect of biochar on soil organisms after several years of field incorporation was assessed. In Chapter 6, we collected soil from four biochar field trials and investigated microbial activity, biomass and community structure. In this way the multi-year effects of biochar onto soil biological properties were assessed (**Objective 5**). We hypothesized that biochar added to soil continues to influence soil microorganisms even long after the easily-available biochar compounds have been degraded, through the aging of biochar during soil incorporation.

Over the different chapters four research questions can be distilled. Chapter 7, brings together the results from each chapter and tries to find an answer to following general research questions:

(i) How do biochar characteristics influence biochar degradation?

(ii) Methodological issue: Are microbial assays compatible with biochar-amended soil?

(iii) How does short-term biochar stability differ from multi-year biochar stability? Does presence of biochar in the soil still significantly affect microbial activity after multi-year field incorporation and is this effect different than observed in short-term incubations?

(iv) Does biochar have an effect on native SOM mineralization and what is the role of soil microorganisms herein?

Chapter 2:

Biochar stability and its molecular-level biochemical composition

Abstract

The objective of this chapter was to investigate if degradability of biochar could be linked to its molecular-level biochemical composition and to the thermal stability of individual OM components. Six biochars were produced during slow pyrolysis at 400°C and 500°C from pine wood, peanut hull and poultry litter and were added at an application rate of 10 t ha^{-1} to a sandy loam soil. C mineralization was monitored during a 25 days incubation and a first order kinetic model was fitted to the data. The molecular-level biochemical composition of the samples was assessed by pyrolysis field-ionization mass spectroscopy (Py-FIMS). Weight loss during analytical pyrolysis was found to decrease with increasing biochar production temperature, in line with the volatile matter content of the biochars. Likewise, for the peanut hull and poultry litter biochars net C mineralization decreased with increasing pyrolysis temperature. However, this was not the case for the pine biochars, which were characterized by intense signals of aromatic building blocks like phenols, lignin monomers and alkylaromatics, often linked to reduced substrate degradation. Specifically, we observed significant negative correlations between the ion intensities of naphthalene and several phenols and lignin monomers and the net C mineralization. sThe ion intensities of several N containing compounds were positively correlated to the net C mineralization. Compared to the 400°C biochars, we observed very low total ion intensities in the 500°C biochars, which manifested into noisy 500°C thermograms. We conclude that Py-FIMS can yield predictors for biochar degradability, although analysis of higher temperature (≥500°C) biochars is problematic due to very limited volatilization of OM fragments.

2.1 Introduction

The application of biochar to the soil has recently been put forward as a possible means for climate change mitigation through C sequestration. After soil addition, biochar is assumed to become part of the recalcitrant soil organic matter (SOM) pool (Skjemstad et al., 2002; de la Rosa and Knicker, 2011). However, recent studies have pointed out that, at least in the short-term, some labile biochar compounds may be mineralized. These compounds, created during pyrolysis, are most likely residues of volatile and semi-volatile organic compounds that condense on the biochar during cooling (Graber et al., 2010; Smith et al., 2010). It is thus important to gain more insight into the chemical nature and the microbial consumption of these labile biochar compounds. Since biochar properties vary widely as a function of the many types of feedstocks, pyrolysis techniques, residence times and temperatures, thorough characterization should be essential to predict their degradability when applied to soils. Already quite some research has looked into either the chemical characterization of biochars or to its stability, however, the link between both is not yet fully understood. Perhaps one constraint in preceding work has been the narrow biochemical window offered by various analytical techniques used. Recent examples include fourier transformed infrared spectrophotometry (FT-IR) analysis (Schnitzer et al., 2007a; Keiluweit et al., 2010; Ozcimen and Ersoy-Mericboyu, 2010), NMR (Schnitzer et al., 2007a; Brewer et al., 2009; de la Rosa and Knicker, 2011), Curie point X-ray diffraction (Keiluweit et al., 2010), Curie-point pyrolysis-gas chromatography/mass spectrometry (Cp Py-GC/MS) (Schnitzer et al., 2007b) and synchrotron-based near-edge X-ray absorption fine structure (NEXAFS) (Keiluweit et al., 2010). All these methods have advantages and disadvantages, a major disadvantage being that none of these techniques allow to determine the strength of chemical bonds. Particularly, it is not only the biochemical composition as such, but also the strength of cross linkages between individual OM building blocks that determines their degradability. This generally applies to any organic compound including, substrates, soil organic matter and biochar.

Pyrolysis field ionization mass spectroscopy (Py-FIMS) provides information on the biochemical composition, while the volatilization temperature of molecular markers is related to their binding strength (Leinweber et al., 2009a). As such, Py-FIMS has frequently been used to assess the molecular-level biochemical composition of biowastes (Hummel et al., 1985; Smidt et al., 2005), wood (Šimkovic et al., 1993), rhizodeposits (Kuzyakov et al., 2003; Melnitchouck et al., 2006), dissolved organic matter (Schulten and Gleixner, 1999) and SOM

(Leinweber and Schulten, 1999; Sleutel et al., 2008). This technique has in addition been used to analyse bio-oil and biochar from fast pyrolysis at 330°C of chicken manure (Schnitzer et al., 2008). Schnitzer et al. (2008) analysed chicken manure char by a myriad of techniques. They concluded that in comparison to Cp Py-GC/MS the Py-FIMS technique enabled the detection and analysis of a much wider molecular weight range and is thus an effective technique to characterize biochars (Schnitzer et al., 2008). However, to the best of our knowledge there are no studies which link molecular-level characteristics of biochar produced by slow pyrolysis to its stability in the soil. In this chapter, we assessed the short-term C mineralization of soil amended with six slow pyrolysis biochars from different feedstocks (pine wood, poultry litter and peanut hulls) produced at different pyrolysis temperatures (400°C and 500°C). We also analyzed these materials with Py-FIMS to link biochar degradability to its molecular-level biochemical composition and thermal stability of individual OM components.

2.2 Material and methods

Soil characteristics

An Inceptisol (WRB classification) with sandy loam soil texture (USDA classification, clay <2µm: 7%, silt 2-50µm: 44% and sand 50-2000 µm: 49%), representative for intensive agriculture in Flanders was collected from an arable field in Lendelede, Belgium. The soil had a SOC content of 0.73 %, a total N content of 0.063 %, pH_{H2O} (1:5) of 6.4.

Biochar production and characterization

Six biochars were produced from three different feedstocks, namely pine chips, peanut hull and poultry litter, at two different slow pyrolysis temperatures (400 °C and 500 °C). The holding time once the target temperature was reached was 0.5h and N_2 was used as a carrier gas. The biochar and oil yields were measured by weighing the fractions after pyrolysis. The concentrations of C, N, H and O were analyzed using a LECO brand (Model CHNS-932) elemental analyzer. The volatile matter, fixed C and ash content was measured by the proximate analysis method (ASTM D1762-84 standard testing method) using a LECO TGA-701 proximate analyzer (Leco Corp., St. Joseph, MI). The remaining ash was destructed by microwave digestion followed by nitric acid and hydrochloric acid destruction and the P, Al,

Fe, K, Mg, Na and Ca content of the digestate was determined by inductively coupled plasma – mass spectrometry (ICP-MS) using a Perkin-Elmer Elan 6000 ICP-MS (Waltham, Mass.) The biochar characterization data are given in Table 2-1. The surface area (SA) and total pore volume (TPV) of the biochars were determined by the BET method with a BELsorp – mini II analyzer (BEL Japan Inc., Osaka, Japan).

Pyrolysis-field ionization mass spectroscopy (Py-FIMS)

Temperature-resolved Py-FIMS was carried out at the Institute for Land Use, Rostock University. About 2–5 mg biochar material was thermally degraded in the ion-source of a modified Finnigan MAT 731 high-performance mass spectrometer. The samples were heated in three replicates under a high vacuum from ambient temperature to 700°C at a heating rate of 10 K per magnetic scan (~1.7 K s^{-1}). After about 20 min, 60 magnetic scans were recorded for the mass range 16-1000 Da (single spectra). The single scan spectra were integrated to obtain one summed spectrum. In general, the summed spectra of three replicates were averaged to give the final survey spectrum. In addition, the volatilization temperature is also considered for identification. For each of the 60 single scans, the ion intensities of these marker signals were calculated. All samples were weighted before and after Py-FIMS to normalize ion intensities per mg sample.

We tentatively assigned mass signals to compound classes, according to Schulten and Leinweber (1999); carbohydrates with pentose and hexose subunits (*m/z* 60, 72, 82, 84, 96, 98, 110, 112, 114, 126, 132,144 and 162), phenols and lignin monomers (*m/z* 94, 108, 110, 122, 124, 138, 140, 150, 152, 154, 156, 164, 166, 168, 178, 180, 182, 194, 196, 208, 210 and 212), lignin dimers (*m/z* 246, 260, 270, 272, 274, 284, 286, 296, 298, 300, 312, 314, 316, 326, 328, 330, 340, 342 and 256), lipids, alkanes, alkenes, fatty acids and n-alkyl esters (*m/z* 202, 216, 230, 244, 256, 258, 270, 272, 284, 286, 298, 300, 312, 314, 326, 328, 340, 342, 354, 368, 380, 382, 394, 396, 408, 410, 422, 424, 438, 452, 466, 480 and 494), alkylaromatics (*m/z* 92, 106, 120, 134, 142, 148, 156, 162, 170, 176, 184, 190, 192, 198, 204, 206, 218, 220, 232, 234, 246, 260, 274, 288, 302, 316, 330, 344, 358, 372 and 386), heterocyclic N containing compounds and peptides (57, 59, 67, 70, 73, 74, 75, 79, 81, 84, 87, 91, 95, 97, 99, 103, 109, 111, 115, 120, 123, 125, 129, 135, 137, 139, 153, 161, 167, 181, 183, 195, 203, 233, 245, 255, 257, 271, 285, 333, 359, 363 and 393).

C mineralization experiment

The short-term C mineralization was determined during a 25-days incubation experiment. Soil mesocosms with 250 g of air-dried soil were prepared in 6.8 cm diameter PVC tubes. Dry soil was sieved using a 2 mm sieve. Per mesocosm, 3.63 g of biochar (sieved with a 2mm sieve) was added (biochar to soil ratio of 1:69 on a mass basis), this was equivalent to the addition of 10 Mg fresh biochar ha^{-1} (based on surface:area ratio). Soil was thoroughly mixed with the biochar and the mixture was filled in the tubes, and slightly compacted to obtain a bulk density of 1.4 g cm^{-3}. There were three replicates per biochar treatment. A control treatment (i.e. soil without biochar addition) in triplicate was also included. After biochar addition, deionized water was added to the soils to achieve a fixed moisture content of 50 % water-filled pore space (WFPS). The soil columns were placed in closed glass jars and kept in an incubation cabinet at 25°C. The emitted CO_2 was trapped in 15 ml 1M NaOH. At day 1, 2, 3, 8, 10, 11, 14, 16, 18, 21, 23 and 25 the vials with NaOH were removed and titrated with HCl in the presence of $BaCl_2$. The water content of the mesocosms was adjusted weekly in order to maintain a WFPS of 50 %.

Data analysis

The cumulative C mineralization, was plotted against the time (t) and a parallel first order kinetic model was fitted to the data using the Levenberg-Marquardt algoritm:

$$C_{\min} = C_0\left(1 - EXP\left(-k * t\right)\right) \quad (2\text{-}1)$$

This model assumes a mineralizable C pool (C_0), which is mineralized according to first-order kinetics, at a mineralization rate k. Because we fitted through all replicates at each measuring time, the fit yields the parameters C_0 and k ± its standard error.

Net C mineralization ($C_{min\ net}$) was calculated as follows:

$$C_{min\ net} = \frac{C_{min\ biochar\ treatment} - C_{min\ control}}{C_{biochar}} \quad (2\text{-}2)$$

with $C_{min\ biochar\ treatment}$ and $C_{min\ control}$ the amount of CO_2 emitted from the biochar and control treatment, and $C_{biochar}$ the C content of the corresponding biochar.

Since multiple predictors were highly correlated to each other, we could not use ordinary least squares regression to investigate relations between C mineralization kinetic model parameters

and the py-FIMS mass peaks, as this would lead to very imprecise estimates. Therefore 'lasso' (least absolute shrinkage and selection operator) regressions (Tibshirani, 1996) were carried out between the $C_{min\ net}$ on the one hand and the recorded ion intensities of the individual Py-FIMS masses (expressed as % of TII) (higher than *m/z* 59) and the O:C and H:C biochar ratios on the other with the R software (R, version 3.0.1., the R foundation for statistical computing, Vienna, Austria). This type of regression fits a linear model between the response Y ($C_{min\ net}$) and multiple predictor variables X's (individual mass peaks (expressed as % of TII), the biochar O:C and H:C ratio), while a penalty is used to shrink the least important variables to zero. In this way the lasso regression is able to perform selection of predictors in a linear model, whilst fully accounting for the high degree of multicollinearity amongst the 'independents'.

2.3 Results and discussion

The elemental composition of the six produced biochars and the yields of slow pyrolysis products are summarized in Table 2-1. With increasing pyrolysis temperature oil yield increased, while char yield decreased. Char yield did not exceed 37%. The volatile matter content decreased while fixed C contents increased with increasing pyrolysis temperature. Decreases in volatile matter content to the benefit of fixed C content with increasing pyrolysis temperature have been reported frequently (Smith et al., 2010) and may be attributed to lower recondensation of volatile compounds when pyrolysis temperature increases (Cross and Sohi, 2011) or a lower degree of devolatilization of the biomass during pyrolysis (Ronsse et al., 2013). For all biochars, H:C ratio decreased with increasing pyrolysis temperature, as was also found by Ronsse et al. (2013). Additionally, they found a significant positive correlation between the C:H ratio and the fixed C content, suggesting that devolatilization removes most of the biomass H, which increases biochar stability in the soil (Ronsse et al., 2013). O:C ratio also decreased with increasing temperature. According to Spokas (2010) the O:C ratio determines the biochar stability in the soil. So we would thus expect increasing biochar stability with increasing pyrolysis temperature.

Table 2-1 Chemical properties of the pine chips (P), peanut hull (PH) and poultry litter (PL) biochars at 400°C and 500°C

	Oil yield	Char yield	Gas yield	Volatiles	Fixed C	Ash	C	H	N	O	H:C ratio	O:C ratio
	%	%	%	%	%	%	%	%	%	%		
P400	51.03	33.41	15.56	30.23	68.72	1.05	74.38	4.06	0.25	14.59	0.055	0.196
P500	66.96	17.26	15.78	19.00	79.89	1.11	81.71	3.10	0.22	8.76	0.038	0.107
PH400	45.21	35.87	18.92	26.09	67.95	5.96	76.73	3.26	2.55	6.88	0.042	0.090
PH500	45.45	32.20	22.35	17.08	75.69	7.23	85.41	2.40	2.64	2.23	0.028	0.026
PL400	40.97	36.97	22.07	24.40	32.53	43.07	41.87	2.43	4.29	16.17	0.058	0.386
PL500	44.40	31.73	23.88	18.08	39.03	42.90	44.35	1.64	4.02	12.19	0.037	0.275

Table 2-2 Total ion intensity (TII) and percentage of volatilized matter (± standard deviations) (or weight loss upon analytical pyrolysis: WL) in the biochar samples

Biochar	TII/mg biochar 10^6 counts mg^{-1}	TII/mg OC 10^6 counts mg^{-1} OC	WL %
P400	86.2 ± 7.8^{b}	115.9 ± 10.5^{c}	27.2 ± 6.2^{bc}
P500	2.8 ± 1.3^{a}	3.4 ± 1.6^{ab}	22.2 ± 1.6^{b}
PH400	153.5 ± 24.4^{c}	200.1 ± 31.8^{d}	23.1 ± 2.8^{b}
PH500	1.6 ± 0.1^{a}	1.9 ± 0.1^{a}	21.1 ± 4.0^{ab}
PL400	66.5 ± 10.9^{b}	158.8 ± 26.0^{d}	35.1 ± 5.7^{c}
PL500	4.3 ± 2.0^{a}	9.7 ± 4.5^{b}	15.0 ± 2.8^{a}

Different letters indicate differences between the TII and volatilized matter during analytic pyrolysis of the biochars (ANOVA, with post-hoc Tukey).

Detailed information on the presence of biodegradable components was derived from Py-FIMS analysis of the different biochars. The total ion intensity (TII, expressed as TII per mg OC) differed significantly between the biochar types (PH400, PL400 > P400 > PL500 ≥ P500 ≥ PL500) (Table 2-2). All the 400°C biochars had a higher TII than their corresponding 500°C biochar from the same feedstock. Py-FIMS analysis of the 500°C biochars was problematic due to very limited volatilization of OM fragments, in spite that during Py-FIMS temperature was brought up to 700°C, i.e. well above the biochar production temperature. Consequently, interpretation of the Py-FIMS data for the 500°C biochars is tentative and we restricted the analysis to identification of the main mass peaks only. The weight loss (WL) during Py-FIMS decreased with increasing production pyrolysis temperature. This is in accordance with the lower content of volatile matter in the higher temperature biochars, observed during proximate analysis (Table 2-1). A substantial part of the volatilized matter upon Py-FIMS (WL%) was not detected as TII (Table 2-2). Sorge et al. (1993a) investigated the relationship between organic C content of whole soil samples, soil particle-size fractions and litter materials, and the behaviour of each group during Py-FIMS (weight loss and the

TII). TII (expressed per mg OC) of the 400°C and 500°C biochars was higher and lower, respectively, than the values for whole soil samples found by (Sorge et al., 1993a). This indicates thus that py-FIMS of 500°C biochars is less efficient than of whole soil samples (Sorge et al., 1993a). They concluded that TII depended not only on the organic C content of the samples, but also on structural features of the C (Sorge et al., 1993a). Therefore we suggest that the discrepancy between WL during Py-FIMS and the TII of the 500°C biochars, may be caused by a difference in chemical C structure. Indeed, at higher pyrolysis temperatures the crystallite content of the biochars increases, whereby amorphous C is transformed into ordened graphene packets (Amonette and Joseph, 2009).

In the PH400 and PL400 biochars the detection of ion intensity started at 310 °C and 280°C and in the P400 biochars only at 360 °C (Figure 2-1). The temperature course of the detected ion intensity of all mass signals, as derived from Py-FIMS, gives an indication of the thermal energy required for the volatilization of detected biomarkers. The P400, PH400 and PL400 thermograms all had a single broad slightly skewed peak around 520°C and 500°C. The counts of TII of the 500°C biochars were much lower than of the 400 °C biochars (Table 2-2) and noisy thermograms were observed. The PH500, and to a lesser extent the P500 thermograms showed a bimodal profile, with a first volatilization maximum occurring at 400-450°C and a second and larger maximum at approximately 620°C. The peak at 400-450°C suggests that during slow pyrolysis at 500°C some compounds are not fully pyrolysed, probably due to the incomplete heat transfer through the feedstock or recondensation of some volatile compounds. Likewise, Haas et al. (2009) reported that during pyrolysis at 500°C, some tar pyrolysis compounds may be entrapped in the biochar. For the PL500 biochar only one single peak of highly thermostable matter (>600°C) was found with maximum beyond the Py-FIMS's 700°C temperature range. There thus was a tendency for higher temperature volatilization, i.e. at >550°C in the P500, PH500 and PL500 biochars and this may be logically linked to the higher slow pyrolysis temperature which apparently led to the preferential loss of volatile matter.

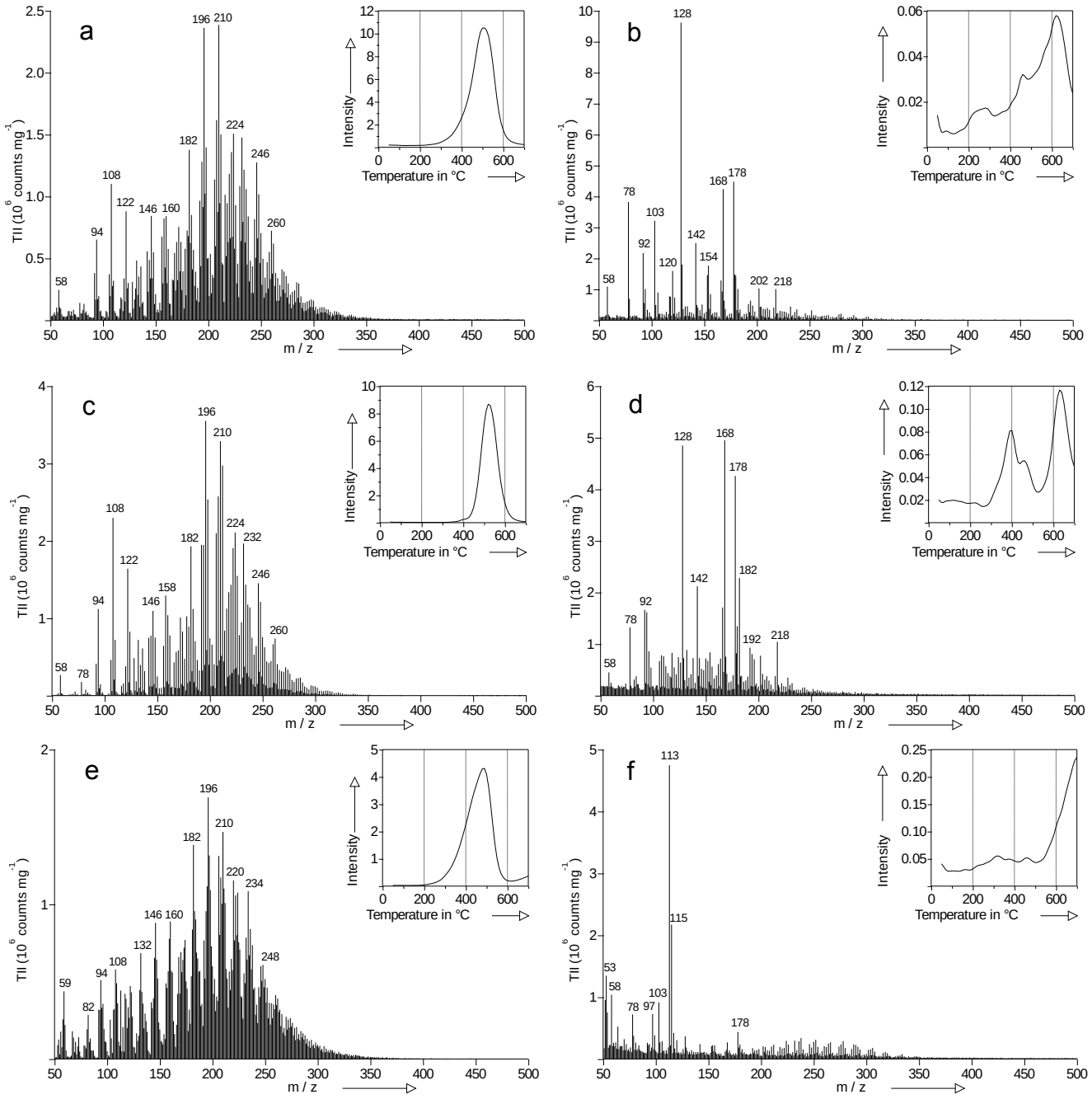


Figure 2-1 Py-FIMS spectra of the six biochars of pine chips pyrolized up to 400°C (a) and 500°C (b), peanut hull (c,d) and poulty litter (e,f). Note that not all axes have the same scale.

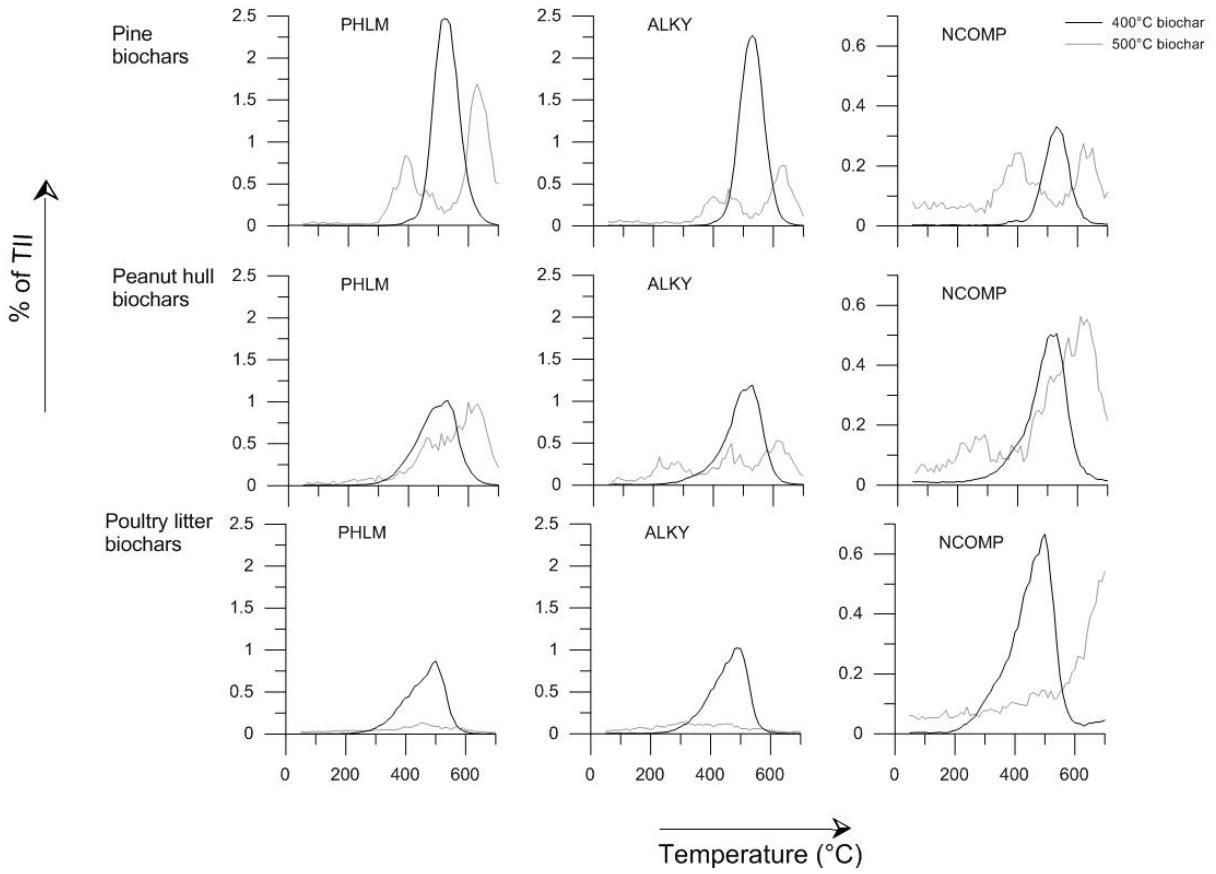


Figure 2-2 Thermograms for the volatilization of selected tentatively assigned compound classes (ALKY: alkylaromatics; PHLM: Phenols and Lignin Monomers; NCOMP: N-containing compounds including proteinaceous and heterocyclic N) of the six biochars.

Specific thermograms of integrated masses were tentatively assigned to the major molecular building blocks present in the investigated biochars, namely alkylaromatics, phenols and lignin monomers and N-containing compounds (Figure 2-2). The peak at 600°C in the P500 and PH500 thermograms (Figure 2-1), was mainly caused by a high thermostability and intensity of the phenols and lignin monomers, and to a lesser extent of the alkylaromatics and N-containing compounds (Figure 2-2). In the PH500 thermogram, the peak at 550°C was induced by a large volatilization of N-containing compounds at a peak temperature of 550°C. Especially, the volatilization maximum of *m/z* 115 contributed strongly to the increase in thermostability of the NCOMP class. According to Sorge et al. (1993b) this signal can be derived from the amino acid proline ($C_5H_9NO_2$) and fragments of asparagine ($C_4H_8N_2O_3$). Considering the 400°C biochars, peanut hull biochar had a significantly higher carbohydrate content than the other biochar types (Table 2-3). The mass spectra of the P400 biochar showed more pronounced peaks indicative of phenols and lignin monomers (Figure 2-

2). This was also clear from a high calculated %TII of the mass signals tentatively assigned to phenols and lignin monomers of the P400 biochar compared to the biochars from the other feedstocks (Table 2-3). Although the pine and peanut hull feedstocks had a comparable lignin content, this indicates that after slow pyrolysis at 400°C a substantial share of the lignin in pine chips was still present in relatively untransformed form in the pine biochars, while the same cannot be concluded for the PH400. Additionally, we observed the highest proportion of tentatively assigned alkylaromatics in the P400 biochars, compared to the other 400°C biochars (Table 2-3). It is well known that polyphenolic compounds like flavanols and tannins are widespread in coniferous trees, including pine (Waterman and Mole, 1994). Sleutel et al. (2008) from Py-FIMS mass spectra of heathland soils, suggested that polyphenols and their fragments may have similar masses as alkylaromatics and could thereby incorrectly contribute to the 'alkylaromatics' compound class. It should be taken into account that these misclassified signals for polyphenols partly explain a higher assignment of alkylaromatics in the pine wood biochars. The PL400 biochars contained more N-containing compounds than the P and PH biochars, with heterocyclic N-containing compounds and peptides representing 9.1% and 1.7% of the TII. This subdivision between both N-containing compound classes is tentative though as it is known that upon analytical pyrolysis part of proteinaceous N is transformed into heterocyclic moieties. A higher TII% of N-containing compounds in the PL400 biochar is obvious, since the N content of the poultry litter biochars was twice and twenty times higher than of the PH400 and P400 biochars (Table 2-1), respectively. For both peanut hull biochars the intensities of mass peaks indicative of N-containing compounds, were low compared to the other biochar types. On the contrary, the proportions of lipids and carbohydrates, were higher in the peanut hull biochars compared to the other types.

Table 2-3 Mean proportions (± standard deviations) of tentatively assigned OM compound classes* (% of total ion intensity: %TII) and the weight loss (WL) during py-FIMS (%) in the six biochar samples

Biochar	CHYDR	PLIM	LDIM	LIPID	ALKY	NCOMP	WL
P400	3.5 ± 0.2^{a}	26.7 ± 1.4^{c}	4.3 ± 0.7^{c}	5.8 ± 0.3^{c}	23.0 ± 0.4^{e}	3.6 ± 0.2^{b}	27.2 ± 6.2^{bc}
P500	7.0 ± 0.4^{a}	26.7 ± 5.6^{c}	0.4 ± 0.1^{a}	2.7 ± 0.2^{ab}	12.9 ± 1.6^{c}	7.4 ± 0.7^{c}	22.2 ± 1.6^{b}
PH400	16.8 ± 1.0^{b}	4.7 ± 0.7^{a}	6.8 ± 0.6^{d}	17.5 ± 1.1^{e}	6.8 ± 0.1^{a}	0.2 ± 0.1^{a}	23.1 ± 2.8^{b}
PH500	20.1 ± 5.3^{b}	0.4 ± 0.2^{a}	3.0 ± 0.2^{b}	14.6 ± 1.8^{d}	9.0 ± 0.3^{b}	0.3 ± 0.2^{a}	21.1 ± 4.0^{ab}
PL400	3.5 ± 0.6^{a}	12.1 ± 0.4^{b}	2.8 ± 0.6^{b}	4.4 ± 0.4^{bc}	14.6 ± 0.3^{d}	10.7 ± 0.6^{d}	32.4 ± 1.2^{c}
PL500	2.2 ± 0.3^{a}	3.8 ± 1.1^{a}	0.3 ± 0.2^{a}	2.1 ± 0.7^{a}	5.2 ± 1.6^{a}	9.5 ± 1.9^{d}	15.0 ± 2.8^{a}

* tentatively assigned compound classes of carbohydrates with pentose and hexose subunits (CHYDR); phenols and lignin monomers (PHLM); lignin dimers (LDIM); lipids, alkanes, alkenes, bound fatty acids and alkyl-esters, free fatty acids and sterols (LIPID); alkylaromatics, with alkylbenzenes, alkylnaphthalenes and alkylphenols subunits (ALKY); heterocyclic N-containing compounds and peptides (NCOMP)
Different letters indicate differences between the mean proportions of tentatively assigned OM compound classes of the biochars (ANOVA, with post-hoc Tukey)

Table 2-4 Modelled C mineralization parameters (± standard error) of the first-order kinetic model and the simulated cumulative C mineralization (C_{min}) of the control soil and the soil amended with different biochars and net C mineralization ($C_{min\ net}$) after 1 month of the biochar amended soil.

Treatment	C_0 (mg CO_2-C g^{-1} soil)	k (h^{-1})	R^2	C_{min} (mg 100 g^{-1} soil)	$C_{min\ net}$ mg CO_2-C g^{-1} biochar-C
Control	52.7 ± 1.6	0.003 ± 0.000	0.99	45.9 ± 1.9^{a}	-
P400	57.3 ± 1.6	0.003 ± 0.000	0.99	49.7 ± 1.6^{ab}	0.349 ± 0.145 *
P500	59.5 ± 1.7	0.004 ± 0.000	0.99	49.8 ± 1.2^{ab}	0.326 ± 0.102 *
PH400	64.4 ± 2.4	0.004 ± 0.000	0.97	59.2 ± 3.6^{c}	1.199 ± 0.323 *
PH500	57.9 ± 1.8	0.003 ± 0.000	0.99	50.5 ± 1.4^{ab}	0.371 ± 0.117 *
PL400	66.0 ± 1.6	0.004 ± 0.000	0.98	63.8 ± 0.5^{c}	2.944 ± 0.077 *
PL500	59.8 ± 1.7	0.004 ± 0.000	0.99	53.8 ± 0.2^{b}	1.326 ± 0.140 *

Different letters indicate significant differences between the short-term cumulative C mineralization of the different biochar treatments (ANOVA, Tukey's with post-hoc test)
* indicates a significant net C mineralization (t-test, H_0 $C_{min\ net}=0$, $P<0.05$)

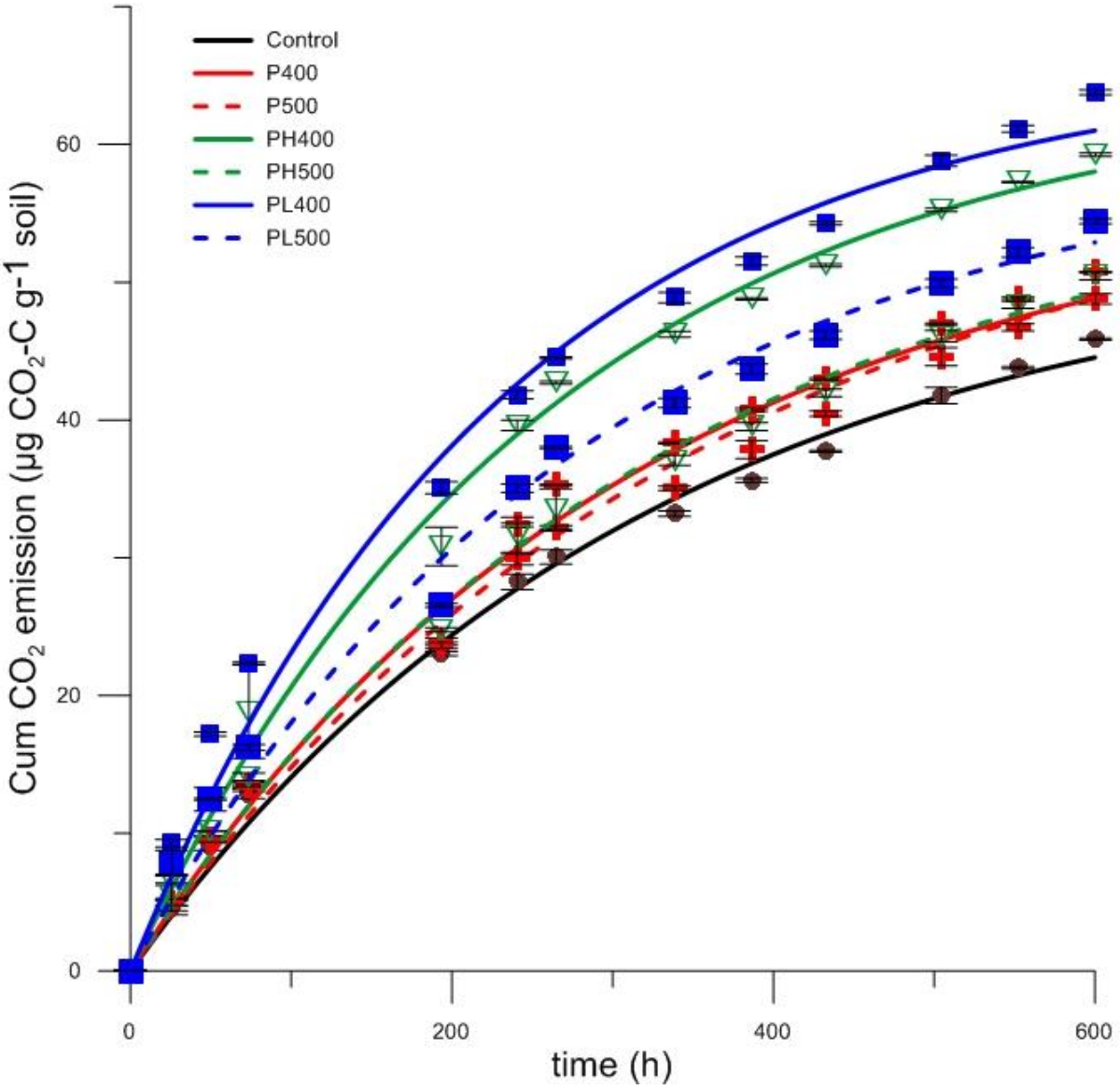


Figure 2-3 Short-term C mineralization (µg CO_2-C g^{-1} soil) (± standard deviations) and the fitted first-order model of the sandy loam control soil and amended with the six biochars

As an important comment, one should bear in mind that not all CO_2 emitted in the C mineralization experiment may be originating from biologically mediated biochar degradation. Also abiotic biochar degradation and priming of SOM might influence the emission of CO_2.. A significant 'net C mineralization' was observed in all biochar treatments (Figure 2-3; Table 2-4). An increase of the pyrolysis temperature from 400°C to 500°C furthermore led to a significant reduction of the cumulative C mineralization in the poultry litter and peanut hull biochar treatments. Lower biochar mineralization rates with increasing pyrolysis temperatures have been reported frequently (Keith et al., 2011; Zimmerman et al., 2011) (see also Chapter

4 and 6) and may be attributed to the higher degree of aromatic condensation with higher pyrolysis temperatures (Baldock and Smernik, 2002; McBeath and Smernik, 2009; Spokas, 2010). Moreover this reduction was accompanied by lower values of the easily-mineralizable C pool (C_0). It seems thusthat more labile OM was mineralized in the 400°C slow pyrolysis temperature treatments. Indeed, the 400°C biochars had higher volatile matter contents, as found during proximate analysis (Table 2-1). The short-term C mineralization from the pine biochar treatments was the lowest of all biochar treatments and neither did pyrolysis temperature influence the C mineralization. Both pine biochars had high phenols and lignin monomer contents. So it appears that the phenols and lignin monomer content of the biochars are inversely proportional to the short-term C mineralization. As lignin monomers are one of the most slowly decomposing and thus stable compounds (Leinweber et al., 1994; Schulten and Leinweber, 1999), it is not surprising that the biochars with the highest lignin monomer content had the slowest C mineralization rates.

