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Compost and biochar amendments to agricultural soils: Alteration of soil water conditions and organic matter composition.

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“The struggle against poverty in the world and the challenge of cutting wealthy country emissions all has a single, very simple solution... Here it is: Put a price on carbon”.

Al Gore

Compost and biochar amendments to agricultural soils: Alteration of soil water conditions and organic matter composition.

Summary

Agricultural crop productivity is closely linked to the quality and quantity of soil organic matter (SOM). The strong coupling of biogeochemical cycles and water supply combined with predicted increasing extreme hydrological events such as droughts due to climate change and high losses of SOM due to intensive land use practices are a major challenge for agricultural productivity. This study focuses on the effects of organic matter (OM) amendments derived from urban wastes on biogeochemical carbon (C)-cycles and its effects on the soil water regime.

Rising temperatures and droughts will increase the risk of crop failure. Due to the positive effect of OM content to soil water holding capacity, the research interest in OC-sequestration in terrestrial ecosystems increased, especially by using urban wastes as soil amendment. However, amending soils with urban waste compost is still under criticism due to contamination concerns. Thus, more sophisticated separation techniques were established to strongly decrease harmful substances, offering valuable C- and nutrient-rich composts aimed as soil additive (as used in study I). In study II and III, biochar (BC) – a waste residue of heat production – was used to investigate its potential as a soil amendment. Research is urgently needed to understand transformation and degradation processes of waste amendments to soil as a long-term OC-sequestration tool, as well as its effects on the native soil organic matter.

Empirical data about BC effects on SOM are mainly derived from laboratory incubation experiments using finely ground model BC, rather than commercial scale BC. Field studies are scarce; however, they are crucially important to validate results obtained by laboratory studies. Moreover, the comparability of field studies suffers from large differences of the BC as only recommendations for BC production and feedstock exist but no legally valid provisions. Apart from changes of chemical and structural composition of BC due to aging, BC-induced medium-term effects on microorganisms have been mainly neglected in soil BC research. However, research on BC is analytically challenging due to the complexity of soil and organic amendments and the slow degradation of BC. To study the effects of different organic matter amendments on agricultural soils, a detailed analysis of the physical and chemical composition is necessary. The aims of this thesis were to investigate the amount and chemical composition of soil OC and its stabilization, as effected by various waste amendments, as well as its usage by soil microorganisms to shed light on the soil water and SOM transformations due to these OC waste amendments.

Based on the three studies, this thesis showed that the amendment with urban waste composts and BC increased the long-term OC storage, but not the water holding capacity (studies I and II). The clay fraction served as main C-sink for urban waste composts (study I) and the free and occluded particulate OM fraction for the BC amended soil, respectively (study II). However, due to the composts, only quantitative (OC storage) effects on the clay fraction were detected but no effects on the chemical composition. Although, the BC was only minimally degraded, the BC-C label was present in all physical fractions after only one year (study II). The degradation of the BC-C by mineralization and incorporation into the microbial biomass further changed depending on soil moisture and time of field exposition of the BC (fresh or 3 years *in-situ* aged). On the one hand, field exposure had an increasing effect on the available water under drought, which in turn raised the microbial activity and C use efficiency. On the other hand, the proportion of BC-C on total respired CO_2 changed with time of field exposure as well as the respired native soil OC derived CO_2 (study III).

Zusammenfassung

Ertragreiche Landwirtschaft und damit die Lebensmittelsicherheit, stehen in engem Zusammenhang mit dem organischen Bodenkohlenstoff. Da der biogeochemische C-Kreislauf stark von der Wasserverfügbarkeit abhängt, stellen die prognostizierten häufigeren Dürren bedingt durch den Klimawandel kombiniert mit C-Verlusten durch intensive Landnutzung, eine große Herausforderung für die landwirtschaftliche Produktivität dar. Deshalb befasst sich diese Dissertation vorrangig mit den Effekten verschiedener organischer Bodenhilfsstoffe aus urbanen Abfällen auf biogeochemische C-Kreisläufe und den Bodenwasserhaushalt.

Ansteigende Temperaturen und häufigere Dürreperioden erhöhen das Risiko für Ernteausfälle. Da der organische Bodenkohlenstoff mit der Bodenwasserhaltekapazität verbunden ist, stieg in den letzten Jahren das Forschungsinteresse an der Speicherung von organischem Kohlenstoff in terrestrischen Ökosystemen, insbesondere durch das Einbringen organischer, urbaner Abfallstoffe. Ein Hauptkritikpunkt für die Einbringung dieser Substanzen in Böden ist das Risiko der Bodenkontaminierung. Dank verbesserter Trennungsverfahren in der Abfallindustrie, können Schadstoffe jedoch weitgehend entfernt und ein wertvoller kohlenstoff- und nährstoffreicher Kompost zur Verfügung gestellt werden, der die Anforderungen eines Bodenhilfsstoffs erfüllt (verwendet in Studie I). Für die Studien II und III wurde Biokohle (BC), ein Abfallprodukt der Wärmeerzeugung, verwendet und ihr Potenzial als Bodenhilfsstoff untersucht. Umsetzungsprozesse der Bodenhilfsstoffe aber auch des bodenbürtigen Kohlenstoffs, erfordern dringend weitere Forschung besonders in Bezug auf deren Stabilisierungsprozesse und Langzeitspeicherung.

Empirische Daten über BC Effekte auf den organischen Bodenkohlenstoff stammen meist aus Laborexperimenten, in denen feingemahlene Modell-BC anstatt großtechnisch produzierter BC benutzt wird. Feldstudien hingegen sind eher selten, allerdings notwendig um Ergebnisse aus Laborversuchen zu überprüfen. Erschwerend kommt hinzu, dass die wenigen Feldstudien schwer vergleichbar sind, da große Unterschiede bei den BC auftreten können, vor allem, weil keine rechtsgültigen Regelungen, sondern nur Empfehlungen für die Produktion und Ausgangsmaterial von BC existieren. Abgesehen von abbaubedingten Änderungen der chemischen und strukturellen Zusammensetzung der BC selbst, sind mittelfristig induzierte BC-Effekte auf Mikroorganismen ebenfalls wenig erforscht.

Das Ziel dieser Arbeit war, die Menge sowie die chemische Zusammensetzung des zugegebenen als auch bodenbürtigen Kohlenstoffs und dessen Stabilisierung im Boden, sowie die Nutzung durch Bodenmikroorganismen zu untersuchen um Bodenwasser- und Bodenkohlenstoffveränderungen zu verstehen. Die Komplexität des Bodens und der Bodenhilfsstoffe stellt jedoch besondere Anforderungen an analytische Methoden, insbesondere der geringe Abbau der BC.

Basierend auf drei Teilstudien konnte mit dieser Arbeit gezeigt werden, dass die Zugabe urbaner Abfallkomposte und Biokohle in landwirtschaftlich genutzten Böden die Langzeitspeicherkapazität von organischem Bodenkohlenstoff erhöht, jedoch keinen signifikanten Effekt auf die Wasserhaltekapazität hat (Studie I und II). Als wichtigste Kohlenstoffsenke für die Komposte fungierte die Tonfraktion (Studie I) und die freie und okkludierte partikuläre OM Fraktionen für die Biokohle (Studie II). Die Tonfraktion profitiert quantitativ durch einen Anstieg des C Speichers, jedoch ändert sich nicht die chemische Zusammensetzung durch die Komposte (Studie I). Obwohl die verwendete Biokohle nur minimal abgebaut worden ist (Studie II), fand sich die ^{13}C-Markierung bereits nach einem Jahr in allen Fraktionen wieder. Die Mineralisierung und Einlagerung der BC in die mikrobielle Biomasse änderte sich in Abhängigkeit von der Bodenfeuchte und BC-Expositionszeit im Feld (frische oder 3 Jahre *in-situ* gealterte BC) (Studie III). Zum einen verbesserte sich mit der Expositionszeit die Wasserverfügbarkeit unter Trockenstress und führte zu einem Anstieg der mikrobiellen Aktivität und der Kohlenstoffnutzungseffizienz. Zum anderen änderte sich mit der Expositionsdauer der BC sowohl der Anteil des BC-C an dem respirierten CO_2 als auch der respirierte bodenbürtige Kohlenstoff.

Content

List of Tables and Figures

Abbreviations

BC	biochar
BC_{aged}	field-aged biochar
BC_{fresh}	non-weathered biochar
BIO	organic waste compost
C	carbon
EBC	European Biochar Certificate
fPOM	free particulate organic matter
FYM	farmyard manure compost
GWS	green waste and sewage sludge compost
IRMS	isotope ratio mass spectrometer
MSW	municipal solid waste compost
N	nitrogen
OC	organic carbon
oPOM	occluded particulate organic matter >20µm
$oPOM_{small}$	occluded particulate organic matter <20µm
ppm	parts per million
SOC	soil organic carbon
SOM	soil organic matter
SPT	sodium polytungstate
qCO_2	metabolic quotient

List of publications and contributions

This scientific doctoral dissertation is based on the following research articles:

Study I:	Lydia Paetsch, Carsten W. Mueller, Cornelia Rumpel, Sabine Houot, Ingrid Kögel-Knabner (2016): Urban waste composts enhance OC and N stocks after long-term amendment but do not alter organic matter composition of a loamy agricultural soil under temperate climate. Agriculture Ecosystems & Environment 223, 211 – 222.
Contribution	I conducted fieldwork, carried out laboratory analyses, collected and evaluated the data, and wrote the manuscript.
Objectives	To investigate the fate of urban waste composts compared to conventional amendments in distinct SOM fractions.
Methods	Agricultural loamy soils were amended with three urban waste composts and conventional farmyard manure every other year since 1998. In 2013, disturbed soil samples were collected and a combined density and particle-size fractionation was applied to obtain different soil organic matter fractions which were analyzed by ^{13}C NMR spectroscopy to detect changes of chemical composition.
Results	The urban waste compost amendments increased the SOC stocks in bulk soils, $\text{oPOM}_{\text{small}}$ and clay fractions in a similar magnitude as farmyard manure. Although, no qualitative impact were found on the long-term SOM fractions were detected, the chemical composition of the OM amendments was still reflected in the labile SOM fractions and increased parameters like the hydrophobicity.
Conclusions	Farmyard manure and urban waste composts with longer transformation times (BIO, GWS) have higher potentials for SOC storage in long-term OC fractions, despite normalized OC inputs of all amendments. The clay fraction as main OC sink for all OM amendments underlines the long-term OC storage potential as it plays the key role for OC stabilization. The differing effect of MSW underlines that OM amendments should aim at stimulating microbial uptake in order to achieve efficient C-sequestration.

Study II: Lydia Paetsch, Carsten W. Mueller, Cornelia Rumpel, Šárka Angst, Alexandra C. Wiesheu, Cyril Girardin, Natalia P. Ivleva, Reinhard Niessner, Ingrid Kögel-Knabner (2017): A multi-technique approach to assess the fate of biochar in soil and to quantify its effect on soil organic matter composition. Organic Geochemistry 112, 177 – 186.

Contribution: I conducted fieldwork, carried out laboratory work and analyses, evaluated the data, and wrote the manuscript.

Objectives To i) assess chemical changes of the biochar in SOM fractions and of the biochar itself and to ii) validate the accuracy of ^{13}C NMR spectroscopy on highly condensed OC.

Methods A single biochar amendment to loamy grassland soil took place in 2012 with maize derived BC produced by gasification at 1200°C. Disturbed and undisturbed soil samples were taken for soil hydraulic measurements and a combined density and particle-size fractionation was applied. The physical fractions and pure BCs were analyzed by using ^{13}C NMR spectroscopy, IRMS and Raman-microspectroscopy to detect structural and quantitative changes.

Results Quantification by isotopic measurements and ^{13}C NMR spectroscopy for aromatic C yielded similar estimates of BC in our soils. The low qualitative changes of the BC due to weathering could not be detected by using ^{13}C NMR but by Raman microspectroscopy. Unordered graphitic-like structures of the BC surfaces decreased after one year of field exposure, indicating higher chemical recalcitrance of the BC residue. After only one year of field exposure, BC was detected in all SOM fractions. Most BC was found in free and aggregated structures but minor contributions were also found in the clay fraction.

Conclusions ^{13}C NMR spectroscopy is suggested for samples with high BC contributions but low BC amounts require additionally more sensitive analytical methods. The trend to native SOC stock increases in the clay fraction of BC-amended soils after only one year of field exposure suggests increased sequestration of OM as mineral-bound SOM in longer terms. Biochar amendment shifted the soil system from organo-mineral associated to POM-dominated OC storage with positive effects on soil air capacity.