Table 2-5 Maximum lasso coefficients of the withheld predictor variables obtained via lasso regression between the Cmin net and individual mass peaks (expressed as % of TII), the biochar O:C and H:C ratio

Predictor variable	Max. Lasso coefficient
m/z 197	1.4
m/z 103	0.1
m/z 128	-0.2
m/z 178	-0.3
m/z 210	-0.5

Lasso regressions were carried out between the mass signals and the $C_{net\ min}$ and the parameters of the first order kinetic model (Table 2-5). Net C mineralization was negatively associated (conditional on the other predictors in the model) with *m/*z 128 (naphtalene (Schnitzer and Schulten, 1992)), 178, and 210 (the latter two are part of the phenols and lignin monomers (Schulten and Leinweber, 1999)). This confirms the hypothesis that aromatic compounds in the biochar, such as naphthalene, phenols and lignin monomers, are stable compounds and do not contribute to short-term biochar degradation. The net C mineralization was positively associated with *m/*z 197 (4-hydroxy-3-methoxy-5-nitro- benzaldehyde) and to a lesser extent with *m/z* 103 (benzonitrile), both heterocyclic N-containing compounds

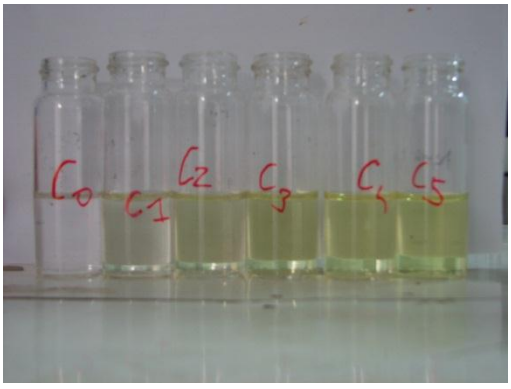
(Schulten and Leinweber, 1999; Leinweber et al., 2009b). Heterocyclic N-containing compounds have often been considered to be very resistant against degradation in the soil (Mengel, 1996). Yet, decomposion of some heterocyclic N-containing compounds (atrazine and cloransulam-methyl) has been found in N-limited environments (Sims, 2006). In our soil incubation experiment we did not add an external source of mineral N and possibly degradation of C-rich biochar constituents (alkylaromatics, carbohydrates, lipids, lignin monomers and dimers) was limited by N-availability. Microbial utilization of non-proteinaceous N-containing compounds from biochar may have been a significant N-supply required for decomposition of native SOM or biochar to proceed.

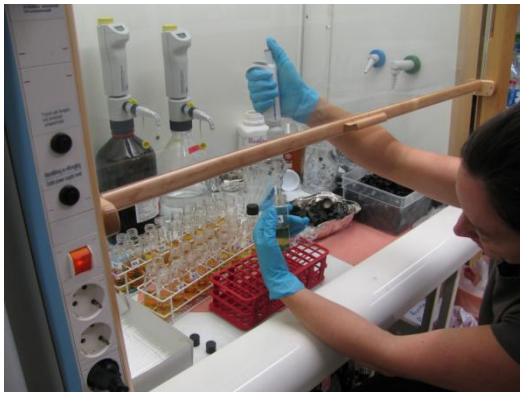
2.4 Conclusions

When pyrolysis temperature was raised from 400°C to 500°C, the weight loss during py-FIMS decreased, suggesting that the lost compounds are part of the easily-degradable biochar fraction and are likely to be linked with short-term C mineralization when the biochars were added to the soil. With increasing pyrolysis temperature we also observed decreasing short-term mineralization, except for the pine biochar treatments. An explanation can be found in the fact that both pine biochars had a higher lignin monomer content, suggesting that the phenols and lignin monomer content was inversely proportional to the C mineralization. Indeed the soil net C mineralization rate were negatively associated with the ion intensities of individual marker signals for phenols and lignin monomers. In addition the net C mineralization rates were positively correlated with total ion intensities of several N-containing compounds. This suggests that heterocyclic N-containing biochar compounds, about whose behaveour in soils very little is known, may thus be degraded in the short-term. However, their contribution to net-C mineralization was probably limited and instead N-release upon biochar decomposition enabled decomposition of other SOM or biochar components. In the 500°C biochars, total ion intensities detected dropped compared to the 400°C biochars, which is manifested in noisy 500°C thermograms.

Chapter 3:

Compatibility of extraction-based microbial assays with biochar-amended soil





Abstract

We tested the compatibility of soil microbial measurements with biochar-amended soil. Biochars were produced at 500-550°C by slow pyrolysis of poultry litter, maize, willow and pine wood and by fast pyrolysis (500°C) of pine wood. The biochars were added to either a silt loamy or a loamy sand soil just before measurement of microbial biomass C (fumigation-extraction), activity (dehydrogenase and β-glucosidase enzyme activity) and community structure (PLFA extraction). Apart from decreased dehydrogenase activity in the presence of poultry litter biochar in the silt loam soil, measured enzyme activities were not influenced by the presence of the biochars. Canonical discriminant analysis (CDA) of the extracted PLFAs revealed that the abundance of the fungal PLFA biomarker 18:1ω9 was higher after the addition of the pine wood and poultry litter biochars. Over the included microbial assays, measurement of microbial biomass C was the most affected by the presence of the biochar. Increased 0.5M K_2SO_4 extracted OC before fumigation of biochar vs. non-amended soil demonstrates extraction of biochar compounds from poultry litter and maize slow pyrolysis and pine fast pyrolysis biochars. Lower 0.5M K_2SO_4 extracted OC contents after fumigation in biochar compared to non-amended soils might have resulted from adsorption of chloroform-lysed soil microbial compounds onto the maize, willow and pine slow pyrolysis biochars. The K_2SO_4-extracted OC contents of poultry litter and pine fast pyrolysis biochars following fumigation on the contrary were increased relative to the unamended control soil and this may be due to adsorption of chloroform or dissolution of some biochar compounds by chloroform. We conclude that fumigation-extraction is incompatible with biochar-amended soils, alternatively microbial biomass can be represented by the sum of the PLFAs.

3.1 Introduction

Biochar has recently gained much attention as a stabile soil ameliorant, capable of sequestering C and boosting soil productivity (Jeffery et al., 2011). Additionally, biochar has been shown to impact soil biological properties (Lehmann et al., 2011). In biochar-amended soils microbial biomass, activity and community structure have often been measured by fumigation-extraction (Kuzyakov et al., 2009; Dempster et al., 2012; Zimmermann et al., 2012), microbial enzyme activity measurements (Bailey et al., 2011; Ameloot et al., 2013a) and phospholipid fatty acid analysis (PLFA) (Santos et al., 2012; Ameloot et al., 2013a), respectively. Lehmann et al. (2011) listed a number of methodological challenges that may arise when assessing soil microbial properties in the presence of biochar. Examples include the possible sorption to the biochars' reactive surface of microbial components upon cell lysis by chloroform (Durenkamp et al., 2010), extracellular enzymes and substrates (Bailey et al., 2011) and phospholipids. On the other hand some biochar compounds might be extracted along during extraction-based microbial assays, e.g. the fumigation-extraction-based microbial biomass assay and PLFA-analysis. Both may lead to either under- or overestimations of microbial measures in the presence of biochar. However, up to now such possible interferences of biochar on measurement of soil microbial biomass, activity and community structure have not been assessed. In this chapter, five biochars, produced from a wide range of feedstocks were added to two soil types just before analysis of microbial biomass, activity and community structure. We wanted to test the hypothesis that the presence of biochar does not influence (i.e. is compatible with) microbial measurements by comparing the results from biochar treated soils with those from corresponding unamended control soils.

3.2 Material and methods

We selected four slow pyrolysis biochars produced from poultry litter (L) (Gaskin et al., 2008), maize (M), willow wood (W) and pine wood (P) and one fast pyrolysis pine biochar (F), all produced within the same temperature range of 500-550°C (Table 3-1). Two Inceptisols (WRB classification) (Table 3-2) were sampled, homogenized, dried at room temperature and passed through a 2mm sieve. Both soils were then compacted to a bulk density of 1.43 g cm^{-3} and pre-incubated at 15°C for one week at a water filled pore space content of 50%. The biochars were then added to the pre-incubated soils (2% w:w), and

immediately thereafter the biomass (microbial biomass C, MBC) (via fumigation-extraction), activity (dehydrogenase and β-glucosidase enzyme activity) and structure (PLFA analysis) of the soil microbial community was analyzed in triplicate or quadruplicate according to the methods described in Chapter 4. Soil samples fumigated with chloroform and non-fumigated samples (10 g fresh soil) were extracted with 30 ml 0.5 M K_2SO_4 and C contents of the extracts were determined with a TOC analyzer (TOC-VCPN, Shimadzu Corporation, Kyoto, Japan). β-glucosidase enzyme activity was measured according to the methods described in Alef and Nannipieri (1995). One gram of moist soil was weighted in glass vials and four ml of modified universal buffer pH 6 and 1 ml 25mM p-nitrophenyl-β-D-glucoside were added. After incubation for 1h at 37°C, 1 ml of 0.5M $CaCl_2$ and 4 ml Tris buffer pH 12 were added. A p-nitrophenol standard series was measured together with the filtrates at 400 nm with a Hitachi 150-20 spectrophotometer (Hitachi Ltd., Tokyo, Japan). Canonical discriminant analysis was undertaking on the nmol % composition of all PLFAs present in a proportion of more than 1 % of the total amount of PLFAs. To investigate significant differences between control and biochar amended treatments, per soil type ANOVA analyses with Dunnett post-hoc tests were carried out.

Table 3-1 Characteristics of the used biochars; feedstock, pyrolysis method, pyrolysis temperature and residence time. C, H and N content, moisture content, ash content and volatile matter content

Biochar type	Feedstock	Pyrolysis method	Temperature (°C)	Residence time at highest temperature	C (%)	H (%)	N (%)	Ash (%)	Volatile matter (%)	Reference
L	Poultry litter	Slow pyrolysis	500	60 min	44.4	1.64	4.02	42.9	18.1	Gaskin et al. (2008)
M	Maize	Slow pyrolysis	550	30 min*	72.1	2.21	1.52	10.9	12.1	Nelissen et al. (2012) Nelissen (2013)
W	Willow wood	Slow pyrolysis	550	23 min	86.3	1.95	0.85	3.2	6.7	Nelissen (2013)
P	Pine wood	Slow pyrolysis	550	60 min	91.6	2.13	0.19	1.0	7.3	Nelissen (2013)
F	Pine wood	Fast pyrolysis	500	<1 s	60.4	nd	0.04	1.4	24.5	Yildiz et al. (2011)

nd: not determined

* total residence time in the pyrolysis unit, residence time at highest temperature is unknown

Table 3-2 Texture and OC content of the loamy sand and silt loam soil

	silt loam	loamy sand
< 2µm (%)	4	18
2 µm- 50 µm (%)	13	74
> 50 µm (%)	83	8
USDA texture class	Silt loam	Loamy sand
OC (%)	1.29	0.80

3.3 Results and discussion

MBC is calculated from the difference between the OC content in a 0.5M K_2SO_4 soil extract before and after fumigation. Thereafter the difference is multiplied by a factor (k_{EC}), which corrects for the incomplete extraction of microbial biomass C (Joergensen, 1996). Before fumigation, significantly more OC was extracted compared to the unamended control of the silt loam soil in case of the L, M, W and F treatments and in case of the L, M and F treatments in the sand loam soil, indicating that 0.5M K_2SO_4 is capable of extracting biochar-derived compounds. After fumigation TOC contents in the K_2SO_4 extracts were either significantly lower (W in silt loam and P in loamy sand treatments) or higher (L in loamy sand and F treatments in both soils) compared to the control treatments (Figure 3-1). These observations may be explained by a number of mechanisms. Microbial biomass C lysed through fumigation can adsorb to the biochar surface, as was observed for activated C by Durenkamp et al. (2010) and in terra preta soils by Liang et al. (2010). Such binding would lead to a decrease of OC extracted after fumigation. To account for adsorption of microbial biomass in terra preta soils, Liang et al. (2010) calculated an additional correction factor *E* for terra preta soils. Our results confirm the need to determine specific MBC correction factors, representing the extractable part of microbial biomass C, for biochar-amended soils. An increase in OC extracted after fumigation on the other hand, suggests that chloroform is capable of extracting significant amounts of organic compounds from some biochars, and/or that chloroform adsorbs significantly to some biochars and is then (partially) co-extracted by K_2SO_4, much in the same fashion as described for clay soils by Alessi et al. (2011).

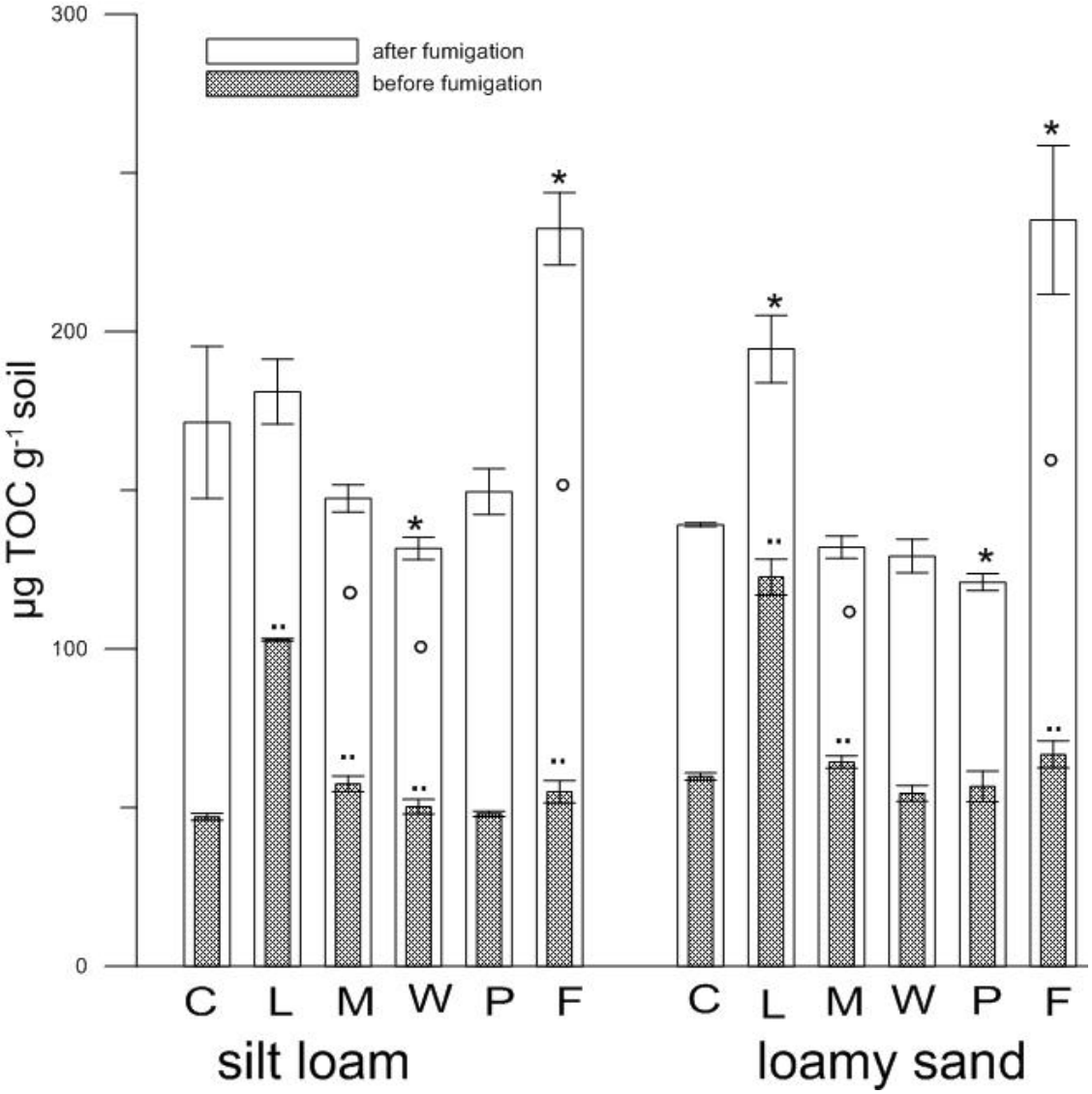


Figure 3-1 Mean TOC content (n=3) (± standard deviations) of the control soil and biochar-amended soil samples in 0.5M K_2SO_4 extracts before and after fumigation with chloroform (bars indicating TOC contents before (dotted bar) and after (open bar) fumigation both start at zero, the microbial biomass is considered as the difference between both bars) (L: poultry litter, M: maize, W: willow, P: pine and F: fast pyrolysis pine) (°Difference between the microbial biomass C of the control and biochar treatment is significant (P<0.05); ¨difference between the TOC content before fumigation of the control and biochar treatment is significant (P<0.05); *difference between the TOC content after fumigation of the control and biochar treatment is significant (P<0.05))

Extracellular enzymes such as β-glucosidase are considered to be "proximate agents of organic matter decomposition" (Sinsabaugh et al., 2008). Dehydrogenase is an intracellular enzyme linked to microbial respiratory processes of recalcitrant compounds and is correlated with active cells (Burns et al., 2013). The use of dehydrogenase and β-glucosidase to characterize soil microbial activity are thus of interest to assess the stability of biochar against

microbial degradation in soil. Adsorption of purified enzymes and substrate to biochar has been reported by Bailey et al. (2011), however possible biochar adsorption of enzymes and substrate has not been assessed in the soil yet. In both soils, the presence of biochar did not affect the β-glucosidase enzyme activity compared to the control treatment (Table 3-3). From this analysis it consequently appears that no adsorption onto the biochar surfaces took place during the β-glucosidase enzyme measurements. However, we observed a decrease in dehydrogenase activity with poultry litter biochar in the silt loam soil. Since dehydrogenase is an intracellular enzyme, adsorption of the enzyme is not a likely explanation. Dehydrogenase enzyme activity is measured as the rate of microbial transformation of soil applied tetrazolium chloride (TTC) into triphenylformazan (TPF) during 24 hours of incubation at 37°C (Moeskops et al., 2010). The decreased dehydrogenase activity in the soil samples with poultry litter biochar, could be due to adsorption of TTC or TPF onto the L biochar surface. In the loamy sand soil, there was not any microbial transformation of TTC to TPF, so the possible adsorption of TTC or TPF onto the L biochar could not be detected. In both soils, total PLFA content was not significantly different between the control samples and the soil samples that received biochar (Table 3-3).

Table 3-3 The mean values and standard deviations of dehydrogenase (n=3), β-glucosidase enzyme activityand of the total PLFAs (n=4) of the control treatments and the treatments that received biochar (L: poultry litter, M: maize, W: willow, P: pine and F: fast pyrolysis pine) just before measurements in the silt loam and loamy sand soil

Treatment	Dehydrogenase activity µg TPF g^{-1} dry soil		β-glucosidase activity µg PNP g^{-1} dry soil		Total PLFAs nmol g^{-1} dry soil	
	silt loam	loamy sand	silt loam	loamy sand	silt loam	loamy sand
Control	207.4 ± 14.5	-1.1 ± 1.8	117.8 ± 5.7	28.8 ± 8.4	25.8 ± 4.8	8.9 ± 1.3
L	170.8 ± 3.3*	1.0 ± 1.9	115.6 ± 14.3	21.2 ± 3.0	23.8 ± 4.9	7.5 ± 1.0
M	196.2 ± 23.6	2.4 ± 3.4	114.4 ± 8.2	23.8 ± 4.3	20.5 ± 6.9	8.9 ± 1.3
W	198.2 ± 27.5	1.0 ± 3.3	121.9 ± 13.2	32.0 ± 6.2	22.7 ± 3.3	8.5 ± 1.5
P	214.5 ± 6.3	3.8 ± 5.2	125.7 ± 6.2	21.1 ± 2.4	24.0 ± 6.9	8.5 ± 1.9
F	241.9 ± 71.7	1.8 ± 1.7	112.4 ± 16.5	38.1 ± 7.6	25.8 ± 2.9	8.7 ± 1.4

* Difference between control and biochar treatment is significant (ANOVA, with Dunnett post-hoc test, $P<0.05$)

Canonical discriminant analysis (CDA) was undertaken on all PLFAs of the different treatments per soil type. In this way, we assessed multivariate differentiation between *a priori*

specified groups. In both soils, the first discriminant function (D1) explained over 57% of the total variation in the PLFA dataset (Fig. 2). D1 in both soil was negatively loaded by the mol% of the 10Me17:0 and 10Me16:0 PLFAs, respectively (Standardized Canonical Discriminant Function Coefficients (SCDFC) were -20.2 and -12.7, respectively), which are both biomarkers for actinomycetes. D1 was positively loaded by 18:1ω9 (SCDFC 26.4 and 10.9, respectively), a presumed indicator for saprotrophic fungi (Buchan et al., 2012; Santos et al., 2012). In both soils, the D1 revealed little differentiation between the control and the W and M biochar treatments, implying that the presence of W and M biochar did not change the PFLA fingerprints. However, the F, P and L biochars all had more positive scores along the D1 compared to the control treatment and were clearly differentiated from the control treatments. Analysis of the concentrations of biomarker PLFAs revealed however that there were no significant differences between concentration of biomarker PLFAs, except for a decreased 18:1ω9c concentration in the L treatment of the loamy sand soil. This difference was possibly due to artefacts during analysis.

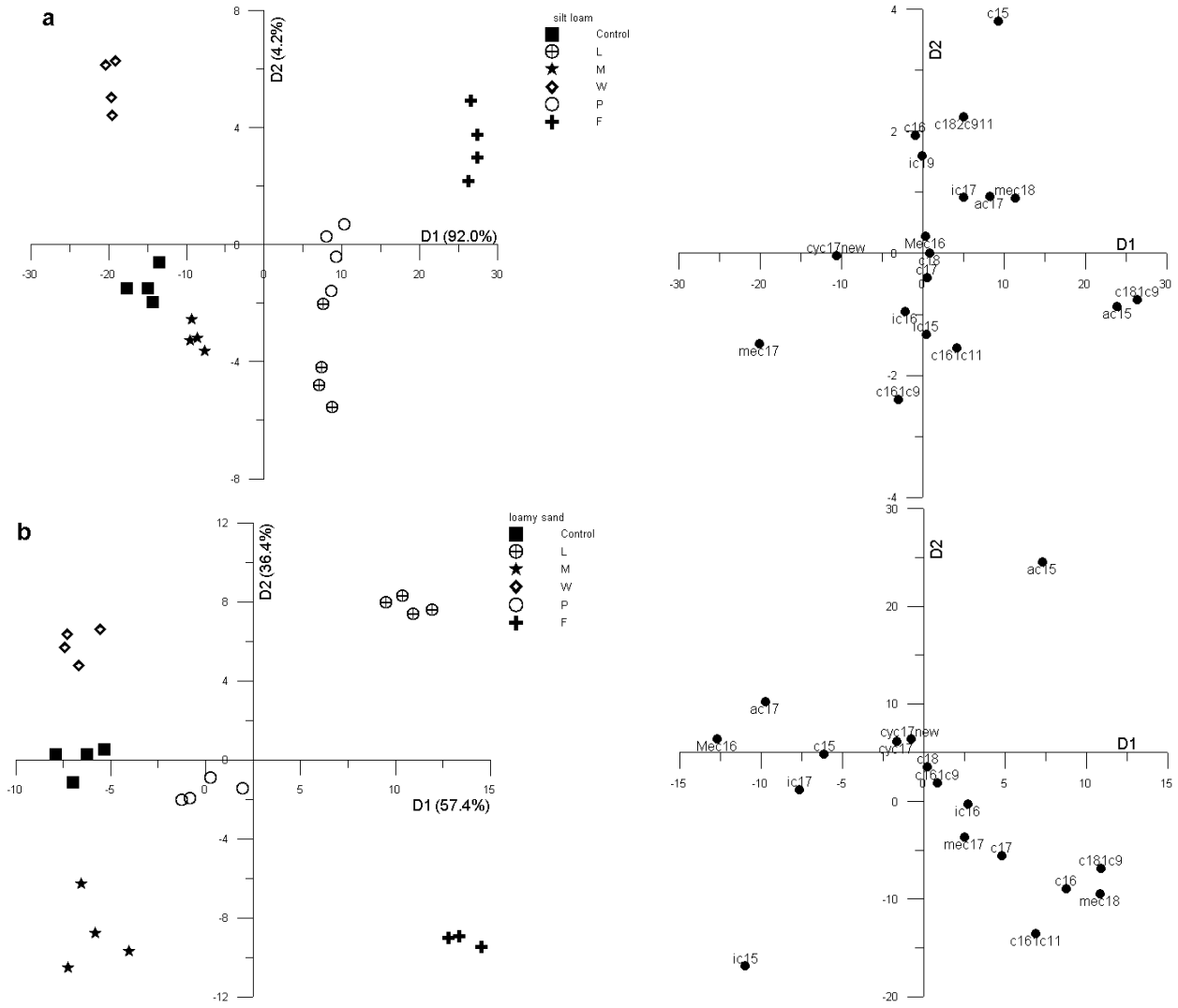


Figure 3-2 Left: CDA ordination based on mol % PLFA of individual PLFAs a) from the sandy loam soil and b) from the loamy sand soil with biochar (L: poultry litter, M: maize, W: willow, P: pine and F: fast pyrolysis pine) added just before measurement (n=4); the first and second discriminant function (D1 and D2) are depicted. Percentage of variance explained by each component is indicated within parenthesis on each axis. Right: Plot of correlation of the primary loading PLFAs with D1 and D2

Table 3-4 Concentrations of marker PLFAs (nmol g^{-1} dry soil) of the silt loam and loamy sand control soils and amended with the five biochar types (L: poultry litter, M: maize, W: willow, P: pine and F: fast pyrolysis pine)

Soil	Treatment	Concentration of marker PFLAs (nmol g^{-1} dry soil)						
		Gram-positive	Gram-negative	18:1ω9c	18:2ω6,9c	actinobacteria	AMF	Protozoa
Silt loam	control	4.72 ± 0.68	2.55 ± 0.17	2.41 ± 0.16	0.72 ± 0.06	2.56 ± 0.18	1.10 ± 0.11	0.11 ± 0.02
	L	4.38 ± 0.71	2.29 ± 0.20	2.26 ± 0.17	0.67 ± 0.07	2.28 ± 0.21	1.01 ± 0.12	0.09 ± 0.02
	M	3.85 ± 0.92	1.80 ± 0.15	1.90 ± 0.28	0.59 ± 0.09	1.99 ± 0.30	0.88 ± 0.14	0.09 ± 0.02
	W	4.07 ± 0.50	2.30 ± 0.13	2.14 ± 0.09	0.68 ± 0.07	2.21 ± 0.16	0.90 ± 0.06	0.10 ± 0.02
	P	4.48 ± 0.94	2.34 ± 0.27	2.19 ± 0.24	0.68 ± 0.08	2.32 ± 0.29	1.02 ± 0.13	0.10 ± 0.02
	F	4.74 ± 0.43	2.49 ± 0.12	2.46 ± 0.09	0.76 ± 0.07	2.53 ± 0.13	1.07 ± 0.09	0.10 ± 0.01
Loamy sand	control	2.33 ± 0.50	1.25 ± 0.06	0.44 ± 0.01	0.20 ± 0.01	0.86 ± 0.03	0.06 ± 0.01	0.01 ± 0.00
	L	1.87 ± 0.21	1.14 ± 0.07	0.35 ± 0.01*	0.18 ± 0.01	0.76 ± 0.05	0.05 ± 0.01	0.01 ± 0.00
	M	2.34 ± 0.22	1.29 ± 0.09	0.41 ± 0.01	0.19 ± 0.01	0.88 ± 0.06	0.07 ± 0.01	0.01 ± 0.00
	W	2.19 ± 0.27	1.23 ± 0.09	0.39 ± 0.02	0.19 ± 0.01	0.82 ± 0.06	0.05 ± 0.01	0.01 ± 0.00
	P	2.29 ± 0.39	1.16 ± 0.07	0.40 ± 0.03	0.17 ± 0.01	0.82 ± 0.07	0.06 ± 0.01	0.01 ± 0.00
	F	2.47 ± 0.25	1.30 ± 0.06	0.45 ± 0.01	0.22 ± 0.01	0.92 ± 0.03	0.06 ± 0.01	0.01 ± 0.00

* Indicates significant difference between biochar treatment and corresponding control (ANOVA, with post-hoc dunnett test, $P<0.05$)

3.4 Conclusions

In conclusion, the impact of the presence of biochar onto the determination of microbial soil properties was limited. Fumigation-extraction appeared to be most affected, but the effect was biochar dependent. It would appear that there is a possibility to correct for such an artefact by means of a factor (k_{EC}) for biochar-amended soils, in line with the known incomplete efficiency of the fumigation-extraction method. On the other hand, increased OC contents after fumigation in the poultry litter and fast pyrolysis pine biochars may indicate adsorption of chloroform and/or dissolution of specific biochar compounds by chloroform. This would render the fumigation/extraction MBC method incompatible with biochar-amended soil, but specific in depth spectroscopic analysis would be required for confirmation. No differences between the total PLFAs were observed between the control and the biochar-amended treatments. Therefore, we advise that the sum of the PLFAs is a better parameter than fumigation-extraction to express total microbial biomass. By canonical discriminant analysis little or no difference between the control and the maize and willow biochars was observed. Additionally, no significant differences between the concentration of biomarker PLFAs were observed between the control and the biochar-amended treatments, except for a decreased concentration of 18:1ω9c in the L treatment of the loamy sand soil. We would therefore suggest to use PLFA 18:2 ω6,9c as a fungal biomarker in future research. The presence of biochar had only a limited impact on the efficiency of microbial activity measurements of dehydrogenase and β-glucosidase. It is clear that the compatibility of soil microbial experiments with biochar therefore depends on biochar type. An expansion to this research is needed to see whether these effects can be extrapolated to other types of biochars, and whether these effects fade out or increase with biochar aging in soil.

Acknowledgement

We acknowledge the Interreg IVb North Sea Region project: ‘Biochar climate saving soils’ for providing us with the W and P biochars. We thank Greet Ruysschaert and Victoria Nelissen of the ILVO (Merelbeke, Belgium) for providing the M biochar and Dane Dickinson and Güray Yildiz for the provision of the F biochar.

Chapter 4:

CO_2 emissions and microbial community shifts after biochar addition – Short-term effects

Compiled from:

Ameloot, N., De Neve, S., Jegajeevagan, K., Yildiz, G., Buchan, D., Funkuin, Y.N., Prins, W., Bouckaert, L., Sleutel, S., 2013. Short-term CO_2 and N_2O emissions and microbial properties of biochar-amended sandy loam soils. Soil Biology and Biochemistry 57, 401-410.

Abstract

Biochar produced during pyrolysis of biomass has the potential to reduce greenhouse gas (GHG) emissions from soils. In order to evaluate the effect of four different biochar additions on the emission of CO_2 an incubation experiment was established in a temperate sandy loam soil. Digestate, a waste-product of the wet fermentation of swine manure, and willow wood was slowly pyrolyzed at 350°C and 700°C, yielding four biochar types (DS350, DS700, WS350 and WS700). In the first incubation experiment (117 days), C mineralization was monitored in soil amended with biochar at a quantity of 10 Mg ha^{-1} on an area-basis (biochar to soil ratio of 1:69 on a mass basis) at 50% water filled pore space (WFPS). CO_2 emissions from the 350°C biochar treatments were significantly higher than the control (no biochar) treatment, while we observed no significantly different net C mineralization in the treatments with the 700°C biochars compared to the control. After fitting a combined first plus zero order model to the cumulative C mineralization data, the parameter for the easily-mineralizable C pool (C_0) positively correlated with the volatile matter (VM) contents of the biochars. Microbial biomass C consistently increased due to all biochar additions, while the dehydrogenase activity increased in the 350°C biochar treatments but decreased in the 700°C biochar treatments. Principal component analysis (PCA) of the extracted phospholipid fatty acids (PLFAs) demonstrated that divergent microbial community structures established after the addition of all biochars. The markers for Gram-positive and Gram-negative bacteria and actinobacteria were more abundant in the 350°C biochar treatments compared to the control and to the other biochar treatments. This study suggests that volatile matter content could be an important property of biochars in explaining short-term CO_2 emissions from biochar-amended soils.

4.1 Introduction

The search for new technologies to mitigate climate change has led to a number of creative ideas, among which the pyrolysis of biomass residues seems to hold considerable potential. During this pyrolysis process in the absence of oxygen biomass residues are converted into bio-oil, biochar and syngas, which are used as renewable energy sources. Recently, biochar has gained much attention as a soil amendment (Forbes et al., 2006; Lehmann et al., 2006; Fowles, 2007). Next to the high C sequestration potential of this product, several studies have shown improved chemical, physical and biological soil conditions and related higher productivity rates in biochar-amended soils (Glaser et al., 2002; Chan et al., 2007; Jeffery et al., 2011; Lehmann et al., 2011). Although biochar was initially considered to be inert to biological and chemical degradation, recent studies suggest that biochar experiences a biphasic decomposition when added to soil (Kuzyakov et al., 2009; Cross and Sohi, 2011). For example, two months after rye grass biochar additions degradation rates dropped from 2% to 0.2% (Kuzyakov et al., 2009). In general, labile compounds in the biochars consist of a wide array of relatively small molecules, including *n*-alkanoic acids, hydroxy and acetoxy acids, benzoic acids, diols, triols, and phenols (Graber et al., 2010) and are degraded rapidly, while the stable aromatic compounds are decomposed at a slower rate (Zimmerman, 2010; Foereid et al., 2011). Biochar stability and the interaction between biochar and soil biota varies depending on the biochar feedstock, the production method (gasification vs. pyrolysis (slow and fast), hydrothermal carbonization, catalytic fast pyrolysis), the pyrolysis residence times and the pyrolysis temperature (Lehmann et al., 2011).

As the soil micropores (<3µm) provide protection against bacterial grazing (Hassink et al., 1993; Strong et al., 2004), it has been suggested that the porous structure of biochar likewise provides an aerated habitat in which soil bacteria are able to flourish safely (Lehmann et al., 2011). Additionally, the adsorption of allelopathic molecules from the soil solution that may otherwise inhibit microbial activity onto the biochar surface likewise influences soil microorganisms (Wardle et al., 1998; Elmer and Pignatello, 2011).

To produce the biochars, a digestate feedstock from a biogas installation and a woody feedstock (willow wood) were used. Manure processing is rapidly increasing in many regions with intensive livestock production, like Flanders, the Netherlands and Denmark in order to reduce nutrient inputs to soil, nitrate leaching and eutrophication problems (Lemmens et al., 2007). Anaerobic digestion or wet fermentation of manure for biogas production, probably is

the most frequently used manure processing technique and produces a digestate (Lemmens et al., 2007). Via the wet fermentation of manure 813.000 Mg digestate $year^{-1}$ is produced, of which on a yearly basis 22.000 Mg is dried by recuperating the heat produced during the fermentation process (Vlaco, 2011). Given the rapid increase in production of these manure digestates, the pyrolysis of these feedstocks to biochar is a potential further step in the processing of manure. While hydrothermal carbonization (typically at low temperatures over an aqueous solution of biomass) of maize digestate (from the biogas production via wet fermentation) has been reported (Mumme et al., 2011), pyrolysis of manure digestate to biochar and application of these biochar types to soils has not been done before.

The first objective was to test if CO_2 emissions and microbial properties from soils amended with biochar were determined by the type of feedstock and the pyrolysis temperature. The second objective was to link the CO_2 emissions under the different scenarios to the biochar properties and the microbial and chemical soil properties. To this end, we measured the CO_2 emissions from unamended soil and soil amended with biochar from two contrasting feedstocks (manure digestate and wood), produced at two contrasting temperatures (slow pyrolysis at 350°C and 700°C). After the incubation experiment we also measured microbial biomass, activity and community structure of the treatments with the four biochars and the control.

4.2 Material and methods

Soil characteristics

An Inceptisol (WRB classification) with sandy loam soil texture (clay <2µm: 7%, silt 2-50µm: 44% and sand 50-2000 µm: 49%), representative for intensive agriculture in Flanders was collected to a depth of 30 cm from an arable field in Lendelede, Belgium. The soil had a SOC content of 0.73 %, a total N content of 0.063 %, pH_{H2O} (1:5) 6.4, and a bulk density of 1.6 g cm^{-3}.

Biochar production and characterization

For this study, biochar samples were produced from a wood feedstock from the municipal park maintance service of the city of Ghent, namely willow wood (*Salix dasyclados*)

and from one swine manure digestate feedstock (Biogas Tec, Belgium). The feedstock samples were oven dried at a temperature of 60°C for at least 24 hours. Both feedstocks were slowly pyrolyzed at 350°C and 700°C in the laboratory of thermo-chemical conversion of biomass (LTCB) at the department of Biosystems Engineering (Ghent University), yielding four types of slow pyrolysis biochar (DS350, DS700, WS350 and WS700). The temperatures were specifically selected to include the extremes at which slow pyrolysis could be undertaken. The slow pyrolysis unit consisted of a cylindrical furnace with a 30 cm high vertical stainless steel reactor (inner diameter of 3.6 cm). The biomass was added into the reactor tube to a height of about 25 cm and flushed with N_2 at a gas flow rate of 800 ml min^{-1}. After a residence time of 10 minutes at the selected temperature, the reactor tube was cooled and the produced biochar was collected, weighed and stored in polypropylene containers at temperatures of about -18°C. All samples were analyzed for total C and N contents by catalytic combustion (Variomax CNS analyser, Elementar, Germany). The pH_{H2O} was determined by weighing 1 g of biochar and adding 10 ml of H_2O, well mixed and measured after 18 hours with a pH electrode (Thermo Orion, 420A plus). Moisture content, volatile matter and ash content were determined by the ASTM D1762-84 standard testing method. All analyses were carried out in duplicate. The surface area and total pore volume of the biochars were determined by the BET method using a TriStar 3000 analyzer (micromeritics) at the Department of inorganic and physical chemistry, Ghent University.

C mineralization experiment

Soil mesocosms with 250 g of air dried soil were prepared in 6.8 cm diameter PVC tubes. The application rate of 10 Mg fresh biochar ha^{-1} was equivalent to the addition of 3.63g biochar per mesocosm (based on surface area ratio). Given the filling height of 5 cm of soil in the tubes, this corresponded to a biochar to soil ratio of 1:69. Soil was thoroughly mixed with the biochar and the mixture was filled in the tubes, and slightly compacted to obtain a bulk density of 1.4 g cm^{-3}. There were three replicates per biochar treatment. A control treatment (i.e. soil without biochar addition) in triplicate was also included. After biochar addition, demineralized water was added to the soils to achieve a fixed moisture content of 50 % WFPS. The soil columns were placed in closed glass jars and put in an incubation cabinet at 25°C. The emitted CO_2 was trapped in 15 ml 1M NaOH. At day 1, 3, 5, 7, 10, 13, 17, 20, 24, 27 and 31 the vials with NaOH were removed and titrated with HCl in the presence of $BaCl_2$. After

30 incubation days the samples were measured weekly. The water content of the mesocosms was adjusted weekly in order to maintain a WFPS of 50 %.

At the end of the incubation (after 117 days) the microbial biomass (MB), activity and community structure was measured. Dehydrogenase activity (DA) was determined following a procedure described in Moeskops et al. (2010). Phospholipid fatty acids (PLFAs) were extracted using a adjusted Bligh and Dyer (1959)- technique, modified by Moeskops et al. (2010). MB was determined as the sum of the PLFAs. The nomenclature of fatty acids and their contribution as specific biomarkers for the soil microbial groups (Gram-positive and Gram-negative bacteria, fungi, arbuscular mycorrhiza fungi (AMF), protozoa and actinomycetes) were adapted from Moeskops et al. (2010). For Gram-positive bacteria the sum of *i*15:0, *a*15:0, *i*16:0, *a*16:0, *i*17:0 and *a*17:0 was used. The PLFAs cy17:0 and cy19:0 were considered to be typical for Gram-negative bacteria, while the 20:4 and 20:5 PLFAs were indicative for protozoa (Buchan et al., 2012). The sum of 10Me16:0 and 10Me18:0 was regarded as a measure for the actinomycetes. The total bacterial community was proportional to the sum of the marker PLFAs for Gram-positive and Gram-negative bacteria, plus15:0 and 17:0 (Buchan et al., 2012). The PLFA 18:2ω6,9c was considered as an indicator for saprotrophic fungi and 16:1ω5 as the indicator for AMF (Joergensen and Wichern, 2008). At the end of the incubation, pH_{H2O} (1:5) was measured.

Data analysis

The cumulative C mineralization, C(t) was plotted against the time (t) and a parallel first plus zero order kinetic model was fitted to the data using the Levenberg-Marquardt algoritm:

$$C(t) = C_0\left(1 - EXP\left(-k_f * t\right)\right) + k_s * t \qquad (4\text{-}1)$$

This model assumes that the organic matter consists of an easily-mineralizable C pool (C_0), which is mineralized according to first-order kinetics, and a more resistant fraction that is mineralized according to zero-order kinetics (Sleutel et al., 2005). In equation 4-1, k_f is the mineralization rate of the fast degradable C pool and k_s the mineralization rate of the slow C pool (Sleutel et al., 2005). Because we fitted through all replicates at each measuring time, the fit yields the parameters ± its standard error. Net C mineralization ($C_{min\ net}$) was calculated as explained in Chapter 2 (equation 2-2).

Differences between biochar properties were assessed via one-way ANOVA and Tukey's post-hoc tests. Principal component analysis (PCA) was also undertaken on the nmol % composition of all PLFAs present in a proportion of more than 1 % of the total amount of PLFAs. Treatment effects on cumulative CO_2 emissions, dehydrogenase activity and MB were assessed by one-way ANOVA and Tukey's post-hoc testsPearson correlation analyses were carried out between the net C mineralized and the soil biological measures and biochar characteristics. All statistical analyses were carried out with IBM SPSS Statistics 21 (SPSS inc., Chicago, USA).

4.3 Results

Biochar production and characterization

The fraction recovered as biochar after pyrolysis (biochar yield), C and N content, ash and volatile matter content, pH and the results of the BET analysis for the four biochars are given in Table 4-1. Biochar yield decreased with increasing pyrolysis temperature. A higher recovery at 350°C may be attributed to a minimal condensation of aliphatic compounds, and limited losses of CH_4, H_2 and CO (Demirbas, 2004). The C:N ratios of the digestate biochars were lower than the C:N ratios of the willow wood biochars, while the C:N ratios of the biochars were highest at the highest pyrolysis temperature. The ash contents were highest in the digestate biochars, while the volatile matter contents of biochars decreased with increasing pyrolysis temperature. Volatile matter contents of the biochars, produced under the same pyrolysis temperatures, were in the same range (20-30% for the 350°C biochars, 10-15% for the 700°C biochar). The pH_{H2O} of the digestate and WS700 biochars was highest, while the WS350 had a lower pH_{H2O}. The surface area and pore volume was lower in the wood biochars than in the digestate biochars, while the average pore size was higher in the wood biochars. The surface area, pore volume and average pore size increased with increasing pyrolysis temperature.

Table 4-1 Percentage biochar yield, C and N content, C:N ratio, moisture content, ash content, volatile matter, and pH_{H2O} of the produced biochars (mean values±standard deviation, n=2), surface area and average pore size (measured via BET analysis). (Different letters indicate significant differences between the C and N contents, C:N ratio, moisture, ash and volatile matter contents and pH of the different biochars).

Biochar type	Biochar yield (%)	C content (%)	N content (%)	C:N (-)	Moisture content (%)	Ash content (%)	Volatile matter (%)	pH_{H2O} 1:10 (-)	Surface area (m^2 g^{-1})	Average pore size (nm)	Pore volume (mm^3 g^{-1})
DS350	61	39.7±0.6^{b}	2.15±0.02^{b}	18.4±0.6^{a}	4.23±0.24^{b}	2.48±0.64^{b}	29.19±0.44^{b}	10.1±0.27^{b}	1.32	3.89	4.35
DS700	31	35.6±1.9^{a}	1.03±0.02^{a}	34.5±1.9^{b}	3.04±0.06^{ab}	2.75±0.22^{b}	10.37±1.89^{a}	11.6±0.04^{c}	9.02	6.74	15.66
WS350	59	67.1±0.05^{c}	1.06±0.006^{a}	63.2±0.05^{c}	4.20±0.33^{b}	0.23±0.03^{a}	35.64±1.33^{c}	8.1±0.19^{a}	0.68	4.21	1.01
WS700	35	80.3±0.02^{d}	1.11±0.05^{a}	74.9±0.06^{d}	2.85±1.00^{ab}	1.28±0.15^{ab}	14.07±0.01^{a}	11.1±0.42^{bc}	2.17	7.06	5.09

C mineralization from biochar-amended soils

After 117 days of incubation, the cumulative CO_2 emissions from the 350°C biochar treatments were higher compared to the control treatment and the other biochar treatments (Figure 4-1). Although the error bars overlap, a lower 117-days cumulative CO_2 emission was observed in the DS700 biochar treatments compared to the control, while the WS700 biochar treatments and the control had a similar CO_2 emission pattern.

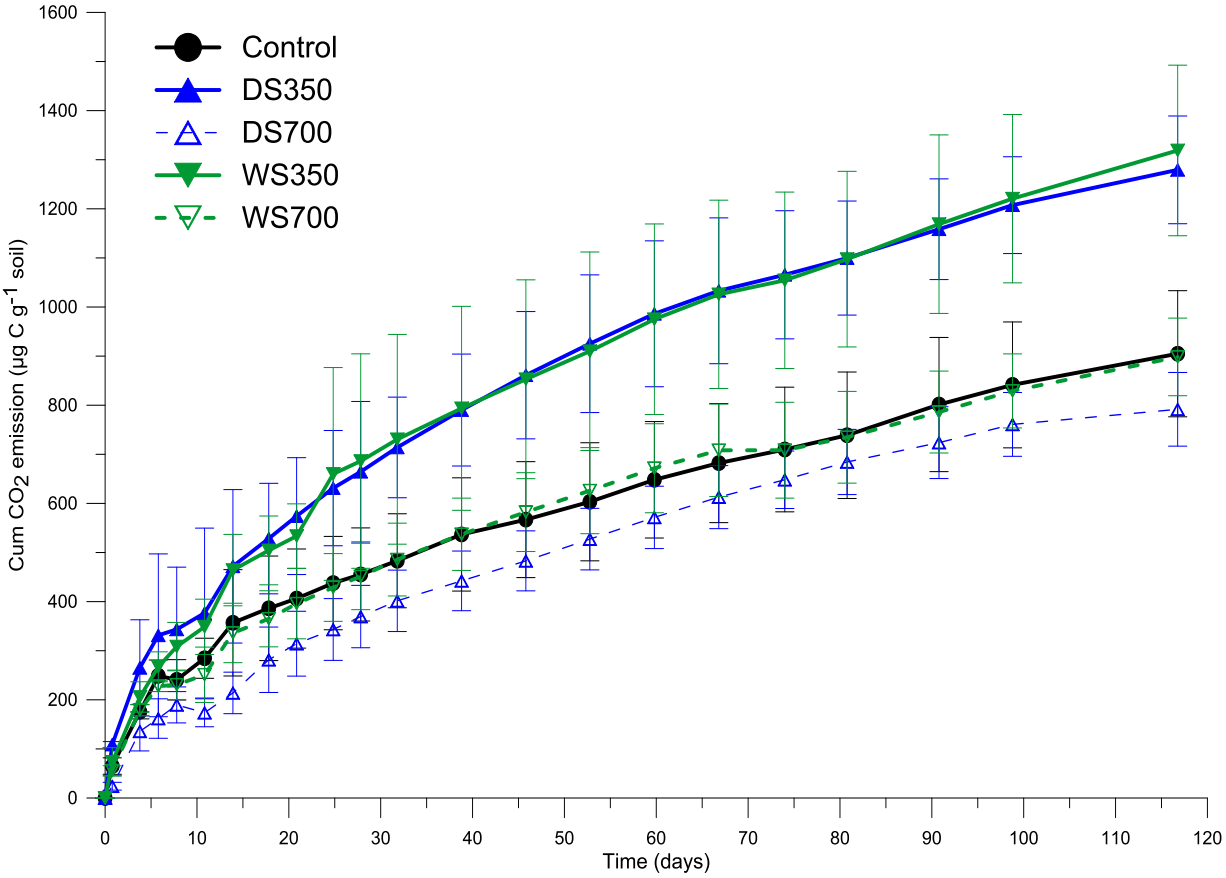


Figure 4-1 Cumulative C mineralization (µg C g^{-1} soil) from the different treatments after 117 days (C mineralization experiment). Error bars indicate standard deviations (n=3).

A significant positive correlation existed between the parameter C_0 (Table 4-2) and the volatile matter content of the biochars (n=4, r=0.99, P<0.01). At the end of the incubation, the net amount of C mineralized (expressed as a percentage C present in added biochar, see

equation 2-2) was significantly higher than zero in the 350°C biochars. $C_{net\ min}$ was highest in the DS350 treatment, followed by the WS350, DS700, and WS700 treatments (Table 4-2).

Table 4-2 Parameters of the parallel first and zero order kinetic model of the C mineralization data±standard errors of the regression (equation 4-1) and the net mineralized C ($C_{net\ min}$) (equation 2-2). Different letters indicate significant differences after one way ANOVA and post-hoc tukey tests (P<0.05), *indicate that $C_{net\ min}$ is significantly different from 0 (P<0.05)

Biochar type	C_0 (mg 100 g^{-1})	k_f (day^{-1})	k_s (mg 100 g^{-1} day^{-1})	$C_{net\ min}$ (%)
Control	32.2±3.2	3.63±0.33	1.04±0.28	-
DS350	55.4±2.1	4.65±0.19	0.62±0.05	7.82±0.33*[c]
DS700	33.9±2.8	3.57±0.26	0.70±0.12	0.72±1.29[a]
WS350	61.0±10.5	4.25±0.87	0.46±0.13	3.77±0.20*[b]
WS700	35.5±3.1	3.37±0.29	0.71±0.13	0.22±0.51[a]

PCA of the major PLFAs (all PLFAs present in a proportion of more than 1 % of the total amount of PLFAs) satisfactorily discriminated the different biochar treatments from each other and from the control. The first two principal components (PCs) together explained 65.8 % of the total variation in the data (Figure 4-2a). The PCs correlated meaningfully with the individual PLFAs (loading plot in Figure 4-2b). High negative loadings on the PC1 axis, as was the case for the DS350, WS350, corresponded to high contents of a15:0, i15:0 and i16:0 all marker PLFAs for Gram-positive bacteria. The PLFAs 10Me16:0 (an indicator for actinomycetes), and 20:4 and 20:5 (proposed indicators for protozoa) positively loaded PC1 (48.7% of total variation). PC2 explained 17.1 % of the variation in the data with mainly positive loadings from 16:1ω5 (AMF), 16:1ω7 and 18:1ω7, and negative loadings from 16:0. PC2 primarily discriminated both digestate biochar treatments from all other treatments, including the control. The biomarker for Gram-negative bacteria cy19:0 was absent in all treatments, while cy17:0 was absent in the 700°C biochar treatments.

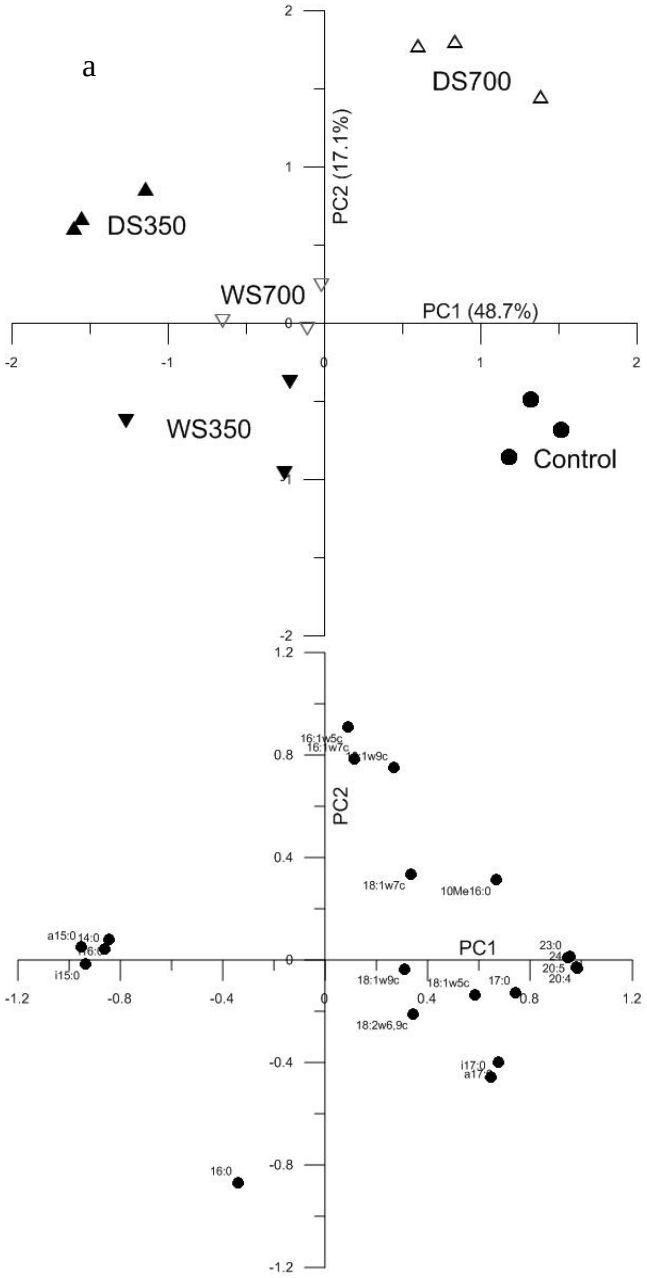


Figure 4-2 a) PCA ordination based on mol % PLFA of individual PLFAs from the biochar-amended and control samples; the first and second principle components are given. Percentage of variance explained by each component is indicated within parenthesis on each axis. b) Plot of correlation of the primary loading PLFAs with PC1 and PC2

Compared to the control, dehydrogenase enzyme activity was significantly ($P<0.05$) higher in the 350°C biochar treatments, while it was lower in the 700°C biochar treatments (Figure 4-3).

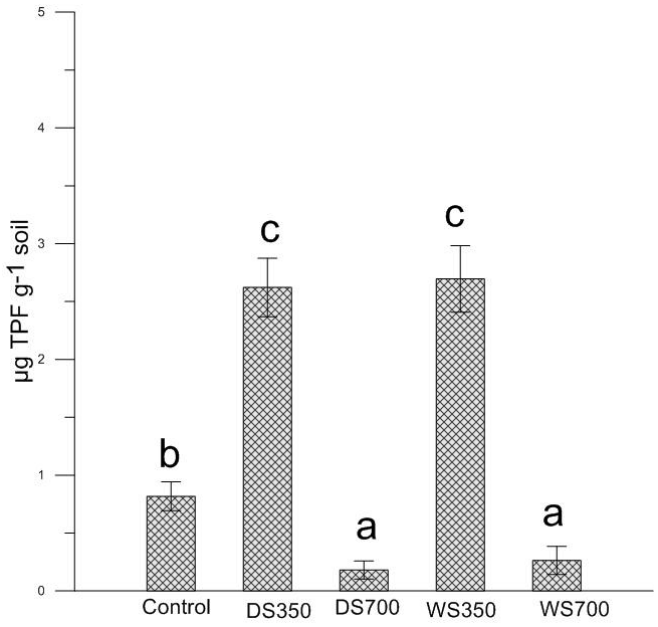


Figure 4-3 Dehydrogenase enzyme activity (µg TPF g^{-1} soil) of the different biochar treatments and the control treatments after 117 days. Error bars indicate standard deviations (n=3).

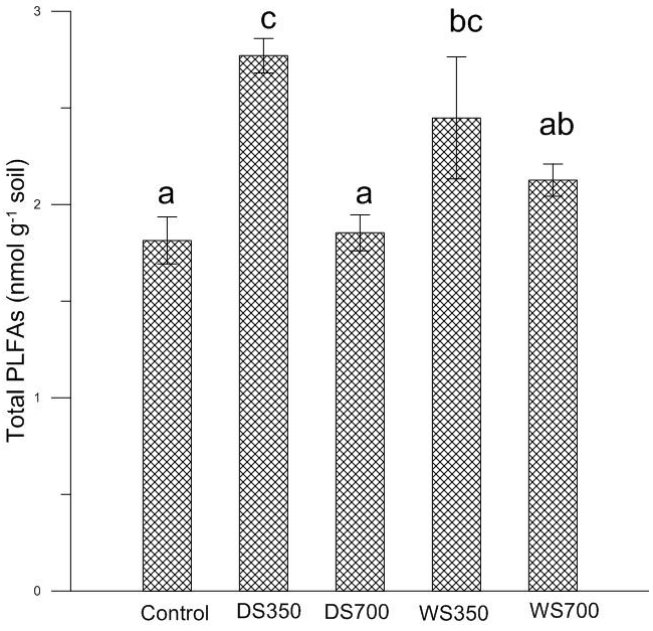


Figure 4-4 Microbial biomass represented as sum of the PLFAs (nmol PLFAs g^{-1} soil) of the different biochar treatments and the control after 117 days. Error bars indicate standard deviations (n=3).

Table 4-3 The pH_{H2O} (1:5). Different letters indicate significant differences between the treatments (P<0.05)

Treatment	pH_{H2O}
control	5.97±0.01[a]
DS350	7.15±0.04[e]
DS700	7.51±0.01[e]
WS350	6.45±0.03[b]
WS700	6.88±0.07[c]

At the end of the incubation experiment pH had increased in all biochar treatments (Table 4-4), with the lowest pH in the WS350 treatment, followed by the WS700, DS350 and DS700 treatments.

Table 4-4 Pearson's r correlations (n=15) between the dehydrogenase activity (DA) (µg TPF g^{-1}), the microbial biomass (MB) (nmol PLFAs g^{-1}), the cumulative C mineralization (Cmin) (µg CO_2-C g^{-1}) and the pH_{H2O} at the end of the incubation experiment.

	C-min	MB	pH_{H20}
DA	0.929**	0.792**	-0.200
Cmin		0. 896**	0.194
MB			0.167

**Correlation is significant (P<0.01) (no additional correlations were significant at the P<0.05 level).

The dehydrogenase activity correlated positively with the cumulative amount of emitted CO_2 and with the MB (Table 4-3). Biochar application only significantly increased MB (P<0.05) for the DS350, WS350 (Figure 4-4). The cumulative amount of emitted CO_2 was positively correlated with the MB (Table 4-3).

4.4 Discussion

Feedstocks for producing biochar will have alternative uses in most cases. It is therefore important to question the rationale for using a particular feedstock for making biochar. We used feedstocks from willow wood and swine manure digestate. The willow wood was obtained from a municipal park maintenance service and its alternative use is in the

production of green waste compost that is commonly applied to agricultural land. In this sense, the final destination is similar as for the biochar, and there is no competition for e.g. bioenergy production. The swine manure digestate needs to be dried prior to pyrolysis, which obviously is an energy consuming step. However, drying is a mandatory step in manure processing in Flanders, irrespective of its final destination. At this moment, much of the dried digestate is exported, and biochar production from digested swine manure does not require additional energy consumption (as compared to alternative uses) prior to pyrolysis.

The application rate of 10 Mg of biochar ha^{-1} was area-based, and was equivalent to a biochar to soil ratio of 1:69 on a mass basis in the incubation tubes (5 cm). Field incorporation of biochar at the same concentration to a depth of e.g. 25 cm would result in an application rate of 50 Mg ha^{-1}. Other authors have applied similar or even higher amounts of biochar, e.g. 40 Mg ha^{-1} (Augustenborg et al., 2012), 50 Mg ha^{-1} (Chan et al., 2008), 90 Mg ha^{-1} (Zimmerman et al., 2011) and 180 Mg ha^{-1} (Zavalloni et al., 2011). Such application rates are high, but biochar application does not need to be repeated yearly because of its recalcitrance in soil.

Enhanced C mineralization of biochar-amended soils may be due to (i) biochar consumption by microorganisms, to (ii) increased native SOM mineralization (priming) or to (iii) abiotic release of biochar-C (Bruun et al., 2008; Cross and Sohi, 2011; Foereid et al., 2011; Jones et al., 2011). In our study, we observed higher net C mineralization from low temperature biochars compared to the control and the treatments with high temperature biochars, which has been previously observed by others others (Zimmerman, 2010; Cross and Sohi, 2011). Furthermore, volatile matter content decreased with increasing pyrolysis temperature. According to Ronsse et al. (2013) slow pyrolysis subjects the original feedstock to a series of devolatilization reactions, while progressively leaving behind an increasingly condensed biochar matrix. In this way lower amounts of volatile compounds are retained in the biochar matrix at higher pyrolysis temperatures. Additionally, some initially devolatilized compounds may recondense in the aromatic biochar matrix (Imam and Capareda, 2012; Kloss et al., 2012).

The model parameter for the easily-mineralizable C pool (C_0) was positively correlated with the higher volatile matter content in these biochars. This correlation should be interpreted with caution, because the volatile matter content was much larger than both the modeled easily-mineralizable C pool and the amount of C mineralized over the entire incubation. However, if this correlation is causal, it suggests labile biochar components were

the driver of increased (net) C mineralization in soils amended with low temperature biochars. The higher net C mineralization in the low temperature treatments could be both abiotically and microbially driven. For the treatments with the low temperature biochars, the higher C mineralization was accompanied by an increase in MB and in dehydrogenase activity. These higher values suggest that the higher CO_2 emissions were due to microbial decomposition of biochar. Dehydrogenase is an intracellular enzyme participating in the processes of oxidative phosphorylation of microorganisms, assumed to be linked with microbial respiratory processes (Alef and Nannipieri, 1995; Insam, 2001) and has often been correlated to the availability of organic matter in the soil (Serra-Wittling et al., 1995; Moeskops et al., 2010). It is probably the higher availability of volatiles in the low temperature biochars, which triggered these higher dehydrogenase enzyme activities. In a sandy loam soil with a texture comparable to the soil we used, increased enzyme activities of β-glucosidase and β-N-acetylglucosaminidase were observed after the addition of 2 % (w:w) fast pyrolysis 500°C biochar, with 40 % volatile compounds (Smith et al., 2010; Bailey et al., 2011). These and our results suggest volatile compounds of the biochars to be involved in the enzymatic stimulation after biochar additions to the soil. Bailey et al. (2011) suggested that enzymes may be inactivated in biochar-amended soils, by blocking or absorption of the substrate. A much lower dehydrogenase activity in the high temperature biochar treatments compared to the unamended control soil would support this hypothesis. Priming of native SOM might just as well explain the higher emissions in the lower temperature biochar treatments. Unfortunately, the study set-up did not allow us to discriminate between native SOM mineralization and biochar mineralization.

The PCA of the relative PLFA concentrations after 117 days of incubation resulted in a clear discrimination between most treatments, showing that different microbial communities were established after the addition of the different biochars. The PCA showed that Gram-positive bacteria were relatively more abundant in the low temperature slow pyrolysis biochar treatments. Actinomycetes and protozoa were relatively more abundant in the control treatment and the DS700 treatment. The second PC differentiated the digestate biochar treatments from the other treatments. It is likely that lower values of the 16:0 PLFA, universally occurring in the membranes of all organisms (Denef et al., 2009), in the digestate biochar treatments were mainly responsible for the high positive loadings on the PC2. The few published studies on the effect of biochar on PLFA soil profiles showed divergent microbial community structures after several biochar additions compared to control

treatments (Birk et al., 2009; Steinbeiss et al., 2009). Unlike Birk et al. (2009), we did not find a significant positive correlation between the soil pH and the Gram-negative bacterial abundance, but the absence of Gram-negative bacteria in the 700°C treatments matches with the decreased Gram-negative abundance in charcoal amended tropical soils (Birk et al., 2009). Steinbeiss et al. (2009) reported increasing Gram-negative bacterial abundance in an agricultural loamy soil with hydrothermal biochars compared to the control, with a higher increase in the treatment with the least stable material. Likewise we observed higher Gram-negative abundance in the treatments with 350°C biochars. This suggests that the occurrence of Gram-negative bacteria in biochar treatments is closely related to the content of easily-available substrate (VM) in the biochar. All biochar treatments increased the Gram-positive bacterial abundance in our study, except the DS700 treatment. Increased abundance of Gram-positive bacteria due to the presence of biochar was also observed by Birk et al. (2009) and (Steinbeiss et al., 2009). Despite the absence of plants, we observed a remarkable increase in the 16:1ω5 AMF marker PLFA in the low temperature biochar treatments compared to the control treatment. Furthermore, a positive non-parametrical correlation between the concentration of 16:1ω5 PLFA and the pH ($n=15$; $r=0.713$; $P<0.01$), suggests that the AMF stimulation was linked to the liming potential of the biochars. Similar conclusions have been drawn by Warnock et al. (2007) through reviewing the available literature dealing with the effect of biochar on AMF. However, as a cautionary remark, it should be emphasized that the PLFA 16:1ω5 is a poor indicator of AMF in soils with a high bacterial biomass (Frostegård et al., 2011), as was the case in our data.

4.5 Conclusions

This study demonstrates that temperatures of slow pyrolysis shape the differences in CO_2 emission from slow pyrolysis biochar-amended soils, independently from the biochar feedstock. CO_2 emissions were higher in the 350°C biochar treatments compared to the 700°C biochar treatments. Pyrolysis at 350°C resulted in biochars with higher volatile matter contents than the corresponding 700°C biochars. This volatile matter content of the biochar was correlated to the short-term CO_2 emissions from soils amended with biochar. Moreover microbial biomass and activity was higher in the soils with the 350°C biochars compared to the other treatments, which suggests that the soil microorganisms were stimulated by the provision of a readily available substrate. Therefore we suggest that this is an important biochar property, influencing the differences in the short-term emissions from biochar-amended soils. The correlations observed here between volatile matter and CO_2 emissions, however, should be interpreted with caution and are only tentative. Microbial biomass and dehydrogenase enzyme activities were clearly correlated to C mineralization. All biochars, except the DS700, increased the abundance of Gram-positive bacteria, while there was a higher abundance of Gram-negative and Gram-positive bacteria and actinobacteria in the 350°C biochar treatments than in the corresponding 700°C biochar treatments.

Chapter 5:

Biochar to soils with contrasting SOM levels: effects on N mineralization and biological properties

Compiled from

Ameloot, N.; Sleutel, S.; Das, KC; Jegajeevagan, K., De Neve, S. 2013. Biochar amendments in soils with contrasting SOM levels: effects on N mineralization and biological properties. Global Change Biology Bioenergy. In press.

Abstract

Four biochar types, produced by slow pyrolysis of poultry litter (PL) and pine chips (P) at 400 or 500°C, were added to two adjacent soils (L and H) with contrasting soil organic matter (SOM) content (8.9 vs. 16.1 g C kg^{-1}, respectively). The N mineralization rate was determined during 14-weeks incubations and assessments were made of the microbial biomass C, dehydrogenase activity and the microbial community structure (PLFA-extraction). The addition of PL biochars increased the net N mineralization (i.e. compared to the control treatment) in both soils while for treatments with P biochars net N immobilization was observed in both soils. Increasing the pyrolysis temperature of both feedstock types led to a decrease in net N mineralization. The ratio of Bacterial to Fungal PLFA biomarkers also increased with addition of biochars, and particularly in the case of the 500°C biochars. Next to feedstock type and pyrolysis temperature, SOM content clearly affected the assessed soil biological parameters, viz. net N mineralization or immobilization, microbial biomass (MB) and dehydrogenase activity (DA) were all greater in the H soil. These increased rates of N mineralization and biological soil parameters in the H soil compared to the L soil might be explained by increased biochar degradation and/or priming of native SOM. However, when considering the H soil's double C and N content, these responses were disproportionally small, which may be partly due to the L soil's somewhat more labile SOM. Nonetheless increasing SOM content and microbial biomass and activity generally appears to result in greater mineralization of biochar. Additionally, higher N mineralization after PL addition to the H soil with lower pH than the L soil can be due to the liming effect of the PL biochars.

5.1 Introduction

Aromatic compounds formed during pyrolysis of biomass, are the main components responsible for building up a stable C pool in the soil upon biochar addition. Yet, biochar is not biologically inert when added to the soil and follows a biphasic mineralization pattern, with the more labile biochar compounds being mineralized rapidly, after which biochar degradation continues at a much slower rate (Cross and Sohi, 2011; Ameloot et al., 2013b). Due to the consumption of these labile biochar compounds and presumed changes of the physicochemical soil habitat (Lehmann et al., 2011), soil biological parameters, such as microbial enzyme activity (Bailey et al., 2011; Ameloot et al., 2013a), biomass (Durenkamp et al., 2010) and community structure are affected (Steinbeiss et al., 2009; Santos et al., 2012).

Soil nitrogen (N) mineralization rates have been found to be affected by biochar amendments. Biochar, and particularly manure based biochars, can be a source of N for plants (Gaskin *et al.*, 2008; Nelson *et al.*, 2011). The addition of manure based biochar, with high N contents has been found to result in net N mineralization (Schouten et al., 2012; Wang et al., 2012). However, N mineralization rates might be reduced by the adsorption of NH_4^+ or NO_3^- onto the biochar surface due to increased cation exchange capacity (CEC) or anion exchange capacity (AEC) (Clough and Condron, 2010). On the other hand, net N immobilization is possible when biochar compounds with a high C:N ratio are microbially degraded, as has been shown many times for other organic amendments (e.g. by Mengel (1996)) and for biochar by Bruun et al. (2012) and Dempster et al. (2012). The type of biochar and especially the C:N ratio of biodegradable biochar compounds seems thus to be important when assessing N mineralization/immobilization after biochar additions to soils. Besides the C:N ratio of organic amendments, also the pH seems to play an important role in the stimulation of microorganisms involved in N mineralization (Curtin et al., 1998). As N mineralization rates have frequently been found to be positively correlated with microbial biomass (Robertson et al., 1988; Burket and Dick, 1998), N mineralization rates may therefore be increased by the addition of biochars with a high pH in acidic soils.

While some authors did not find an effect of soil organic matter (SOM) content onto N mineralization rates (Gunapala et al., 1998; Lundquist et al., 1999), strong positive correlations between the N mineralization rates and soil C and N content have been observed by others (Bending et al., 2002; Camargo et al., 2004; Schomberg et al., 2009), and were attributed to higher availability of microbial substrate. Bending et al. (2002) assessed N mineralization rates and microbial soil properties after addition of several organic residues in two similar soils with contrasting soil organic matter (SOM) levels. They concluded that SOM content affects the functioning of soil microorganisms and N mineralization. Yet, assessment of N mineralization rates after the addition of biochars to soils with contrasting SOM levels and the role of soil microbial properties has not been addressed up to now.

In regions with intensive animal production like Flanders there is considerable overproduction of animal manure in relation to the available arable land, which has resulted in nitrate leaching and ground and surface water eutrophication (van Grinsven et al., 2012). Pyrolysis of manure to biochar may be a valuable manure processing technique to reduce risk of excessive fertilization, besides producing bioenergy. Song and Guo (2012) reported that pyrolysis of poultry litter at 400°C and 500°C decreased the water extractable N in the resulting biochar to 19.1% and 1.62%, of the water extractable N in the feedstock, respectively. Therefore, producing biochar from manure can be an option in regions with large manure excesses and related problems.

In this study, we sampled two adjacent arable soils with similar texture and crop rotation, but with contrasting organic matter content. To quantify possible differences in SOM quality, we used a physicochemical fractionation procedure and compared the composition of the SOM of both soils. We amended both soils with four biochar types, produced from two contrasting feedstocks, namely pine chips and poultry litter at two slow pyrolysis temperatures (400°C and 500°C). By doing so, we tried to unravel the interactions between biochar, native SOM and soil microorganisms. In particular:

(i) Does biochar application result in a net increased or decreased release of mineral N in soils?

(ii) What is the effect of pyrolysis temperature and feedstock type on this response?

(iii) Is there an interaction between SOM quality and the effect of biochar on N availability and soil microbial parameters?

We hypothesized that biochar addition to the soil with a high SOM level would lead to higher microbial stimulation and consequent higher N mineralization than in the soil with a low SOM content. Secondly, we expect higher N mineralization rates from the poultry litter biochar-amended soils than from the pine biochar treatments, where possible N immobilization might be expected.

5.2 Material and methods

Soil characteristics

Two arable Luvisols (WRB classification) were sampled in Otegem (West-Flanders, Belgium) with similar texture and crop rotation, but with contrasting organic Ccontent. Soil was collected from the 0-30 cm soil layer with an auger by following a zig zag pattern over the whole field. Total C and N contents were measured with a CNS elemental analyser (Variomax), according to the principle of catalytic tube combustion under excess oxygen supply and high temperature (850 – 1150 °C). General soil characteristics are summarized in Table 5-1.