Study III: Lydia Paetsch, Carsten W. Mueller, Ingrid Kögel-Knabner, Margit von Lützow, Cyril Girardin, Cornelia Rumpel (submitted, under revision): Effect of *in-situ* aged and fresh biochar on soil water holding capacity and microbial C use under drought conditions. Nature Scientific Reports.

Contribution: I designed the experiment together with the co-authors, carried out laboratory work and analyses, evaluated the data, and wrote the manuscript.

Objectives To investigate the effect of fresh and field-aged BC on soil water holding capacity and microbial parameters under drought conditions.

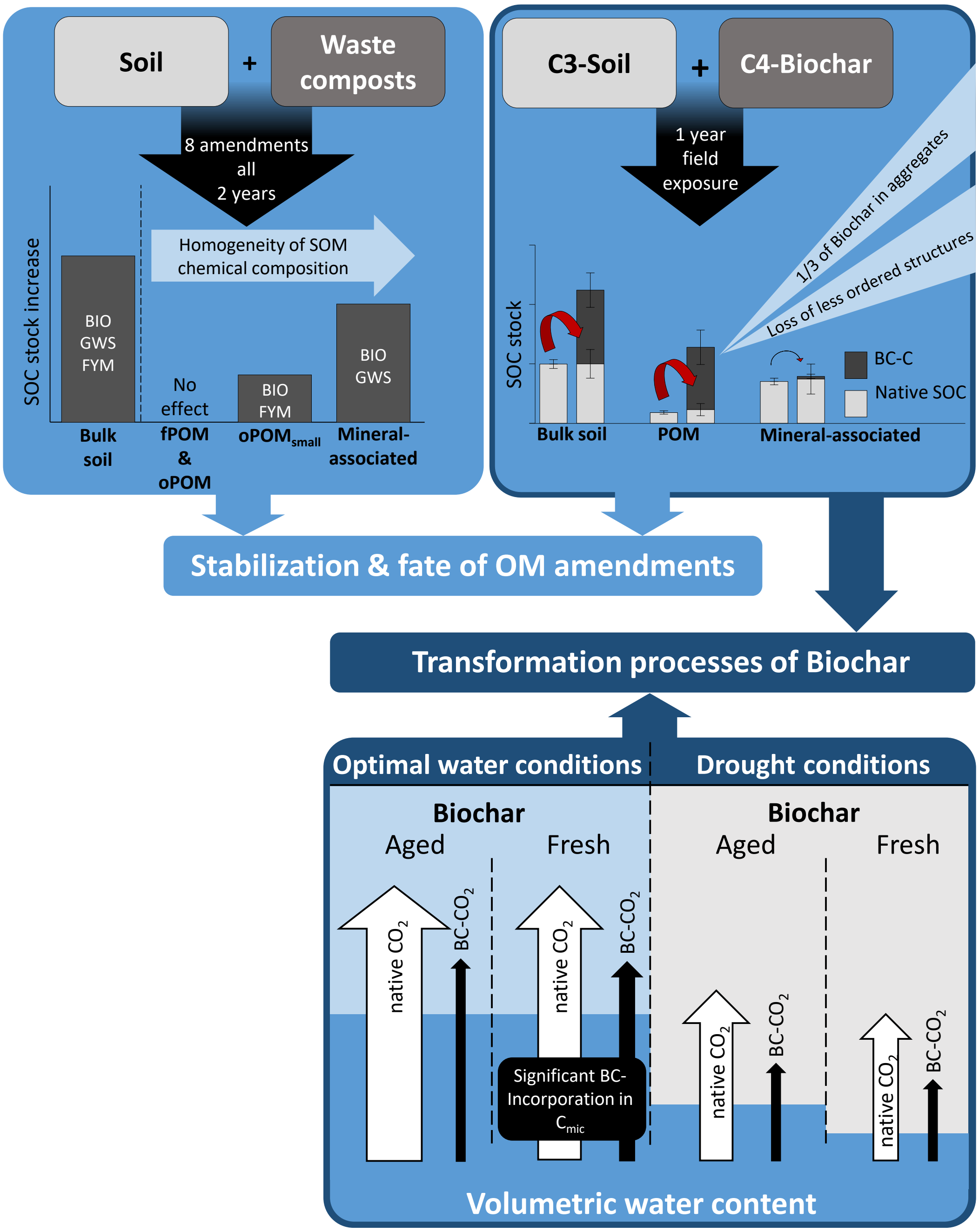
Methods Fresh BC, 3 years field-aged BC and an untreated control were incubated under optimal water and drought conditions. The C mineralization and microbial biomass was measured and the proportion of native and BC- derived C was calculated. With these parameters, the biomass-based metabolic quotient during the 3-months incubation was assessed.

Results Native and total mineralization rates were highest in aged BC amended soils, followed by fresh BC and the control soils with significant higher mineralization under optimal water conditions than under drought conditions. Fresh BC decomposed in two phases with a first flush in the beginning of the experiment and slower mineralization until the end of the incubation experiment. Biochar-C was incorporated into the microbial biomass only in fresh BC amended soils and under optimal water conditions.

Conclusions Biochar addition can improve the available water in loamy soils, especially under drought conditions. Aging of BC further increased this effect and improved native substrate availability, as indicated by higher metabolic quotients.

The research papers and publication permissions are attached in the appendices.

Thesis at a glance



1 Introduction

1.1 Background: Soil organic matter amendments for climate change mitigation soil quality

For the first time in history, the “4 per 1000 initiate” from the Paris Climate Agreement (21st Conference of the Parties to the United Nations Framework Convention on Climate Change) brought soil management in focus to a broad community as a climate change mitigation tool. Yearly increases of 0.4 % of global soil carbon stocks were proposed to not only withdraw CO_2 from the atmosphere, but also to improve important ecosystem services of soils like soil water retention and soil fertility. This approach is supposed to reduce the global temperature increase to +1.5 to +2 °C until 2100, the threshold beyond no significant effects of climate change are indicated (Meinshausen et al., 2009).

There are different strategies to increase soil organic carbon (SOC) stocks of agricultural soils. A fast and economically interesting approach is the amendment with exogenous organic carbon from e.g. urban waste composts or pyrolized organic matter (biochar). The usages of easily available wastes that occur in high and constant amounts seem to be a win–win–situation for climate change mitigation. Composted urban wastes enable an increase of medium-term C stocks. Additionally, pyrolized OM, due to its recalcitrant aromatic composition, offers a long-term organic carbon source, remaining in soils for decades to millennia (Laird, 2008).

Composts, as well as biochar (BC) have long been used as soil amendment in agriculture. Pre-Columbian inhabitants in the Brazilian Amazon basin amended their agricultural used soils with a mixture of charcoal and different organic wastes around 500 to 2500 years ago. They created a soil, which is now classified as *hortic Antrosol and* known as “Terra Preta do Indio” (Terra Preta) or “Black Earth of the Indian” (Glaser and Birk, 2012). It was estimated that these Terra Preta sites cover an area of 500 km² in Central Amazonia (Smith, 1980; Woods and McCann, 1999). The enhanced fertility of “Terra Preta” soils is expressed by higher levels of SOM, nutrients, such as nitrogen (N), phosphorus (P), calcium (Ca) and potassium (K), higher pH-values and higher water holding capacity than in the surrounding Ferralsols or Acrisols (Smith, 1980; Sombroek, 1966; Zech et al., 1990). With the discovery of the enormous potential for improving soils, many studies focused on researching BC-soil mixtures (Ding et al., 2016; Lehmann and Rondon, 2006; Sohi et al., 2010). However, depending on feedstock, composting or pyrolysis conditions of the OM amendment (Tripathi et al., 2016), resulting chemical and physical soil parameters strongly differ and thus often result in opposite conclusions about BC effects. These BC properties affect soil habitat properties for microorganisms, as reflected by differing microbial activity, biomass and metabolic efficiency (Lehmann et al., 2011; Zhou et al., 2010). Additionally, weathering of the amendments changes their effects on soil over time (Kuzyakov et al., 2009; Naisse et al., 2015b).

Due to the qualitative uncertainties, BC production standardization and regulations are urgently needed. A first voluntary European industrial standard for BC was developed in 2012, the so called “European Biochar Certificate” (EBC). Based on the latest scientific data and practices, the EBC aims at certifying a sustainable production of the BC feedstock, BC with a high energy efficiency and low emission pyrolysis technique. Furthermore, the EBC defined BC quality parameters which guarantee a low hazardous BC application in agronomic systems. With these guidelines, customers have a reliable quality basis, while producers have well-defined standards to provide high quality products.

However, the EBC was still in progress when the experiment in Lusignan was planned at which my studies were based. The BC which was used in my studies did not meet all requirements of the EBC, especially with regard to the production temperature. However, the main requirements of the EBC on the sustainable production, the positive carbon footprint, ecologically sustainable procurement, and environmentally safe storage were fulfilled.

1.2 State of the art

1.2.1 Stabilization of soil organic matter

Living and dead inputs from plants, animals and soil microorganisms determine the soil organic C cycle. Due to their decomposition and transformation processes, OC is recycled, and a large portion of C is stored in the soil or released as CO_2. The functional fractions of the highly heterogenic soils are stabilized by specific mechanisms with differing turnover times (von Lützow et al., 2007). The main SOM stabilization mechanisms were identified as 1) inaccessibility of OM due to physical protection in aggregates, 2) interactions with minerals and metal ions (Martin and Haider, 1986; Sollins et al., 1996; von Lützow et al., 2008), as well as 3) the chemical composition of the OM (von Lützow et al., 2006). Fresh plant and animal residues show very short turnover times of less than 10 years (Jenkinson and Ladd, 1981). In this initial phase of OM decomposition the chemical recalcitrance seems to play an important role (von Lützow et al., 2006). The intermediate pool preserves SOM by biogenic aggregation (turnover 10-100 years), whereas the passive pool is controlled by interactions with the mineral soil matrix (Cotrufo et al., 2013) and by spatial inaccessibility (e.g. microaggregation and formation of hydrophobic surfaces) (von Lützow et al., 2008).

Depending on the ‘quality’ of the OM amendments, the response of microorganisms (mineralization rate and microbial biomass) strongly vary, as various soil stabilization and destabilization mechanisms of OM take place simultaneously. There are many possibilities to modify OM. When OM is composted or pyrolized, more labile compounds become degraded, which increases the ‘quality’ of the amendment with regard to its C-sequestration potential (Bastida et al., 2008; Peltre et al., 2010; Ryals et al., 2014). Detailed research on stabilization pathways of amendments is crucial to understand the effects to the SOM pools.

1.2.2 Fractionation as a tool to identify stabilization pathways of OM amendments in soils

The analysis of bulk soil can be insufficient to identify stabilization pathways of OM amendments to follow their fate, whereas distinct SOM fractions might strongly reflect effects of OM amendments by their qualitative and quantitative changes. Depending on the research question, the common physical fractionation protocols include aggregate fractionation, particle-size fractionation or density fractionation (von Lützow et al., 2007). However, there is no standardized fractionation procedure (Christensen, 2001; Elliott and Cambardella, 1991). The physical SOM fractions and their quantification enable determining active, intermediate and passive OM pools and gaining information about the effects of amendments and of environmental changes on SOM storage (Christensen, 2001; von Lützow et al., 2007). Furthermore, isotopic analysis of these fractions allows for following the fate of BC-C within the SOC pools and distinguishing between BC-induced C and native SOC. The association of soil particles and their spatial arrangement play a crucial role in SOM dynamics, as they are indicators for C bioavailability by accessibility, which is a prerequisite for decomposition (von Lützow et al., 2007). The sorption behavior is an important stabilization mechanism of SOM, allowing the allocation of SOM to an active pool, and SOM in silt and clay fractions to an intermediate and passive pool. This classification is based on the concept that bonding affinities to SOM in the sand fraction (quartz particles) is very weak, whereas clay-sized particles (e.g. sesquioxides, layer silicates) with a large surface area and many reactive sites adsorb SOM by strong ligand exchange and polyvalent cation bridges (Sposito et al., 1999; von Lützow et al., 2007). In study I and II, a combined density and particle-size protocol according to Mueller et al. (2009) was used (see 2.3.2). In contrast to aggregate fractionation, or particle-size fractionation without separation of density fractions, the usage of a combined density and particle-size protocol enables robust conclusions about effects of OM amendments and of environmental changes.

The low-density fractions, fPOM and oPOM, are differentiated by, inter alia, their degradation stage and their embedding into the soil matrix. The fPOM fraction is not associated with soil minerals from organo–mineral complexes (uncomplexed fraction), whereas the oPOM fraction is particularly protected from degradation in aggregated structures and decomposed more strongly than the fPOM fraction. Consequently, the fPOM fraction is defined as active, labile pool, which responds quickly to OM inputs and environmental changes (von Lützow et al., 2007). The oPOM fraction belongs to the intermediate pool (von Lützow et al., 2007). However, compounds, as for example BC, are low in density and mostly detected in the free and occluded POM fraction. The attribution to the 'lable' or 'active' pool might be incorrect as the complex, highly condensed aromatic structure of the BC makes this substance comparatively resistant to decomposition. Hence, BC is commonly attributed to the passive pool (von Lützow et al., 2008).