Table 5-1 General soil characteristics of two silt loam cropland soils with comparatively low (L) and high (H) soil organic carbon

Soil	Total C	Total N	C:N	pH(KCl)	Textural class (USDA)	Sand	Silt	Clay
	(g C kg^{-1})	(g N kg^{-1})	(-)	(-)		(%)	(%)	(%)
L	8.86±0.39	0.71±0.02	12.06±0.17	5.3	Silt loam	23	66	11
H	16.09±0.51	1.40±0.06	11.52±0.24	4.7	Silt loam	20	68	12

Table 5-2 Chemical properties of the poultry litter (PL) and pine chips (P) biochars produced at 400°C and 500°C (SA: surface area and TPV: Total pore volume, measured by BET-N_2 adsorption)

Biochar	Oil yield	Char yield	Gas yield	Volatiles	Fixed carbon	Ash	C	H	N	S	O	P	SA	TPV	pH(H_2O) 1:10
	%	%	%	%	%	%	%	%	%	%	%	µg.g^{-1}	m^2 g^{-1}	cm^{-3} g	
P400	51.03	33.41	15.56	30.23	68.72	1.05	74.38	4.06	0.25	0.00	14.59	29	0.22	1.79 10^{-3}	7.56
P500	66.96	17.26	15.78	19.00	79.89	1.11	81.71	3.10	0.22	0.01	8.76	36	22.77	2.53 10^{-2}	8.27
PL400	40.97	36.97	22.07	24.40	32.53	43.07	41.87	2.43	4.29	0.61	16.17	9904	4.85	2.69 10^{-2}	10.09
PL500	44.40	31.73	23.88	18.08	39.03	42.90	44.35	1.64	4.02	0.54	12.19	9514	6.55	3.17 10^{-2}	9.74

Physicochemical fractionation of soil organic matter

The scheme of the physicochemical SOM fractionation method is presented in Figure 5-1. An ultrasonication-wet sieving method (Amelung et al., 1998) was used to fractionate SOM into sand size (>53µm) (sand C and N) and silt+clay size (<53µm) (silt+clay C and N) fractions. In order to breakdown macro and micro aggregates, samples were dispersed by ultrasonication (20 g soil in 100 ml deionized water) using an ultrasonic vibrator (Sonics Vibracell 600 with Sonotrode CV 26). The ultrasonic probe was first calibrated by heating 150 ml deionized water in a Dewar vessel for 2 minutes. A low energy input of 60 J ml^{-1} was used to protect particulate organic matter (POM) from disruption, whilst achieving dispersion of sand sized aggregates (Amelung and Zech, 1999). After ultrasonic dispersion, the >53µm fraction was isolated by wet sieving the dispersed soil slurry through a 53µm mesh sieve. POM which had been retained on the sieve together with sand was collected in pre-weighed aluminium cups. The water and soil fraction passing through the 53 µm sieve (i.e. the silt+clay size fraction) was separated. The dissolved organic C content (DOC) of the water phase was determined on a TOC analyzer (TOC-VCPN, Shimadzu Corporation, Kyoto, Japan). Total dissolved nitrogen (DN) of the water phase was determined after alkaline persulfate oxidation as NO_3^- with a continuous flow autoanalyzer (Chem-lab 4, Skalar Anaytical, Breda, the Netherlands). The silt+clay size fraction was dried for 24h at 50°C and collected in pre-weighed aluminium cups. The silt+clay size fraction was then subjected to a chemical fractionation procedure, according to Mikutta et al. (2006) and slightly modified by Sleutel et al. (2009), which involves sequential oxidation by 6%NaOCl and mineral extraction by 10%HF-acid. The procedure results in the isolation of a chemically stable 6%NaOCl resistant SOM fraction composed of mineral-protected as well as biochemically recalcitrant OM, and a biochemically non-bound recalcitrant SOM fraction resistant to 10%HF treatment (Figure 5-1). A 5g sample of the isolated silt+clay size fraction was reacted three times for 6 hours with 50 ml 6%NaOCl adjusted to pH 8.0 inside 85ml Nalgene centrifuge tubes. Samples were centrifuged and decanted in between oxidation cycles and were ultimately washed one time with 1M NaCl and three times with deionized H_2O. After drying and weighing, a subsample was collected for total C and N analysis. Then, 3g of the oxidation residue was treated four times with 20 ml 10%HF in order to dissolve and remove mineral constituents and mineral-bound OM. Extraction residues were washed five times with deionized water

to remove salts and residual HF and were decanted into pre weighed aluminium cups, dried and weighed. SOM fractionation analyses were carried out on both soils in duplicate.

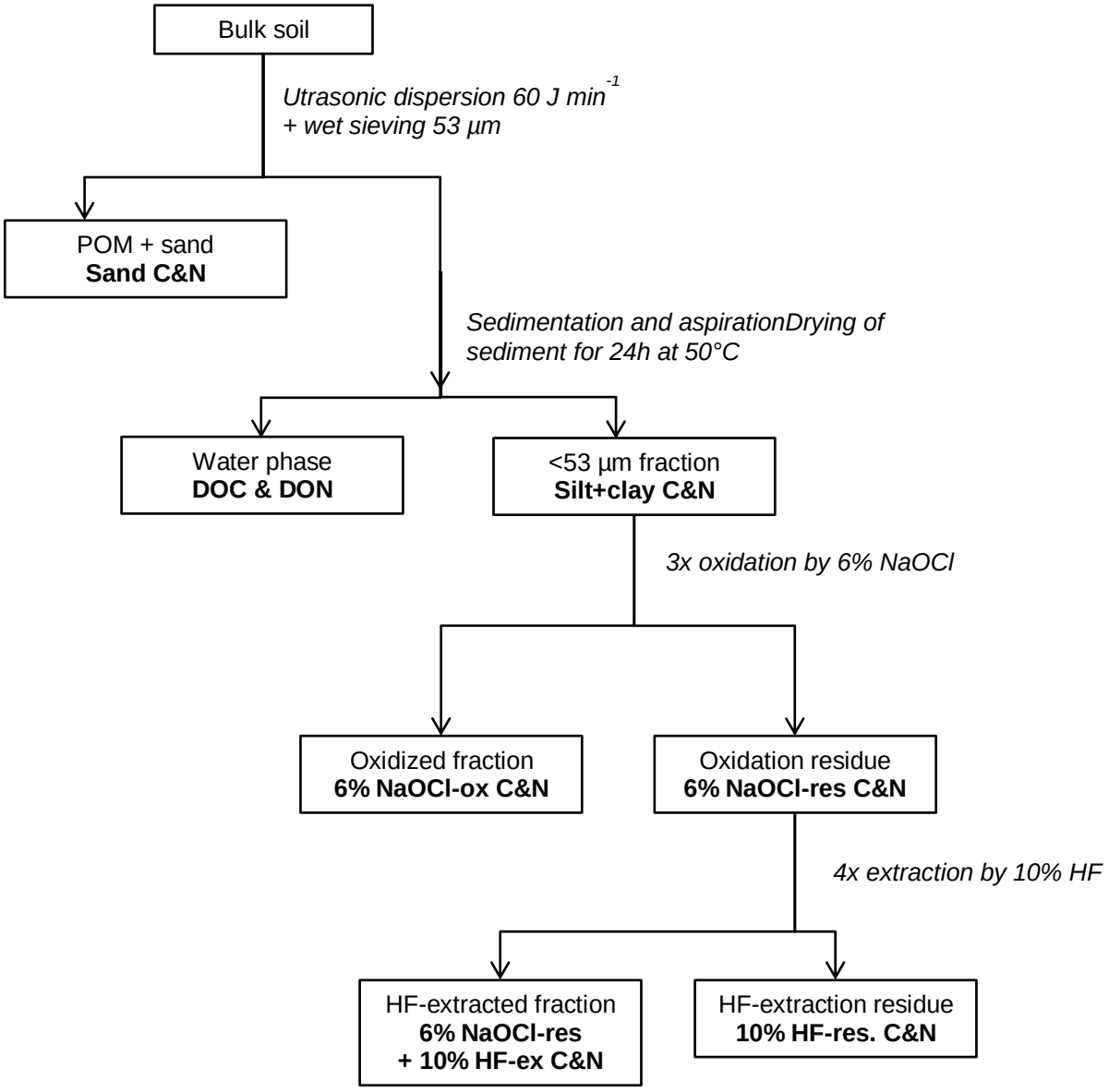


Figure 5-1 Combined physical and chemical fractionation scheme to isolate presumed labile (dissolved C and N, sand C and N, 6% NaOCl-ox C and N), mineral protected (6% NaOCl+10% HF-ex C and N) and recalcitrant organic matter, (10% HF-res C and N)

Biochar production and characterization

Biochar was produced from two different feedstocks, namely pine chips and poultry litter, at two different slow pyrolysis temperatures (400 °C and 500 °C). The holding time once the target temperature was reached was 0.5h and N_2 was used as a carrier gas. The biochar and oil yields were measured by weighing the fractions after pyrolysis. The concentration of C, N, H and O were analyzed using a LECO brand (Model CHNS-932) elemental analyzer. The volatile matter, fixed Cand ash content was measured by the ASTM D1762-84 standard testing method using a LECO TGA-701 proximate analyzer (Leco Corp., St. Joseph, MI). The remaining ash was destructed by microwave digestion followed by nitric acid and hydrochloric acid destruction and the P, Al, Fe, K, Mg, Na and Ca content of the digestate was determined by inductively coupled plasma – mass spectrometry (ICP-MS) using a Perkin - Elmer Elan 6000 ICP - MS equipment (Waltham, Mass.). The biochar characterization data are given in Table 5-2. The surface area (SA) and total pore volume (TPV) of the biochars were determined by the BET method with a BELsorp – mini II analyzer (BEL Japan Inc., Osaka, Japan). pH(H_2O) was measured in using a 1:10 ratio. Samples were thoroughly mixed and allowed to equilibrate for 18 h and measured with a pH electrode (Thermo Orion, 420A plus).

Incubation experiment

A 14-week incubation experiment was set up to estimate soil N mineralization. Thoroughly mixed air dried soil (137 g of dry soil) was filled in PVC tubes with an inner diameter of 0.05 m. The soil was brought to a bulk density of 1.4 Mg m^{-3} by compaction until a predetermined filling height (5 cm) was achieved. The moisture content of the soil was then adjusted to a level of 50% water filled pore space (WFPS) by the addition of deionized water and temperature was kept constant at 19 °C. Biochar was added equivalent to a 20 t ha^{-1} dose, calculated on surface area basis, corresponding to 1:35 (w biochar w^{-1} soil). One unamended control treatment was included per soil. We prepared 21 tubes per treatment (7 sampling events x 3 replicates), resulting in a total number of 210 tubes. The moisture content was monitored regularly during incubation by weighing the tubes and was kept constant by adding deionized water as required. Every two weeks three replicates per treatment were destructively sampled and analyzed for mineral nitrogen (N) content.

Mineral N (as NO_3^- and NH_4^+) was determined from 10g fresh soil extracted with 50 ml 1 M KCl and measured colorimetrically by a continuous flow auto-analyzer (Chem-lab 4, Skalar Anaytical, Breda, the Netherlands). At the end of the 14-week incubation, the activity of the dehydrogenase enzyme (DA), microbial biomass (MB), and microbial community structure and pH(KCl) (1:2) were determined. The procedure for dehydrogenase activity was modified from Casida et al. (1964). Five gram fresh soil was weighed in glass vials, and 2 ml 3% solution of triphenyltetrazolium chloride and 2ml Tris buffer pH 7.8 were added. Soil suspensions were incubated in the dark for 24 h at 37 °C. After incubation, 18 ml of methanol was added to each vial and the vials were shaken in the dark for 2 h with a linear shaker (125 rev min−1). Filtrates were collected in 50 ml volumetric flasks. To extract all produced triphenyl formazan (TPF), the remaining soil in the vials was washed twice with methanol, following which filter papers were also washed twice. Filtrates in the volumetric flasks were made up to 50 ml with methanol. The colour intensity of the filtrates was measured at 485nm with a Hitachi 150-20 spectrophotometer. All measurements were carried out in triplicate with one blank.

The structure of the microbial community was described by the fatty acid composition of the phospholipids (PLFA) in the soil (see below). PLFAs were extracted using a modified Bligh and Dyer (1959)-technique, described in Moeskops et al. (2010). Microbial biomass (MB) was determined as the sum of the PLFAs. For Gram-positive bacteria the sum of *i*15:0, *a*15:0, *i*16:0, *a*16:0, *i*17:0 and *a*17:0 PLFAs was used. The PLFAs cy17:0 and cy19:0 were used to quantify Gram-negative bacteria. The sum of 10Me16:0 and 10Me18:0 were used to quantify actinobacteria. The total bacterial community was assumed to be represented by the sum of the marker PLFAs for Gram-positive and Gram-negative bacteria, in addition to 15:0 and 17:0. The PLFA 18:2ω6,9c was considered as an indicator for saprotrophic fungi and 16:1ω5 as the indicator for AMF following Joergensen and Wichern (2008).

Data analysis

A zero order model ($N_{min} = N_0 + k*t$) was fitted to the N mineralization data (amounts of NO_3^- plus NH_4^+) with IBM SPSS statistics 21 (SPSS inc., Chicago, USA), yielding a zero order N mineralization rate *k*. Because we fitted through all replicates at each measuring time, the fit yields the parameter *k* ± its standard error. Net N mineralization ($N_{net\ min}$) was calculated as the difference between mineral N contents of the biochar treatments minus the control at the last extraction date. The experiment was designed with two fixed factors: 1) treatment (five levels: control, P400, P500, PL400 and PL500), 2) soil type (two levels: L and H soil). The differences between the treatments in pH, $N_{net\ min}$, DA, MB and the PLFA biomarkers were assessed by two-way ANOVA. When the interaction term was significant ($P<0.05$), we applied one way ANOVA by combining the levels of each factor. If the interaction was not significant, the main effects of the different treatments were assessed via post-hoc Tukey's tests. Principal component analysis (PCA) was undertaken on the percentage distribution of all PLFA compounds using IBM SPSS Statistics 21 (SPSS inc., Chicago, USA).

5.3 Results

Physicochemical fractionation of soil organic matter

Five C & N fractions were isolated via the combined physical and chemical fractionation method, namely C&N in the sand fraction, dissolved C&N, 6% NaOCl-ox C&N, 6% NaOCl-res + 10% HF-ex C&N and 10% HF-res C&N. In the low SOM soil (L), 94.86 ± 5.09 % C and 101.48 ± 6.44 % N was recovered. In the high SOM soil (H), the recovery was 102.25 ± 2.32 % C and 93.65 ± 3.61 % N.

The relative proportion of the 10%HF-res C fraction was significantly ($P<0.05$) higher in the H soil than in the L soil (Figure 5-2a). There were no significant differences in the proportions of the other fractions between both soils. The dissolved N and 6% NaOCl-res + 10% HF-ex N fraction was significantly ($P<0.05$) lower in the H soil than in the L soil (Figure 5-2b). The sand N, 6%NaOCl-ox N and 10%HF-res N fractions did not differ between both soils. The C:N ratios of the 6%NaOCl-res+10%HF-ex fraction and the

10%HF-res fraction were significantly ($P<0.05$) lower in the H soil than in the L soil (5.85 ± 0.10 vs. 9.06 ± 0.33 and 18.76 ± 0.00 vs. 27.32 ± 0.00, respectively). There were no significant differences in the C:N ratios of the other soil fractions.

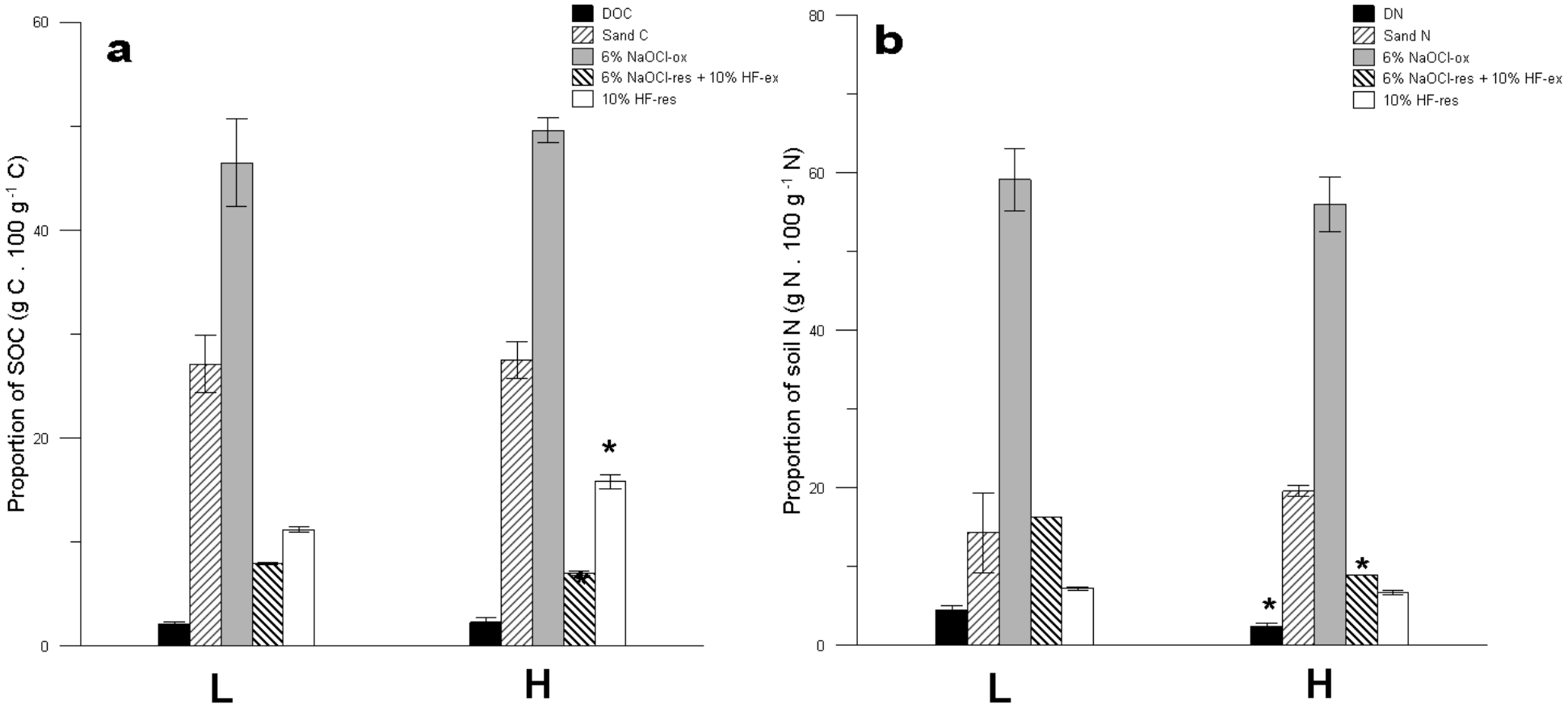


Figure 5-2 Distribution of OC (a) and N (b) of the low (L) and high (H) SOM soil over physicochemical soil fractions. *indicate statistically significant differences (t-test with $P<0.05$) in one fraction between both soils. (Error bars indicate standard deviation, n=2)

N mineralization

The amounts of NO_3^- increased in both soils with all biochar treatments with time (Figure 5-3). However there was a trend towards lower NO_3^- mineralization from the pine biochar treatments compared to the control, while the poultry litter biochars were significantly higher than the control. Although starting from a lower initial NO_3^- content in H soil, the relative increase in NO_3^- contents was over all treatments higher than their corresponding treatment in the L soil. The contents of NH_4^+ showed a decreasing trend during the mineralization experiment. Therefore the relative share of NH_4^+ (expressed to total mineral N) decreased from over average 20% in the L soil and 9% in the L soil initially to 3% in both soils at the end of the incubation.

Considering the net N mineralization data, there was a significant interaction between the factors soil type and biochar treatments (Table 5-3). The amount net N mineralization of the poultry litter biochar treatments were higher (although not significantly in the case of PL400) in the H soil than in the corresponding treatments in the L soil (Table 5-3). Pine biochar amendment decreased N mineralization rates compared to the corresponding control. Net negative N mineralization was observed for P500 for both soils, and P400 in the H soil. In both soils and with both feedstocks increasing pyrolysis temperature to 500°C decreased the N mineralization rates compared to the corresponding 400°C biochar treatments (Table 5-3). There was a significant interaction ($P<0.05$) between the treatments and the soil type for pH. The amount of mineral N at the end of the incubation was positively correlated to the soil pH(KCl), measured at the end of the incubations ($n=30$, $P<0.01$, Pearson's $r=0.78$) and to dehydrogenase enzyme activity ($n=30$, $P<0.01$, Pearson's $r=0.61$) .

Table 5-3 The calculated linear N mineralization rates (*k* ± standard error) (with determination coefficient (R^2)) and net mineralized amount of N (± standard deviation) and the pH(KCl) (1:2) (± standard deviation) at the end of the incubation (H: high SOM soil, L: low SOM soil, P: pine chip biochar, PL: poultry litter biochar, 400: pyrolysis at 400°C, 500: pyrolysis at 500°C)

Treatment	k (mg N. kg^{-1} .$week^{-1}$)	R^2	$N_{net\ min}$ (mg N. kg^{-1})	pH(KCl) (-)
L control	0.92±0.11	0.88	-	5.30±0.03 [abc]
L P400	0.80±0.11	0.84	0.3±1.3[b]	5.41±0.03 [cd]
L PL400	2.09±0.24	0.89	25.7±2.25[de]*	7.08±0.03 [e]
L P500	0.38±0.11	0.60	-7.4±0.8[a]*	5.54±0.02 [bc]
L PL500	1.45±0.12	0.94	9.4±1.7[c]*	7.16±0.05 [e]
H control	1.21±0.11	0.92	-	4.62±0.09 [ab]
H P400	0.89±0.16	0.76	-6.6±1.0[ab]*	4.59±0.02 [a]
H PL400	2.83±0.27	0.92	32.0±3.5[e]*	6.64±0.06 [de]
H P500	0.53±0.11	0.72	-10.7±0.6[a]*	4.74±0.03 [ab]
H PL500	2.34±1.62	0.95	19.6±5.0[d]*	6.60±0.08 [de]

[a] Different small letters indicate significant differences ($P<0.05$) between treatments in the L and H soils after two-way ANOVA.

* denotes the net mineralized amount of N to be significantly different from zero ($P<0.05$)

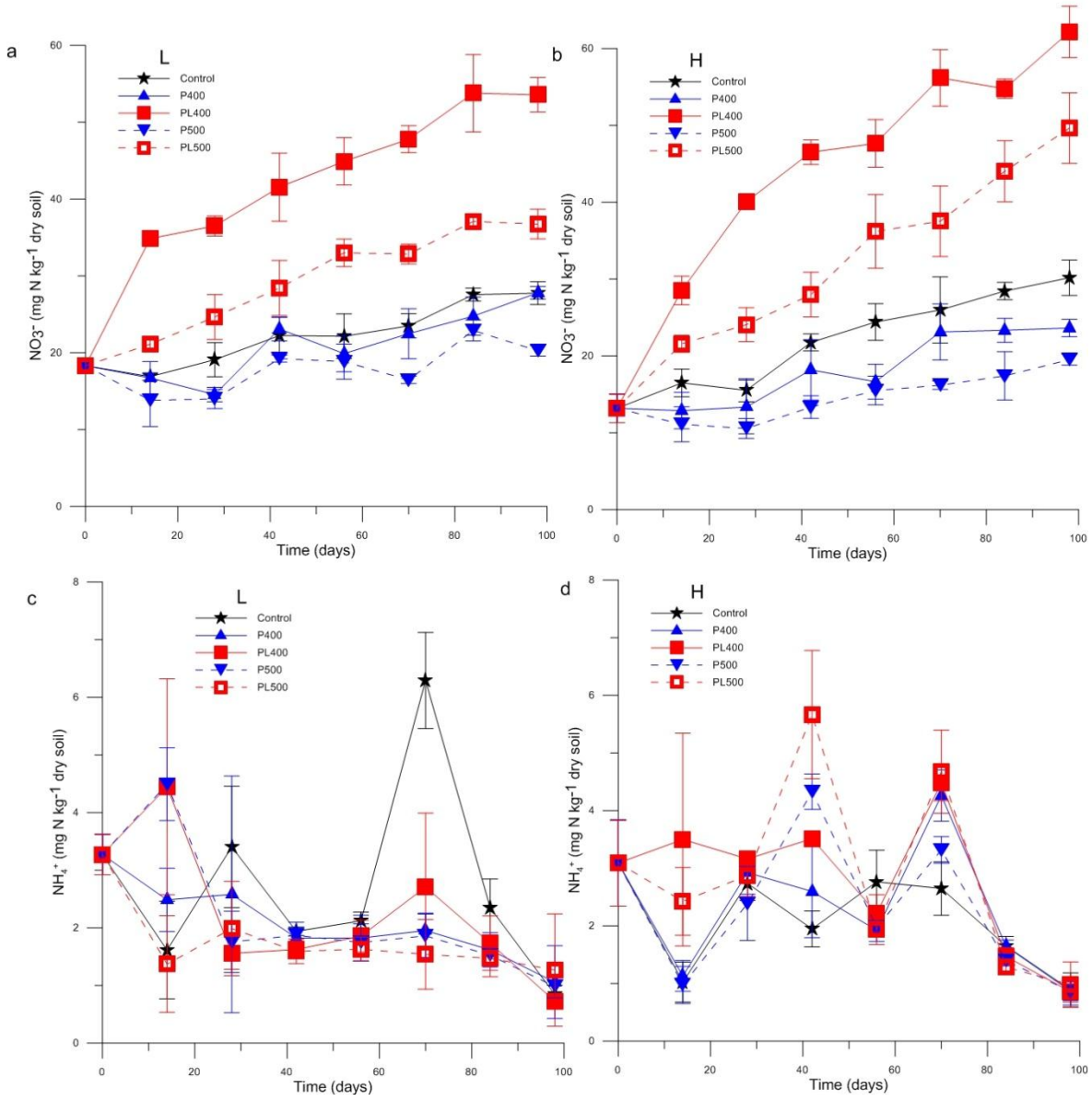


Figure 5-3 Amounts (mg N kg^{-1} dry soil) of NO_3^- (plots above, a and b) and NH_4^+ (plots below, c and d) in the L (plots left, a and c) and and H soil (plots right, b and d) plotted against the time. (H: high SOM soil, L: low SOM soil, P: pine chip biochar, PL: poultry litter biochar, 400: pyrolysis at 400°C, 500: pyrolysis at 500°C)

Biological soil properties

There was a significant interaction ($P<0.05$) between the factors soil and treatment for dehydrogenase enzyme activity. In the L soil, dehydrogenase activity was higher (although not significantly) in the PL400 treatment compared to the control. For the H soil, amendment of PL400 or PL500 (although not significantly) biochar significantly increased the dehydrogenase activity compared to the control, while pine wood biochar amendment did not (Figure 5-4). In the L soil, the dehydrogenase activity was consistently lower when amended with 500°C pyrolysis temperature biochar (P500 and PL500) compared to with the P400 and PL400 biochars, but this difference was only significant for the PL treatments (Figure 5-4). In the H soil, dehydrogenase activity of the PL500 treatment was significantly lower than in the PL400 treatment.

The interaction between the factors soil and treatment was not significant ($P<0.05$) for MB. Over all treatments, MB was on average 1.5 times higher in the H soil than in the L soil (Figure 5-4). In both soils, MB did not increase due to the addition of biochar to the soils. PLFA concentrations in soil samples after 98 days of incubation were expressed as percentages of the total extracted PLFA. Separate principal component analyses of the PLFA concentrations were conducted for both soils. For the L soil, a first component (PC1) explained 32.6% of the variance and discriminated the control and pine biochar treatments with positive loadings on the one hand from the poultry litter biochar treatments with negative loadings on the other (Figure 5-5a). A second component (PC2) explained an additional 23.2% of the variance and the 400°C and 500°C biochar treatments had positive and negative scores on PC2, respectively. For the H soil, PC1 and PC2 explained 32.7% and 15.7% of the variance, respectively and similar patterns as for the L soil could be observed (Figure 5-5b).

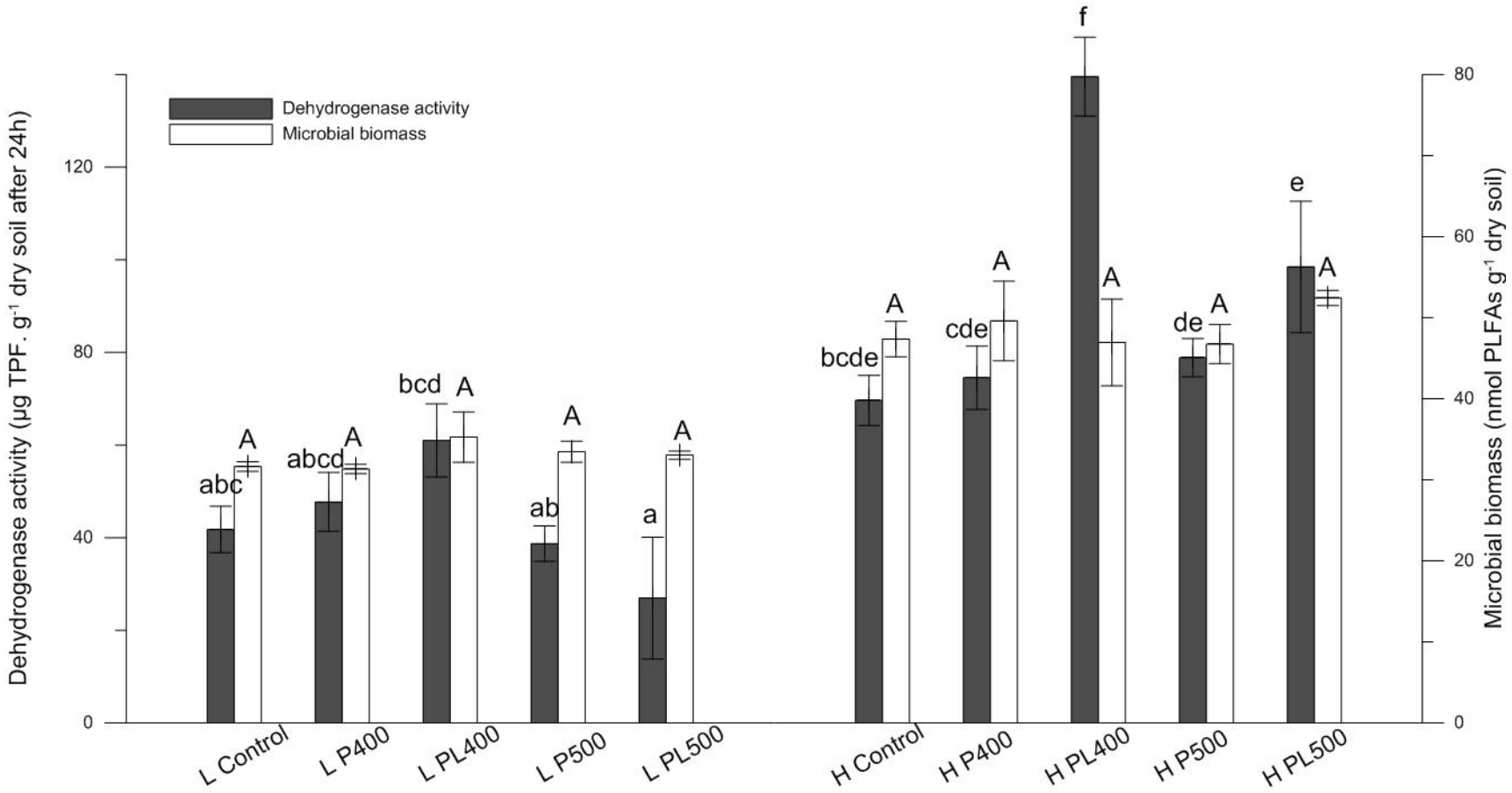


Figure 5-4 Dehydrogenase activity (full bars) and microbial biomass (white bars) of two unamended (control) and biochar-amended soils with low (L) and high SOM content (H). Different small letters indicate significant differences in dehydrogenase activity (significant interaction between factors), while different capital letters indicate significant differences ($P<0.05$) in microbial biomass between the different treatments per soil (no significant interaction between factors).

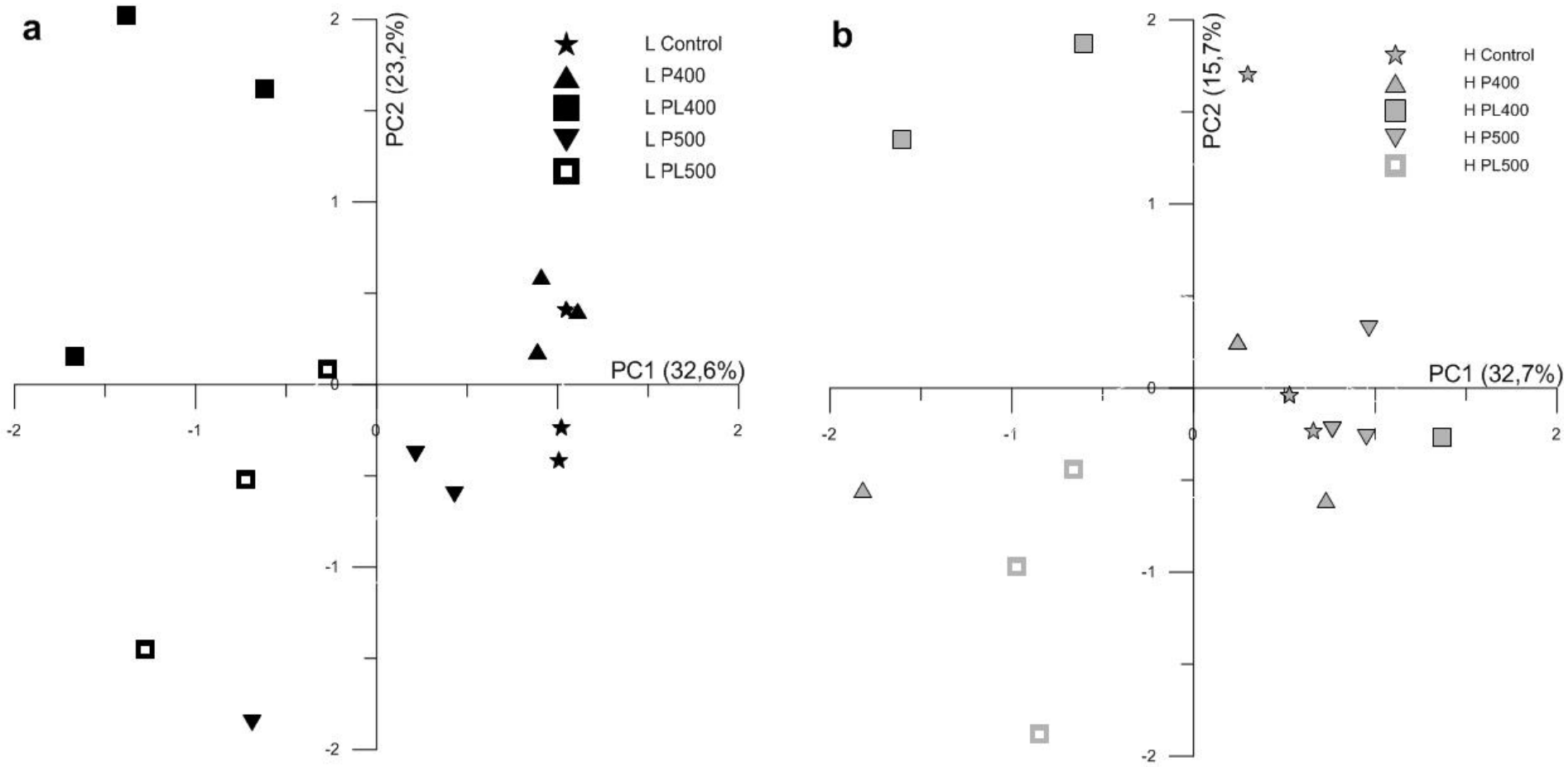


Figure 5-5 PCA ordination based on mol % PLFA of individual PLFAs from the biochar-amended and control samples a) in the low soil (L) and b) in the high SOM (H) soil; the first and second principle components are given. Percentage of variance explained by each component is indicated within parenthesis on each axis.

The addition of the 500°C biochars increased the ratio of bacterial to fungal PLFA biomarkers (B:F ratio) in both soils (Table 5-4), although not significantly. In both soils the PL amendments resulted in an increase in abundance of PLFA biomarkers for Gram+ bacteria, but only significantly ($P<0.05$) for the PL500 biochar in the H soil. Amendment of PL biochars to the L soil, significantly lowered proportions of biomarkers for AMF. P biochar addition did not affect any of the PLFA biomarkers compared to the control treatments, except for a significant decrease in fungal and AMF biomarkers in L soil amended with P500 biochar.

Table 5-4 Relative concentrations (expressed as % of total extracted PLFAs) of marker PLFAs for Gram-positive and Gram-negative bacteria, Fungi, Actinobacteria and AMF and the ratios of bacteria:fungi marker PLFAs of the different treatments (H: high SOM soil, L: low SOM soil, P: pine chip biochar, PL: poultry litter biochar, 400: pyrolysis at 400°C, 500: pyrolysis at 500°C).