1.2.3 Elucidating the chemical composition and structure of SOM by ^{13}C CPMAS NMR spectroscopy and Raman microspectroscopy

Solid-state cross polarization magnetic angle spinning (CPMAS) ^{13}C Nuclear magnetic resonance (NMR) spectroscopy is a well-established tool for the characterization of the chemical composition of SOM in solid soil samples and soil fractions. The semi-quantitative, non-destructive technique enables analyzing the entire sample without a pre-treatment like extraction, which is considered to contain only a part of the total SOM (Kögel-Knabner, 1997). The NMR phenomenon is based on the magnetic properties of nuclei of atoms, which are forced by an external electromagnetic field to redistribute themselves among different energy levels and consecutively are detected as a resonance signal at a specific resonance frequency in a spectrum (Kögel-Knabner, 2002). The magic angle spinning (MAS) technique improves the quality of the signal by compensating the chemical shift anisotropy. Therefore, a fast rotation of the sample in an angle of 54.74° during the measurement was applied. The cross-polarization from the abundant ^{1}H and the less abundant ^{13}C spins lead to a signal enhancement that enables investigations of solid samples at natural ^{13}C abundance (Kögel-Knabner, 1997; Wilson, 1987).

However, the quality of the spectra depends on the content of C and paramagnetic materials (Kögel-Knabner, 1997). To avoid long acquisition times, low resolution, and low signal-to-noise ratios of the spectra, the clay fractions were treated with 10 % hydrofluoric acid (HF) (Schmidt et al., 1997). This treatment concentrates the content of OC as it removes paramagnetic material (Schmidt et al., 1997). Whereas HF treatment is widely used and several authors postulated only minor changes in the chemical composition

(Schmidt et al., 1997), a preferential loss of considerable C amounts, as well as alterations in the chemical composition of distinct C compounds were observed (Rumpel et al., 2006).

Several indicators have been developed from NMR spectra to determine the degree of degradation of plant material and SOM, and other soil properties. In study I and II, the alkyl/O-alkyl-C ratio was utilized as a sensitive index of the extent of decomposition, as described by Baldock et al. (1997). This indicator is based on the observation that aliphatic components are more resistant to degradation than cellulose, hemicellulose, and proteins and thus accumulate during decomposition. Furthermore, the hydrophobicity index was calculated by dividing aromatic-C and alkyl-C by the sum of O-N-alky and carboxyl C (Kögel-Knabner, 1997). Using this index, we determined the surface wettability of the samples, and thus, deduce information about the accessibility of OM for microorganisms (von Lützow et al., 2006).

In addition to direct spectra interpretation, a molecular mixing model (MMM), as described by Nelson and Baldock (2005), was utilized in study II. This mathematical model enables an estimation of the relative content of molecular components – such as char. Combined with results from isotopic measurements, we could quantitatively compare the BC contributions to the soil physical fractions. This approach allowed direct estimates for the accuracy of ^{13}C NMR spectroscopy.

In study II, ^{13}C CPMAS NMR spectroscopy and Raman microspectroscopy were combined to obtain a comprehensive view of the chemical composition and structure of BC and SOM in soil physical fractions. The accuracy of BC detection in soil has been questioned because difficulties were encountered for highly condensed polyaromatic networks of kerogen (Smernik et al., 2006). This is especially important for materials that were produced at high temperature, as the BC that was used in the study I and II. Thus, to detect small changes of the BC structure, RM was used. Comparably to ^{13}C CPMAS NMR spectroscopy, RM is a non-invasive method and enables analysis of the molecular structure in solid samples. The combination of a Raman spectroscope with an optical microscope offers label-free Raman-images with high molecular selectivity. In contrast to the bulk soil method (NMR spectroscopy), selective measurements gain information about small structural changes of BC surfaces that may occur due to decomposition. Unordered carbonaceous substances contain two main peaks, indicating graphitic like (G) and defect structures (D). The relative intensity of the peaks (I(D)/I(G) ratio) serves as a measure for the structural order of the BC and SOC (Ferrari and Robertson, 2000). One of the first combination of ^{13}C NMR spectroscopy and Raman spectroscopy for SOM characterization of soil amended with OM was presented by Francioso et al. (2000). In 2013, Ribeiro-Soares et al. (2013) studied the structure of Terra preta soils and showed that the crystallite size of the compounds was in the range between nanographite and amorphous carbons. Consequently, they were able to differentiate between ancient BC and freshly produced BC.

1.2.4 Isotope tracing to follow the fate of BC-C in soils and to validate the accuracy of ^{13}C NMR spectroscopy

Analysis of total OC and N contents or of the chemical composition of soils are not sufficient to disentangle the fate of OM amendments when studying the complex OM dynamics in soils. The use of stable isotopes allows for comprehensively following the pathways of OM within SOM fractions and for obtaining a process-oriented understanding of OM transformation dynamics. Both, naturally- and radioactively-labeled OC in OM amendments, have been utilized to study OM decomposition mechanisms in soil and the contribution of biotic and abiotic factors (Kuzyakov and Domanski, 2000). Partitioning of native and amendment-induced OM changes, are crucial to identify inhibiting and accelerating effects by the inputs (priming effects) (Kuzyakov et al., 2000). Further, the label has been used to trace the microbial incorporation and respiration of exogenous C (Kuzyakov et al., 2009). This, in turn served as a proxy for biogeochemical C cycling. The main tracer techniques to estimate the C input and its functioning in soils are 1) pulse labeling, 2) continuous labelling and 3) ^{13}C natural abundance (C_3/C_4 change). The first two techniques are artificial labelling techniques, in which plants become exposed to a ^{11}C-, ^{13}C-, or ^{14}C-enriched atmosphere (Kuzyakov and Domanski, 2000). In contrast, the ^{13}C natural abundance method utilizes the differing ^{13}C contents from C_3 and C_4 plants, discriminating ^{13}C in different intensities (see section 2.3.5). All of these techniques require the use of a partitioning model during data analysis. The isotopic mixing model, a simple partitioning model, provides quantitative results about the proportion of C derived from the input material (BC) within SOM pools or within the microbial biomass and their respired CO_2.

1.3 Research aim and objectives

Study I:

The aim of this study was to elucidate the fate of urban waste compost in distinct soil organic matter fractions to determine its potential for long-term SOC sequestration potential compared to conventional OM amendments.

The main objectives were:

i) to study how similar amounts of C inputs with contrasting urban waste compost amendments affect SOM forming processes in the fPOM, oPOM, and organo-mineral fraction compared to conventional soil amendments

ii) to investigate how the OM amendments affect SOM fractions sequestered over long term with regard to their chemical composition

iii) to evaluate, if there is a relative accumulation of hydrophobic components resistant to decomposition or effects on water holding capacity

Study II:

The aim of this study was to use a multi-technique approach to assess the chemical and structural changes of SOM, physical fractions and the pure BC amendments after one year after field exposure.

The main objectives were:

i) to validate the accuracy of BC detection by ^{13}C NMR spectroscopy

ii) to quantify chemical and physical effects of BC on distinct SOM fractions

iii) to study how one year of field exposure induces structural changes of SOC and of the BC surface due to weathering

Study III:

The aim of this study was to investigate the effect of physico-chemical changes in BC amended soils with time of field exposure on the soil physical structure and its effects on microbial parameters under drought conditions.

The main objectives were:

i) to quantify the effect of fresh and three years field-aged BC on soil water holding capacity

ii) to investigate the effect of the BC on native SOC mineralization

iii) to study the effect of the BC to microbial biomass, activity and metabolic efficiency

2 Materials and methods

2.1 Experimental sites and amendments

Both sampling sites were research sites owned by the French National Institute of Agricultural Research (INRA). The study site for study I is located in Feucherolles (48° 87'27'' N, 1° 97'37'' E), close to Paris, with a mean annual precipitation of 696 mm and a mean annual temperature of 11 °C. The soil type is hortic glossic Luvisol (IUSS Working Group WRB, 2014). The study site for studies II and III is located in Lusignan (46°25"12.91"N, 0°07"29.35" E), close to Poitier, with a mean annual precipitation of 800 mm and a mean annual temperature of 11 °C. The soil at this site was classified as a dystric Cambisol (IUSS Working Group WRB, 2014). All plots of study site I received conventional N fertilizer (1:1 urea and ammo-nitrate), and were cropped rotationally with maize and wheat. After harvesting, OC inputs from crop residues and roots accounted for 0.39 kg m^{-2} to 0.42 kg m^{-2} C per year. The study site II was used as grassland with fourfold mowing per year and removal of the biomass.

The randomized plots of study site I (Fig. 1) were amended with similar amounts of OC (0.4 kg OC m^{-2}) every other year for a 15-year period. These amendments were 1) residual municipal solid wastes after separate collection of packaging (MSW), 2) organic wastes issued from a separate collection and co-composted with green wastes (BIO), and 3) green wastes and sewage sludge mixture (GWS). As control amendment, conventional cattle manure (FYM) was used, which was obtained from a nearby farm. Green wastes were added in all compost mixtures with 40 %, 33 % and 17 % for GWS, BIO and MSW, respectively. MSW contained 25 % kitchen wastes and 21 % paper and cardboard, whereas BIO contained 66 % kitchen wastes. GWS contained 20 % sewage sludge and 20 % wood chips. Inorganic components in MSW were glass (12 %), plastics (12 %) and other components (8 %) (values in % of fresh weight) (ADEME, 2015; Annabi et al., 2007). The BC experiment of study site II (studies II and III) included a single BC amendment (3 kg m^{-2}) in 2012 (Fig. 1). The BC was produced by gasification of maize (*Zea mays* L.) (C_4 plant) silage (~10 mm). The feedstock pellets were pyrolized for 40 minutes at 1200 °C in a commercial reactor (©A.G.T. – Advanced Gasification Technology s.r.l., Cremona, Italy). Main physicochemical characteristics of the fresh and one year field-aged BC are given in Table 1. The BC was incorporated into the upper 10 cm of the soil.

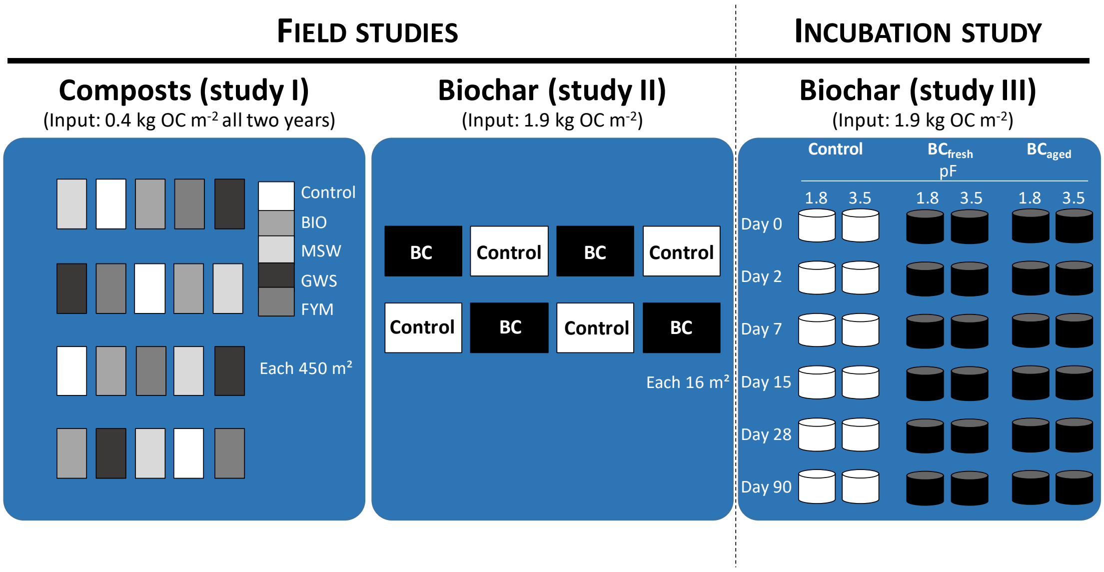


Fig. 1: Block design and incubation set up of studies I, II and III.

Table 1: Chemical characteristics of non-weathered ($BC_{pure\ fresh}$) and one year field-aged ($BC_{pure\ aged}$) pure biochar. Analysis were performed in triplicates and standard deviations of weathered BC were conducted from four field replicates with n = 3, respectively (not analyzed = N.A., dry matter=DM).