Treatment	Relative concentration of marker PFLAs (% of total PLFA)					B:F ratio
	Gram-positive*	Gram-negative	Fungi*	Actinobacteria	AMF*	
L control	26.9±0.5^{ab}	8.68±0.23^{a}	1.36±0.07^{c}	7.79±0.09^{a}	5.80±0.05^{c}	28.2±1.5^{a}
L P400	26.9±0.1^{ab}	8.74±0.15 ^{a}	1.26±0.08^{bc}	7.90±0.06^{a}	5.60±0.11^{c}	30.4±1.2^{a}
L PL400	25.7±0.9^{a}	9.23±0.40^{a}	1.22±0.04^{bc}	7.87±0.31^{a}	5.09±0.19^{a}	30.5±0.8^{a}
L P500	27.5±0.4^{b}	8.67±0.15^{a}	1.17±0.09^{b}	7.39±0.13^{a}	5.37±0.04^{b}	33.4±2.7^{a}
L PL500	26.7±0.1^{ab}	8.64±0.23^{a}	1.15±0.01^{b}	7.43±0.26^{a}	5.26±0.08^{b}	32.9±0.7^{a}
H control	28.8±0.1^{b}	9.37±0.17^{a}	0.83±0.07^{a}	8.04±0.15^{a}	5.81±0.25^{c}	48.6±4.2^{a}
H P400	28.4±0.3^{b}	9.50±0.17^{a}	0.81±0.05^{a}	8.10±0.44^{a}	5.66±0.12^{c}	49.7±3.5^{a}
H PL400	28.0±0.5^{b}	9.05±0.41^{a}	0.82±0.04^{a}	8.48±0.68^{a}	5.76±0.06^{c}	47.9±2.6^{a}
H P500	29.0±0.4^{bc}	8.97±0.02^{a}	0.76±0.04^{a}	7.72±0.03^{a}	5.73±0.03^{c}	53.1±2.9^{a}
H PL500	29.2±0.4^{c}	8.79±0.08^{a}	0.80±0.07^{a}	8.25±0.10^{a}	5.68±0.12^{c}	50.8±4.1^{a}

*indicates that interaction between soil type and treatment was significant after two-way ANOVA

5.4 Discussion

The SOM composition of the high (H) and low (L) SOM soils was compared by means of physicochemical fractionation. Although the total C and N content in the H soil was twice as high as in the L soil, overall differences in the relative distributions of the SOM fractions between both soils were small. Sieving at 53µm and subsequent treatment with 6% NaOCl of the <53µm fraction was used to quantify labile SOM fractions. Uncomplexed OM in the sand fraction is well known to be a readily-available food or energy source for the soil microbial biomass (Gregorich et al., 2006). Kader et al. (2010) found significant linear relationships between N mineralization from temperate cropland soils and their contents of NaOCl oxidizable N. Hence we considered both the sand OM and silt+clay NaOCl oxidizable OM as measures of labile C and N. However, between our soils we did not find significant differences in the proportions of the labile C and N fractions. The N mineralized after 14 weeks was only 1.3 times higher in the control treatment of the H soil compared to the L soil, despite a 2.3 times higher N content in the H soil. In line, dehydrogenase activity and MB in the H soil control treatment were respectively only 1.3 and 1.5 times higher than in the L soil. The content of labile presumed C and N fractions failed to explain these trends. However, the more stable C fraction (10% HF-ex) was higher in the H soil than in the L soil. Since microbial enzymatic degradation and the size of its biomass are dependent on substrate availability and quality (Fliessbach and Mader, 2000; Aon and Colaneri, 2001) these data point to a relatively lower degradability of the SOM in the H soil compared to the L soil. In the H soil (more recently converted from grassland to arable land than the L soil) we observed a lower fungal abundance and a higher bacterial (Gram-positive, Gram-negative and actino-bacteria) abundance and consequent larger B:F ratio (Table 5-4). This is in accordance with results from Bardgett et al. (2007). They investigated microbial community composition along a 150-year chronosequence of exposed soils under a glacier. In the most recently exposed sites with older and more recalcitrant C they found greater bacterial abundance relative to fungi than in soils which were ice-free for 150 years with more modern C-substrates. Also Feng and Simpson (2009) and Billings and Ziegler (2008) found higher B:F ratios and actinobacterial abundances, respectively, in soils with kinetically more stable OM.

In both soils, PL biochar application increased the N mineralization rates compared to the control and pine biochar treatments. This is in line with previous observations of increased N mineralization after addition of manure-derived biochars to soils (Gaskin et al., 2008; Nelson

et al., 2011). Additionally, enhanced native SOM mineralization due to biochar amendment could just as well explain the higher net N release of mineral N in case of the PL treatments, as was also observed as increased gross mineralization rates after the addition of maize biochars by Nelissen et al. (2012). Changes in the turnover rate of SOM due to the addition of various organic amendments have been attributed to a stimulation of the soil microbial community, the so-called 'priming effect' (Kuzyakov et al., 2000). Due to the substrate-induced microbial growth native SOM might be co-metabolized (Kuzyakov et al., 2000). Several studies have indeed demonstrated increased SOM mineralization after the addition of isotopically labeled biochars (Luo et al., 2011; Zimmerman et al., 2011). Through investigating priming effects of 19 different biochar types in six soils, Zimmerman et al. (2011) determined that priming of native SOM in the presence of biochar ranged between -59 and 89% of SOM mineralization without biochar. In our study, N mineralization rates after the addition of PL biochar were between 1.5 and 3 times higher than in the soils without biochar, indicating that both biochar mineralization and SOM mineralization contributed to these high rates. Unfortunately, we were unable to discriminate between native SOM N mineralization and N mineralization from biochar. In addition, PL biochar addition increased pH in both soils, and there existed a positive correlation between the pH and the amount of mineral N at the end of the incubation. The liming potential of the biochars may thus play an important role in stimulating soil microorganisms in these relatively acidic soils.

The PL400 biochar had a higher volatile matter content, a higher N content and lower C:N ratio than the PL500 biochar (Table 5-2). As could be expected, net N mineralization rates were significantly higher in the PL400 treatments. A lower biochar N content and N supply with increasing pyrolysis temperature has been documented previously and was attributed to increased loss of volatile fractions of N (Gundale and DeLuca, 2007; DeLuca et al., 2009). Microbial community composition as expressed by PLFA biomarkers (Table 5-4) was quite similar in both soils after PL500 or PL400 addition. Further analysis of the individual PLFAs demonstrated that PL500 addition increased the proportion of cy17:0 PLFA, which is a marker for Gram-negative bacteria.

Addition of P biochar to the H soil resulted in a significant net negative N mineralization compared to the unamended control treatment. One possible explanation could be the inhibition of microbial activity due to the presence of toxic biochar compounds (Graber et al., 2010). However, in MB and dehydrogenase enzyme activity did not decrease, suggesting that N immobilization occurred. Also Nelissen et al. (2012) observed increased gross N

immobilization rates (from NO_3^- to the microbial biomass pool) after addition of maize biochar. In the L soil, the N immobilization effect was less pronounced and only significant in the P500 treatment. Moreover, N immobilization increased with increasing pyrolysis temperature and consequently increasing C:N ratio. Several authors have suggested that microbial consumption of volatile biochar components with a large C:N ratios resulted in N immobilization (Clough and Condron, 2010; Ippolito et al., 2012).

Both N mineralization and N immobilization with addition of the PL and P biochars, respectively, were more pronounced in the H soil compared to the L soil. We also found significant interaction between the soil type and the treatment, indicating that there was an effect of the soil type on the net N mineralization. Several mechanisms might be responsible for this observation. First, priming effects of biochar onto native SOM can be higher in the soil with a high SOM content than in the low SOM soil, through the greater availability of microbial substrate. In the H soil, with a relatively more stable SOM and higher B:F ratio, soil organisms were possibly better adapted to break down the less easily-degradable biochar compounds than the microbial community in the L soil. Though not significantly, B:F ratios increased in both soils after biochar addition (Table 5-4). However, B:F ratios in both soils were affected in a different way. In the L soil fungal abundance decreased due to biochar addition, while in the H soil bacterial abundance (sum of Gram-negative, Gram-positive plus PLFA 15:0 and 17:0) increased. As bacteria have been found to be strongly related to microbial decomposition and N mineralization (Lundquist et al., 1999; Bittman et al., 2005), this may explain the enhanced N mineralization/immobilization patterns in the H soil compared to the L soil treatments. In the L soil we observed decreased fungal abundance in the high temperature treatments together with lower mineralization rates compared to the low temperature biochar treatments. One proposed mechanism may be that the inhibition of fungi by biochar resulted in lower bacterial activity as was suggested by Bodé et al. (2013). They investigated residue degradation in combination with inhibition of either fungi or bacteria. Fungal inhibition reduced the capacity of bacteria to degrade organic substrates (Bodé et al., 2013). Increasing N mineralization rates by PL biochar amendment in the H soil might also have been influenced by the soil pH. In the H soil, with an initial lower pH, PL additions increased the pH to a higher extent than in the L soil.

5.5 Conclusions

Both feedstock and pyrolysis temperature strongly determined whether a net N release or immobilization occured upon biochar application. While amendment with poultry litter (PL) biochar resulted in net mineral N release (compared to the control), net N immobilization was observed after the addition of pine chips (P) biochars. Regardless of biochar type, the net cumulative N release decreased with increasing pyrolysis temperature (400 vs. 500°C). Net N mineralization and N immobilization, as well as microbial biomass stimulation and dehydrogenase enzymes activities were higher in the H soil than in the L soil. The more widespread presence of SOM and active microbial biomass in the H soil may have resulted more SOM priming or higher biochar mineralization than in the L soil. However, the magnitude of these microbial responses is dependent of the SOM quality. From this study it clearly appeared that SOM content and quality has an interactive control on the microbial response to biochar applications to soil.

Chapter 6:

CO_2 emissions and microbial community shifts after biochar addition – multi-year effects

Compiled from

Ameloot, N.; Sleutel, S.; Case, S.; Alberti, G.; McNamara, N.P.; Zavalloni, C.; Vervisch, B.; delle Vedove, G.; De Neve, S. Biochar inhibits soil microorganisms: analysis of four biochar field experiments. Submitted.

Abstract

In recent years, several biochar field experiments have been established to determine the effect of biochar on crop yields, nutrient dynamics, physical soil properties and greenhouse gas emissions. As laboratory studies are typically short-term experiments, investigating soil microbial properties among these multi-year experiments can help us establishing the effect of biochar on soil organisms after several years of biochar incorporation into soil. Soil was sampled from biochar-amended and control plots of four biochar field trials in Lincoln (UK), Rivignano (IT), Rocca Bernarda (IT) and Beano (IT). All soil samples were air-dried, pre-incubated at 15°C and WFPS of 50% for one week and then incubated to measure CO_2 emissions. At the end of the experiment (Lincoln 63 days; Italian sites 56 days), soil cores were destructively sampled to determine β-glucosidase and dehydrogenase enzyme activity, total microbial biomass and microbial community structure (PLFA). Soil OC contents of biochar-amended plots were higher than of control plots, however, only in the most recently established site (Rivignano) and the field that had received large amounts of biochar (49 t ha^{-1}, incorporation depth of 10 cm) (Lincoln) was this trend was significant. Lower CO_2 emissions from biochar-amended plots than from unamended plots indicate that native soil organic matter (SOM) decomposition was depressed (negative priming). Cumulative C mineralization expressed as % of OC content (Relative C mineralization) was lower in biochar-amended plots compared to control plots of all sites, except in the longest established site (Beano). Relative C mineralization rates were positively and significantly correlated to the microbial biomass and β-glucosidase enzyme activity. This may indicate that soil microbial community activity was inhibited in biochar-amended plots. Toxic effects of PAH and heavy metals in the biochar towards microorganisms were unlikely to have occurred, as even in the field site with the highest microbial reduction no evidence for biochar toxicity was found. In the field site with the highest application of biochar (Lincoln), a microbial community composition shift towards lower fungal abundance was observed. Possibly, the Lincoln plots amended with huge amounts of biochar may be less suitable for fungi due to the unsuitability of biochar as a substrate and also due to the adsorption of labile SOM on the biochar surface.

6.1 Introduction

The effect of biochar on soil biological properties has been reviewed by Lehmann et al. (2011) and by Ameloot et al. (2013b) with a specific focus on biochar stability. Both direct and indirect effects determine the interaction between biochar and soil organisms. Indirectly, biochar changes soil chemical and physical properties, e.g. soil porosity, pH, cation exchange capacity (CEC) and adsorption properties. Microorganisms can directly utilize a number of labile biochar compounds as an energy source (Cross and Sohi, 2011). These volatile compounds are biomass compounds, that have not yet been subjected to devolatilization during pyrolysis (Ronsse et al., 2013) or volatilized compounds that have recondensed in the biochar matrix (Imam and Capareda, 2012; Kloss et al., 2012) . They consist of relatively small molecules, such as *n*-alkanoic acids, hydroxyl- and acetoxy-acids, benzoic acids, diols, triols, and phenols (Graber et al., 2010). Ameloot et al. (2013a) found that the volatile matter content of biochar was positively correlated to the short-term CO_2 emissions from soils amended with biochar through the provision of an available substrate. In most studies the effect on soil biota was assessed during laboratory incubations, with unweathered biochar added to soil in mesocosms (Novak et al., 2010; Zavalloni et al., 2011; Ameloot et al., 2013a). The duration of these experiments ranges from several weeks (Cross and Sohi, 2011) to less than two years (Kuzyakov et al., 2009), allowing only for understanding the short-term effects of biochar on soil organisms. Moreover, during laboratory incubations temperature and soil moisture can be controlled, while under field conditions experiment conditions are more complex, but in the same time more realistic. Hence the question remains whether and how microbial activity is affected in biochar-amended soils after several years of application and under field conditions. After its incorporation in the field, volatile biochar compounds may be degraded (Cross and Sohi, 2011; Ameloot et al., 2013b) and biochar ages through both biotically and abiotically mediated oxidation (Cheng et al., 2006; Hale et al., 2011). This aging process may alter the biochar pH, CEC and O:C ratio, and other properties, that likely influence the microbial community in biochar-amended soils.

In recent years, a number of field experiments have been established to assess the multi-year effect of biochar addition on plant productivity (Jeffery et al., 2011; Hammond et al., 2013), physical soil properties (Major et al., 2010), nutrient dynamics (Major et al., 2012) and greenhouse gas emissions (Castaldi et al., 2011; Afeng et al., 2012; Liu et al., 2012; Case et al., 2013). Case et al. (2013) studied CO_2 and N_2O emissions via in-situ measurements, and

concluded that CO_2 emissions decreased in the biochar-amended plots compared to unamended plots. The mechanism underlying this suppression was uncertain, but the authors suggested that the soil microbial community played a central role in the stabilization of soil organic matter (SOM) by biochar addition. Investigating the soil microbial properties of field trials with biochar is thus an important research priority. To the best of our knowledge, to date no thorough investigation of soil microbial properties in multi-year field trials have been published.

In this study, we investigated microbial biomass, activity and community structure in the different treatments of four biochar field experiments (United Kingdom and Italy). We hypothesized that biochar added to soil continues to influence soil microorganisms even after the readily-available biochar compounds have been degraded, through the ageing of biochar during soil incorporation.

6.2 Material and methods

Soil characteristics

Soil was collected in September 2012 from one site in the UK (Lincoln) and from three sites in Italy (Rocca Bernarda, Rivignano and Beano). All sites had a light texture (sandy loam to silt loam) and all applied biochars were produced from woody feedstocks at 400°C or 500°C (Table 6-1). The application rates varied between 20 and 49 t ha^{-1} and the application depth ranged from 10 to 35 cm. The Rivignano site was a recently established experiment (February 2012), while the Lincoln , Rocca Bernarda, and Beano trials were established in 2010, 2010 and 2008 respectively. Twenty five soil samples were taken from three control and three biochar-amended plots at the Rocca Bernarda and Beano sites, from four control and four biochar-amended plots at Rivignano and from five control and five biochar-amended plots at the Lincoln site (Figure 6-1). Soil samples were randomly taken with a soil auger to the depth corresponding to the biochar incorporation depth. Thereafter soil samples from each plot were homogenized, air dried and sieved using a 2mm sieve. Soil pH was determined by weighing 5g of air dried soil and adding 25ml of 1M KCl. Samples were shaken for 2 hours and after one hour pH was measured with a pH electrode (Thermo Orion, 420A plus). For the Lincoln site (with low pH) the C and N content was determined with a CNS analyser (Variomax, Elementar Analysensysteme, Hanau, Germany). For the Italian sites (with higher

pH and thus possible presence of carbonates), organic C (OC) content was determined as the difference between the total C content and the total inorganic C content of the soil samples, with the solid module of a TOC analyzer (TOC-VCPN, Shimadzu Corporation, Kyoto, Japan).

Table 6-1 Coordinates, soil texture, last crop, last fertilization rate and biochar application rate (t dry biochar ha^{-1}), depth and date, biochar characteristics (feedstock, pyrolysis circumstances, and C & N content) at each site.

	Lincoln	Rivignano	Rocca Bernarda	Beano
Coordinates	53°14'13.92"N 0°32'15.58"W	45°53'16.59"N 13°02'03.85"E	46° 1'22.96"N 13°25'40.23"E	46° 00' 00" N 13° 01' 00" E
Mean air temperature	9.4 °C	13.2 °C	13 °C	13 °C
Sand %	49	53	24	27
Silt %	23	36	57	58
Clay %	28	11	18	15
Soil texture (USDA)	sandy clay loam	sandy loam	silt loam	silt loam
Crop	Miscanthus -not harvested at sampling	Maize - harvested at sampling	Grapes (vineyard) - no undercover	Maize -not harvested at sampling
Biochar application rate	49 t ha^{-1}	30 t ha^{-1}	30 t ha^{-1}	2x10 t ha^{-1}
Application depth	10 cm	15 cm	15 cm	15 cm
Application date	2010	February 2012	July 2010	2008 (10 t ha^{-1}) 2009 (10 t ha^{-1})
Biochar feedstock	Thinnings of hardwood trees (oak, cherry and ash greater than 50mm diameter)	Woody: Pruning orchard	Woody: Pruning orchard	Coppiced woodlands (beech, hazel, oak, birch)
Pyrolysis method	400°C for 24h	Retort kiln at atmospheric pressure at 500°C	Retort kiln at atmospheric pressure at 500°C	Charcoal kiln at 500°C
Biochar C	72.3 %	42.5 %	42.5 %	87 %
Biochar N	0.172 %	0.62 %	0.62 %	1.25 %

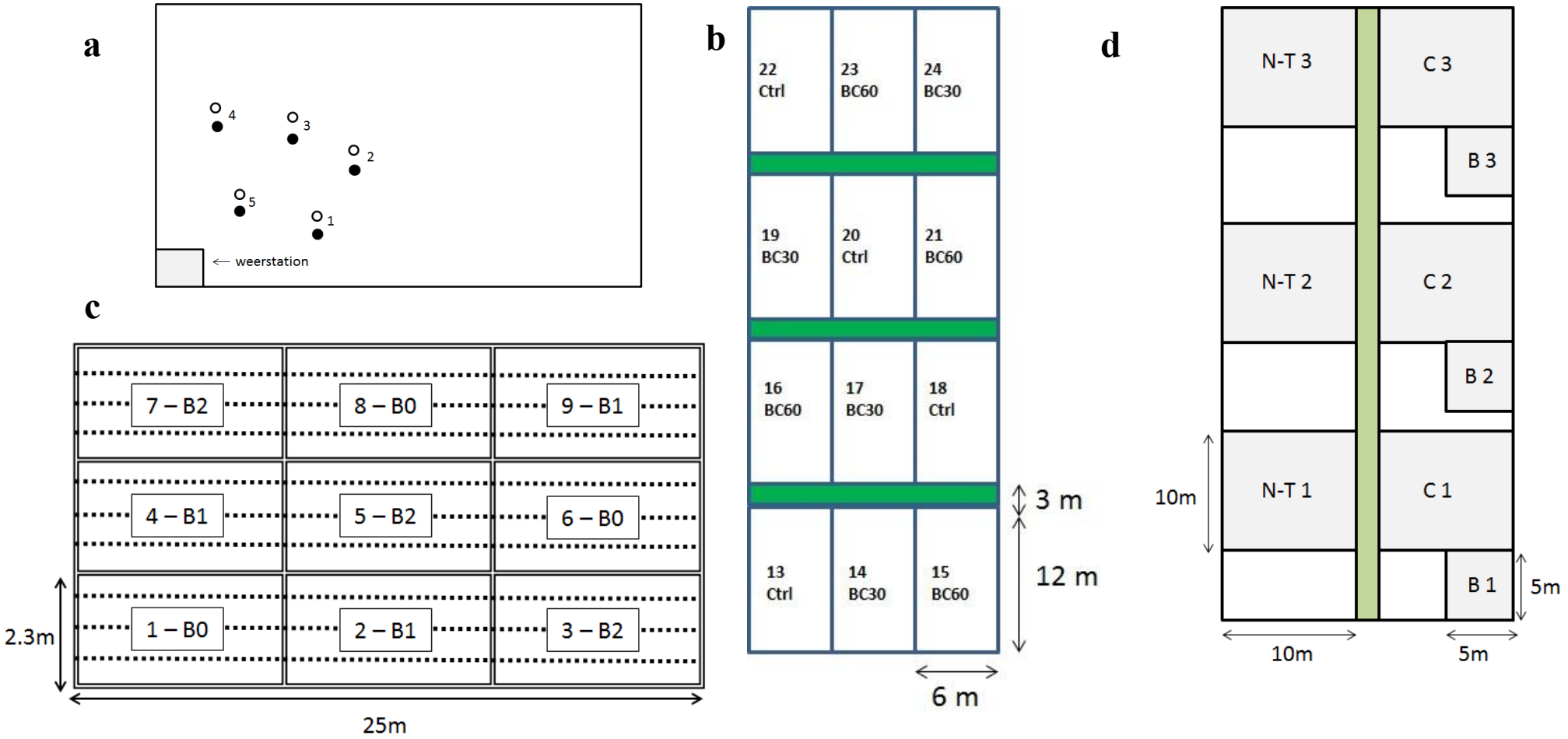


Figure 6-1 Site description of the four field trials. (a) Lincoln: open dots represent control plots (n=5), black dots represent biochar amended plots (n=5) (49 t ha^{-1}). (b) Rivignano: plots 13, 16, 20 and 24 are control plots (n=4), plots 14, 17, 19 and 22 are amended with 30 $t.ha^{-1}$ biochar (n=4). (c) Rocca Bernarda: plots 1-B0, 6-B0 and 8-B0 are control plots (n=3), plots 2-B1, 4-B1 and 9-B1 are amended with 30 $t.ha^{-1}$ biochar (n=3). (d) Beano: Plots C1, C2 and C3 are control plots (n=3), plots B1, B2 and B3 are biochar amended plots (20 t ha^{-1})

Incubation experiment and analyses

For each sampled plot soil mesocosms were prepared with 110 g of air dried soil inside PVC columns (diameter 6.8 cm). Upon filling, the soil from all sites was compacted to achieve the same bulk density of 1.4 g cm^{-3}. Deionized water was then added to each container to achieve a fixed moisture content of 50 % water filled pore space (WFPS). The soil columns were placed in closed glass jars (with a volume of 1.5 l) and put in an incubation cabinet at 15°C. After one week of pre-incubation, the emitted CO_2 was trapped in 15 ml 1M NaOH. At 14 and 8 different measuring dates for the Lincoln and Italian fields, respectively, the vials containing NaOH were removed and titrated with HCl in the presence of $BaCl_2$. At each measuring date the water content of the mesocosms was adjusted in order to maintain a moisture content of 50% WFPS.

After the incubation (for Lincoln after 63 days and for the three Italian field trials after 56 days) soil was removed from the tubes and analyzed for dehydrogenase and β-glucosidase enzyme activity, microbial biomass and community structure. The procedure for dehydrogenase enzyme activity analysis was adapted from Casida et al. (1964). Five grams of fresh soil was weighed in glass vials, then 2 ml 3% triphenyltetrazolium chloride solution and 2 ml of Tris buffer (pH 7.8) were added. Soil suspensions were incubated in the dark for 24 h at 37°C. After the incubation, 20 ml of methanol was added to each vial and the vials were shaken in the dark for 2 h with a linear shaker (125 rev min−1). Filtrates were collected in 50 ml volumetric flasks. To extract all the triphenyl formazan (TPF) produced, the remaining soil in the vials was washed twice with methanol, after which the filter papers were also washed twice. Filtrates in the volumetric flasks were made up to 50 ml with methanol. The color intensity of the filtrates was measured at 485 nm with a Hitachi 150-20 spectrophotometer (Hitachi Ltd., Tokyo, Japan). All measurements were carried out in triplicates with one blank. β-glucosidase enzyme activity was measured according to the methods described in Alef and Nannipieri (1995). One gram of moist soil was weighed in glass vials, then 4 ml of modified universal buffer (pH 6) and 1 ml of 25mM p-nitrophenyl-β-D-glucoside were added. After incubation for 1h at 37°C, 1 ml of 0.5M $CaCl_2$ and 4 ml of Tris buffer (pH 12) was added. A p-nitrophenol standard series was measured together with the filtrates at 400 nm with a Hitachi 150-20 spectrophotometer (Hitachi Ltd., Tokyo, Japan).

The structure of the microbial community was described by the fatty acid composition of the phospholipids (PLFA) in the soil. PLFAs were extracted using a modified Bligh and Dyer (1959)-technique, described in Moeskops et al. (2010). Microbial biomass (MB) was quantified by the sum of the PLFAs. For Gram-positive bacteria the sum of *i*15:0, *a*15:0, *i*16:0, *a*16:0, *i*17:0 and *a*17:0 PLFAs was used. The PLFAs cy17:0 and cy19:0 were used to quantify Gram-negative bacteria. The sum of 10Me16:0 and 10Me18:0 were used to quantify actinobacteria. The total bacterial community was assumed to be represented by the sum of the marker PLFAs for Gram-positive and Gram-negative bacteria, in addition to 15:0 and 17:0. The PLFA 18:2ω6,9c was considered as an indicator for saprotrophic fungi and 16:1ω5c as the indicator for arbuscular mycorrhizal fungi (AMF) following Joergensen and Wichern (2008).

Data analysis

Cumulative C mineralization was plotted against time (t) and a combined first-plus-zero order kinetic model was fitted to the data using the Levenberg-Marquardt algoritm (Eq. 4-1). Because we fitted through all replicates at each measuring time, the fit yields the parameters ± its standard error. Cumulative C mineralization for each plot was calculated using the first-plus-zero order model per plot at the final measuring date. Relative C mineralization was calculated as the cumulative C mineralization relative to the soil OC content of the soil. Independent t-tests were used to assess significant differences among the biochar and control treatments for soil pH, OC and N content and C:N ratio, the cumulative amount of emitted CO_2-C, enzyme activities and contents of PLFA biomarkers. All statistical tests were conducted with IBM SPSS Statistics 21 (SPSS inc., Chicago, USA). Principal component analysis (PCA) was also conducted on the nmol % composition of all PLFAs present in a proportion of more than 1 % of the total extracted amount of PLFAs.

6.3 Results

The OC (i.e. native SOC + C in aged biochar) contents of biochar-amended plots of the Lincoln and Rivignano sites were significantly higher than the OC content of control plots, however they were not significantly different at the Rocca Bernarda and Beano sites (Table 6-2). Soil nitrogen (N) contents of the control plots were not significantly different from the respective biochar-amended plots at any of the four sites (Table 6-2). Due to biochar addition, C:N ratios significantly increased at the Lincoln, Rivignano and Rocca Bernarda sites. Soil pH was higher in biochar-amended plots than in the control plots at the Lincoln and Rocca Bernarda site (Table 6-2).

Table 6-2 Soil pH(KCl) 1:5, OC and N content and C:N ratio of the control (C) and biochar-amended plots (B). Mean ± standard deviation.

Site		pH(KCl) 1:5	OC content %	N content %	C:N ratio
Lincoln	C	6.18±0.34	1.86±0.20	0.15±0.01	12.4±0.5
	B	6.81±0.27*	4.00±0.46*	0.17±0.01	23.5±2.8*
Rivignano	C	7.71±0.08	0.66±0.05	0.12±0.02	5.4±1.2
	B	7.77±0.04	1.15±0.03*	0.13±0.00	8.9±0.0*
Rocca Bernarda	C	7.51±0.05	1.18±0.20	0.11±0.02	11.1±0.5
	B	7.39±0.04*	1.36±0.10	0.11±0.01	12.6±0.7*
Beano	C	7.24±0.06	1.43±0.08	0.14±0.00	10.4±0.6
	B	7.23±0.01	1.46±0.15	0.15±0.01	10.1±0.8

*indicates significant differences between control and biochar-amended plots ($P<0.05$)

We observed significantly lower cumulative CO_2 emissions from mesocsoms prepared from the Lincoln and Rivignano sites biochar-amended plots compared to control plots (Figure 6-2). At the Lincoln site, the difference in emitted CO_2 between the biochar and control plots was the largest. The amount of CO_2-C emitted per gram OC (relative C mineralization) was lower in biochar-amended plots than in control plots at all sites; however, this decrease was not significant at the Beano site (Figure 6-3).

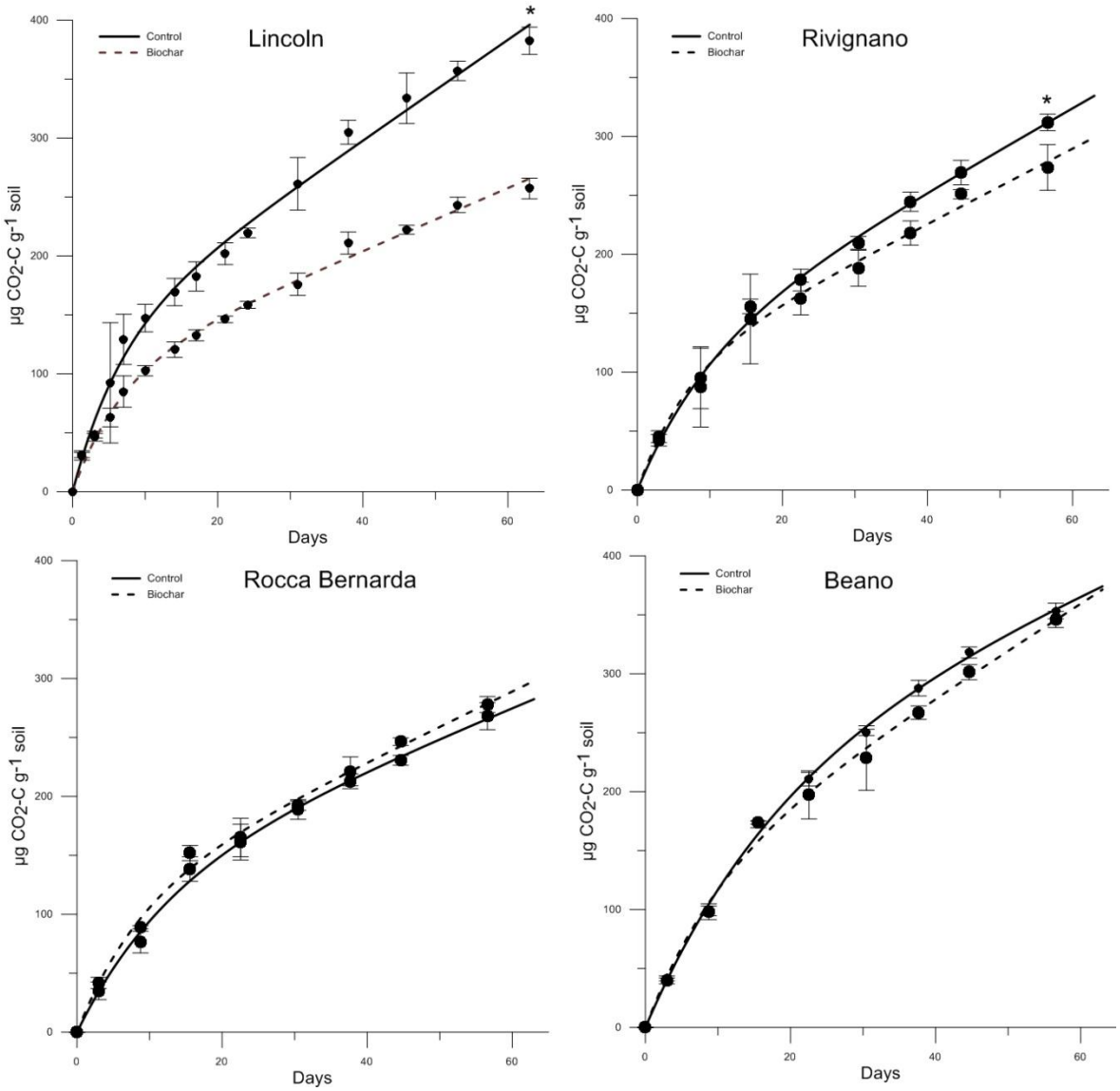


Figure 6-2 Cumulative C mineralization (µg CO_2-C g^{-1} soil) and a fitted first- and zero-order model of the control and biochar-amended plots at the four sites. Vertical bars indicate standard deviations; *indicates significant differences ($P<0.05$) between cumulative CO_2 emission at the end of the incubation of the control and biochar-amended plots.

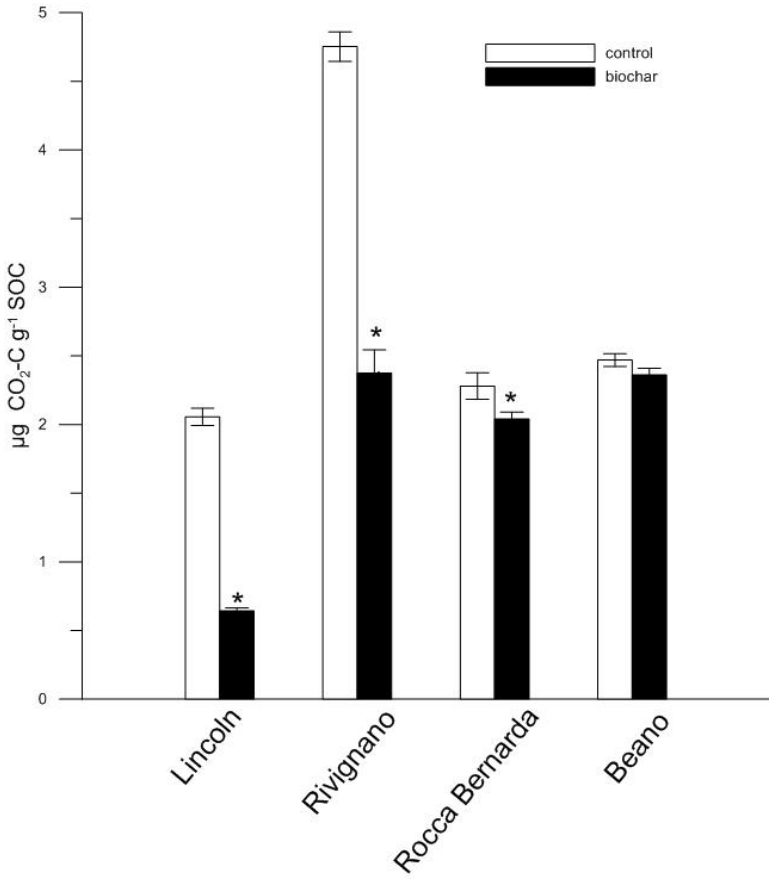


Figure 6-3 Cumulative amounts of emitted CO_2-C (after 63 days for the Lincoln sites and 56 days for the other sites) relatively to the soil C content (relative C mineralization) in the four sites. Vertical bars indicate standard deviation; * indicates significant differences ($P<0.05$) between relative C mineralization of the control and biochar-amended plots.

At each site, the size of the easily-mineralizable C pool (C_0) was lower in the biochar-amended plots compared to the control plots (Table 6-3). Mineralization rates of the slow C pool (k_s) were lower for the biochar-amended plots than for control plots at the Lincoln and Rivignano sites.

Table 6-3 Parameters (± standard error) and R^2 of the first-plus-zero order kinetic model fitted to the cumulative C mineralization data. C_0 is the size of the easily-mineralizable C pool, k_s and k_f are the mineralization rates of the slow and fast C pools, respectively.

Site		C_0 (mg C g^{-1})	k_f (day^{-1})	k_s (mg C g^{-1} day^{-1})	R^2
Lincoln	C	127.2±13.1	0.15±0.03	4.27±0.30	0.994
	B	97.2±7.7	0.15±0.02	2.67±0.18	0.995
Rivignano	C	113.3±22.0	0.10±0.03	3.51±0.45	0.997
	B	98.2±13.0	0.15±0.04	3.19±0.30	0.996
Rocca Bernarda	C	124.2±26.9	0.08±0.02	2.52±0.51	0.997
	B	110.1±18.4	0.12±0.03	2.98±0.40	0.996
Beano	C	220.9±53.4	0.05± 0.01	2.55±0.82	0.999
	B	122.4±27.4	0.10±0.03	3.96±0.56	0.996

*indicate significant differences between biochar and control plots ($P<0.05$)

Except for the Beano site, dehydrogenase enzyme activity was significantly lower in the biochar-amended plots when compared to control plots (Figure 6-4a). β-glucosidase activity was significantly lower in the biochar-amended soil than in the control soil at the Lincoln site, whereas no significant differences were observed at the other sites (Figure 6-4b).

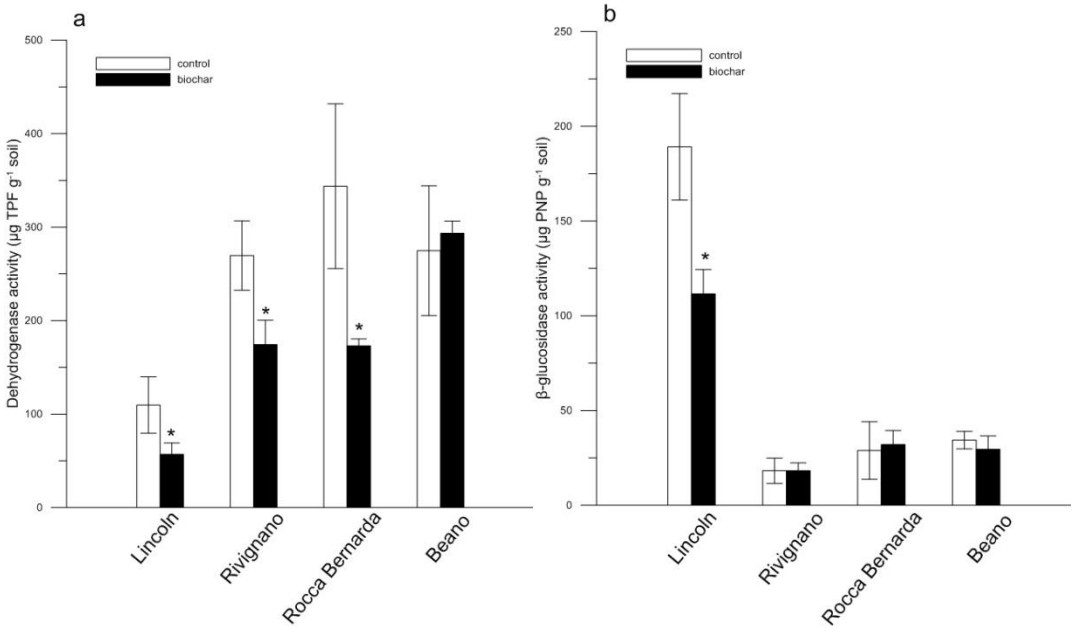


Figure 6-4 Dehydrogenase enzyme activity (a) and β-glucosidase enzyme activity (b) of the four selected sites, Vertical bars indicate standard deviation; * indicates significant differences ($P<0.05$) between control and biochar-amended plots.