Parameter	Unit	$BC_{pure\ fresh}$	$BC_{pure\ aged}$
pH (0.01 *M* $CaCl_2$)	–	9.7	N.A.
Salinity	mS cm^{-1}	9.6	N.A.
OC content	mg g^{-1}	633.3	707.6 ± 18.1
N content	mg g^{-1}	13.5	16.5 ± 0.9
C/N ratio	–	46.9	43.0 ± 2.2
Ash content (550°C)	% DM	24.6 ± 1.7	N.A.

2.2 Sampling and pre-treatment

Samples of urban waste compost (study I) and BC amended soils (study II) were collected in September and October 2013 and for study III in September 2015. The last amendment with urban waste composts took place in 2011. Ten soil samples were randomly obtained from the top 28 cm (Ap horizon) of the soil profile with a shovel and composited together to form a representative sample from each urban waste compost amended and control plot. In the BC-amended and control plots (studies II and III), the disturbed samples were collected from the upper 10 cm, representing the BC-amended layer. Additionally, three undisturbed soil samples (100 cm^3 sampling rings, 10 cm depth) were sampled per plot. In total, 28 plots with urban waste compost or BC amendments and unamended control soils were sampled. Four field replicates for each amendment were obtained. Furthermore, pure composts and BC were sampled to compare the chemical composition of the pure substrates with the density and particle-size fractions.

For study I, the urban waste compost amended soils were sieved <2 mm after air-drying. For study II and III, the BC amended soils were not sieved to retain the BC pellets. Soils for study II were air-dried, but were used field-fresh for study III. For both studies, plant residues and living roots were removed manually. Inorganic material of urban waste compost amended soils (glass, plastic, stones) accounted for less than 1 % of the dry mass, organic material for less than 0.05 % of the dry mass.

2.3 Analytical Methods

2.3.1 C and N measurements

For data processing and interpretation, all bulk soil samples, SOM fractions, and amendments were analyzed for their C and N contents. Aliquots of each sample were measured in duplicate using dry combustion with an elemental analyzer EA3000 (Hekatech, Wegberg, Germany). Total C contents were considered as OC because no evidence of inorganic C contribution was detected by treating the soils with 10 % hydrochloric acid (HCl).

2.3.2 Density and particle-size fraction as SOM stabilization indicators

The density and particle-size fractionation protocol was performed, following Christensen (2001) with adaptation from Mueller et al. (2009) (Fig. 2). The fPOM and oPOM fractions were obtained by using sodium polytungstate (SPT) (TC Tungsten compounds, Grub am Forst, Germany) used as heavy solution with a density of 1.8 g cm^{-3}. The fPOM fraction was saturated with SPT over night and the floating material was removed by a water jet pump. Afterwards, the sample was dispersed ultrasonically with an energy input of 400 J ml^{-1} to release the oPOM fraction. The oPOM fraction was sieved into >20µm (oPOM) and <20µm (oPOM$_{small}$). After several centrifugation-decantation steps, salinity of the mineral residue was low enough (conductivity < 50 µS cm^{-1}) to start the particle-size fractionation. By wet sieving, the sand fraction (63 µm to 2000 µm) and the coarse silt fraction (20 µm to 63 µm) were separated. The medium (>6.3 µm) and the combined fine silt and clay fraction (<6.3 mm) were separated by sedimentation in settling columns. All fractions were freeze-dried (-40°C) (Martin Christ Gefriertrocknungsanlagen GmbH, Osterode, Germany) for further analysis and storage.

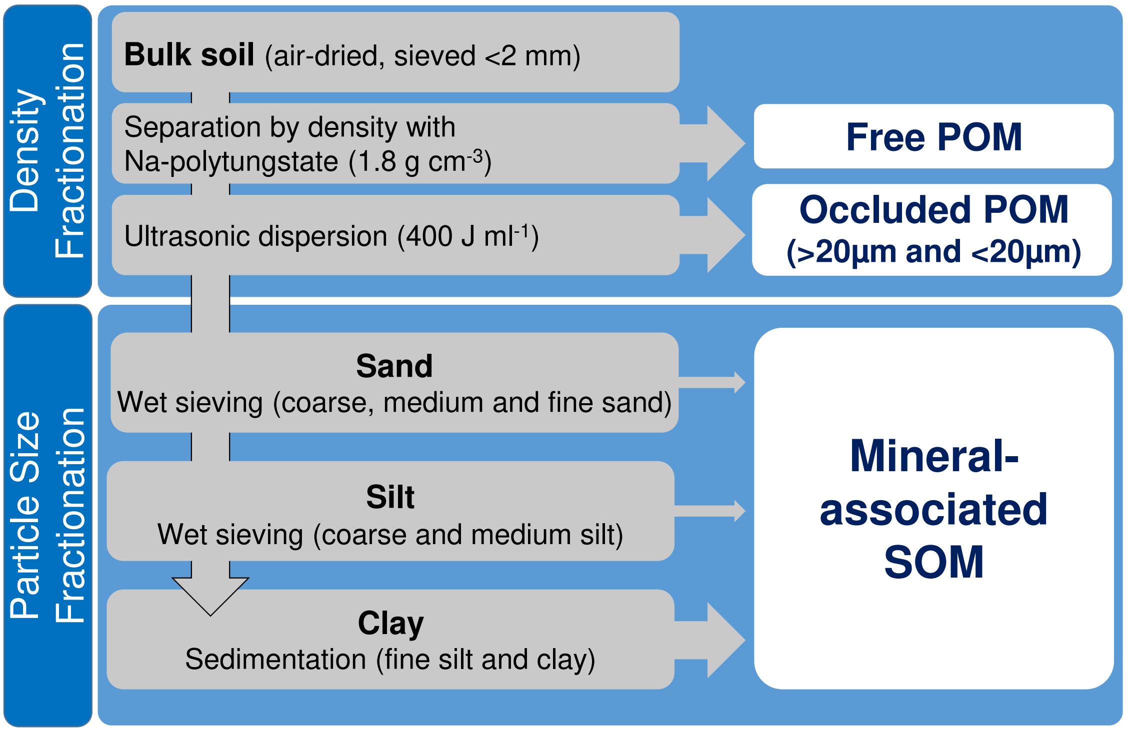


Fig. 2: Fractionation scheme of the combined density and particle-size fractionation protocol.

2.3.3 Solid-state ^{13}C NMR spectroscopy

The chemical composition of the amendments, POM, as well as HF-treated clay fractions were obtained using a Bruker Avance III 200 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). All samples, expect the fine clay fractions, were grinded and placed in zirconium rotors and spun with a rotation speed of 5 kHz in the magnetic field of the NMR spectrometer. Cross-polarization magic angle spinning technique with a contact time of 1 ms and a recycle delay of 0.4 s for clay or 1 s for the POM fractions and amendments was applied. Depending on the C content of the samples, between 2000 – 250,000 scans were accumulated. A line broadening between 0 and 50 Hz was applied. For the integration of the spectra, the integration routine of the spectrometer was used. The most important integration regions for further interpretation were: 0 – 45, 45 – 110, 110 – 160 and 160 – 220 ppm, assigned to alkyl-C, O/N-alkyl-C, aryl-C and carboxyl-C, respectively (Wilson, 1987). Signals derived from lignin are assigned to 56, 72, 128, 150 to 155 ppm. Signals attributed to polysaccharides are located at 72 ppm and as a shoulder at 82 ppm. Lipids and proteins show signals at 21 ppm, 32 ppm and 56 ppm, respectively (Kögel-Knabner, 2002; Wilson, 1987). For the calibration of the ^{13}C chemical shifts, tetramethylsilane was utilized and set to 0 ppm.

2.3.4 Raman microspectroscopy

Structural changes of the BC and BC amended soil fractions due to weathering and degradation were determined by RM. Expected low surface changes of the BC might be 'diluted' by grinding the samples to analyze bulk chemical changes by ^{13}C NMR spectroscopy. In study II, RM was used in addition to ^{13}C NMR spectroscopy to detect surface changes occurring within the outer micrometers of the BC – the penetration depth of the Raman microscope.

Briefly, RM relies on inelastic scattering, or Raman scattering, of monochromatic light. Usually, a laser beam is used causing excitation of the molecule by a photon in either the ground (lowest energy) or excited electronic state (a rotational and vibrational energy level within an electronic state) (Fig. 3). For a short time, the molecule is set in a virtual energy state before the photon scatters. There are three possibilities, how the photon can behave:

1) the photon passes the samples or is reflected (main part),

2) the photon stays without any changes of the energy level and scatters in various directions (elastic, Mie or Rayleigh scattering) (~10^{-4} – 10^{-3} compared to the irradiated light) or

3) an energy exchange between sample and photons take place (inelastic scattering), where the photons either 'absorb' vibrational energy from the sample (Anti-Stokes-scattering) or release energy to the sample (Stokes-scattering) (~10^{-8} – 10^{-6} compared to the irradiated light). During this process, wavelengths in the visible range occur, enabling a coupling of RS with an optical microscope (Raman microspectroscopy).

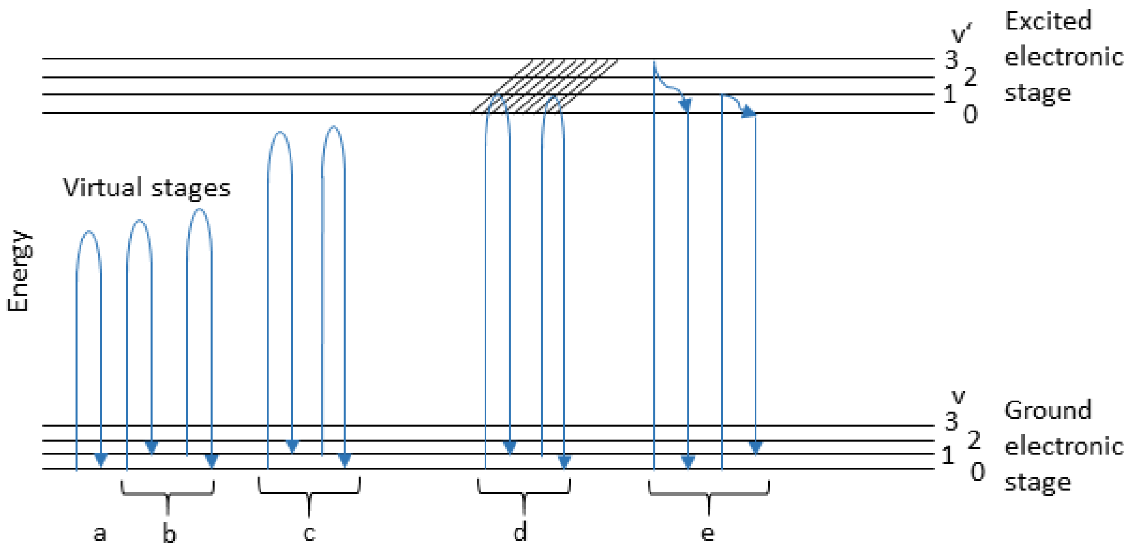


Fig. 3: Mechanisms of light-scattering processes with (a) Rayleigh, (b) non-resonance Raman, (c) pre-resonance Raman, (d) resonance Raman and (e) resonance fluorescence (according toTurrell and Corset, 1996).

In general, the Raman spectra of (unordered) carbonaceous substances contain two main peaks. The G ("graphitic")-peak (E2g-symmetry) around 1580 cm^{-1}, and the D ("defect")-peak around 1350 cm^{-1} (A1g-symmetry). Their relative intensity (I(D)/I(G) ratio) is a measure for the structural order of the sample. The relative intensity is directly proportional to the order of the nanostructure (Ferrari and Robertson, 2000). For the RM analysis, a LabRAM HR Raman microscope system (Horiba Jobin Yvon, Japan) with a HeNe-laser (633 nm, 0.14 mW at the sample) and an integrated Olympus BXFM microscope with a 50× objective (Olympus MPlan N, NA= 0.75) was used. All soil fractions and the pure BC were measured at five spots for 100 s. As measurement points, five black spots were selected optically. The spectra were baseline-corrected, normalized and the mean spectra were calculated.

2.3.5 Isotopic measurements

To follow the fate of the amendment within SOC fractions (study II), the microbial biomass, and the CO_2 emissions (study III), were used. Furthermore, the accuracy of the ^{13}C NMR analysis was validated by using the different isotopic signatures of C_3 (native SOC) and C_4 plants (maize-derived BC). The differing natural abundance of ^{13}C isotopes is based on the discrimination of ^{13}C isotopes during assimilation of the different C_3 and C_4 photosynthesis types. Both types discriminate ^{13}C from atmospheric CO_2 but varying degrees: whereas the enzyme Rubisco in C_3 plants leads to a depletion of about -27‰

compared to the atmospheric CO_2, Phosphoenol pyruvate carboxylase in C_4 plants results in a lower depletion of about -13 ‰ (Farquhar et al., 1989).

In studies II and III, the native SOM (bC_3) developed under a C_3 vegetation and the proportion of C_4 BC was calculated by the isotopic mixing model:

$$bC_3 = \frac{\delta C_{mixture} - \delta C_4}{\delta C_3 - \delta C_4}$$

Where δC_4 is the $\delta^{13}C$ signature of the pure BC and δC_3 of the native SOC of the control (‰). $\delta C_{\mathrm{mixture}}$ is the $\delta^{13}C$ signal of the bulk soils and the specific values of each physical fraction. Solid samples of physical fractions (study II) were analyzed with an elemental analyzer (Fisons Instruments EA/NA, Italy), coupled to an isotopic ratio mass spectrometer (VG IOSOGAS, England). Due to the much lower concentration of C in the fumigation extracts of the microbial biomass experiment, the samples were analyzed in a more sensitive isotope ratio mass spectrometer (Delta V Advantage Thermo Fisher Scientific, Bremen, Germany), coupled with an elemental analyzer (Flash 2000, Thermo Fisher Scientific, Bremen, Germany). The $^{13}CO_2$ contribution was analyzed in an isotopic mass spectrometer (Microgas, Hawlett 500P).

2.3.6 Microbial biomass and activity

The soil microbial biomass plays a crucial role for the regulation of carbon cycles (Horwath, 1994). Due to their energy demand for growth and metabolism, microbes rely on OC from plants and SOM. They can rapidly adapt their energetic performance and activity rates to changing environmental conditions (Schloter et al., 2003). The microbial biomass is hence used as a short- and long-term indicator for the microbial habitat and amendment quality.

In study III, results from the fumigation extraction method and mineralization measurements were combined to calculate the metabolic quotient (qCO_2) to evaluate the microbial C use efficiency and the substrate availability.

The microbial biomass was determined by a modified chloroform fumigation-extraction method from Vance et al. (1987) at different times of incubation. Chloroform is used as an effective biocide that does not solubilize non-microbial soil organic matter or render it decomposable (Jenkinson and Powlson, 1976). After 16 h in a chloroform-saturated atmosphere, the major proportion of the microorganisms is assumed to be killed off (~90 %).

Afterwards, the dead microbial biomass was extracted by a 0.03 *M* potassium sulfate solution after 30 minutes of shaking and 10 minutes of centrifugation at 10,000 RPM.

The microbial biomass is defined as the difference between OC content of the fumigated sample and OC content of the non-fumigated sample. As OC and ^{13}C contents of the microbial biomass were determined, the supernatants were freeze-dried and the solid residue was analyzed in an isotope ratio mass spectrometer (Delta V Advantage Thermo Fisher Scientific, Bremen, Germany), coupled with an elemental analyzer (Flash 2000, Thermo Fisher Scientific, Bremen, Germany). All δ^{13}C values were expressed relative to the Pee Dee Belemnite (PDB) international isotope standard.

Apart from the information about the microbial biomass, the microbial activity was determined by measuring the mineralization rates. Just the active microorganisms drive biogeochemical processes and the ratio between biomass and mineralization enables to draw conclusions about the microbial C use efficiency.

To determine the microbial activity, which depends on water content and aging state of the BC, an incubation experiment was conducted. For 90 days, soil samples at 100 % and 50 % water holding capacity were incubated at 20 °C in darkness. The decomposition of the OM was analyzed by measuring the released of CO_2 with a gas chromatograph (Agilent MICROGC, Santa Clara, USA). The specific mineralization, which was induced by the BC, was determined by comparison of the stable isotope signature of the BC to the signature of native SOC by using an isotopic mass spectrometer (Microgas, Hawlett 500P). After each gas measurement, the jars were flushed for 20 minutes with moistened CO_2-free air to receive a strong CO_2 depletion in the jar's atmosphere. To avoid anaerobic conditions between the defined measuring dates, gases were additionally measured and the jars were flushed at least every 14 days throughout the incubation period.

Using the microbial biomass and the microbial activity, the biomass-based metabolic quotient (qCO_2) was calculated. The ratio of incorporation (microbial biomass) to CO_2 mineralization (microbial activity) allows for evaluating the microbial C use efficiency and the substrate availability. A high metabolic quotient indicates a low efficiency of C mineralization and a higher substrate availability (Bimüller et al., 2014). The qCO_2 was calculated by using the equation

$$qCO_2 = \left(\frac{CO_2 - C}{C_{mic}}\right)$$

where CO_2-C is the cumulative respired CO_2-C in mg kg^{-1} soil h^{-1} and C_{mic} the corresponding microbial biomass C in mg kg^{-1} soil.

Table 2: Overview of the soil parameters and the applied methods, as utilized in study I.

Study I **Urban waste composts enhance OC and N stocks after long-term amendment but do not alter organic matter composition** **15 year ongoing field experiment with urban waste composts and conventional compost amendments every other year to an Ap horizon (Luvisol)**
Soil parameters
pH in 0.01 *M* CaCl$_2$ solution
SOC and N contents by using an elemental analyzer and calculation of SOC and N stocks
density and particle-size fractionation (SPT with a density of 1.8 g cm^{-3}, ultrasonically dispersion with 400 J ml^{-1}) to receive fPOM, oPOM, oPOM<20µm, sand, silt and clay fractions
Water holding capacity
Soil fraction parameters
SOC and N contents by using an elemental analyzer and calculation of SOC and N stocks
Chemical composition of all fractions and pure amendments by using solid-state ^{13}C NMR spectroscopy
Data presentation and statistical analyses
Data presentation by using SigmaPlot
Statistical analyses by using RStudio

Table 3: Overview of the soil parameters and the applied methods, as utilized in study II.

Study II **A multi-technique approach to assess the fate of biochar in soil and to quantify its effect on soil organic matter composition** **One year ongoing field experiment with a single biochar amendment to the A horizon (Cambisol) of a grassland soil**
Soil parameters
pH in 0.01 *M* CaCl$_2$ solution
SOC and N contents by using an elemental analyzer and calculation of SOC and N stocks
density and particle-size fractionation (SPT with a density of 1.8 g cm^{-3}, ultrasonically dispersion with 400 J ml^{-1}) to receive fPOM, oPOM, oPOM<20µm, sand, silt and clay
Water holding capacity
Soil fraction parameters
SOC and N contents by using an elemental analyzer and calculation of SOC and N stocks
^{13}C of soil fractions and pure BC by using isotope ratio mass spectrometer
Chemical composition of all fractions and pure BCs by using solid-state ^{13}C NMR spectroscopy
Structural composition of all fractions and pure BCs by using Raman microspectroscopy
Data presentation and statistical analyses
Data presentation by using SigmaPlot and Excel
Statistical analyses by using RStudio

Table 4: Overview of the determined soil parameters and the applied methods in study III.

Study III **Effect of in-situ aged and fresh biochar on soil water holding capacity and microbial C use under drought conditions** **90-days incubation experiment with three year field aged and fresh BC in an A horizon (Cambisol) of an grassland soil**
Soil parameters
pH in 0.01 *M* $CaCl_2$ solution
SOC and N contents by using an elemental analyzer
^{13}C of bulk soil and BC by using isotope ratio mass spectrometer
Microbial parameters
Microbial biomass by using fumigation-extraction method
^{13}C of microbial biomass by isotope ratio mass spectrometer
CO_2 soil respiration by using gas chromatography
^{13}C-CO_2 measurements by using an isotopic mass spectrometer
Data presentation and statistical analyses
Data presentation by using Excel and InDesign
Statistical analyses by using RStudio

3 Discussion

3.1 The impact of urban waste compost and BC (studies I and II) on the C-sequestration potential

The discussion section focusses on a general discussion and only summarizes results from the three studies performed for this doctoral thesis. For more detailed presentations of the results, please see the attached publications and the manuscript in the appendices.

With regard to total SOC stocks, the results indicated significantly higher SOC stocks for all compost and BC amendments, except for MSW. Eight repeated amendments with BIO or GWS of 0.4 kg OC m^{-2} increased the SOC stock in a similar magnitude as conventional FYM (about 30 %). The single BC amendment of 3 kg m^{-2} BC (1.9 kg OC m^{-2}) increased the total SOC stock by 125 %.

Composts

The compost-amended soils store up to one third of the total OC in the POM fractions but the major proportion of OC was stored in the clay fraction (article 1, Fig. 3 a). This is due to SOC content and mass increases of the $oPOM_{small}$ (BIO and FYM) and the clay fractions (BIO and GWS) – the intermediate and long-term C pools (article 1, Table 3 and supplementary material Fig. A1). Soil OC stock increases of these SOM fractions accounted for 55 % (MSW) to 94 % (BIO) of the total SOC stock increases. The significant SOC stock increases between 42 % - 46 % in the clay fraction after amendment with BIO, GWS or FYM clearly show that their amendment build up a mineral-associated SOM pool, which was considered by Scholes and Scholes (2013) as relevant to reduce the carbon release to the atmosphere. Significant mass changes of the fPOM fraction, as found by Carter et al. (2003) and Leroy et al. (2008), might be already passed, indicating that their experiments gave only a very short-term insight into the biogeochemical C-cycle. These authors have already criticized that their experiments were too short, as no effects to the oPOM fraction was detected.

Peltre et al. (2012) attributed the low SOC stock increase after MSW amendments of their experiment to the chemical composition of the amendment. They argued that the high amount of e.g. paper in MSW resulted in higher contents of quickly degradable polysaccharides and lower contents of lignin compared to the other urban waste composts and the FYM. However, the ^{13}C NMR spectra showed neither for the pure amendments (article 1, Fig. 1), nor for the physical fractions of the amended soils significant differences in their chemical composition (article 1, Fig. 5). Furthermore, the POM and the clay fraction showed no distinctive lignin accumulation. Thus, it is proposed that the C-to-N ratio of the pure amendments, which was highest for MSW (article 1, Table 1), is a more suitable indicator for the lower OC-sequestration potential. Combined with the lower OC contents in the clay fraction compared to the other amended clay fractions (article 1, Table 3), both results support the hypothesis of microbial use efficiency as control of C-stabilization in soils (Cotrufo et al., 2013; Rumpel et al., 2015).

Biochar

In various soils, the clay fraction was found to represent the dominating SOC and N fraction in cropland soils (Christensen, 2001; Jagadamma and Lal, 2010; von Lützow et al., 2007). With the amendment of BC, however, the main storage shifts from a clay- to a POM-dominated system (article 2, Fig. 2). The SOC stock increase in the POM fractions is fully explained by BC-derived C, as no significant changes in native SOC stocks were detected (article 2, Table 3). In contrast to the compost study, the difference in the natural ^{13}C abundance allows for a detection of the BC-C. One third of the BC was present in aggregated structures isolated as oPOM and oPOM$_{small}$, one year after application (article 2, Table 3). As described in studies using low-temperature BC (Kimetu and Lehmann, 2010), a rapid rate of incorporation of BC into aggregates occurs after only one amendment. Four percent of the BC-C were associated with minerals of the clay fraction (article 2, Table 3). This implies that all physical fractions contained BC after only one year of field exposure. Such a rapid comminution and incorporation of BC into the mineral-associated OM fraction has not been reported before, neither from low- nor from high-temperature BC. It can be assumed that fine BC particles serve as binding agent, and are at the same time protected from degradation (Brodowski et al., 2006; Yang et al., 2016).

After one year of field exposure, BC did not significantly affect the native SOC storage, but a trend to increased SOC storage in the clay fraction occurred (article 2, Fig. 2). This remarkable effect and the possible involvement of microorganisms needed to be studied with BC which was field exposed for a longer time period. With study III, the microbial use of BC and the protection of microbial products by mineral interactions were studied (see section 3.4).

3.2 Chemical composition of composts and BC and its effects on physical fractions (studies I, II, and III)

Composts

The strikingly similar solid-state ^{13}C NMR spectra of the pure urban waste compost amendments (study I) were dominated by polysaccharides, showing a high proportion of cellulose, as indicated by the high resolution for the crystalline C at 72 and 89 ppm (article 1, Fig. 1) (Earl and VanderHart, 1981; Newman and Hemmingson, 1995). The MSW and the FYM inputs resulted in a slightly lower alkyl C contribution than BIO and GWS amendments.

The POM fractions, isolated from urban waste compost amended soils, slightly differed from the OM amendments themselves (article 1, Fig. 1 and 5). Two years after the last amendment, the compounds, which originated from urban waste composts, were almost completely decomposed. Polysaccharides, derived from incorporated crop residues from wheat and maize, are known to further dilute the compost effects to these fractions (Bolinder et al., 1997; Kögel-Knabner, 2002). Only MSW inputs increased the aromatic signal at 128 ppm in the POM fractions which likely originated from black C or soot (article 1, Fig. 5) (Kögel-Knabner, 1997; Mueller and Kögel-Knabner, 2009; Pichler et al., 2000). This is in agreement with other MSW studies that presented repeated MSW amendments (Almendros et al., 2003; De la Rosa et al., 2008; González-Vila et al., 2002).