For the Italian sites (Rivignano n=4; Rocca Bernarda and Beano n=3), no significant shifts in microbial groups were observed between biochar-amended and control plots (Figure 6-5 and Table 6-3); a significant shift was only found at the Lincoln site (n=5). For the latter site, the sum of all extracted PLFAs (indicative for the microbial biomass) was higher in the soil mesocosm from control plots than from biochar-amended plots (Table 6-4). Additionally, fungal abundance was significantly lower, and B:F ratios were higher in biochar-amended plots than in control plots.

Table 6-4 Proportions of marker PLFAs for Gram-positive, Gram-negative, Actinomycetes, Fungi and AMF (% of total extracted PLFAs), sum of all PLFAs (nmol g^{-1}soil) and the B:F ratio (-). Mean ± standard deviation.

Site		Sum of the PLFAs	Gram-positive	Gram-negative	Actinomyc.	Fungi	AMF	B:F ratio
Lincoln	C	44.85±3.26	22.39±0.64	10.84±0.11	9.09±0.66	2.82±0.14	6.58±0.58	12.35±0.85
	B	34.96±3.07*	22.43±0.51	11.56±0.27	8.90±0.52	2.07±0.39*	6.02±0.27	17.50±3.33*
Rivignano	C	26.70±2.40	23.65±1.77	10.59±0.32	9.51±0.37	1.99±0.19	4.53±0.39	17.91±2.05
	B	28.67±3.06	23.42±0.78	10.44±0.44	9.39±0.41	1.89±0.13	4.75±0.23	18.57±1.48
Rocca	C	28.32±12.61	19.19±1.58	8.80±0.86	7.73±0.82	4.55±0.88	4.64±0.50	6.64±1.57
Bernarda	B	27.82±3.92	18.74±1.37	8.42±0.64	7.80±0.68	4.75±1.06	5.23±0.11	6.24±1.82
Beano	C	32.19±13.31	19.25±3.59	9.14±2.05	8.20±2.21	3.04±2.21	4.17±0.79	13.78±8.60
	B	25.77±2.54	21.59±0.82	10.83±1.01	8.99±0.22	1.87±0.52	4.30±0.28	19.21±7.16

*indicates significant differences (P<0.05) between control and biochar-amended plots

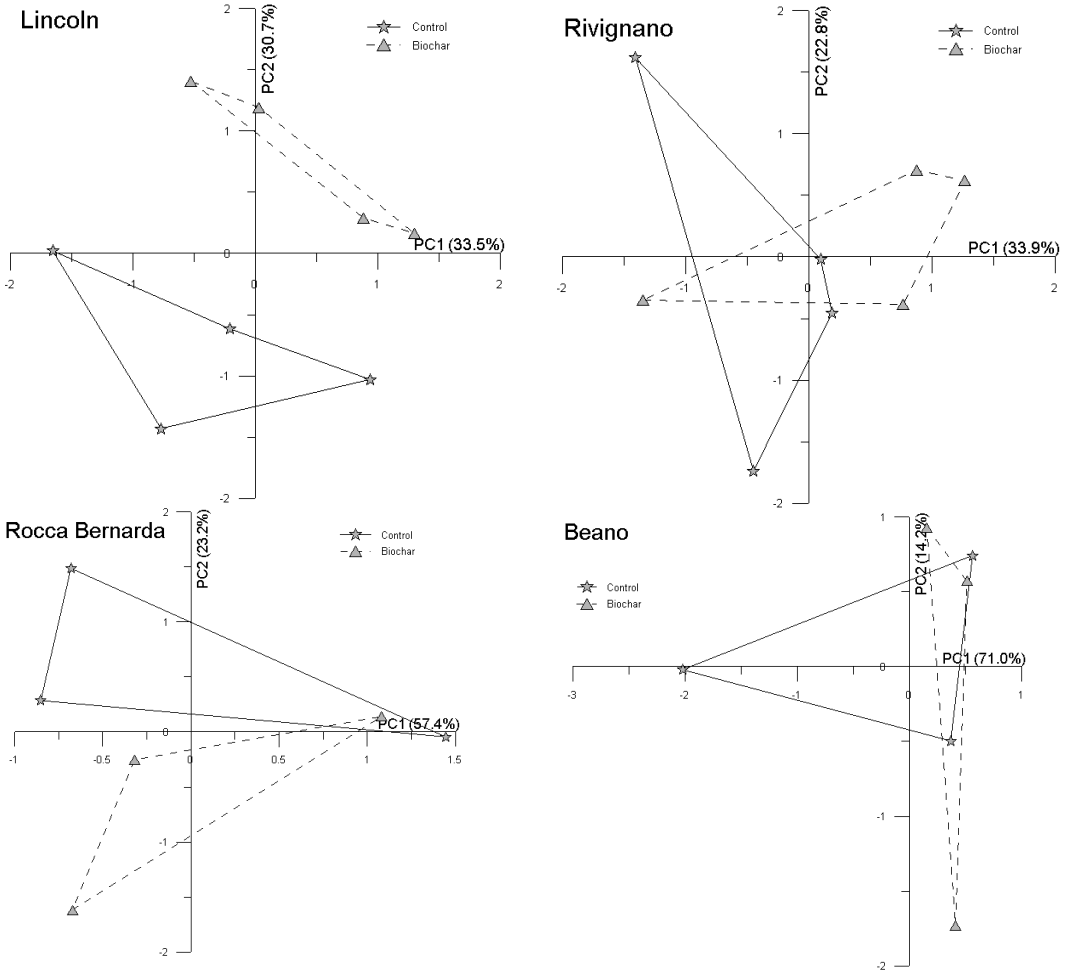


Figure 6-5 Scatter plots of the first two principal components (PC) of the PCA on the PLFAs of the four sites (full lines: control plots, dotted lines: biochar amended plots).

6.4 Discussion

Soil C content and C mineralization

In this study we assessed the effects of biochar addition on microbial communities, soil respiration and OC content in four established field experiments located in the UK and Italy. Only at the Lincoln and Rivignano sites the biochar-amended plots had significantly higher organic C contents compared to their corresponding control plots. At the Lincoln field experiment, 49 t biochar ha^{-1} was incorporated to a depth of 10 cm, leading to an increase in soil OC content of 115%. Lower biochar rates were applied to the other field trials.

Although the air-dried soil sampled from the field sites were pre-incubated for one week, we still observed an initial flush of CO_2 emission, most likely derived from decomposition of easily-degradable SOM components. To account for this initial exponential flush followed by a linear increase, we chose to model data by means of a parallel first-plus-zero-order kinetic model. In Chapter 4 a significant net C mineralization was observed after the addition fresh biochar produced at 350°C (10 t ha^{-1}), through a 117 days incubation experiment, but no net C mineralization with 700°C biochar. Additionally, they found a positive correlation between the easily-mineralizable C pool (C_0) and the volatile matter contents of the biochars, indicating these volatile compounds may form a microbial substrate in the short-term. In the multi-year experiments, however, we found lower relative C mineralization rates in biochar-amended plots compared to control plots of all sites (not significantly in the Beano site). Additionally, the easily-mineralizable C pool was smaller in all biochar-amended plots compared to their corresponding control plots. It would appear that the volatile C compounds contained within the biochar had already been microbially decomposed during 1-4 years of biochar incorporation in the field. At Lincoln and Rivignano, the sites with the highest difference in soil C content between biochar and control plots, there was a lower mineralization rate of the slow C pool (k_s) in biochar-amended plots compared to control plots. This was also found for soil cores amended with fresh biochar by Ameloot et al. (2013a), and would reflect the low degradability of the polyaromatric biochar matrix in the soil. Several authors have reported that volatile biochar compounds may mineralize in the short-term, where after the polyaromatic recalcitrant matrix may remain in the soil for tens to hundreds of years (Smith et al., 2010; Cross and

Sohi, 2011). However, this does not explain the net lower C mineralization in soil amended with biochar at these sites. Several mechanisms might explain a lower C mineralization in soil amended with biochar:

i) Abiotic precipitation of soil-borne CO_2 can reduce the apparent amount of CO_2 emitted from the biochar-amended soils and this process increases with pH (Joseph et al., 2010; Lehmann et al., 2011; Case et al., 2013). However, at all field sites both biochar-amended and control plots had pH values that were close to neutral conditions (Table 2), under which conditions CO_2 precipitation would not occur. This suggests that abiotic CO_2 precipitation was not of importance in reducing CO_2 emissions from biochar-amended plots.

ii) A shortage of N in the biochar-amended sites might have occurred, due to nitrate (NO_3^-) and/or ammonium (NH_4^+) adsorption on the biochar surface through increased anion exchange capacity (AEC) and cation exchange capacity (CEC), respectively. Clough et al. (2013) found that NO_3^- adsorption only occurred in biochars produced at a temperature of at least 600°C, suggesting that NO_3^- adsorption did not occur in our study. However, NH_4^+ adsorption was found to be less dependent of high pyrolysis temperatures (Clough et al., 2013) and may reach up to 75% of the NH_4^+ in the added solution (Dempster et al., 2012). This limitation of N may have inhibited soil microorganisms to mineralize native SOM substrates, since they need substantial amounts of N to maintain their tissue C/N ratios.

iii) At sites where we observed lower respiration rates in biochar-amended plots compared to control plots, another explanation might be that less native SOM was respired than in the control plots, i.e. that negative priming occurred. Native SOM decomposition has in laboratory incubation experiments been found to be retarded by the presence of biochar (Cross and Sohi, 2011; Zimmerman et al., 2011). This was ascribed to a stabilization of native SOM, which may have been due to adsorption of labile SOM components onto the reactive surfaces of biochar (Cross and Sohi, 2011; LeCroy et al., 2013) or physical trapping within biochar pores (Major et al., 2009).

Microbial community structure

Over all experiments, measures of microbial activity and biomass generally decreased in biochar-amended plots compared to control plots. These results suggest inhibition of the activity of soil organisms in biochar-amended plots, which can be caused by a toxic effect of biochar compounds to microorganism (Graber et al., 2010) or by decreased substrate availability in biochar-amended plots. Across the investigated biomarkers, most strikingly there was a consistent higher B:F ratio in biochar plots compared to control plots and a lower fungal abundance, even if a no significant decrease in fungal abundance was detected also at Rivignano and Beano. Wiedner and Glaser (2013) reviewed biochar-fungi interactions in soils and concluded that saprophytic fungi can be both promoted or inhibited by the presence of biochar in the soil. Possible toxic effects of biochar towards fungi may be an explanation for the observed shift towards lower fungal abundance in biochar-amended plots. Only at the Lincoln site data on the PAH and heavy metal content of the used biochar were available. The PAH content (16 hazardous EPA listed PAHs) of the biochar used in the Lincoln site was 8 mg kg^{-1} biochar (Case et al., 2012). Considering the application rate of 49 t ha^{-1}, the incorporation depth of 10cm and soil density of 1.4 t m^{-3}, this leads to a soil PAH content of 0.28 mg kg^{-1} soil. Several fungi have been reported to have the ability to metabolize PAHs, which makes them attractive for the bioremediation of contaminated soils with PAH concentrations much higher than the concentrations in the Lincoln site (Sack and Gunther, 1993; Cerniglia, 1997). Therefore, it is unlikely that the relatively low concentration of PAHs present in biochar-amended soils could be toxic to fungi. Moreover, fungi were found to be less sensitive to PAH contamination than Gram-negative bacteria (Yang et al., 2007); while no decrease in the abundance of the latter was observed in biochar-amended plots. Also, heavy metals have been reported to be toxic towards fungi (Baldrian, 2010). The calculated heavy metal contents were of the Lincoln site 0.21 mg Pb kg^{-1} soil, 0.39 mg Cr kg^{-1} soil, 0.63 mg Cu kg^{-1} soil and 2.84 mg Zn kg^{-1} soil (Case et al., 2012). These concentrations are low compared to the concentrations that have been found to be toxic for microbial activity (Kuperman and Carreiro, 1997) and fungi (Baldrian, 2010). For these reasons, it is unlikely that toxic effects of PAH and heavy metals towards fungi may have occurred. Since no evidence for biochar toxicity towards fungi was found at the Lincoln site (with the greatest shift in microbial community structure and the highest reduction in microbial abundance

and activity), it seems unlikely that at the other field sites considered in this study with biochar derived as well from woody biomass was toxic towards soil microorganisms.

Another possible explanation for the decreased relative fungal abundance in biochar-amended plots may be the unsuitability of biochar as a substrate for fungi. Ascough et al. (2010) reported no colonization of two saprofythic fungi on wood-derived biochar, apart from a small number of cracks within the biochar. They concluded that biochar was an unsuitable substrate for fungi. However, in the presence of biochar the fungal hyphae were actively growing and searching for other easily-available substrates (Ascough et al., 2010). A decreased fungal to bacteria ratio in the biochar-amended plots compared to their corresponding controls may be explained by this mechanism.

The coinciding of lower soil C mineralization with a lower fungal abundance in biochar-amended plots compared to control plots may indicate that both are related. Bodé et al. (2013) investigated residue degradation in combination with microbial inhibition of fungi and bacteria. Fungal inhibition reduced the capacity of bacteria to degrade organic substrates. Therefore they suggest that bacteria need fungi to decompose organic substrates. In biochar-amended plots the available substrate, modelled as the C_0 parameter was found to be lower than in control plots. Indeed, one suggested mechanism to explain suppressed CO_2 emissions in biochar-amended plots, other than direct inhibition, is the adsorption of labile SOM components on the biochar surface (Cross and Sohi, 2011; LeCroy et al., 2013). The chemical nature of these labile SOM components is not yet fully understood and needs to be addressed in future research. Whether labile SOM was adsorbed onto the biochar could not be confirmed in this study and requires further investigation. In the most recently established field site (Rivignano) this shift towards lower fungal abundance was not observed, suggesting that available substrate was still sufficient to maintain fungal biomass. Possibly, volatile biochar compounds may still have acted as a substrate for fungi by the time of sampling (7 months after biochar addition) (Smith et al., 2010; Cross and Sohi, 2011; Ameloot et al., 2013a). Alternatively, labile SOM adsorption onto the biochar may not have occurred at Rivignano a significant degree after only 7 months of field incorporation, as this adsorption has been found to increase when biochar ages and oxidizes following soil incorporation (LeCroy et al., 2013).

6.4.1 Conclusions

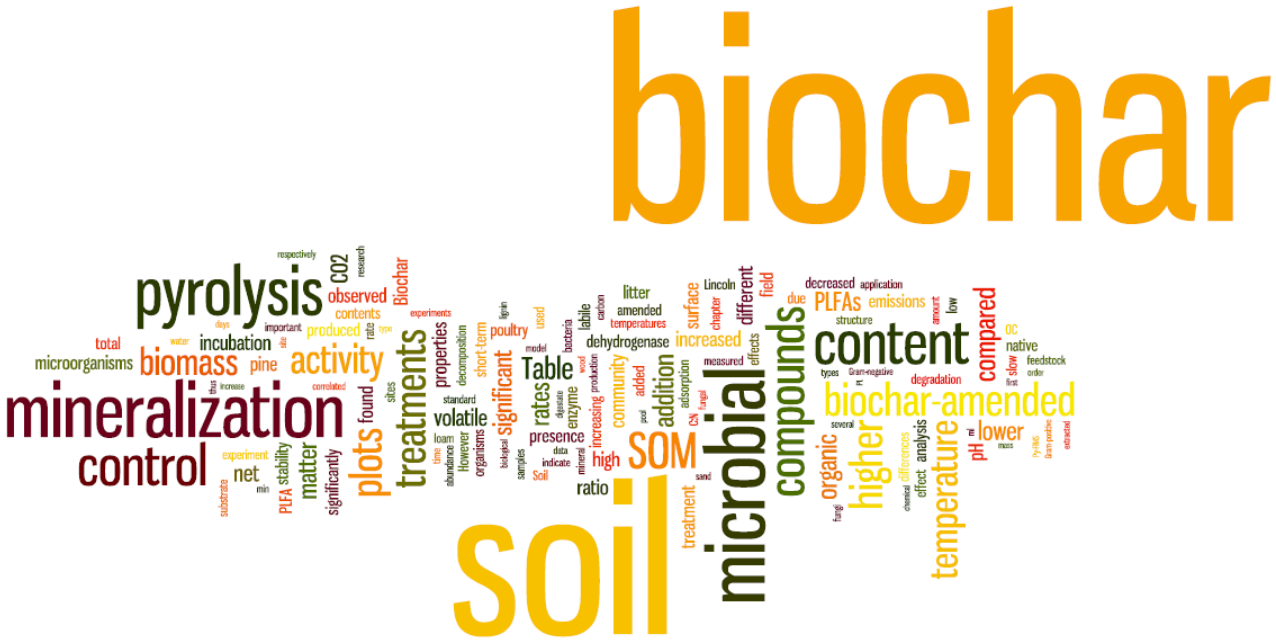
While most studies have assessed the effect of biochar on soil microbial properties during short-term incubations, analysis of multi-year biochar field experiments in this study enabled the understanding of microbial communities after several years of biochar incorporation. Negative priming of SOM was found in all biochar-amended plots and was accompanied by decreased microbial biomass and activity and decreased substrate availability. We found no indications for toxic effects of biochar towards soil organisms but a general inhibition of microbial activity and soil respiration was apparent in all fields. It thus seems unlikely that biochar would still function as a substrate after 1-4 years of aging in the field, in contrast to many short-term lab studies. The potential adsorption of labile SOM compounds or mineral-N on the reactive biochar surface would be a likely mechanism explaining 'negative priming' of native SOM mineralization. Further mechanistic research will be required to investigate the significance of such a mechanism.

Acknowledgement

The sampling campaign at the Italian sites was supported by the European community's Seventh Framework programme (FP2007-2013) under grant agreement no. 262060 (ExpeER). For Lincoln, we acknowledge the Natural Environment Research Council for providing a PhD studentship award to Sean Case (NE/H525346/1) and additional support from CEH project number NEC03487. Thanks to Dr. Jeanette Whitaker (Centre for Ecology and Hydrology, Lancaster, UK) and Dr. David Reay (University of Edinburgh, UK) for assistance with the design and implementation of the Lincoln field experiment. Furthermore we acknowledge the biochar COST action (TD1107).

Chapter 7:

General Discussion and conclusions



7.1 Introduction

Biochar has the potential to sequester C in the long term, however the role and fate of soil organisms in biochar-amended soils remains unclear. In this thesis we investigated the effect of biochar on soil microorganisms, through both short-term (incubation) and multi-year (field) experiments. Furthermore, we used an advanced molecular-level analysis technique to identify the biochemical nature of biochar compounds and investigated the effect of biochar on soils with different native SOM levels. In this last chapter the main findings of the research carried out are synthesized and answers are formulated for the four major research questions:

(ii) How do biochar characteristics influence biochar degradation? (section 7.2)

(iii) Methodological issue: Are microbial assays compatible with biochar-amended soil? (section 7.3)

(iv) How does short-term biochar stability differ from multi-year biochar stability? Does presence of biochar in the soil still significantly affect microbial activity after multi-year field incorporation and is this effect different than observed in short-term incubations? (section 7.4)

(v) Does biochar have an effect on native SOM mineralization and what is the role of soil microorganisms herein? (section 7.5)

As biochar research is still at an early stage of development, quite some research questions remain unanswered and therefore we will conclude with suggestions for further research (section 7.6).

7.2 Biochar characteristics and biochar stability

From the review, carried out in Chapter 1, it is clear that pyrolysis temperature strongly influences the C content of biochars. In our incubation experiments (Chapter 4 and 5) we found lower C and N mineralization rates and microbial activities in the soils amended with high temperature biochars compared to soils with the corresponding biochars produced at lower temperature. It would thus appear that biochar becomes more biologically inert with increasing pyrolysis temperature. In Chapter 4, we measured CO_2 emissions from a sandy loam soil amended with four biochar types, produced at 350°C and 700°C and from two very different feedstocks, namely willow wood and digestate. We found comparable CO_2 emission

rates from the soils amended with biochar produced at the same pyrolysis temperature, independently from the biochar feedstock. Also in Chapter 2 and 5 the C and N mineralization rates were always lower in soil amended with biochar produced at higher temperatures, compared to soil with lower temperature biochar. With increasing pyrolysis temperature, volatile matter content decreases to the benefit of fixed C content (Cross and Sohi, 2011; Ronsse et al., 2013). This fixed C is less degradable and remains thus more stable when applied to the soil.

In Chapter 4, we found a significant positive correlation between the modelled parameter for the easily-degradable C pool (C_0) and the biochar's volatile matter content. This gave clear indications for the importance of volatile biochar compounds to supply an easily-available C source for soil microorganisms. Detailed biochemical characterization of biochars in combination with short-term C mineralization experiments, revealed the chemical nature of volatile biochar compounds. Py-FIMS characterization of the 500°C biochars was difficult due to limited volatilization of OM fractions in the biochars. Still several N-containing compounds positively correlated with the net C mineralization. Probably microorganisms degraded heterocyclic N compounds indirectly in their search for N in soils with little N, due to the addition of biochar with a high C:N ratio.

In Chapter 2 and 5, we observed lower C mineralization rates from soils amended with pine biochars compared to the other biochars. These biochars were found to have higher phenols and lignin monomer contents compared to other biochars (Chapter 2). Moreover, the content of phenols and lignin monomore compounds were negatively associated to the C mineralization (Chapter 2). These results suggest that these compounds are important in biochar stabilization from microbial degradation.

7.3 Methodological issues

Biochar is known to have a considerable surface area, covered with functional C groups. Immobilization through adsorption of organic molecules or ions may introduce at present poorly known methodological issues when assessing microbial soil assays in the presence of biochar. For example, substrates added to soil for enzyme activity assays may be bound or the extraction efficiency of PLFA biomarkers may be reduced by presence of biochar in soil samples. Therefore, the compatibility of several widely used microbial assays in biochar-

amended soils was investigated (Chapter 3). In this section we will reconsider the results from the microbial measures determined in the other chapters with the compatibility results in our mind.

In this compatibility experiment we found that enzyme activity assays were not influenced by the presence of biochar, apart from a lower dehydrogenase enzyme activity in a loam soil in the presence of 500°C poultry litter biochar. In Chapter 5, the same biochar was incubated in two silt loam soils and after 14 weeks dehydrogenase enzyme activity was determined. Although no significant decrease was observed in the PL500 treatments compared to the control, possible increased dehydrogenase activity might have been masked. Fumigation-extraction of microbial C appeared to be signifiantly influenced by the presence of biochar in different ways (Chapter 3). Reasons for this may be that the K_2SO_4 extractant is capable of extracting biochar compounds from biochars, that adsorption of chloroform lysed microbial soil compounds onto the biochar surface may occur or that choloform is capable of extracting OC compounds from the biochar amended to the soils. If, in future biochar research, the fumigation-extraction technique is used as a measure for microbial biomass, the k_{EC} factor should be carefully re-assessed for biochar-amended soils. Without the assessment of a new k_{EC} factor, results from fumigation-extraction studies in the presence of biochar should be interpreted with caution. Zelles (1999) meta-analysed available studies on PLFA profiles and found a good correlation between the sum of the PFLAs and the microbial biomass. Moreover, in Chapter 3 we did not find an influence of the presence of biochar on the sum of the PLFAs. For these reasons, we quantified microbial biomass by the sum of the PLFAs in Chapters 4, 5 and 6 of this thesis. We found a lower extraction efficiency of 18:1ω9c PLFA, which can be used as a fungal biomarker (Buchan et al., 2012) in the presence of poultry litter biochar (Chapter 3). Therefore, in this thesis the PFLA 18:2 ω6,9c was taken as fungal biomarker.

Dehydrogenase enzyme activity was used as a proxy for microbial substrate degradation in Chapters 4, 5 and 6 and enabled the better interpretation of the microbial response in biochar-amended soils. However, when considering the magnitude of the dehydrogenase enzyme activities measured in Chapter 4, 5 and 6, it seems that for this assay there was a considerable difference between the batches. Especially the results of Chapter 4 and 5 gave evidence of a batch problem. In Chapter 4 the data ranged from 0.2 till 2.7 µg TPF g^{-1} soil, while in Chapter 5 data ranged from 27 till 130 µg TPF g^{-1} soil. Although both experiments were carried out in a comparable soil and during a comparable incubation experiment, dehydrogenase enzyme activities were many times higher in Chapter 5 than in

Chapter 4. Therefore comparisons of the absolute amounts of dehydrogenase activities from the different chapters are not possible. Yet, the comparisons between the different biochar and control treatments measured within the same chapter, and thus within the same batch are still valid.

7.4 Short-term degradation versus multi-year biochar stability

In Chapters 2, 4 and 5 we carried out short-term incubation experiments, with biochar freshly added to soil tubes to assess short-term effects of biochar. In Chapter 4, a C mineralization experiment was carried out by measuring CO_2 emissions from a sandy loam soil amended with several biochar types and from an unamended control. CO_2 data were plotted against the incubation time and a first-plus-zero-order model was fitted to the data. The parameter representing the easily-degradable C pool (C_0) was positively correlated with the volatile matter content of the biochars ($n=4$, $r=0.99$, $P<0.01$). We found thus indications for the importance of volatile biochar compounds to supply an easily-degradable C source to soil microorganisms. Gram-positive and Gram-negative bacteria were more abundant in the low temperature biochar treatments, suggesting that these bacteria may use the biochar compounds as an energy source. However, in Chapter 5 we did not find a higher Gram-positive abundance in the low temperature biochars when compared to the high temperature biochar treatments. In Chapter 4, volatile matter contents of the 700°C digestate and willow biochars were about 66 and 61% lower (respectively) than in the corresponding 350°C biochars. In contrast, volatile matter contents of the 500°C biochars used in Chapter 5 were only 37 and 25% lower than the 400°C pine and poultry litter biochars, respectively. Possibly the pyrolysis temperatures at which the biochars were produced in this experiment (400 and 500°C) and the consequent volatile matter contents did not differ enough from each other, which resulted in no difference in the Gram-positive bacterial abundance.

In Chapter 6 the multi-year effects of soil applied biochar on microbial activity were examined. In the short-term incubation experiments soil organisms will mostly be directly influenced by the presence of volatile biochar compounds, that can act as a substrate for microorganism. Instead, in the multi-year experiments these direct effects are expected to fade and in addition ‘aging’ of the biochar (i.e. alterations in pH, CEC, O:C ratio and functional

groups through biotically and abiotically mediated oxidation) becomes important in influencing the microbial community. Soil was sampled from four biochar field experiments, C mineralization was measured and modelled by a first-plus zero-order model. In the biochar-amended plots the C_0 parameter was always lower than in the unamended control plots, indicating that C in the biochar plots was less available to soil organisms. In contrast in most field sites, C content of the biochar-amended plots was higher than in the unamended control plots, and consequently the relative C mineralization was lower in the biochar plots. Relative C mineralization rates were significantly positively correlated to dehydrogenase enzyme activity measurements, indicating that metabolic activity decreased in the biochar plots compared to the unamended plots. It seems thus that in soils amended with biochar microbial activity is inhibited, probably due to decreased substrate availability or N limitations, due to adsorption of mineral N on the biochar. A shift towards less fungi was observed, except in the most recently established field experiments. Possible suggested explanations for this were the unsuitability of aged biochar as a fungal substrate and adsorption of lable SOM compounds on the biochar.

7.5 Effect of biochar on native SOM mineralization

In biochar-amended soils both increases as well as decreases of native SOM mineralization have been reported, the so-called positive or negative priming effect (Hamer et al., 2004; Novak et al., 2010; Cross and Sohi, 2011; Keith et al., 2011; Luo et al., 2011; Zimmerman et al., 2011). Enhanced mineralization of native SOM can be attributed to several mechanisms. Firstly, the native SOM can be co-metabolized due to substrate-induced microbial growth (Kuzyakov et al., 2000) or or microorganisms may decompose native SOM in order to acquire N needed for the decomposition of biochar (Blagodatskaya and Kuzyakov, 2008).

In Chapter 6 we found evidence of negative priming in the biochar-amended plots. In some biochar-plots amended there were lower C mineralization rates than in the unamended control plots. It is thus likely that less native SOM was respired in the biochar-amended plots than in the control plots. Stabilization of native SOM compounds in the presence of biochar was suggested also by Cross and Sohi (2011) and Zimmerman et al. (2011). Toxic effects of biochar on soil organisms were unlikely, since even in the field site with the highest microbial

reduction no evidence of biochar toxicity was found. This suggests that microorganisms were inhibited due to decreased substrate availability in the biochar-amended plots compared to the control plots. Possibly labile SOM compounds were physically trapped (Major et al., 2009) or adsorbed in the biochar's micropores (Cross and Sohi, 2011), which are then inaccessible to microorganisms.

We found in Chapter 5 evidence for an interactive control of native SOM content on the effect of biochar application on soil microbial activity. Both N mineralization and N immobilization after the addition of the poultry litter and pine biochars, respectively, were more pronounced in a soil with a high SOM level (H) compared to a soil with a nearly twice as low SOM level (L). In line, the addition of biochar induced higher microbial biomass and activity increases in the H soil compared to the L soil. At first sight a possible explanation would be that positive priming of native SOM mineralization were enhanced with increasing SOM level. However, when considering the H soil's double C and N content, the responses in the H soil were in fact disproportionally smaller than in the L soil. Physicochemical fractionation pointed to a somewhat more labile character of the native SOM in the L soil when compared to the H soil and this may partly explain the disproportional relation between priming of N mineralization by biochar application and soil C and N content. Yet the differences in SOM quality between both soils were relatively limited and other mechanistic explanations should be looked into.

7.6 Future research

7.6.1 Effect of biochar on soil fauna

Like soil microorganisms, soil fauna will be affected in biochar-amended soils. This thesis and the vast majority of the literature assessing the impact of biochar on soil biology deals only with microbial communities, and the impact of biochar on soil fauna is clearly under-researched. This seriously impairs our ability to fully understand the fate and stability of biochar added to soils. Most authors reporting on earthworms in biochar-amended soils could not draw conclusions about the factors important in earthworm preference and survival (Liesch et al., 2010; Beesley and Dickinson, 2011; Li et al., 2011); this emphasises the need for better understanding of the interactions between different types of biochar and earthworms in a variety of soils. Biochar particles also can be broken down physically, thereby facilitating

abiotic and biotic decomposition. In this way, earthworms are likely to influence biochar stability.

Additionally, there is an urgent necessity to address the role of soil fauna other than earthworms in biochar degradation as part of soil C cycling. Apart from earthworms, other soil fauna such as protozoa, nematodes, collembola, microarthropods and termites may play an important part in fate of biochar after incorporation in the soil. Some research has been published on the effect of biochar on nematode abundance and functional groups (Zhang et al., 2013) and collembola (Salem et al., 2013). However the link between biochar stability and soil macro-organisms is far from understood. Elucidating the impacts of soil fauna directly and indirectly on biochar stability is an important research priority.

In the soil from the biochar field experiments (Chapter 6), we investigated nematode abundance and this was found to decrease in the biochar-amended plots compared to the control plots (Figure 7-1). By monitoring the expression level of crucial genes, indicative for species sensitivity to external stress in the nematode *Caenorhabditis elegans*, Liuzzi et al. (2012) reported that the presence of PAHs has a detrimental effect on these genes and on the survival of nematodes. We suggest that this observed decrease might be due with the abundance of toxic compounds, such as PAHs. However, more research is needed to confirm this hypothesis.

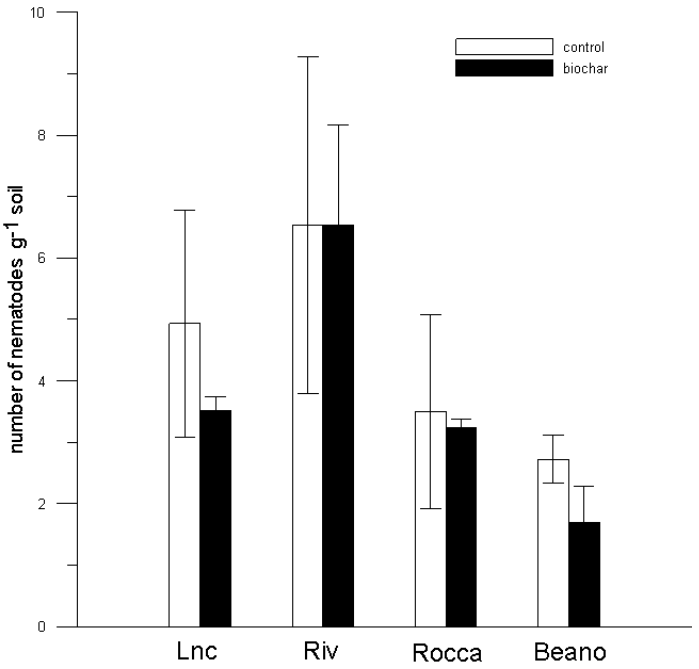


Figure 7-1 Number of nematodes per gram soil of the control plots (white bars) and the biochar-amended plots (black bars) of the four selected field trials in Lincoln, Rivignano, Rocca Bernarda and Beano.

7.6.2 Indirect effects

While we found evidence for the microbial consumption of apparently bio-available biochar components, it is much more difficult to investigate indirect effects of biochar on soil microorganism. The porous nature of biochar has often been suggested to be of importance in influencing soil microbial communities through physical protection agains bacterial predators. However, it has to be borne in mind that the average pore size of biochars (nm scale) is much smaller than that of the smallest soil organisms (µm scale) (Hassink et al., 1993). Therefore, the ability of biochar to protect microorganisms against grazers may be insignificant. A second potential indirect control of biochar on the soil microbial activity could be through water retention in the biochar pores which would alter the soil water balance. However, many biochars have a hydrophobic nature. Finally, some biochars have the capacity to retain pesticides, mineral N sources or other organic compounds, such as labile SOM compounds, through adsorption on its reactive surface or through physical protection in biochar micropores, too small for soil organisms (Loganathan et al., 2009; Qiu et al., 2009; Major et al., 2010; Graber et al., 2011a; Graber et al., 2011b). Unravelling how these indirect effects

influence soil microorganisms requires a mechanistic and well-thought-out research approach, and is also an important research priority.

7.6.3 N_2O emissions in biochar-amended soils

N_2O is an important GHG and is produced in the soil as a by-product during denitrification, in which specialized microorganisms reduce nitrate (NO_3^-) to N_2. Alternatively, N_2O is produced in the soil during the process of nitrification, whereby nitrite is used as an alternative electron acceptor while it is reduced to N_2O. Denitrification is the most important N_2O emission process in agricultural soils (Dalal et al., 2003). The enzymes involved in denitrification are produced by anaerobic heterotrophic bacteria that are inhibited by the presence of O_2 (Aulakh et al., 1991; Richardson et al., 2009). This implies that dentrification occurs in soils with little O_2, but with sufficient NO_3^- and available C. Denitrifier activity increases above 70% WFPS, and as a consequence N_2O emissions increase. However, in soils approaching full saturation (and thus complete anoxic conditions) complete denitrification to N_2 is likely to occur .

Most authors have observed decreased N_2O emissions from soils amended with biochar of various types. Many mechanisms for these decreased N_2O emissions have been hypothesized. The increased porosity and consequent increased O_2 levels in biochar-amended soils may influence the anaerobic denitrification process. A higher soil porosity implies increased O_2 diffusion, which suppresses N_2O and N_2 emissions. In contrast, higher water retention in biochar pores might create anaerobic 'hot spots' at which complete denitrification till N_2 is likely to occur, thereby preventing N_2O emission. More alkaline soil circumstances stimulate the activity of the enzyme N_2O reductase. Increased pH due to the addition of alkaline biochar may in this way decrease N_2O emissions. Another possible hypothesized mechanism for lowered N_2O emissions, might be a decreased NO_3^- availability due to enhanced microbial N-immobilisation resulting from microbial consumption of N-depleted volatile biochar compounds. Furthermore, adsorption of NH_4^+ and NO_3^- through increased CEC and AEC, would also reduce the amount of NO_3 that is available for denitrification. A shortage of available C, that can be used as a substrate by denitrifiers in soils with recalcitrant biochar might also decrease the denitrification potential, leading to lower N_2O emissions rates. Finally chemical reduction of N_2O has been forwarded as a potential mechanism alleviating its emission. Adsorbed aromatic and aliphatic compounds onto the biochar surface might be oxidized by the presence of N_2O,. Alternatively, N_2O can be removed by chemisorption onto

active sites at the biochar's reactive surface. It is clear that the processes influencing N_2O emissions in biochar-amended soils are far from unravelled and future mechanistic research should address this knowledge gap.

Summary

Biochar addition to soil influences soil micoorganisms in different ways. Indirectly, biochar's porous nature and reactive internal surface changes the microbial habitat. Additionally, some biochar compounds can be a food source for soil microorganisms. In this PhD dissertation we tried to find an answer to four emerging research questions: 1) How do biochar characteristics influence biochar degradation?; 2) Methodological issue: Are microbial assays compatible with biochar-amended soil?; 3)How does short-term biochar stability differ from multi-year biochar stability? Does presence of biochar in the soil still significantly affect microbial activity after multi-year field incorporation and is this effect different than observed in short-term incubations?; 4) Does biochar have an effect on SOM decomposition, and what is the role of soil microorganisms herein?