In the more altered $\text{oPOM}_{\text{small}}$ and clay fraction, the distinct aromatic peak did not appear and the spectra were uniform (article 1, Fig.5). The clay fraction was dominated by signals of polysaccharides and alkyl C, similar to a clay fraction which has been isolated from loamy, arable topsoils (Mueller and Kögel-Knabner, 2009). During decomposition of plant residues and OM amendments, microorganisms and their residues determine the OM of the clay fraction, which is associated with soil minerals (Cotrufo et al., 2013; Simpson et al., 2007). Consequently, the chemical composition of the OM in the clay fraction is independent from the chemical composition of the input material (Kelleher and Simpson, 2006; Kleber et al., 2015; Miltner et al., 2012; Schurig et al., 2013). For all soils, increasing alkyl/O-N-alkyl ratio from fPOM to $\text{oPOM}_{\text{small}}$ fractions were noted (article 1, Table 3), indicating increasing decomposition degrees (Baldock et al., 1997). The hydrophobicity of the urban waste compost amended POM fractions increased in the order control < FYM < MSW < GWS < BIO (from 0.6 to 0.7) (article 1, Table 3) which is comparable to conventional amendments like pig manure and crop straw (Zhou et al., 2010) (H ~ 0.79). This enhanced hydrophobicity lead to a quantitative increase of the proportion POM fraction. The clay fraction was not affected by this phenomenon and showed even lower hydrophobicity in MSW-amended soils than the control. However, the generally low proportion of POM did not have an impact on the bulk soil wettability which is in agreement with results of Annabi et al. (2007) and Doerr et al. (2006).

The larger C storage in the clay fraction after BIO, GWS, and FYM amendments compared to MSW (indicated by their C-to-N ratio and chemical composition), as presented in study I, further support the hypothesis of microbial use efficiency as control of C-stabilization in soil (Cotrufo et al., 2013; Rumpel et al., 2015).

Biochar detection accuracy and chemical composition of pure BC and BC amended physical fractions

In contrast to conventional SOM studies, BC detection by ^{13}C NMR spectroscopy is often problematic due to its condensed, aromatic structure. The low degree of protonation, the rapid relaxation time (specifically the T1ρH proton spin-lattice relaxation time) (Smernik et al., 2002) and the interference with other aromatic OM present (Hammes et al., 2008) hampers the accuracy of this method. Our BC was produced at high temperatures which generally results in a high degree of aromatic condensation for pyrolysis BC (McBeath et al., 2011). By combining ^{13}C NMR spectroscopy and IRMS, an almost 1:1 line correlation ($n=16$, $R^2=0.98$) was detected with minor underestimations of BC by ^{13}C NMR spectroscopic analysis (article 2, Fig. 4 b). These underestimations can be explained by the parameters of the MMM that was used for the ^{13}C NMR spectra. In contrast to an aryl-C content of 73 % in our BC, Nelson and Baldock (2005) used an aryl-C content of only 65 %. When BC was highly concentrated, as present in the fPOM fraction, BC was almost completely detected by the ^{13}C CPMAS NMR analysis (underestimation accounted for 8 %). With decreasing BC proportions, this underestimation increased, indicating that ^{13}C NMR spectroscopy BC detection and quantification accuracy is limited to higher BC proportions in soil. A combination with other methods, like RM or IRMS (if possible), is therefore recommended.

The chemical composition of the BC amended SOM fractions was only affected by the increasing contribution of aromatic C (article 2, Fig. 3). In higher concentrations, as in the fPOM fraction, the aromatic C peak dominated the spectrum and its contribution decreased with decreasing BC proportion in the order fPOM < oPOM < $\text{oPOM}_{\text{small}}$ < clay fraction (article 2, tab. 3). The BC contribution to the clay fraction was only marginal (4 %, determined by the IMM) and no distinct aromatic peak was detected by NMR spectroscopy. An explanation could be microbial BC degradation and BC-C incorporation transformed into non-aromatic compounds (Luo et al., 2013; Singh et al., 2014) not identifiable as BC anymore (see section 3.4.2.). Other functional groups were not affected by the BC amendment.

A more detailed assessment of the pure BC before and after weathering revealed that both BCs were not altered in their bulk chemical composition (article 2, supplementary material Fig. A.2). The spectra were dominated by an aryl-C peak and minor contributions of O-N-alkyl-C and carboxyl-C. This is in line with findings of dos Anjos Leal et al. (2015) and McBeath et al. (2013) for low-temperature BC.

However, by using RM, changes in the nanostructure of the weathered and non-weathered BC were detected (article 2, Table 2 and Fig. 1 a). A preferential loss of less ordered and amorphous structures was demonstrated, resulting in higher-ordered structures of the weathered BC. Artificial weathering of the same BC support the findings of study II: Naisse et al. (2015b) found, after chemical and physical weathering in a laboratory experiment, that the biological stability of the residual material increased. Mukome et al. (2014) showed a two-phase degradation in their laboratory incubation study with low-temperature BC, This degradation was associated with labile, aliphatic components of BC, in contrast to a more recalcitrant aromatic residue. The loss of less-ordered structures of the BC indicates that two differently available fractions for weathering or degradation are also present in our BC. The loss of less-ordered structures further resulted in residual highly-ordered graphitic-like structures, presumably more recalcitrant than the non-weathered BC.

The BC amended oPOM and clay fraction showed no significant differences in the nanostructures (article 2, Table 2 and Fig. 1 b), which indicates only physical fragmentation and protection of the BC from weathering within the soil structure. The structural order was the same as in the pure unweathered BC. To our knowledge, this study is the first successful combination of ^{13}C NMR and RM for the analysis of SOM fractions. Due to the combination of non-destructive bulk and surface analysis and the different nature of information gained, RM and CPMAS can be used as complementary methods.

3.3 Soil water holding capacity changes due to amendments (studies I, II, and III)

The soils of both study sites (Feucherolles and Lusignan) were characterized by very good available water capacities (Cambisol and Luvisol). An improvement of the soil physical conditions, hence, focused on an increase of the air capacity and the strongly bound water which is still plant available (pF 3.5 to 4.2).

The amendments with composts (study I) or one year field-aged BC (study II) did not affect total soil water holding capacities, but the BC positively affected the air capacity. With ongoing aging of the BC (3 years of field exposure, study III), an increased potential to store water between pF 3.5 and 4.2 was detected which is crucial for plant water supply under drought (manuscript 1, Fig. 2). Responsible for the difference between fresh, one and three year field-aged BC might be fragmentation due to mechanical stress of freeze-thaw cycles during field exposure which form new cracks and fractures. These new pores are known to increase the pore connectivity of BC_{aged}, compared to BC_{fresh} particles (Spokas et al., 2014). Additionally, retained plant available water reduces water stress, and hence, retard drought effects to plants (Koide et al., 2015). Moreover, microorganisms benefit from the improved water conditions during droughts due to BC amendments, indicated by increased microbial biomass and microbial activity, as compared to the control (see section 3.4).

3.4 Biological impact of BC (study III)

3.4.1 Microbial activity

In the studies I and II only assumptions were possible about the microbial contribution to SOC-sequestration. Thus, study III focused on BC effects on microorganisms. The strongest mineralization was detected in pF 1.8 sets in BC_{aged} samples, followed by BC_{fresh} and the control (manuscript 1, Fig. 3 a). Due to water stress in pF 3.5 sets, the mineralization rates strongly decreased, but remained highest in BC_{aged}, followed by BC_{fresh}, and control sets (manuscript 1, Fig. 3 a). Decreasing mineralization by decreasing water contents have already been presented in previous studies (Manzoni et al., 2012a; Manzoni et al., 2012b). As reported in several studies with low- or high-temperature BC under optimal water conditions (Ameloot et al., 2013; Liao et al., 2016; Luo et al., 2011; Maestrini et al., 2015), a “first flush” of CO_2 in BC_{fresh} amended soils was also detected in our study (pF 1.8) (manuscript 1, Fig. 3 b and c). This flush was probably related to the mineralization of labile C compounds of the BC, and the stimulation of native SOC mineralization (Jones et al., 2011; e.g. Smith et al., 2010), supporting our assumption of study II that losses of less ordered structures were caused by labile C compounds like aliphatic C with a remaining more recalcitrant aromatic residue. In this context, the flush in the first days of the experiment indicates an immediate adaption of microorganisms to the usage of these labile BC compounds (Jiang et al., 2016). However, due to the fast subside of this effect, the effect on SOM is most likely very short (e.g. Kuzyakov et al., 2009). The effect is induced by ‘r-strategist’ microbes, which are adapted to respond quickly to newly-available C sources (Cross and Sohi, 2011; Farrell et al., 2013). This assumption is supported by the absence of a BC-C mineralization flush in BC_{aged} amended soils, which are most likely already depleted in labile BC-C compounds (manuscript 1, Fig. 3 c) or protected from degradation (Jones et al., 2011; Naisse et al., 2015a). A lowered native SOC mineralization compared to control levels was not detected. No lowered native SOC mineralization under the level of control samples was found as presented by (Luo et al., 2013; Ventura et al., 2015). They assume lowered mineralization of native SOC due to toxic compounds of the BC or mineral adsorptive protection. In contrast, sorption of potential inhibitors in SOM by BC_{aged} is assumed, which might increase extracellular reactions and therefore induce the breakdown of native SOM (Jan et al., 2009).

3.4.2 Microbial biomass growth and incorporation of BC-C

During the incubation experiment of study III, the general trend of decreasing microbial biomass at pF 1.8 (decrease of 22 % in BC_{fresh} and 38 % in the control) was detected for all amendments, whereas it remained rather constant at pF 3.5 (-7 % in BC_{fresh} and 9% in the control, manuscript 1, Table 3). Negative microbial biomass dynamics at optimal water conditions are supposedly related to a rapid consumption of easily available substrate, causing its depletion. The decline between the start and the end of the experiment was more pronounced in BC_{aged} and the control than in BC_{fresh} (38 % and 35 %, compared to 22 %). Drought conditions, however, inhibit microbial biomass losses, as indicated by stagnating values. The moderate increase of microbial biomass in BC_{fresh} compared to the control is similar to the findings of Zhou et al. (2017). However, Hale et al. (2011) and Kuzyakov et al. (2009) observed that this trend decreases with increasing duration of the experiment. This two-phased development was again explained with, firstly, an increase of microbial biomass due to the labile carbon pool (Lou et al., 2015) of the BC, and secondly, with the exhaustion of bioavailable C. In this context, it is unlikely that BC serves as a suitable habitat for microbes (Lehmann et al., 2011), even though microsites and protection from predators were presented as possible benefits for microbes (Pietikäinen et al., 2000). Nonetheless, BC aging is known to increase microsites and therefore microbial biomass, but no significant effects were detected in BC_{aged} on microbial biomass, compared to the control.

However, the state of aging played a crucial role for the BC-C incorporation into microbial biomass. Labile BC-C of BC_{fresh} is assumed to be used by microorganisms as an energy source and substrate (Kuzyakov et al., 2009; Luo et al., 2013; Smith, 2016). At pF 1.8, the incorporation rate is comparable to the findings of Kuzyakov et al. (2009), who presented values from 1.5 to 2.6 %. Aged BC, however, seems to be mineralized preferentially, because the ^{13}C content of the microbial biomass was depleted (manuscript 1, Table 3). Under water stress, the BC-C proportion to OC respired significantly increased compared to pF 1.8. Due to continuous BC-C mineralization throughout the experiment in BC_{aged} treatments, it is likely that microbes persistently used BC-C. In contrast, Ameloot et al. (2014) found that one to four years aged, wood-derived BC was not used by microbes anymore. These findings support the assumption of study II that parts of the 4 % BC-C detected in the clay fraction, is microbial incorporated BC-C, which has been transformed into non-aromatic compounds (Luo et al., 2013; Singh et al., 2014), and was not identifiable by ^{13}C NMR spectroscopy.