In a first study six biochars, produced from three feedstocks and at two pyrolysis temperatures (400 and 500°C) were characterized by pyrolysis field ionization mass spectrometry (py-FIMS) and the resulting mass signals were assigned to different compound classes (carbohydrates with pentose and hexose subunits, phenols and lignin monomers, lignin dimers, lipids, alkanes, alkenes, fatty acids and n-alkyl esters, alkylaromatics, and heterocyclic N containing compounds and peptides). In the 500°C biochars, total ion intensities detected dropped compared to the 400°C biochars, as manifested in noisy 500°C thermograms. When pyrolysis temperature was raised from 400°C to 500°C, the weight loss during py-FIMS decreased, logically suggesting that thermolabile compounds were preferentially lost when producing biochar at 500°C. The net C mineralization was negatively correlated with the ion intensities of individual marker signals of aromatic compounds. Hence, the aromaticity of biochar C, which is linked to feedstock and pyrolysis temperature, determines the degradability. In addition the net C mineralization rate was positively correlated with total ion intensities of several heterocyclic N-containing compounds. This suggests that heterocyclic N-containing biochar compounds, about whose behaveour in soils very little is known, may thus be degraded in the short-term. However, their contribution to net-C mineralization was probably limited and instead N-release upon microbial consumption of these N-containing compounds enabled decomposition of other SOM or biochar components.

After this short-term incubation experiment it was clear that assessments of biological properties other than CO_2 respiration of biochar-amended soils were necessary to better understand biochar stability. But before employing several extraction-based microbial measurements in further incubation experiments, these assays had to be tested for their

compatibility with biochar-amended soils. To test this, five biochars, produced from a wide range of feedstocks were added to two soils just before analysis of microbial biomass (via fumigation-extraction), activity (via dehydrogase and and β-glucosidase enzyme activity) and community structure (via PLFA extraction). We wanted to test the hypothesis that the presence of biochar does not influence (i.e. is compatible with) microbial measurements by comparing the results from biochar treated soils with those from corresponding unamended control soils. The impact of the presence of biochar on the determination of microbial soil properties was limited. Chloroform based fumigation-extraction of microbial C appeared to be most affected, but the effect was biochar dependent. Willow wood and pine biochar had a lower TOC content after fumigation, which may be due to adsorption of lysed compounds to the biochar. It would appear that there is a possibility to correct for such an artefact by means of a factor (k_{EC}) specific for biochar-amended soils, in line with the known incomplete efficiency of the fumigation-extraction method. On the other hand, increased OC contents after fumigation of the poultry litter and fast pyrolysis pine biochars may indicate adsorption of chloroform and/or dissolution of specific biochar compounds by chloroform. This would render the fumigation/extraction MBC method incompatible with biochar-amended soil, but specific in depth spectroscopic analysis would be required for confirmation. We observed no differences between the total PLFAs extracted from control and the biochar-amended treatments. Therefore, we recommend that the sum of the PLFAs is a better parameter than the fumigation-extraction based MBC to express total microbial biomass. By canonical discriminant analysis little or no differences between the control and the maize and willow biochars were observed. However, the pine (fast and slow pyrolysis) and poultry litter biochars were capable of influencing the extraction efficiency of the fungal biomarker 18:1ω9c. The presence of biochar had only a limited impact on the efficiency of microbial activity measurements of dehydrogenase and β-glucosidase. It is clear that the compatibility of soil microbial experiments with biochar therefore depends on biochar type. An expansion to this research is needed to see whether these effects can be extrapolated to other types of biochars, and whether these effects fade out or increase with biochar aging in soil.

In a next step, we measured the CO_2 emissions and microbial properties from soil amended with biochar from two contrasting feedstocks (manure digestate and wood), produced at two contrasting temperatures (slow pyrolysis at 350°C and 700°C). The first objective was to test if CO_2 emissions and microbial properties from soils amended with biochar were determined by the type of feedstock and the pyrolysis temperature. The second

objective was to link the CO_2 emissions under the different scenarios to the biochar properties and the microbial and chemical soil properties. CO_2 emissions were higher in the 350°C biochar treatments than in the 700°C biochar treatments. Pyrolysis at 350°C resulted in biochars with higher volatile matter contents than the corresponding 700°C biochars. These volatile matter contents of the biochar were correlated to the short-term CO_2 emissions from soils amended with biochar. Moreover microbial biomass and activity was higher in the soils with the 350°C biochars compared to the other treatments, which suggests that the soil microorganisms were stimulated by the provision of a readily available substrate. Therefore we suggest that this is an important biochar property, influencing the differences in the short-term emissions from biochar-amended soils. The correlations observed here between volatile matter content and CO_2 emissions, however, should be interpreted with caution and are only tentative. There was a higher abundance of Gram-negative and Gram-positive bacteria and actinobacteria in the 350°C biochar treatments than in the other biochar treatments. Additional research involving more types of biochar and/or different pyrolysis conditions are needed to confirm the observations in this study.

To investigate if the native SOM content has an interactive control exerted by biochar on the soil microbial community and its activity, we sampled two adjacent arable soils (L and H) with similar texture and crop rotation, but with contrasting organic matter content (8.9 vs. 16.1 g C kg^{-1}, respectively). To quantify possible differences in SOM quality, we used a physicochemical fractionation procedure to compared SOM quality of both soils. We amended both soils with four biochar types, produced from two contrasting feedstocks, namely pine chips and poultry litter at two slow pyrolysis temperatures (400°C and 500°C). Both feedstock and pyrolysis temperature strongly determined whether a net N release or immobilization occured upon biochar application. While amendment with poultry litter (PL) biochar resulted in a net mineral N release compared to the control, net N immobilization was observed after the addition of pine chips (P) biochars. Regardless of biochar type, the net cumulative N release decreased with increasing pyrolysis temperature (400 vs. 500°C). Net N mineralization and N immobilization, as well as MBC stimulation and dehydrogenase enzymes activities were higher in the H soil than in the L soil. After biochar addition to the H soil increased rates of N dynamics and dehydrogenase enzyme activity compared to the rates in the L soil. However, the magnitude of these microbial responses is dependent of the SOM quality. From this study it was apparant that SOM content exerts an interactive control on the microbial response to biochar applications to soil.

Experiments based on addition of fresh biochar to soils during incubations only target short-term effects of biochar onto the soil microbial community. While labile biochar compounds seem to be important in providing an available food source for soil microorganisms in the short-term, it remains largely unknown what would happen to the microbial community when these volatile compounds are degraded. Moreover, in the soil biochar ages through oxidation, both biologically and abiotically mediated. By investigating soil microbial properties of four multi-year field experiments the medium-term effect of biochar onto soil organisms was assessed. Our research hypothesis was that biochar addition remains to change soil microbial properties even after consumption of the readily available biochar compounds. Relative C mineralization was lower in the biochar-amended plots than in the control plots and this was accompanied by decreased microbial biomass and activity. To the C mineralization data a first plus zero order model was fitted and the parameter for the easily available C source decreased in all biochar-amended plots compared to the control plots. This indicates that substrate availability of the biochar-amended soil was lower than in the control plots. In most field sites, in line suppression of native SOC mineralization occured in the biochar-amended plots. In the field site where massive amounts of biochar were added this suppression was the highest and was accompanied by the highest decreases in enzyme activity and microbial biomass. Additionally, a microbial shift towards less fungi in this field site may suggest that these high amounts of biochar were not easily colonized by fungi.

This PhD work shows clearly that biochar addition influences the soil microbial community in the short-term and remains to do so after several years of incorporation. In the short-term labile biochar compounds seem to provide an available food source for soil organisms. Pyrolysis temperature determines the amount of these labile compounds and thus the degradation by soil organisms; the higher the pyrolysis temperature, the lower the amounts of labile compounds and the lower C mineralization rates. When these labile compounds were degraded after several years of field incorporation, C mineralization rates remained lower in biochar-amended plots compared to the control plots. Suggested mechanisms for this reduction were adsorption of labile SOM compounds or mineral N onto the biochar surface. This indicates that biochar has a potential to sequester C in the long term.

Future research should address the role of soil fauna into the biochar stability, unravel how indirect effects influence soil organisms and focus on the mechanisms behind N_2O emission reductions in biochar-amended soils.

Samenvatting

De toediening van biochar aan bodems beïnvloeden bodemmicro-organismen op verschillende manieren. Op een indirecte manier zorgt biochar met zijn poreuze structuur en zijn reactief interne oppervlak voor een verandering van de microbiële habitat. Bovendien kunnen sommige componenten in de biochar ook direct geconsumeerd worden door micro-organismen. In deze thesis wordt het effect op micro-organismen van verschillende types biochar, met variërende chemische structuur, biologische stabiliteit en duur van inwerking in de bodem onderzocht. Meer specifiek werd een antwoord gezocht op vier onderzoeksvragen: 1) Wat is de invloed van biochareigenschappen op zijn stabiliteit: 2) Methodologische problemen: zijn technieken om microbiële bodemparameters te bepalen compatibel met biochar?; 3) Is er nog steeds een effect van biochar op de microbiële gemeenschap na verscheidene jaren van biocharincorporatie en is dit effect verschillend van kortetermijneffecten?; 4) Heeft biochar een effect op de afbraak van bodem organische stof (BOS), en wat is de rol van bodemmicro-organismen hierin?

In een eerste studie werden zes types biochar geproduceerd op twee verschillende pyrolyse-temperaturen (400°C en 500°C) vanuit drie uitgangsmaterialen (dennenhout, pindanootomhulsels en kippenmest). Deze werden dan gekarakteriseerd via de ‘pyrolysis field ionization mass spectrometry’ (py-FIMS) techniek. De resulterende massa-signalen werden toegewezen aan verschillende componentenklassen (koolwaterstoffen met pentose en hexose onderdelen; fenolen en lignine monomeren; lignine dimeren; lipiden; alkanen, alkenen, vetzuren en n-alkyl esters; alkylaromaten; en heterocyclische N-bevatten componenten en peptiden). De totale ionintensiteit (TII) van de 500°C biochars waren significant lager dan die van de 400°C biochars. In de thermogrammen van de 500°C biochars was dan ook veel ruis waar te nemen. De gewichtsafname tijdens py-FIMS was lager voor de 500°C dan voor de overeenkomstige 400°C biochars, wat kan wijzen op een preferentiële afname van thermolabiele componenten. De netto C mineralisatie was omgekeerd evenredig met individuele ionintensiteiten van bepaalde aromatische verbindingen. Dit toont dus aan dat de afbreekbaarheid van biochar bepaald wordt door de aanwezigheid van aromatische verbindingen in de biochar en afhankelijk is van het type uitgangsbiomassa en de pyrolysetemperaturen. Bovendien was de netto C mineralisatie ook positief gecorreleerd aan individuele ionintensiteiten van bepaalde heterocyclische N-bevattende verbindingen. Dit kan erop wijzen dat deze heterocyclische N-bevattende verbindingen uit de biochars in de korte termijn afgebroken kunnen worden. Waarschijnlijk gaat het hier om een indirect effect,

aangezien micro-organismen deze N-bevattende verbindingen zullen afbreken in hun zoektocht naar N voor de afbraak van BOS of biochar componenten.

Na dit eerste kortetermijnincubatie-experiment was het duidelijk dat er een noodzaak was om andere biologische bodemparameters te meten dan louter CO_2 respiratie. Op deze manier kan echter een betere inschatting van de biologische stabiliteit van biochar gemaakt worden. Voor we konden van start gaan met verschillende microbiële bepalingstechnieken in verder onderzoek, moest getest worden of de aanwezigheid van biochar geen nadelige invloed heeft op deze microbiële technieken. We moesten dus met andere woorden nagaan of deze technieken compatibel zijn met bodems waaraan biochar toegevoegd is. Om dit te testen werden vijf biochars, geproduceerd vanuit uiteenlopende startbiomassa's vlak voor de analyse van microbiële bodemparameters toegediend aan twee bodemtypes (Hoofdstuk 3). De gebruikte technieken waren de bepaling van microbiële biomassa (via de fumigatie-extractie techniek), microbiële activiteit (via dehydrogenase en β-glucosidase enzymactiviteiten) en microbiële gemeenschapsstructuur (via PLFA extractie). De technieken houden allen een extractie-stap in en het risico bestaat dat tijdens deze extractie-stap bepaalde componenten uit de biochar geëxtraheerd worden of dat bepaalde geëxtraheerde bodemcomponenten geadsorbeerd worden aan biochar. De hypothese die getest werd in dit onderzoek was dat aanwezigheid van biochar geen invloed heeft op (en dus compatibel is met) deze microbiële bepalingstechnieken. Uit het onderzoek bleek echter dat biochar wel degelijk een invloed had op de microbiële bepalingen. De fumigatie-extractie techniek waarbij chloroform gebruikt wordt om microbiële cellen te lyseren bleek de grootste invloed te ondervinden van de aanwezigheid van biochar. Er werd vastgesteld dat biochars vervaardigd uit wilgen- en dennenhout die geproduceerd werden via 'slow pyrolysis' gelyseerde microbiële bodemcomponenten kunnen adsorberen, waardoor de efficiëntie van de fumigatie-extractie techniek afneemt. Maar aan de andere kant werden ook verhoogde TOC gehaltes opgemeten na de fumigatie van de biochar bodems met chloroform. Dit kan wijzen op een extractie van bepaalde biocharcomponenten. De fumigatie-extractie techniek bleek dus weinig geschikt te zijn om toe te passen in bodems waaraan biochar toegediend werd. Wanneer de som van de PLFAs echter bekeken werd als maat voor de microbiële biomassa, zagen we echter geen verschillen tussen de bodems waaraan biochar werd toegediend en de controle bodems. Daarom werd in het vervolg van de thesis de som van de PLFAs als een maat voor microbiële biomassa genomen in plaats van de microbiële biomassa bepaald via de fumigatie-extractie techniek. Na canonische discriminanten analyse van de individuele PLFAs werden weinig

verschillen waargenomen tussen de controle bodems en de bodems met mais- en wilgenhoutbiochars. Bij de bodems met dennnenhoutbiochars, vervaardigd via 'fast pyrolysis' en 'slow pyrolysis' zagen we echter wel een verminderde efficiëntie van de PLFA 18:1 ω9c. Daarom werd in verder onderzoek slechts gebruik gemaakt van de PLFA 18:2 ω9,11c als biomerker voor de schimmels. De aanwezigheid van de verschillende biochar types had slechts een beperkte invloed op de bepalingen van de enzymactiviteiten.

In een volgende stap (Hoofdstuk 4) werden de CO_2 emissies en microbiële parameters gemeten vanuit een bodem waarin biochar werkt ingewerkt tijdens een incubatie-experiment. De biochar werd vervaardigd uit twee startbiomassa's (wilgenhout en digestaat afkomstig van de natte vergisting van varkensmest voor de productie van biogas) en werd gepyrolyseerd op twee contrasterende temperaturen (350°C en 700°C) via 'slow pyrolysis'. Deze CO_2 emissies werd gecorreleerd aan de biochareigenschappen, de eigenschappen van de microbiële gemeenschap en de chemische bodemeigenschappen na een incubatie van 117 dagen. In de bodems waarin biochar ingewerkt werd die geproduceerd werd op 350°C zagen we een grotere CO_2 emissie dan in de bodems met de overeenkomstige 700°C biochars. De pyrolyse op 350°C resulteerde in biochars met een hoger gehalte aan volatiele componenten dan de 700°C biochars. Deze gehaltes aan volatiele componenten bleken positief gecorreleerd te zijn met de korte termijn CO_2 emissie vanuit de bodem waaraan de biochars toegediend werd. Bovendien zagen we een verhoogde microbiële biomassa en microbiële activiteit in de bodems waaraan de 350°C biochar toegediend werden ten opzichte van de andere behandelingen. Dit duidt op een stimulatie van bodemmicro-organismen doordat deze gemakkelijk afbreekbare C-bron in de biochar kan dienen als een voedselbron voor micro-organismen. In alle bodems met biochar, behalve in de D700 behandeling, zagen we een verhoogde biomassa aan Gram-positieve bacteriën. Bovendien zagen we een hogere biomassa aan Gram-negatieve and Gram-positieve bacteriën in de bodems met 350°C biochars dan in de bodems met 700°C biochars.

In een volgend hoofdstuk (Hoofdstuk 5) werd biochar ingewerkt in twee bodems (L en H), die eenzelfde textuur en gewasrotatie hadden, maar een contrasterende hoeveelheid BOS (8.9 versus 16.1 g C kg^{-1}, respectievelijk). De kwaliteit van de BOS van beide bodems werd gekarakteriseerd via een fysico-chemische fractioneringsmethode. Via een incubatie-experiment werden aan beide bodems vier biochartypes toegediend. Deze biochars werden geproduceerd uit dennenhout en kippenmest en werden gepyrolyseerd aan 400 en 500°C via 'slow pyrolysis'. Zowel het uitgangsmateriaal als de pyrolyse temperatuur bepaalden of er een

netto N-vrijstelling of N-immobilisatie (ten opzichte van de controle bodems) optrad na biochar inwerking in de bodem. Kippenmestbiochars resulteerden in een netto N-vrijstelling, terwijl netto N-immobilisatie optrad in de bodems met dennenhout biochars. Onafhankelijk van het type biochar, verminderde de netto N-vrijstelling met toenemende pyrolyse temperatuur (400 versus 500°C). In de H bodem was de N-mineralisatie en N-immobilisatie hoger dan in de L bodem, ook de microbiële biomassa en de dehydrogenase enzymactiviteit was hoger in deze bodem. Na het inwerken van biochar zagen we ook een verhoogde dehydrogenase enzymactiviteit en toegenomen N-mineralisatie (bij kippenmestbiochars) en N-immobilisatie (bij dennenhoutbiochars) in de H bodems. De grootte van deze respons was echter afhankelijk van de kwaliteit van BOS. Hoewel het BOS gehalte in de H bodem dubbel zo hoog was als in de L bodem waren de verhogingen in N-mineralisatie/immobilisatie en microbiële parameters disproportioneel kleiner. Dit kan te wijten zijn aan het feit dat de BOS in de H bodem moeilijker afbreekbaar was dan in de L bodem, met meer labiele BOS componenten. Deze studie toont dus duidelijk aan dat het gehalte aan BOS een interactieve controle uitoefent op de microbiële respons na biocharinwerking in de bodem.

In de voorgaande hoofdstukken werd dus gekeken naar de microbiële respons na inwerking van verse biochar in de bodem. Uit deze kortetermijnexperimenten bleek dat labiele biocharcomponenten een belangrijke voedselbron vormen voor bodemmicro-organismen. Wat er gebeurt met de microbiële bodemgemeenschap nadat deze labiele componenten afgebroken zijn, blijft nog grotendeels onzeker. Bovendien kan biochar in de bodem oxideren, wat opnieuw een effect op de micro-organismen teweegbrengt. Om het effect van biochar op micro-organismen in de middellange termijn na te gaan (Hoofdstuk 6) werden microbiële bodemparameters bepaald van grond verzameld uit vier veldexperimenten, waarin biochar reeds gedurende 7 maanden tot 4 jaar in de bodem ingewerkt was. De hypothese was dat de inwerking van biochar de bodemmicro-organismen blijft beïnvloeden zelfs nadat de labiele componenten afgebroken zijn. Relatieve C mineralisatie (relatief t.o.v. de hoeveelheid OC in de bodem) was telkens lager in de plots waarin biochar ingewerkt was dan in de corresponderende controle plots. Deze vermindering ging telkens gepaard met een lagere microbiële biomassa en activiteit. Een nulde plus eerste orde model werd gefit aan de cumulatieve C mineralisatie uitgedrukt t.o.v. de tijd en hieruit bleek dat de parameter die overeenkomt met de hoeveelheid gemakkelijk beschikbare C telkens lager was in de biochar plots dan in de controle plots. Dit kan dus duiden op een verminderde beschikbaarheid van microbieel substraat in deze bodems. In de meeste veldexperimenten, zagen we een

verminderde CO_2 emissie in de biochar plots dan in de control plots, wat kan duiden op een verminderde afbraak van BOS. In het veldexperiment waarin grote hoeveelheden biochar (49 ton ha^{-1}) ingewerkt werden, zagen we de grootste vermindering van CO_2-mineralisatie en microbiële enzymactivteiten. Bovendien zagen we enkel in dit veldexperiment een lagere biomassa aan bodemschimmels, wat erop wijst dat deze grote hoeveelheden biochar niet gemakkelijk gekoloniseerd kunnen worden door schimmels.

Dit doctoraat toont dus aan dat de microbiële bodemgemeenschap wel degelijk beïnvloed wordt door de aanwezigheid van biochar en dit zowel in de korte als in de middellange termijn. In de korte termijn kunnen de labiele biochar componenten een gemakkelijk afbreekbaar substraat vormen voor microbiële afbraak. De pyrolysetemperatuur bepaalt de hoeveelheid labiele componenten en dus de afbreekbaarheid in de korte termijn. Hoe hoger de pyrolyse temperatuur, hoe lager het gehalte aan volatiele componenten en hoe lager dus de C-mineralisatie. Nadat deze labiele componenten afgebroken zijn, blijft de C-mineralisatie lager in bodems met biochar dan in de controle bodems. Mogelijks wordt deze reductie veroorzaakt door de adsorptie van labiele BOS componenten of minerale N aan het biochar oppervlak en duidt dus op het belang van biochar om op de lange termijn C op te slaan in de bodem.

In dit onderzoek werd slechts gekeken naar microbiële bodemparameters, in de toekomst moet daarom ook nog de rol van de macro-organismen onderzocht worden. Ook zou mechanistisch onderzoek naar de indirecte effecten van biochar op micro-organismen en naar N_2O emissie reductie uitgevoerd moeten worden.

References

Afeng, Z., Rongjun, B., Genxing, P., Liqiang, C., Qaiser, H., Lianqing, L., Jinwei, Z., Jufeng, Z., Xuhui, Z., Xiaojun, H., Yu, X., 2012. Effects of biochar amendment on soil quality, crop yield and greenhouse gas emission in a Chinese rice paddy: a field study of 2 consecutive rice growing cycles. Field Crops Research 127, 153-160.

Alef, K., Nannipieri, P., 1995. Chapter 7 - Enzyme activities, In: Kassem, A., Paolo, N. (Eds.), Methods in Applied Soil Microbiology and Biochemistry. Academic Press, London, pp. 311-373.

Alessi, D.S., Walsh, D.M., Fein, J.B., 2011. Uncertainties in determining microbial biomass C using the chloroform fumigation-extraction method. Chemical Geology 280, 58-64.

Ameloot, N., De Neve, S., Jegajeevagan, K., Yildiz, G., Buchan, D., Funkuin, Y.N., Prins, W., Bouckaert, L., 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., 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., Zech, W., Zhang, X., Follett, R.F., Tiessen, H., Knox, E., Flach, K.W., 1998. Carbon, nitrogen, and sulfur pools in particle-size fractions as influenced by climate. Soil Science Society of America Journal 62, 172-181.

Amelung, W., Zech, W., 1999. Minimisation of organic matter disruption during particle-size fractionation of grassland epipedons. Geoderma 92, 73-85.

Amonette, J.E., Joseph, S., 2009. Chapter 3 - Characteristics of biochar: microchemical properties, In: Lehmann, J., Joseph, S. (Eds.), Biochar for environmental management. Earthscan, London, pp. 33-53.

Aon, M.A., Colaneri, A.C., 2001. II. Temporal and spatial evolution of enzymatic activities and physico-chemical properties in an agricultural soil. Applied Soil Ecology 18, 255-270.

Ascough, P.L., Sturrock, C.J., Bird, M.I., 2010. Investigation of growth responses in saprophytic fungi to charred biomass. Isotopes in Environmental and Health Studies 46, 64-77.

Atkinson, C.J., Fitzgerald, J.D., 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., Mueller, C., 2012. Biochar and Earthworm Effects on Soil Nitrous Oxide and Carbon Dioxide Emissions. Journal of Environmental Quality 41, 1203-1209.

Aulakh, M.S., Doran, J.W., Mosier, A.R., 1991. Field evaluation of 4 methods for measuring denitrification. Soil Science Society of America Journal 55, 1332-1338.

Bailey, V.L., Fansler, S.J., Smith, J.L., Bolton, H., 2011. Reconciling apparent variability in effects of biochar amendment on soil enzyme activities by assay optimization. Soil Biology and Biochemistry 43, 296-301.

Baldock, J.A., Smernik, R.J., 2002. Chemical composition and bioavailability of thermally altered Pinus resinosa (Red Pine) wood. Organic Geochemistry 33, 1093-1109.

Baldrian, P., 2010. Effect of Heavy Metals on Saprotrophic Soil Fungi, Soil Heavy Metals. Springer Berlin Heidelberg, pp. 263-279.

Bardgett, R.D., Richter, A., Bol, R., Garnett, M.H., Baumler, R., Xu, X.L., Lopez-Capel, E., Manning, D.A.C., Hobbs, P.J., Hartley, I.R., Wanek, W., 2007. Heterotrophic microbial communities use ancient carbon following glacial retreat. Biology Letters 3, 487-490.

Beesley, L., Dickinson, N., 2011. Carbon and trace element fluxes in the pore water of an urban soil following greenwaste compost, woody and biochar amendments, inoculated with the earthworm Lumbricus terrestris. Soil Biology & Biochemistry 43, 188-196.

Bending, G.D., Turner, M.K., Jones, J.E., 2002. Interactions between crop residue and soil organic matter quality and the functional diversity of soil microbial communities. Soil Biology and Biochemistry 34, 1073-1082.

Billings, S.A., Ziegler, S.E., 2008. Altered patterns of soil carbon substrate usage and heterotrophic respiration in a pine forest with elevated CO_2 and N fertilization. Global Change Biology 14, 1025-1036.

Birk, J.J., Steiner, C., Teixiera, W.C., Zech, W., Glaser, B., 2009. Microbial Response to Charcoal Amendments and Fertilization of a Highly Weathered Tropical Soil, In: Woods, W.I., Teixeira, W.G., Lehmann, J., Steiner, C., WinklerPrins, A., Rebellato, L. (Eds.), Amazonian Dark Earths: Wim Sombroek's Vision. Springer Netherlands, pp. 309-324.

Bittman, S., Forge, T.A., Kowalenko, C.G., 2005. Responses of the bacterial and fungal biomass in a grassland soil to multi-year applications of dairy manure slurry and fertilizer. Soil Biology and Biochemistry 37, 613-623.

Blagodatskaya, E., Kuzyakov, Y., 2008. Mechanisms of real and apparent priming effects and their dependence on soil microbial biomass and community structure: critical review. Biology and Fertility of Soils 45, 115-131.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian Journal Biochemistry and Physiology 37, 911-917.

Bodé, S., Fancy, R., Boeckx, P., 2013. Stable isotope probing of amino sugars – a promising tool to assess microbial interactions in soils. Rapid Communications in Mass Spectrometry 27, 1367-1379.

Bolan, N.S., Kunhikrishnan, A., Choppala, G.K., Thangarajan, R., Chung, J.W., 2012. Stabilization of carbon in composts and biochars in relation to carbon sequestration and soil fertility. Science of the Total Environment 424, 264-270.

Brewer, C.E., Schmidt-Rohr, K., Satrio, J.A., Brown, R.C., 2009. Characterization of Biochar from Fast Pyrolysis and Gasification Systems. Environmental Progress and Sustainable Energy 28, 386-396.

Brokhoff, S.R., 2010. Sand-based turfgrass root-zone modification with biochar, Iowa State University. Iowa State University, Ames, p. 40.

Brown, R.A., Kercher, A.K., Nguyen, T.H., Nagle, D.C., Ball, W.P., 2006. Production and characterization of synthetic wood chars for use as surrogates for natural sorbents. Organic Geochemistry 37, 321-333.

Bruun, E.W., Ambus, P., Egsgaard, H., Hauggaard-Nielsen, H., 2012. Effects of slow and fast pyrolysis biochar on soil C and N turnover dynamics. Soil Biology and Biochemistry 46, 73-79.

Bruun, S., Jensen, E.S., Jensen, L.S., 2008. Microbial mineralization and assimilation of black carbon: Dependency on degree of thermal alteration. Organic Geochemistry 39, 839-845.

Buchan, D., Moeskops, B., Ameloot, N., De Neve, S., Sleutel, S., 2012. Selective sterilisation of undisturbed soil cores by gamma irradiation: Effects on free-living nematodes, microbial community and nitrogen dynamics. Soil Biology and Biochemistry 47, 10-13.

Burket, J.Z., Dick, R.P., 1998. Microbial and soil parameters in relation to N mineralization in soils of diverse genesis under differing management systems. Biology and Fertility of Soils 27, 430-438.

Burns, R.G., DeForest, J.L., Marxsen, J., Sinsabaugh, R.L., Stromberger, M.E., Wallenstein, M.D., Weintraub, M.N., Zoppini, A., 2013. Soil enzymes in a changing environment: Current knowledge and future directions. Soil Biology and Biochemistry 58, 216-234.

Camargo, F.A.O., Gianello, C., Tedesco, M.J., 2004. Soil Nitrogen Availability Evaluated by Kinetic Mineralization Parameters. Communications in Soil Science and Plant Analysis 35, 1293-1307.

Case, S.D.C., McNamara, N.P., Reay, D.S., 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.

Case, S.D.C., McNamara, N.P., Reay, D.S., Whitaker, J., 2013. Can biochar reduce soil greenhouse gas emissions from a Miscanthus bioenergy crop? Global Change Biology Bioenergy In press.

Casida, L.E.J., Klein, D.A., Santoro, T., 1964. Soil Dehydrogenase Activity. Soil Science 98, 371-376.

Castaldi, S., Riondino, M., Baronti, S., Esposito, F.R., Marzaioli, R., Rutigliano, F.A., Vaccari, F.P., Miglietta, F., 2011. Impact of biochar application to a Mediterranean wheat crop on soil microbial activity and greenhouse gas fluxes. Chemosphere 85, 1464-1471.

Cerniglia, C.E., 1997. Fungal metabolism of polycyclic aromatic hydrocarbons: past, present and future applications in bioremediation. Journal of Industrial Microbiology and Biotechnology 19, 324-333.

Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2007. Agronomic values of greenwaste biochar as a soil amendment. Australian Journal of Soil Research 45, 629-634.

Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2008. Using poultry litter biochars as soil amendments. Australian Journal of Soil Research 46, 437-444.

Cheng, C.H., Lehmann, J., Thies, J.E., Burton, S.D., Engelhard, M.H., 2006. Oxidation of black carbon by biotic and abiotic processes. Organic Geochemistry 37, 1477-1488.

Chenu, C., Plante, A.F., 2006. Clay-sized organo-mineral complexes in a cultivation chronosequence: revisiting the concept of the 'primary organo-mineral complex'. European Journal of Soil Science 57, 596-607.

Clein, J.S., Schimel, J.P., 1994. Redulction in microbial activity in birch litter due to drying and rewetting events. Soil Biology and Biochemistry 26, 403-406.

Cleveland, C.C., Liptzin, D., 2007. C:N:P stoichiometry in soil: is there a "Redfield ratio" for the microbial biomass? Biogeochemistry 85, 235-252.

Clough, T., Condron, L., Kammann, C., Müller, C., 2013. A Review of Biochar and Soil Nitrogen Dynamics. Agronomy 3, 275-293.

Clough, T.J., Condron, L.M., 2010. Biochar and the Nitrogen Cycle: Introduction. Journal of Environmental Quality 39, 1218-1223.

Cross, A., 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.

Curtin, D., Campbell, C.A., Jalil, A., 1998. Effects of acidity on mineralization: pH-dependence of organic matter mineralization in weakly acidic soils. Soil Biology and Biochemistry 30, 57-64.

Dalal, R.C., Wang, W., Robertson, G.P., Parton, W.J., 2003. Nitrous oxide emission from Australian agricultural lands and mitigation options: a review. Australian Journal of Soil Research 41, 165-195.

Das, K.C., Garcia-Perez, M., Bibens, B., Melear, N., 2008. Slow pyrolysis of poultry litter and pine woody biomass: Impact of chars and bio-oils on microbial growth. Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering 43, 714-724.

de la Rosa, J.M., Knicker, H., 2011. Bioavailability of N released from N-rich pyrogenic organic matter: An incubation study. Soil Biology and Biochemistry 43, 2368-2373.

De Neve, S., Pannier, J., Hofman, G., 1996. Temperature effects on C- and N-mineralization from vegetable crop residues. Plant and Soil 181, 25-30.

DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., 2009. Chapter 14 - Biochar Effects on Soil Nutrient Transformations, In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management: Science and Technology. Earthscan, London, pp. 251-270.

Demirbas, A., 2001. Carbonization ranking of selected biomass for charcoal, liquid and gaseous products. Energy Conversion and Management 42, 1229-1238.

Demirbas, A., 2004. Effects of temperature and particle size on bio-char yield from pyrolysis of agricultural residues. Journal of Analytical and Applied Pyrolysis 72, 243-248.

Dempster, D.N., Gleeson, D.B., Solaiman, Z.M., Jones, D.L., Murphy, D.V., 2012. Decreased soil microbial biomass and nitrogen mineralisation with Eucalyptus biochar addition to a coarse textured soil. Plant and Soil 354, 311-324.

Denef, K., Roobroeck, D., Manimel Wadu, M.C.W., Lootens, P., Boeckx, P., 2009. Microbial community composition and rhizodeposit-carbon assimilation in differently managed temperate grassland soils. Soil Biology and Biochemistry 41, 144-153.

Downie, A., Crosky, A., Munroe, P., 2009. Chapter 2 - Physical properties of biochar, In: Lehmann, J., Joseph, S. (Eds.), Biochar for environmental management. Earthscan, London, pp. 13-32.

Durenkamp, M., Luo, Y., Brookes, P.C., 2010. Impact of black carbon addition to soil on the determination of soil microbial biomass by fumigation extraction. Soil Biology and Biochemistry 42, 2026-2029.

Elmer, W.H., Pignatello, J.J., 2011. Effect of Biochar Amendments on Mycorrhizal Associations and Fusarium Crown and Root Rot of Asparagus in Replant Soils. Plant Disease 95, 960-966.

Emmerich, F.G., Desousa, J.C., Torriani, I.L., Luengo, C.A., 1987. Applications of granular model and percolation theory to the electrical resistivity of heat treated endocarp of babassu nut Carbon 25, 417-424.

Feng, X., Simpson, M.J., 2009. Temperature and substrate controls on microbial phospholipid fatty acid composition during incubation of grassland soils contrasting in organic matter quality. Soil Biology and Biochemistry 41, 804-812.

Fierer, N., Schimel, J.P., 2003. A proposed mechanism for the pulse in carbon dioxide production commonly observed following the rapid rewetting of a dry soil. Soil Science Society of America Journal 67, 798-805.

Fliessbach, A., Mader, P., 2000. Microbial biomass and size-density fractions differ between soils of organic and conventional agricultural systems. Soil Biology and Biochemistry 32, 757-768.

Foereid, B., Lehmann, J., Major, J., 2011. Modeling black carbon degradation and movement in soil. Plant and Soil 345, 223-236.

Forbes, M.S., Raison, R.J., Skjemstad, J.O., 2006. Formation, transformation and transport of black carbon (charcoal) in terrestrial and aquatic ecosystems. Science of the Total Environment 370, 190-206.

Fowles, M., 2007. Black carbon sequestration as an alternative to bioenergy. Biomass and Bioenergy 31, 426-432.

Frostegård, Å., Tunlid, A., Bååth, E., 2011. Use and misuse of PLFA measurements in soils. Soil Biology and Biochemistry 43, 1621-1625.

Gaskin, J.W., Steiner, C., Harris, K., Das, K.C., Bibens, B., 2008. Effect of low-temperature pyrolysis conditions on biochar for agricultural use. Transactions of the American Society of Agricultural and Biological Engineers 51, 2061-2069.

Glaser, B., Lehmann, J., 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, 12.

Glaser, B., 2007. Prehistorically modified soils of central Amazonia: a model for sustainable agriculture in the twenty-first century. Philosophical Transactions of the Royal Society B Biological Sciences 362, 187-196.

Graber, E., Meller Harel, Y., Kolton, M., Cytryn, E., Silber, A., Rav David, D., Tsechansky, L., Borenshtein, M., Elad, Y., 2010. Biochar impact on development and productivity of pepper and tomato grown in fertigated soilless media. Plant and Soil 337, 481-496.

Graber, E., Tsechansky, L., Gerstl, Z., Lew, B., 2011a. High surface area biochar negatively impacts herbicide efficacy. Plant and Soil 353, 95-106.

Graber, E.R., Tsechansky, L., Khanukov, J., Oka, Y., 2011b. Sorption, Volatilization, and Efficacy of the Fumigant 1,3-Dichloropropene in a Biochar-Amended Soil. Soil Science Society of America Journal 75, 1365-1373.

Gregorich, E.G., Beare, M.H., McKim, U.F., Skjemstad, J.O., 2006. Chemical and Biological Characteristics of Physically Uncomplexed Organic Matter. Soil Science Society of America Journal 70, 975-985.

Gunapala, N., Venette, R.C., Ferris, H., Scow, K.M., 1998. Effects of soil management history on the rate of organic matter decomposition. Soil Biology and Biochemistry 30, 1917-1927.

Gundale, M.J., DeLuca, T.H., 2006. Temperature and source material influence ecological attributes of ponderosa pine and Douglas-fir charcoal. Forest Ecology and Management 231, 86-93.

Gundale, M.J., DeLuca, T.H., 2007. Charcoal effects on soil solution chemistry and growth of Koeleria macrantha in the ponderosa pine/Douglas-fir ecosystem. Biology and Fertility of Soils 43, 303-311.

Haas, T.J., Nimlos, M.R., Donohoe, B.S., 2009. Real-Time and Post-reaction Microscopic Structural Analysis of Biomass Undergoing Pyrolysis. Energy and Fuels 23, 3810-3817.