3.4.3 Effects of BC on metabolic efficiency

To evaluate the metabolic efficiency (qCO_2) of the soil microbial biomass, the metabolic quotient was calculated. In the experiment, the qCO_2-values are comparable to values from other disturbed arable soils (Wardle and Ghani, 1995). For BC and control soils, the high qCO_2-values at the beginning decreased until the end of the incubation (manuscript 1, Fig. 4). The only exception was observed at day 7 of the incubation, when the qCO_2 increased by 6 - 39% (pF 1.8) and 9 - 19% (pF 3.5) due to the CO_2 flush. The decreasing qCO_2-values are results of constant mineralization (per h), but decreasing microbial biomass. The high qCO_2-values indicate relatively 'large' C losses (through respiration), ultimately reducing the potential for long-term C-sequestration in organo-mineral complexes (Manzoni et al., 2012b; von Lützow et al., 2007). In contrast, other studies found constant respiration but increasing microbial biomass (Zhou et al., 2017). During my experiment, the mineralization rates decreased not proportional to the decrease of microbial biomass, which could be explained by a shift of the microbial community (Jones et al., 2011; Lehmann et al., 2011). Changes in microbial community composition cannot be excluded and its effects on the qCO_2 by distinct microbial groups are difficult to estimate (Waldrop and Firestone, 2004). Taxa-specific community changes were evidenced by Farrell et al. (2013), Chen et al. (2017), Chen et al. (2013) and Gomez et al. (2014). Gomez et al. (2014) further found an alleviation of the microbial biomass decrease with increasing BC amendments. On the one hand, it could be assumed that the alleviation occur due to a buffering effect of the BC on the microbial community or on the other hand, due to a recycling of labile C from dead microbial biomass. One strategy of the microorganisms in the pF 1.8 sets could be gaining nutrients by overflow respiration or C excretion ('luxury consumption', 'waste metabolism' (Smith, 1979), 'N-mining' (Craine et al., 2007)).

Significant BC effects on the qCO_2 were detected only under water stress. Low qCO2-values can be ascribed to a shift from growth to maintenance respiration or preparation for dormancy stages and not for growth (Herron et al., 2009) due to limited substrate supply and thus slow diffusion rates along the increasingly tortuous paths of thin water films. Another explanation is a change in the physiology of microbes and an adaption to more desiccating conditions (Stark and Firestone, 1995). Generally, low qCO_2-values indicate improved biophysical conditions for microbes, but under stressing conditions, this interpretation seems to be invalid. The closer look on the single parameters of the control and BC_{fresh} samples indicate more stressed conditions by water shortage and starving, as reflected by stagnating microbial biomass and lower the C-mineralization compared to BC$_{aged}$ samples. This implies that lower qCO$_2$-values do not represent better biophysical conditions, but decreasing activity of microorganisms preparing or being in dormant states (Allison et al., 2010). The increasing metabolic efficiency after BC$_{aged}$ amendment suggests improving conditions, in which the dormant stage of microbes is retarded and an active metabolism can be maintained longer.

4 Conclusion

The chief aim of this thesis was to elucidate the fate of urban waste compost and BC amendments on distinct soil organic matter fractions to determine long-term SOC-sequestration potentials and effects on water holding capacity. Field and incubation experiments were used to evaluate aging processes and its effects to physical, chemical and biological properties.

The normalized urban waste and conventional compost amendments of study I provided detailed information about quantitative and qualitative effects on OM composition and stocks in bulk soil and physical fractions of a loamy agricultural soil under temperate climate. Amendments with BIO and GWS every other year over a 15-year period enhanced OC and N stocks in bulk soils, oPOM$_{small}$ and especially the fine mineral fraction in a magnitude similar to conventional FYM application. Municipal soil waste composts had no significant effect on OC and N stocks compared to the control.

The chemical composition of the amendments was reflected in the POM fractions but all urban waste compost amended soils showed a uniform composition of the mineral-associated OM. Decomposition and incorporation of the input materials by microorganisms transform the amendments and sequester them as microbial compounds. This is a possible cause for the similar clay OM composition in soil despite the different chemical composition of the amendments. The higher C storage in the clay fraction after BIO, GWS, and FYM amendments is likely related to better microbial C use efficiency. A high microbial uptake and lower mineralization are crucial to achieve efficient long-term C-sequestration. Despite the long-term amendment, all soils remained hydrophilic, indicating that higher or annual OM amendments are possibly further increasing OC-sequestration of this agricultural soil.

The field experiment with BC amendment resulted in two different central findings. Firstly, combining methods allowed validating the accuracy of BC detection by ^{13}C NMR spectroscopy. Secondly, substantial information was gained about the fate of BC in soil. With regard to stable isotope mass spectroscopy data, high concentrations of the condensed aromatic BC were quantified accurately, whereas low concentrations could not be detected. Raman microspectroscopic analysis underlined that surface aging of BC occurs. The bulk method, NMR spectroscopy, however, was unsuitable to detect these minimal changes of the nanostructure.

The BC was found in all the physical fractions, with highest concentrations in the fPOM and oPOM fractions. Analysis of the chemical structure of the BC, however, revealed no significant aging of the BC in the oPOM and clay fraction, indicating only physical fragmentation and protection from degradation in the soil structure. Consequently, the chemical composition of the OC in the fractions decreased from aryl C-dominated in the fPOM fraction to no-distinct BC-derived aryl C signal in the clay fraction. Furthermore, BC affects native SOM by stimulating the C-sequestration in associations with the fine-sized minerals. This stimulating effect needs to be studied in longer terms to evaluate the total C-sequestration potential of BC. After one year of field exposure, it can be concluded that loamy soils benefit from increasing coarse pore volume, which in turn improves the soil functionality. However, it remained open, if changes of native C stock in the clay fraction occur due to altered inputs to the clay fraction, or due to altered mineralization caused by aging of BC.

To answer these open questions from study II, we conducted an incubation experiment, focusing on the effects of BC *in-situ* aging on water availability and microorganisms in a loamy grassland soil. The 90 days incubation experiment with fresh and three years field aged BC revealed that the BC degradation stage had different effects on water conditions and microbial parameters under drought and optimal water conditions. Biochar significantly increased the volumetric water content in drought-affected soil, which increased upon BC aging. Moreover, the microbial activity under drought was improved compared to control- and BC_{fresh}-treatment. The amendment with BC led to significant increased native SOC mineralization, highest in soils containing aged BC. This effect increased in drought affected soils. The expected C flush after BC_{fresh} amendment did not affect the cumulative mineralized C, though a buffering effect of BC_{fresh} on the microbial biomass decline was found. Furthermore, only fresh BC-C, no aged BC-C, was incorporated into the microbial biomass. In drought-affected soils, only BC_{aged} addition significantly increased the qCO_2. By conducting the study, the assumption from study II was ultimately supported: The BC is incorporated into the microbial biomass and aging plays a crucial role for soil biological processes. Microbial usage of C further strongly depends on the water content of the soil, which can be improved by BC amendment. An amendment with BC therefore offers a suitable strategy for mitigating current and future water shortages on drought-affected soils with major positive effects on soil microorganisms.

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Appendices

Publication I

Lydia Paetsch, Carsten W. Mueller, Cornelia Rumpel, Sabine Houot, Ingrid Kögel-Knabner (2016): Urban waste composts enhance OC and N stocks after long-term amendment but do not alter organic matter composition of a loamy agricultural soil under temperate climate. Agriculture Ecosystems & Environment 223, 211 – 222, DOI:10.1016/j.agee.2016.03.008

Publication II

Lydia Paetsch, Carsten W. Mueller, Cornelia Rumpel, Šárka Angst, Alexandra C. Wiesheu, Cyril Girardin, Natalia P. Ivleva, Reinhard Niessner, Ingrid Kögel-Knabner (2017): A multi-technique approach to assess the fate of biochar in soil and to quantify its effect on soil organic matter composition. Organic Geochemistry 112, 177 – 186, DOI:10.1016/j.orggeochem.2017.06.012

Manuscript III

Lydia Paetsch, Carsten W. Mueller, Ingrid Kögel-Knabner, Margit von Lützow, Cyril Girardin, Cornelia Rumpel (submitted, under revision): Effect of in-situ aged and fresh biochar on soil water holding capacity and microbial C use under drought conditions. Nature Scientific Reports.

Eidesstattliche Erklärung

Curriculum vitae

Presentations

Publication I

The results of study I are summarized and published in the article:

Urban waste composts enhance OC and N stocks after long-term amendment but do not alter organic matter composition

by Lydia Paetsch, Carsten W. Mueller, Cornelia Rumpel, Sabine Houot, Ingrid Kögel-Knabner

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Urban waste composts enhance OC and N stocks after long-term amendment but do not alter organic matter composition

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ABSTRACT

Organic matter (OM) amendments, originating from waste materials, could be used to enhance soil organic carbon (SOC) storage and fertility of cropland soils. However, there is a limited understanding about the long-term effect of different urban waste compost amendments on soil organic matter (SOM) formation processes and their impact on SOC storage. Accordingly, the long-term effects of different OM amendments on the amount and composition of particulate and mineral associated SOM were investigated. Surface soils were sampled from a Luvisol under cropping rotation, which received biannually 0.4 kg organic carbon m^{-2} in form of three different urban composts or cattle manure for a period of 15 years.

Despite similar C input, different urban waste compost amendments resulted in contrasting C storage. While there were no C stock changes for municipal solid waste compost amended soils, composts from organic waste and green waste and sewage sludge increased SOC stocks in a similar range as conventional farmyard manure. In bulk soils, SOC stocks were increased by approximately 30%, in occluded particulate OM <20 μm by 155% (organic waste compost) and 71% (green waste and sewage sludge compost). Carbon storage in clay fractions showed approximately 20% higher values in all treatments. Organic matter amendments result in C–N coupling as for N proportional stock increases were recorded. The high variability of the composition of different amendment types was reflected in the particulate OM fractions but not in the highly uniform composition of the mineral-associated OM. Bulk soil wettability was not affected by the amendments, as only POM fractions showed increased hydrophobicity, but not clay fractions. Clay fractions showed high alkyl/O-alkyl and low C/N ratios, characteristic for microbial material. The OM composition in the clay fraction is determined by the microbial residues that are independent in their composition from the input material. The increased C storage in the clay fractions of the soil amended with organic wastes and green waste and sewage sludge compost and farmyard manure might be promoted by a better microbial use efficiency leading to C sequestration as microbial compounds.

1. Introduction

In recent years, there has been a strong interest in recycling of urban waste through composting and their use as soil amendment Hargreaves et al. (2008). With the development of more sophisticated separation techniques, these materials have been recognized as valuable resources due to their high fertilization and C sequestration potential (Hargreaves et al., 2008) as well as their beneficial impact on many soil functions (Zebarth et al., 1999; Celik et al., 2004). However, most studies on urban waste effects on soil focused on the evaluation of contamination risks (heavy metals and organic pollutants) (e.g. Hernando et al., 1989; Giusquiani et al., 1995; Murillo et al., 1997; Maftoun et al., 2005; Baldi et al., 2010) or fertilization potential (e.g. Iglesias Jiménez et al., 1993; Eriksen et al., 1999; Bar-Tal et al., 2004; Mkhabela and Warman, 2005). Less is known about their influence on soil organic matter (SOM) formation processes and in particular their long-term effect

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Table 1
Input and chemical characteristics of OM amendments with composting procedures and maturation times. The mean inputs are calculated by data from 1998 to 2013 and were implemented in two year intervals. CN = control, BIO = biowaste compost, MSW = municipal solid waste compost, GWS = green waste and sewage sludge compost, and FYM = farmyard manure.

Amendment	Composting procedure	Fermentation time	Maturing time	Mean input (kg m^{-2})	pH (–)	C content (g kg^{-1})	N content	C_{inorg}	C/N ratio (–)	Lignin content[a] (%) TOM	Alkyl C	O-N-alkyl C	Aryl C	Carboxyl C	Alkyl-C/O-N-alkyl C (–)	H index
											Proportion (%)					
BIO	SILODA® – aerobic fermentation accelerated by ventilation through turning	4–12 weeks	8–24 weeks	2.8	7.8 ± 0.1	210.5 ± 47.3	17.42 ± 4.45	0.86	12.0	31.1 ± 8.9	18.0	57.5	17.7	6.9	0.31	0.46
MSW	SILODA® – aerobic fermentation accelerated by ventilation through turning	3–4 weeks	5 days	1.8	7.9 ± 0.1	298.1 ± 18.5	19.10 ± 2.30	2.51	19.6	15.2 ± 2.4	14.7	69.8	10.6	4.9	0.21	0.29
GWS	Covert pile outside	4–12 weeks	4–16 weeks	2.6	7.7 ± 0.1	264.6 ± 44.7	23.35 ± 2.71	1.10	10.2	28.5 ± 11.9	17.4	56.3	18.4	7.8	0.31	0.44
FYM	–	–	–	3.5	8.8 ± 0.3	308.8 ± 91.1	19.73 ± 2.97	1.30	17.2	23.4 ± 2.5	11.7	65.2	16.2	6.8	0.18	0.31

[a] Lignin and cutin-like fractions as determined using the Van Soest fractionation method in % of total OM (% TOM) from Peltre et al. (2012).

on SOM quality and ecological soil functions such as the improvement of the soil structure.