Hale, S., Hanley, K., Lehmann, J., Zimmerman, A., 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., Amelung, W., 2004. Interactive priming of black carbon and glucose mineralisation. Organic Geochemistry 35, 823-830.

Hammond, J., Shackley, S., Prendergast-Miller, M., Cook, J., Buckingham, S., Pappa, V.A., 2013. Biochar field testing in the UK: outcomes and implications for use. Carbon Management 4, 159-170.

Hassink, J., Bouwman, L.A., Zwart, K.B., Brussaard, L., 1993. Relationships between habitable pore space, soil biota and mineralization rates in grassland soils. Soil Biology and Biochemistry 25, 47-55.

Hilscher, A., Heister, K., Siewert, C., Knicker, H., 2009. Mineralisation and structural changes during the initial phase of microbial degradation of pyrogenic plant residues in soil. Organic Geochemistry 40, 332-342.

Hockaday, W.C., 2006. The organic geochemistry of charcoal black carbon in the soils of the university of Michigan Biological Station, Chemistry. Ohio State University, p. 187.

Hofrichter, M., Bublitz, F., Fritsche, W., 1997. Fungal attack on coal: I. Modification of hard coal by fungi. Fuel Processing Technology 52, 43-53.

Hofrichter, M., Ziegenhagen, D., Sorge, S., Ullrich, R., Bublitz, F., Fritsche, W., 1999. Degradation of lignite (low-rank coal) by ligninolytic basidiomycetes and their manganese peroxidase system. Applied Microbiology and Biotechnology 52, 78-84.

Hshieh, F.Y., Richards, G.N., 1989. Factors influencing chemisorption and ignition of wood chars. Combustion and Flame 76, 37-47.

Hummel, D.O., Dussel, H.J., Czybulka, G., Wenzel, N., Holl, G., 1985. Analytical pyrolysis of copolymers. Spectrochimica Acta Part a-Molecular and Biomolecular Spectroscopy 41, 279-290.

Imam, T., Capareda, S., 2012. Characterization of bio-oil, syn-gas and bio-char from switchgrass pyrolysis at various temperatures. Journal of Analytical and Applied Pyrolysis 93, 170-177.

Insam, H., 2001. Developments in soil microbiology since the mid 1960s. Geoderma 100, 389-402.

IPCC, 2007. Contribution of Working Groups I, II and III to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change, In: Team, C.W., R.K., P., Reisinger, A. (Eds.). IPCC, Geneva, Switzerland.

Ippolito, J.A., Novak, J.M., Busscher, W.J., Ahmedna, B.M., Rehrah, D., Watts, D.W., 2012. Switchgrass Biochar Affects Two Aridisols. Journal of Environmental Quality 41, 1123-1130.

Jeffery, S., Verheijen, F.G.A., van der Velde, M., Bastos, A.C., 2011. A quantitative review of the effects of biochar application to soils on crop productivity using meta-analysis. Agriculture, Ecosystems & Environment 144, 175-187.

Joergensen, R.G., 1996. The fumigation-extraction method to estimate soil microbial biomass: Calibration of the kEC value. Soil Biology and Biochemistry 28, 25-31.

Joergensen, R.G., Wichern, F., 2008. Quantitative assessment of the fungal contribution to microbial tissue in soil. Soil Biology and Biochemistry 40, 2977-2991.

Jones, D.L., Murphy, D.V., Khalid, M., Ahmad, W., Edwards-Jones, G., DeLuca, T.H., 2011. Short-term biochar-induced increase in soil CO(2) release is both biotically and abiotically mediated. Soil Biology and Biochemistry 43, 1723-1731.

Joseph, S.D., Camps-Arbestain, M., Lin, Y., Munroe, P., Chia, C.H., Hook, J., van Zwieten, L., Kimber, S., Cowie, A., Singh, B.P., Lehmann, J., Foidl, N., Smernik, R.J., Amonette, J.E., 2010. An investigation into the reactions of biochar in soil. Australian Journal of Soil Research 48, 501-515.

Kader, M.A., Sleutel, S., Begum, S.A., D'Haene, K., Jegajeevagan, K., De Neve, S., 2010. Soil organic matter fractionation as a tool for predicting nitrogen mineralization in silty arable soils. Soil Use and Management 26, 494-507.

Karhu, K., Mattila, T., Bergström, I., Regina, K., 2011. Biochar addition to agricultural soil increased CH4 uptake and water holding capacity - Results from a short-term pilot field study. Agriculture, Ecosystems & Environment 140, 309-313.

Keiluweit, M., Nico, P.S., Johnson, M.G., Kleber, M., 2010. Dynamic Molecular Structure of Plant Biomass-Derived Black Carbon (Biochar). Environmental Science and Technology 44, 1247-1253.

Keith, A., Singh, B., 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.

Kim, J.S., Sparovek, G., Longo, R.M., De Melo, W.J., Crowley, D., 2007. Bacterial diversity of terra preta and pristine forest soil from the Western Amazon. Soil Biology and Biochemistry 39, 684-690.

Kloss, S., Zehetner, F., Dellantonio, A., Hamid, R., Ottner, F., Liedtke, V., Schwanninger, M., Gerzabek, M.H., Soja, G., 2012. Characterization of slow pyrolysis biochars: effects of feedstocks and pyrolysis temperature on biochar properties. Journal of Environmental Quality 41, 990-1000.

Kolb, S.E., Fermanich, K.J., 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.

Kuperman, R.G., Carreiro, M.M., 1997. Soil heavy metal concentrations, microbial biomass and enzyme activities in a contaminated grassland ecosystem. Soil Biology and Biochemistry 29, 179-190.

Kuzyakov, Y., Friedel, J.K., Stahr, K., 2000. Review of mechanisms and quantification of priming effects. Soil Biology and Biochemistry 32, 1485-1498.

Kuzyakov, Y., Leinweber, P., Sapronov, D., Eckhardt, K.-U., 2003. Qualitative assessment of rhizodeposits in non-sterile soil by analytical pyrolysis. Journal of Plant Nutrition and Soil Science 166, 719-723.

Kuzyakov, Y., Subbotina, I., Chen, H.Q., Bogomolova, I., Xu, X.L., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by C-14 labeling. Soil Biology and Biochemistry 41, 210-219.

Laborda, F., Monistrol, I.F., Luna, N., Fernández, M., 1999. Processes of liquefaction/solubilization of Spanish coals by microorganisms. Applied Microbiology and Biotechnology 52, 49-56.

LeCroy, C., Masiello, C.A., Rudgers, J.A., Hockaday, W.C., Silberg, J.J., 2013. Nitrogen, biochar, and mycorrhizae: Alteration of the symbiosis and oxidation of the char surface. Soil Biology and Biochemistry 58, 248-254.

Lehmann, J., Gaunt, J., Rondon, M., 2006. Bio-char sequestration in terrestrial ecosystems - a review. Mitigation and Adaptation Strategies for Global Change 11, 395-419.

Lehmann, J., 2007. Bio-energy in the black. Frontiers in Ecology and the Environment 5, 381-387.

Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D., 2011. Biochar effects on soil biota – A review. Soil Biology and Biochemistry 43, 1812-1836.

Leinweber, P., Schulten, H.R., Korschens, M., 1994. Seasonal-variations of soil organic-matter in a long-term agricultural experiment. Plant and Soil 160, 225-235.

Leinweber, P., Schulten, H.R., 1999. Advances in analytical pyrolysis of soil organic matter. Journal of Analytical and Applied Pyrolysis 49, 359-383.

Leinweber, P., Jandl, G., Eckhardt, K.U., Schulten, H.R., Schlichting, A., Hofmann, D., 2009a. Analytical Pyrolysis and Soft-Ionization Mass Spectrometry, Biophysico-Chemical Processes Involving Natural Nonliving Organic Matter in Environmental Systems. John Wiley & Sons, Inc., pp. 539-588.

Leinweber, P., Walley, F., Kruse, J., Jandl, G., Eckhardt, K.-U., Blyth, R.I.R., Regier, T., 2009b. Cultivation Affects Soil Organic Nitrogen: Pyrolysis-Mass Spectrometry and Nitrogen K-edge XANES Spectroscopy Evidence. Soil Science Society of America Journal 73, 82-92.

Lemmens, B., Ceulemans, J., Elslander, H., Vanassche, S., Brauns, E., Vrancken, K., 2007. Beste Beschikbare Technieken (BBT) voor mestverwerking - derde editie. VITO, Mol, Belgium, 414 pp.

Li, D., Hockaday, W.C., Masiello, C.A., Alvarez, P.J.J., 2011. Earthworm avoidance of biochar can be mitigated by wetting. Soil Biology and Biochemistry 43, 1732-1737.

Liang, B., Lehmann, J., Sohi, S.P., Thies, J.E., O'Neill, B., Trujillo, L., Gaunt, J., Solomon, D., Grossman, J., Neves, E.G., Luizão, F.J., 2010. Black carbon affects the cycling of non-black carbon in soil. Organic Geochemistry 41, 206-213.

Liesch, A.M., Weyers, S.L., Gaskin, J.W., Das, K.C., 2010. Impact of two different biochars on earthworm growth and survival. Annals of Environmental Science 4, 9.

Liu, X.Y., Qu, J.J., Li, L.Q., Zhang, A.F., Zheng, J.F., Zheng, J.W., Pan, G.X., 2012. Can biochar amendment be an ecological engineering technology to depress N_2O emission in rice paddies? A cross site field experiment from South China. Ecological Engineering 42, 168-173.

Liuzzi, V.C., Daresta, B.E., de Gennaro, G., De Giorgi, C., 2012. Different effects of polycyclic aromatic hydrocarbons in artificial and in environmental mixtures on the free living nematode C. elegans. Journal of Applied Toxicology 32, 45-50.

Loganathan, V.A., Feng, Y.C., Sheng, G.D., Clement, T.P., 2009. Crop-Residue-Derived Char Influences Sorption, Desorption and Bioavailability of Atrazine in Soils. Soil Science Society of America Journal 73, 967-974.

Lundquist, E.J., Jackson, L.E., Scow, K.M., Hsu, C., 1999. Changes in microbial biomass and community composition, and soil carbon and nitrogen pools after incorporation of rye into three California agricultural soils. Soil Biology and Biochemistry 31, 221-236.

Luo, Y., Durenkamp, M., De Nobili, M., Lin, Q., Brookes, P.C., 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.

Major, J., Steiner, C., Downie, A., Lehmann, J., 2009. Chapter 15 - Biochar effects on nutrient leaching, In: Lehmann, J., Joseph, S. (Eds.), Biochar for Environmental Management: Science and Technology. Earthscan, London, pp. 271-287.

Major, J., Lehmann, J., Rondon, M., Goodale, C., 2010. Fate of soil-applied black carbon: downward migration, leaching and soil respiration. Global Change Biology 16, 1366-1379.

Major, J., Rondon, M., Molina, D., Riha, S.J., Lehmann, J., 2012. Nutrient Leaching in a Colombian Savanna Oxisol Amended with Biochar. Journal of Environmental Quality 41, 1076-1086.

McBeath, A.V., Smernik, R.J., 2009. Variation in the degree of aromatic condensation of chars. Organic Geochemistry 40, 1161-1168.

Melnitchouck, A., Leinweber, P., Broer, I., Eckhardt, K.-U., 2006. Pyrolysis-field ionization mass spectrometry of rhizodeposits – a new approach to identify potential effects of genetically modified plants on soil organisms. Environmental Biosafety Research 5, 37-46.

Mengel, K., 1996. Turnover of organic nitrogen in soils and its availability to crops. Plant and Soil 181, 83-93.

Mikutta, R., Kleber, M., Torn, M.S., Jahn, R., 2006. Stabilization of soil organic matter: Association with minerals or chemical recalcitrance? Biogeochemistry 77, 25-56.

Moeskops, B., Sukristiyonubowo, Buchan, D., Sleutel, S., Herawaty, L., Husen, E., Saraswati, R., Setyorini, D., De Neve, S., 2010. Soil microbial communities and activities under intensive organic and conventional vegetable farming in West Java, Indonesia. Applied Soil Ecology 45, 112-120.

Mumme, J., Eckervogt, L., Pielert, J., Diakité, M., Rupp, F., Kern, J., 2011. Hydrothermal carbonization of anaerobically digested maize silage. Bioresource Technology 102, 9255-9260.

Nelissen, V., Rütting, T., Huygens, D., Staelens, J., Ruysschaert, G., Boeckx, P., 2012. Maize biochars accelerate short-term soil nitrogen dynamics in a loamy sand soil. Soil Biology and Biochemistry 55, 20-27.

Nelissen, V., 2013. Effect of biochar on soil functions and crop growth under northwestern European soil conditions. Ghent University, Ghent, Belgium.

Nelson, N.O., Agudelo, S.C., Yuan, W.Q., Gan, J., 2011. Nitrogen and Phosphorus Availability in Biochar-Amended Soils. Soil Science 176, 218-226.

Novak, J.M., Busscher, W.J., Watts, D.W., Laird, D.A., Ahmedna, M.A., Niandou, M.A.S., 2010. Short-term CO_2 mineralization after additions of biochar and switchgrass to a Typic Kandiudult. Geoderma 154, 281-288.

O'Neill, B., Grossman, J., Tsai, M.T., Gomes, J.E., Lehmann, J., Peterson, J., Neves, E., Thies, J.E., 2009. Bacterial Community Composition in Brazilian Anthrosols and Adjacent Soils Characterized Using Culturing and Molecular Identification. Microbial Ecology 58, 23-35.

Ozcimen, D., Ersoy-Mericboyu, A., 2010. Characterization of biochar and bio-oil samples obtained from carbonization of various biomass materials. Renewable Energy 35, 1319-1324.

Pietikainen, J., Kiikkila, O., Fritze, H., 2000. Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos 89, 231-242.

Puri, B.R., Singh, D.D., Nath, J., Sharma, L.R., 1958. Chemisorption of oxygen on activated charcoal and sorption of acids and bases. Industrial and Engineering Chemistry 50, 4.

Qadeer, R., Hanif, J., Saleem, M., Afzal, M., 1994. Characterization of activated-charcoal. Journal of the Chemical Society of Pakistan 16, 229-235.

Qayyum, M.F., Steffens, D., Reisenauer, H.P., Schubert, S., 2011. Kinetics of carbon mineralization of biochars compared with wheat straw in three soils. Journal of Environmental Quality.

Qiu, Y.P., Xiao, X.Y., Cheng, H.Y., Zhou, Z.L., Sheng, G.D., 2009. Influence of Environmental Factors on Pesticide Adsorption by Black Carbon: pH and Model Dissolved Organic Matter. Environmental Science and Technology 43, 4973-4978.

Richardson, D., Felgate, H., Watmough, N., Thomson, A., Baggs, E., 2009. Mitigating release of the potent greenhouse gas N_2O from the nitrogen cycle – could enzymic regulation hold the key? Trends in Biotechnology 27, 388-397.

Rillig, M.C., Mummey, D.L., 2006. Mycorrhizas and soil structure. New Phytologist 171, 41-53.

Robertson, K., Schnurer, J., Clarholm, M., Bonde, T.A., Rosswall, T., 1988. Microbial biomass in relation to C and N mineralization during laboratory incubations. Soil Biology and Biochemistry 20, 281-286.

Ronsse, F., van Hecke, S., Dickinson, D., Prins, W., 2013. Production and characterization of slow pyrolysis biochar: influence of feedstock type and pyrolysis conditions. Global Change Biology Bioenergy 5, 104-115.

Sack, U., Gunther, T., 1993. Metabolism of PAH by fungi and correlation with extracellular enzymatic-activities. Journal of Basic Microbiology 33, 269-277.

Salem, M., Kohler, J., Rillig, M.C., 2013. Palatability of carbonized materials to Collembola. Applied Soil Ecology 64, 63-69.

Santos, F., Torn, M.S., Bird, J.A., 2012. Biological degradation of pyrogenic organic matter in temperate forest soils. Soil Biology and Biochemistry 51, 115-124.

Schmidt, M.W.I., Skjemstad, J.O., Gehrt, E., Kögel-Knabner, I., 1999. Charred organic carbon in German chernozemic soils. European Journal of Soil Science 50, 351-365.

Schnitzer, M., Schulten, H.R., 1992. The analysis of soil organic-matter by pyrolysis field-ionization mass-spectrometry. Soil Science Society of America Journal 56, 1811-1817.

Schnitzer, M.I., Monreal, C.M., Facey, G.A., Fransham, P.B., 2007a. The conversion of chicken manure to biooil by fast pyrolysis I. Analyses of chicken manure, biooils and char by C-13 and H-1 NMR and FTIR spectrophotometry. Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes 42, 71-77.

Schnitzer, M.I., Monreal, C.M., Jandl, G., Leinweber, P., Fransham, P.B., 2007b. The conversion of chicken manure to biooil by fast pyrolysis II. Analysis of chicken manure, biooils, and char by curie-point pyrolysis-gas chromatography/mass spectrometry (Cp Py-GC/MS). Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes 42, 79-95.

Schnitzer, M.I., Monreal, C.M., Jandl, G., 2008. The conversion of chicken manure to bio-oil by fast pyrolysis. III. Analyses of chicken manure, bio-oils and char by Py-FIMS and Py-FDMS. Journal of Environmental Science and Health Part B-Pesticides Food Contaminants and Agricultural Wastes 43, 81-95.

Schomberg, H.H., Wietholter, S., Griffin, T.S., Reeves, D.W., Cabrera, M.L., Fisher, D.S., Endale, D.M., Novak, J.M., Balkcom, K.S., Raper, R.L., Kitchen, N.R., Locke, M.A., Potter, K.N., Schwartz, R.C., Truman, C.C., Tyler, D.D., 2009. Assessing Indices for Predicting Potential Nitrogen Mineralization in Soils under Different Management Systems. Soil Science Society of America Journal 73, 1575-1586.

Schouten, S., van Groenigen, J.W., Oenema, O., Cayuela, M.L., 2012. 'Bioenergy from cattle manure? Implications of anaerobic digestion and subsequent pyrolysis for carbon and nitrogen dynamics in soil'. Global Change Biology Bioenergy 4, 751-760.

Schulten, H.R., Gleixner, G., 1999. Analytical pyrolysis of humic substances and dissolved organic matter in aquatic systems: structure and origin. Water Research 33, 2489-2498.

Schulten, H.R., Leinweber, P., 1999. Thermal stability and composition of mineral-bound organic matter in density fractions of soil. European Journal of Soil Science 50, 237-248.

Serra-Wittling, C., Houot, S., Barriuso, E., 1995. Soil enzymatic response to addition of municipal solid-waste compost. Biology and Fertility of Soils 20, 226-236.

Silber, A., Levkovitch, I., Graber, E.R., 2010. pH-Dependent Mineral Release and Surface Properties of Cornstraw Biochar: Agronomic Implications. Environmental Science and Technology 44, 9318-9323.

Šimkovic, I., Alfȍldi, J., Schulten, H.-R., 1993. Effect of pretreatment on pyrolysis-field ionization mass spectrometric data of (4-O-methyl-d-glucurono)-d-xylan from beech wood. Biomass and Bioenergy 4, 373-378.

Sims, G.K., 2006. Nitrogen starvation promotes biodegradation of N-heterocyclic compounds in soil. Soil Biology and Biochemistry 38, 2478-2480.

Sinsabaugh, R.L., Lauber, C.L., Weintraub, M.N., Ahmed, B., Allison, S.D., Crenshaw, C., Contosta, A.R., Cusack, D., Frey, S., Gallo, M.E., Gartner, T.B., Hobbie, S.E., Holland, K., Keeler, B.L., Powers, J.S., Stursova, M., Takacs-Vesbach, C., Waldrop, M.P., Wallenstein, M.D., Zak, D.R., Zeglin, L.H., 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11, 1252-1264.

Skjemstad, J.O., Reicosky, D.C., Wilts, A.R., McGowan, J.A., 2002. Charcoal Carbon in U.S. Agricultural Soils. Soil Science Society of America Journal 66, 1249-1255.

Sleutel, S., De Neve, S., Roibas, M.R.P., Hofman, G., 2005. The influence of model type and incubation time on the estimation of stable organic carbon in organic materials. European Journal of Soil Science 56, 505-514.

Sleutel, S., Leinweber, P., Begum, S.A., Kader, M.A., Van Oostveldt, P., De Neve, S., 2008. Composition of organic matter in sandy relict and cultivated heathlands as examined by pyrolysis-field ionization MS. Biogeochemistry 89, 253-271.

Sleutel, S., Leinweber, P., Begum, S.A., Kader, M.A., De Neve, S., 2009. Shifts in soil organic matter composition following treatment with sodium hypochlorite and hydrofluoric acid. Geoderma 149, 257-266.

Smidt, E., Eckhardt, K.U., Lechner, P., Schulten, H.R., Leinweber, P., 2005. Characterization of different decomposition stages of biowaste using FT-IR spectroscopy and pyrolysis-field ionization mass spectrometry. Biodegradation 16, 67-79.

Smith, J.L., Collins, H.P., Bailey, V.L., 2010. The effect of young biochar on soil respiration. Soil Biology and Biochemistry 42, 2345-2347.

Sohi, S., Shackley, S., Meikle, M., Pratt, K., Lopez-Capel, E., Haszeldine, S., Gaunt, J., Mašek, O., Manning, D., Cook, J., Carter, S., 2009. Biochar: An emerging Technology for climate change mitigation, p. 21.

Sohi, S.P., Krull, E., Lopez-Capel, E., Bol, R., 2010. A review of biochar and its use and function in soil, In: Sparks, D.L. (Ed.), Advances in Agronomy, Vol 105. Elsevier Academic Press Inc, San Diego, pp. 47-82.

Song, W.P., Guo, M.X., 2012. Quality variations of poultry litter biochar generated at different pyrolysis temperatures. Journal of Analytical and Applied Pyrolysis 94, 138-145.

Sorge, C., Muller, R., Leinweber, P., Schulten, H.R., 1993a. Pyrolysis mass-spectrometry of whole soils, soil particle-size fractions, litter materials and humic substances - statistical evaluation of sample weight, residue, volatilized matter and total ion intensity. Fresenius Journal of Analytical Chemistry 346, 697-703.

Sorge, C., Schnitzer, M., Schulten, H.R., 1993b. In-source pyrolysis-field ionization mass-spectrometry and curie-point pyrolysis gas-chromatography/mass-spectrometry of amino-acids in humic substances and soils. Biology and Fertility of Soils 16, 100-110.

Spokas, K.A., Koskinen, W.C., Baker, J.M., 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., 2010. Review of the stability of biochar in soils: predictability of O:C molar ratios. Carbon Management 1, 289-303.

Spokas, K.A., Novak, J.M., Stewart, C.E., Cantrell, K.B., Uchimiya, M., DuSaire, M.G., Ro, K.S., 2011. Qualitative analysis of volatile organic compounds on biochar. Chemosphere 85, 869-882.

Steinbeiss, S., Gleixner, G., 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., Garcia, M., Forster, B., Zech, W., 2008. Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic Ferralsol. Pedobiologia 51, 359-366.

Strong, D.T., De Wever, H., Merckx, R., Recous, S., 2004. Spatial location of carbon decomposition in the soil pore system. European Journal of Soil Science 55, 739-750.

Tamir, G., Shenker, M., Heller, H., Bloom, P.R., Fine, P., Bar-Tal, A., 2011. Can Soil Carbonate Dissolution Lead to Overestimation of Soil Respiration? Soil Science Society of America Journal 75, 1414-1422.

Thies, J., Rillig, M.C., 2009. Chapter 6 - Characteristics of biochar: biological properties, In: Lehmann, J., Joseph, S. (Eds.), Biochar for environmental management. Earthscan, London, UK, pp. 85-106.

Tibshirani, R., 1996. Regression Shrinkage and Selection via the Lasso. Journal of the Royal Statistical Society Series B 58, 267-288.

van Grinsven, H.J.M., ten Berge, H.F.M., Dalgaard, T., Fraters, B., Durand, P., Hart, A., Hofman, G., Jacobsen, B.H., Lalor, S.T.J., Lesschen, J.P., Osterburg, B., Richards, K.G., Techen, A.K., Vertes, F., Webb, J., Willems, W.J., 2012. Management, regulation and environmental impacts of nitrogen fertilization in northwestern Europe under the Nitrates Directive; a benchmark study. Biogeosciences 9, 5143-5160.

Van Zwieten, L., Kimber, S., Morris, S., Chan, K., Downie, A., Rust, J., Joseph, S., Cowie, A., 2010. Effects of biochar from slow pyrolysis of papermill waste on agronomic performance and soil fertility. Plant and Soil 327, 235-246.

Vance, E.D., Brookes, P.C., Jenkinson, D.S., 1987. Microbial Biomass Measurements in Forest Soils - Determination of Kc Values and Tests of Hypotheses to Explain the Failure of the Chloroform Fumigation Incubation Method in Acid Soils. Soil Biology and Biochemistry 19, 689-696.

Verheijen, F., Jeffery, S., Bastos, A.C., van der Velde, M., Diafas, I., 2010. Biochar Application to Soils: A Critical Scientific Review of Effects on Soil Properties, Processes and Functions. Joint Research Centre (JRC) Scientific and Technical Report, Luxembourg. Office for the Official Publications of the European Communities, 166 pp.

Vlaco, v., 2011. Ecological and Economic benefits of digestate (in Dutch).

Wang, T., Camps Arbestain, M., Hedley, M., Bishop, P., 2012. Chemical and bioassay characterisation of nitrogen availability in biochar produced from dairy manure and biosolids. Organic Geochemistry 51, 45-54.

Wardle, D.A., Zackrisson, O., Nilsson, M.C., 1998. The charcoal effect in Boreal forests: mechanisms and ecological consequences. Oecologia 115, 419-426.

Wardle, D.A., Nilsson, M.C., Zackrisson, O., 2008. Response to comment on "fire-derived charcoal causes loss of forest humus". Science 321, 1295.

Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C., 2007. Mycorrhizal responses to biochar in soil - concepts and mechanisms. Plant and Soil 300, 9-20.

Waterman, P., Mole, S., 1994. Analysis of phenolic plant metabolites, In: Lawton, J., Likens, G. (Eds.), Methods in Ecology Blackwell Scientific Publications, Oxford, pp. 36–43.

Wengel, M., Kothe, E., Schmidt, C.M., Heide, K., Gleixner, G., 2006. Degradation of organic matter from black shales and charcoal by the wood-rotting fungus Schizophyllum commune and release of DOC and heavy metals in the aqueous phase. Science of the Total Environment 367, 383-393.

Wiedner, K., Glaser, B., 2013. Biochar-Fungi Interactions in Soils, In: Ladygina, N., Rineau, F. (Eds.), Biochar and Soil Biota. CRC Press, pp. 69-99.

Yamato, M., Okimori, Y., Wibowo, I.F., Anshori, S., Ogawa, M., 2006. Effects of the application of charred bark of Acacia mangium on the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia. Soil Science and Plant Nutrition 52, 489-495.

Yang, H., Su, Y.H., Zhu, Y.G., Chen, M.M., Chen, B.D., Liu, Y.X., 2007. Influences of polycyclic aromatic hydrocarbons (PAHs) on soil microbial community composition with or without vegetation. Journal of Environmental Science and Health Part A Toxic/Hazardous Substances and Environmental Engineering 42, 65-72.

Yildiz, G., Ronsse, F., Prins, W., Assink, D., Gerritsen, L., van Duren, R., Rosso-Vasic, M., 2011. Catalytic fast pyrolysis of biomass, 19th European Biomass Conference and Exhibition, Berlin, pp. 1145 - 1148.

Zavalloni, C., Alberti, G., Biasiol, S., Vedove, G.D., Fornasier, F., Liu, J., 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, X.-K., Li, Q., Liang, W.-J., Zhang, M., Bao, X.-L., Xie, Z.-B., 2013. Soil Nematode Response to Biochar Addition in a Chinese Wheat Field. Pedosphere 23, 98-103.

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., 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.

Zimmermann, M., Bird, M.I., Wurster, C., Saiz, G., Goodrick, I., Barta, J., Capek, P., Santruckova, H., Smernik, R., 2012. Rapid degradation of pyrogenic carbon. Global Change Biology 18, 3306-3316.

Curriculum Vitae

Personal information

Address:
Goedlevenstraat 69
9041 Oostakker

E-mail: n_ameloot@hotmail.com

Date of birth: 24/04/1985
Place of birth: Roeselare, Belgium

Education

1997-2003: Sint-Aloysiuscollege, Diksmuide
Latin-Mathematics
Graduated magna cum laude

2003-2008: Master Bio-engineer, soil and water management, Ghent University
Graduated magna cum laude

Nov 2009-April 2012: Environmental Coordinator type A, Ghent University

Work experience

June 2009 -> until now
Researcher and teaching assistant Ghent University, department of soil management
Biochar additions to soils: effects on soil microorganisms and carbon stability

April 2009 -> June 2009:
Expert pesticides (A2-level), Federal public service health, food chain safety and environment (Brussels)

September 2008 -> April 2009:

Project collaborator Ghent University, department of soil management

Geophysical research of the Moervaart depression, Flanders

Scientific accomplishments

Publications

A1

1. **Ameloot, Nele**; Maenhout, Peter; De Neve, Stefaan; Pascal Boeckx; Sleutel, Steven. Mechanisms of N_2O emission reductions in biochar-amended soil. In preparation.
2. **Ameloot, Nele**; Sleutel, Steven; Leinweber, Peter; Kristof De Beuf; De Neve, Stefaan. Biochar stability and its molecular-level biochemical composition via pyrolysis-field ionization mass spectroscopy (py-FIMS). Submission pending.
3. **Ameloot, Nele**; Sleutel, Steven; Dewaele, Jeroen; Vervisch, Bram; Dickinson, Dane; De Neve, Stefaan. Compatibility of extraction-based soil microbial assays with biochar-amended soil. Submission pending.
4. **Ameloot, Nele**; Sleutel, Steven; Case, Sean; Alberti, Giorgio; McNamara, Niall P.; Zavalloni, Costanza; Vervisch, Bram; delle Vedove, Gemini; De Neve, Stefaan. Biochar inhibits soil microorganisms: analysis of four biochar field experiments. ENVIRONMENTAL SCIENCE AND TECHNOLOGY, submitted. (IF 5.275)
5. Verheijen, Frank; Graber, Ellen; **Ameloot, Nele**; Bastos, Ana; Sohi, Saran; Knicker, Heike. 2013. Biochars in soils: new insights and emerging research needs. EUROPEAN JOURNAL OF SOIL SCIENCE, biochar special issue. (IF 2.34)
6. **Ameloot, Nele;** Sleutel, Steven; Das, KC; Jegajeevagan, Kanagaratnam; De Neve, Stefaan. 2013. Biochar amendments in soils with contrasting SOM levels: effects on N mineralization and biological properties. GLOBAL CHANGE BIOLOGY – BIOENERGY, in press. (IF 4.714)
7. **Ameloot, Nele**; Graber, Ellen; Verheijen, Frank; De Neve, Stefaan. 2013. Biochar (in)stability in soils: the role of soil organisms. EUROPEAN JOURNAL OF SOIL SCIENCE 64 : 379-390. (IF 2.34)
8. Jegajeevagan, Kanagaratnam; Sleutel, Steven; **Ameloot, Nele**; Kader, Muhammed Abdul; De Neve, Stefaan. 2013. Soil organic matter fractions and N mineralization in sandy croplands with various intensity of vegetables in rotation. SOIL USE & MANAGEMENT 29: 333-343. (IF 1.608)
9. Buchan, David; Tsegaye Gebremikael, Mesfin; **Ameloot, Nele**; Sleutel, Steven; De Neve, Stefaan. 2013. The effect of free-living nematodes on nitrogen mineralisation in undisturbed and disturbed soil cores. SOIL BIOLOGY & BIOCHEMISTRY 60 : 142-155. (IF 3.504)
10. **Ameloot, Nele**; De Neve, Stefaan; Jegajeevagan, Kanagaratnam; Yildiz, Güray; Buchan, David; Funkuin, Yvonne Nkwain; Prins, Wolter; Bouckaert, Liesbeth; Sleutel, Steven. 2013. Short-term CO_2 and N_2O emissions from biochar-amended sandy loam soils. SOIL BIOLOGY & BIOCHEMISTRY 57 : 401-410. (IF 3.504)
11. Bouckaert, Liesbeth; Van Loo, Denis; **Ameloot, Nele**; Buchan, David; Van Hoorebeke, Luc; Sleutel, Steven. 2013. Compatibility of X-ray computed tomography

with soil biological experiments. SOIL BIOLOGY & BIOCHEMISTRY 56 : 10-12. (IF 3.504)

12. Buchan, David; Moeskops, Bram; **Ameloot, Nele**; De Neve, Stefaan. 2012. Selective sterilisation of undisturbed soil cores by gamma irradiation : effects on free-living nematodes, microbial community and nitrogen dynamics. SOIL BIOLOGY & BIOCHEMISTRY 47 : 10-13. (IF 3.504)
13. Staelens, Jeroen; **Ameloot, Nele**; Almonacid, Leonardo; Padilla, Evelyn; Boeckx, Pascal; Huygens, Dries; Verheyen, Kris; Oyarzún, Carlos; Godoy, Roberto. 2011. Litterfall, litter decomposition and nitrogen mineralization in old-growth evergreen and secondary deciduous Nothofagus forests in south-central Chile. REVISTA CHILENA DE HISTORIA NATURAL 84 : 125-141. (IF 0.929)
14. Saey, Timothy; Van Meirvenne, Marc; Vermeersch, Hans; **Ameloot, Nele**; Cockx, Liesbet. 2009. A pedotransfer function to evaluate the soil profile textural heterogeneity using proximally sensed apparent electrical conductivity. GEODERMA 150 (3-4) : 389-395. (IF 2.345)

Others

1. van der Putten, Wim; de Vries, Franciska; **Ameloot**, **Nele;** Campbell, Colin; Griffiths, Rob; Lindo, Zoë; Muscolo, Adele Maria; Kelly Ramirez; Ritz, Karl. 2012. Identify key challenges for soil biodiversity science. Result of first open meeting of the Global Soil Biodiversity Meeting.
2. Sleutel, Steven; van De Vijver, Ellen; Moeskops, Bram; Bouckaert, Liesbeth; **Ameloot, Nele**; De Bolle, Sara; Van Meirvenne, Marc; De Neve, Stefaan. 2011. Onderbouwing van een methodiek voor de systematische monitoring van koolstofvoorraden in landbouwbodems. Studie voor LNE. BOD/STUD/2010/05

Congress contributions

Oral presentations

1. **Ameloot, Nele**; Vervisch, Bram; De Neve, Stefaan. 2013. Determination of the biological soil quality of biochar fields. Second EXPEER annual workshop, February 2013, Florence, Italy.
2. **Ameloot, Nele;** Graber, Ellen; Verheijen, Frank; De Neve, Stefaan. 2012. Biochar (in)stability in soils: the role of soil organisms. EUROSOIL 2012 conference, July 2012, Bari, Italy.
3. **Ameloot, Nele;** Funkuin, Yvonne Nkwain; Sleutel, Steven; De Neve; Stefaan. 2011. Greenhouse gas emissions from biochar-amended loamy soils : a lab CO_2, CH_4 and N_2O emission experiment. Soil science in a changing world, September 2011, Wageningen, the Netherlands.

Poster presentations

1. **Ameloot, Nele**; Das, KC; Buchan, David; De Neve, Stefaan. 2011. Biochar amendments change microbial community structure and activity and nutrient dynamics in Flemish loamy soils. UK Biochar conference, May 2011, Edinburgh, UK.
2. Buchan, David; Moeskops, Bram; **Ameloot, Nele**; De Neve, Stefaan. 2010. A comparison of the size and structure of the microbial and free-living nematode

communities between organically and conventionally managed soils. SOM 2010, September 2010, Presqu'île de giens, France.

3. **Ameloot, Nele**; Das, KC; Buchan, David; De Neve, Stefaan. 2010. Biochar amendments change microbial community structure and nutrient cycles in Flemish loamy soils. SOM 2010, September 2010, Presqu'île de giens, France.
4. **Ameloot, Nele**; Buchan, David; Sleutel, Steven; Das, KC. 2010. Biochar amendments change the nutrient cycles in Belgian loamy soils. Soil fertility and Soil productivity conference, March 2010, Berlin, Germany.
5. **Ameloot, Nele**; De Neve, Stefaan; De Bolle, Sara. 2009. N_2O-emissions from soil with different biochar amendments. North American biochar conference, August 2009, Boulder, Colorado, USA.
6. Almonacid, Almonacid; Padilla, Evelyn; **Ameloot, Nele;** Staelens, Jeroen; Godoy, Roberto. 2007. Aporte de litera y mantillo: capital de nutrientes en bosques siempreverdes del sur de Chile. November 2007, Gran Hotel Pucon, Pucon, Chili.

Scientific commitments

- Grantholder of Expeer project fund (Determination of the biological soil quality of biochar fields)
- Belgian Management Committee member of the Biochar COST action (TD 1107) Working group co-leader (WG3)
- Guest editor of the European Journal of Soil Science special issue "Biochar Effects on Soil Properties Processes and Functions"
- Co-convener of biochar session (5.3) in EUROSOIL 2012, July 2012, Bari, Italy
- Member of The Global Soil Biodiversity Initiative
- Guest editor of Acta Horticulturae 958: International Symposium on Sustainable Vegetable Production in Southeast Asia
- Co-organizer of the international symposium on Sustainable Vegetable Production in Southeast Asia, March 2011, Salatiga, Indonesia

Research visits abroad

- Rothamsted research center, dr. Tony Miller, January 2010, Harpenden, UK
- Wageningen University and Research, Nematod identification course, prof. Tom Bongers, February 2010, Wageningen
- September & October 2007, Valdivia, Chile, Universidad Austral de Valdivia