Studies on the effect of organic matter amendments need long-term field experiments with permanent plots that allow to quantify soil change across scales of decades (Richter et al., 2007). Such studies have shown that repeated conventional OM amendments have positive effects on OC contents and stocks after several decades (e.g. Gerzabek et al., 2001; Menichetti et al., 2015). Soil organic carbon (SOC) increased mainly due to an increase of OC associated to soil minerals (Gong et al., 2009), even though the increases of OC concentrations in this fraction were much lower than in the particulate organic matter (POM) fractions (Sleutel et al., 2006). Also, after single urban waste compost amendments increasing bulk SOC concentrations were reported (Giusquiani et al., 1995; Weber et al., 2007). A first study gaining a deeper understanding of OC sequestration after different urban waste compost amendments in a long-term field experiment was carried out by Peltre et al. (2012). The authors showed increases of SOC stocks for all studied amendments (between 0.86 and 1.63 kg m^{-2}), with contrasting ability to increase C stocks. As equal amounts of C were added in all treatments, the differences in C stocks might be explained by contrasting quality of urban waste composts (Peltre et al., 2012).

With respect to OM quality, the chemical composition of SOM after long-term animal manure or compost amendments was found to be altered in bulk soils, micro- and macroaggregates and in the fine fraction (Mao et al., 2008; Zhou et al., 2010; Yu et al., 2015). The amendments increased intensities of alkyl C and decreased aromatic C and carboxyl C in the POM fractions (Mao et al., 2008; Zhou et al., 2010). Moreover, the mineral fraction was characterized by a decrease of alkyl C, O alkyl C and aryl C and an increase of phenol and carboxyl C (Wang et al., 2012; Yu et al., 2015). These changes indicate that the long-term organic amendments may have specific effects on SOM composition and formation processes. Urban waste composts are industrially produced from different types of feedstocks: green wastes, sewage sludge as well as municipal house hold wastes. These composts may thus be distinct in their effects on soil microbial activity and SOM formation as compared to conventional OM amendments. However, their long-term effects on the composition of different SOM pools is still poorly understood.

In terms of SOM chemical composition, OM amendments can lead to enrichment of aromatic and/or aliphatic compounds (Castaldi et al., 2005; Carvalho et al., 2009) or increased methoxyl substituents originating from lignin (Lal, 2014). Hydrophobicity reduces surface wettability and thus the accessibility of OM for microorganisms (Lützow et al., 2006), resulting in decreased decomposition rates of such amendments associated with a relative accumulation of POM residues. Fine particles (<63 mm) exhibit the greatest degree of water repellency, which can partly be explained by the greater OM content in these fractions (de Jonge et al., 1999). Whereas pure clays show hydrophilic behaviour, OM associated with clay minerals increases their hydrophobicity (Chenu et al., 2000). This may increase hydrophobicity (Diacono and Montemurro, 2010), and thus possibly lead to increased aggregate stability (Annabi et al., 2011; Lal, 2014; Yu et al., 2015) in soils receiving such amendments.

The aim of our study was to investigate SOM formation processes after equal C inputs with contrasting urban waste compost amendments by studying the nature of SOM in different soil physical fractions. We followed the degradation path from free OM to mineral protected fractions for cropland soil amended with different urban waste composts isolated by a combined density and particle size fractionation protocol separating OM with different degradation stages (Aoyama et al., 1999; Wang et al., 2012). We used samples from a 15 year ongoing field experiment that was designed to examine the influence and differences of a conventional OM amendment with farmyard manure (FYM) and three industrially produced urban waste composts on loamy cropland soils compared to a control without OM amendments. The amount of OM amendment was normalized to obtain equal C inputs. We hypothesized that long-term amendment with these contrasting materials would have specific effects on SOM composition and thus might lead to increased hydrophobicity of the OM. In particular, we expected effects on the composition of the POM fraction, such as a relative accumulation of hydrophobic components resistant to decomposition as well as in the mineral-associated SOM. We analyzed the elemental and chemical composition of bulk soils and their wettability as well as chemical composition of OM and hydrophobicity in physical fractions deduced from solid-state ^{13}C NMR spectroscopy.

2. Materials and methods

2.1. Study site

The long-term field experiment “QualiAgro” (http://www6.inra.fr/qualiagro) is managed by INRA (National Institute of Agricultural Research) and located in Feucherolles (48°87′27″N, 1°97′37″E) near Paris, France. Mean annual rainfall is 695.5 mm and the mean annual temperature is 11 °C. The soil type is hortic glossic Luvisol (IUSS Working Group WRB, 2014). The initial soil OC content was 10 g kg^{-1} in 1998, at the start of the experiment (Houot et al., 2002). The completely randomized block experiment comprised an area of 6 ha with 40 plots. The plot size is 450 m^2. The plots were separated by 6 m wide strips to avoid any cross contamination. The soils were amended biannually with 4 organic materials (3 urban wastes composts and 1 cattle manure). Organic matter additions were based on similar amounts of OC (0.4 kg OC m^{-2}). Additionally, all plots received 140 ± 37 kg ha^{-1} or 82 ± 34 kg ha^{-1} of conventional N fertilizer (1:1 urea and ammo-nitrate) for wheat and maize, respectively, in spring. During the experiment, the site was cropped conventionally with maize and wheat. According to Peltre et al. (2012), an additional OC input of 0.39 kg m^{-2} to 0.42 kg m^{-2} per year originated from crop residues and roots.

The three composts were residual municipal solid wastes after separate collection of packaging (MSW), biowastes issued form a separate collection and co-composted with green wastes (BIO) and green wastes and sewage sludge mixture (GWS). Cattle manure (FYM) was obtained from a nearby farm. All initial mixtures of the three composts contained green wastes in different proportions: 40%, 33% and 17% for GWS, BIO and MSW, respectively. MSW contained additionally 25% kitchen wastes and 21% paper and cardboard, BIO contained 66% kitchen wastes, and GWS contained 20% sewage sludge and 20% wood chips. Inorganic components were also determined in the initial mixture of MSW including glass (12%), plastics (12%) and other components (8%) (values as % of fresh weight) (Annabi et al., 2007; ADEME, 2015). For each compost type, the composts came from 3 to 4 plants with similar process. Average composting procedures have been described by Annabi et al. (2007). Briefly, all composts underwent accelerated fermentation through enforced aeration then maturation. General parameters of the OM amendments like fermentation and maturation times are given in Table 1.

2.2. Sampling and sample pre-treatment

Soil samples were collected in September 2013, two years after the last amendment. Ten soil samples were randomly taken from the top 28 cm of the soil profile with a shovel and composited together to form a representative sample from each plot. In total, 20 plots were sampled which included 4 field replicates of the 4 soils which received biannually OM amendments and the control soil. Additionally, the OM amendments were sampled in 2013. Samples were stored in plastic bags and transported to the laboratory. Bulk soils were air dried and sieved <2 mm (inorganic material >2 mm (glass, stones) accounted for less than 1% of the dry mass, organic for less than 0.05% of the dry mass (data not shown)).

2.3. Physical fractionation

A combined density and particle-size fractionation protocol was used to separate SOM into particulate OM (POM) and mineral associated OM from bulk soils as well as from the amendments, sampled in 2013 (Amelung and Zech, 1999; Schmidt et al., 1999; Mueller and Kögel-Knabner, 2009). Briefly, 20 g of bulk soil were capillary-saturated with 150 ml sodium polytungstate solution (SPT) (TC Tungsten compounds, Grub am Forst, Germany) with a density of 1.8 g cm^{-3}. After settling overnight the floating free POM (fPOM) was siphoned from the surface using a vacuum pump. The remaining sample was dispersed ultrasonically (Bandelin, Sonopuls HD 2200, Berlin, Germany) with an energy input of 400 J ml^{-1} to release occluded POM (oPOM). Thereafter, the samples were centrifuged (~3700 × g for 30 min) and the floating material (occluded POM) was siphoned and sieved to separate POM <20 μm (oPOM$_{\text{small}}$) and >20 μm (oPOM). To remove SPT, fPOM and occluded POMs were rinsed with de-ionized water using a pressure filtration device until the conductivity was below 1.5 μS cm^{-1}. The mineral residue was also rinsed to remove SPT (conductivity < 50 μS) with de-ionized water using several centrifugation (~4000 × g for 30 min) — decantation steps. Thereafter, the sand fraction (63 μm to 2000 μm) and the coarse silt fraction (20 μm to 63 μm) were fractionated by wet sieving. The medium silt fraction (>6.3 μm) and the combined fine silt and clay fraction (<6.3 μm) were separated with settling columns. This fine fraction (<6.3 μm) is in the following called clay fraction. All fractions were recovered after freeze drying. The particle-size and density fractionation resulted in recovery rates of 95 ± 1% of the initial sample mass and 87 ± 6% recovery of C. For the input materials the

Table 2
Mean bulk density, pH values in 0.01 M $CaCl_2$ solution and texture of samples ($n = 4$). Values within a column with different letters are significantly different (Tukey-HSD, $p < 0.05$). Wettability was measured by water drop penetration time (WDPT) test. CN = control, BIO = biowaste compost, MSW = municipal solid waste compost, GWS = green waste and sewage sludge compost, and FYM = farmyard manure amended soils.

Plot	C content (g kg^{-1})	N content	Bulk density (g cm^{-3})	pH $CaCl_2$ (–)	Wettability (s)	Sand (g kg^{-1})	Silt	Clay
CN	11.28^a ± 2.49	1.20^a ± 0.27	1.41^a ± 0.03	6.0 ± 0.1	<5	34.1 ± 1.0	650.5 ± 20.3	293.3 ± 18.9
BIO	13.25^b ± 3.71	1.57^b ± 0.14	1.30^b ± 0.04	7.0 ± 0.0	<5	41.7 ± 2.5	643.5 ± 14.5	303.3 ± 16.9
MSW	12.16^a ± 0.82	1.28^{ab} ± 0.09	1.31^b ± 0.06	6.9 ± 0.1	<5	37.7 ± 3.0	674.0 ± 73.9	261.8 ± 80.8
GWS	14.88^b ± 0.82	1.56^b ± 0.08	1.27^b ± 0.03	6.3 ± 0.1	<5	39.2 ± 2.8	635.5 ± 38.4	300.0 ± 12.5
FYM	14.18^b ± 2.00	1.46^{bc} ± 0.18	1.31^b ± 0.03	6.6 ± 0.1	<5	34.7 ± 0.8	626.3 ± 21.5	309.0 ± 14.8

same procedure was applied. The fPOM fraction obtained from these materials was additionally washed over a 20 μm sieve, which removed fine mineral components.

Before NMR analysis, the clay fractions were treated with 10% hydrofluoric acid (HF) to concentrate the SOM and to enhance signal-to-noise ratio of the solid state ^{13}C NMR spectra (Schmidt et al., 1997). Briefly, this procedure included 5 additions of HF, shaking during 2 h and centrifugation and decantation of the supernatant. Afterwards, the samples were rinsed with de-ionized water until the pH was buffered to pH 4. The final clay fraction was freeze dried. Carbon recoveries after HF treatment ranged between 82% (amended FYM soils) to 86% (amended BIO soils).

2.4. Water drop penetration time test

The wettability of bulk soils (air dried and sieved <2 mm) was determined by using the water drop penetration time test (WDPT test) (Letey, 1969). Briefly, five drops of de-ionized water (20 °C) were applied to the soil surface using a glass pipette. Infiltration times of the water droplets were measured. An infiltration time <5 s classifies the sample as hydrophilic (Adams et al., 1969; McGhie and Posner, 1981; Ma'shum and Farmer, 1985). The longer the infiltration time the more pronounced is the hydrophobicity. Samples with infiltration times >60 s were described as hydrophobic (Adams et al., 1969; Roberts and Carbon, 1971; Bisdom et al., 1993).

2.5. Chemical analysis

The pH values of the bulk soils were measured in 0.01 M $CaCl_2$ solution (1:2.5 w:vol) (Table 2). The C and N concentrations of bulk soils and OM amendments were measured in duplicate by dry combustion with Vario EL elemental analyzer (Elementar Analysensysteme, Hanau, Germany). The C and N concentrations of the physical fractions and HF treated clay fractions were measured by a similar procedure (EA3000 elemental analyzer; Hekatech, Wegberg, Germany). We consider that total C concentration represent OC because we did not find any evidence of inorganic C contribution to our soils using acid treatment. However, with every OM amendment approx. 0.016 kg m^{-2} (BIO), 0.017 kg m^{-2} (GWS), 0.018 kg m^{-2} (MSW) 0.021 kg m^{-2} (FYM) C_{inorg} were added (C and N contents of ash produced by 6 h heating at 550 °C in a muffle furnace) (Table 1).

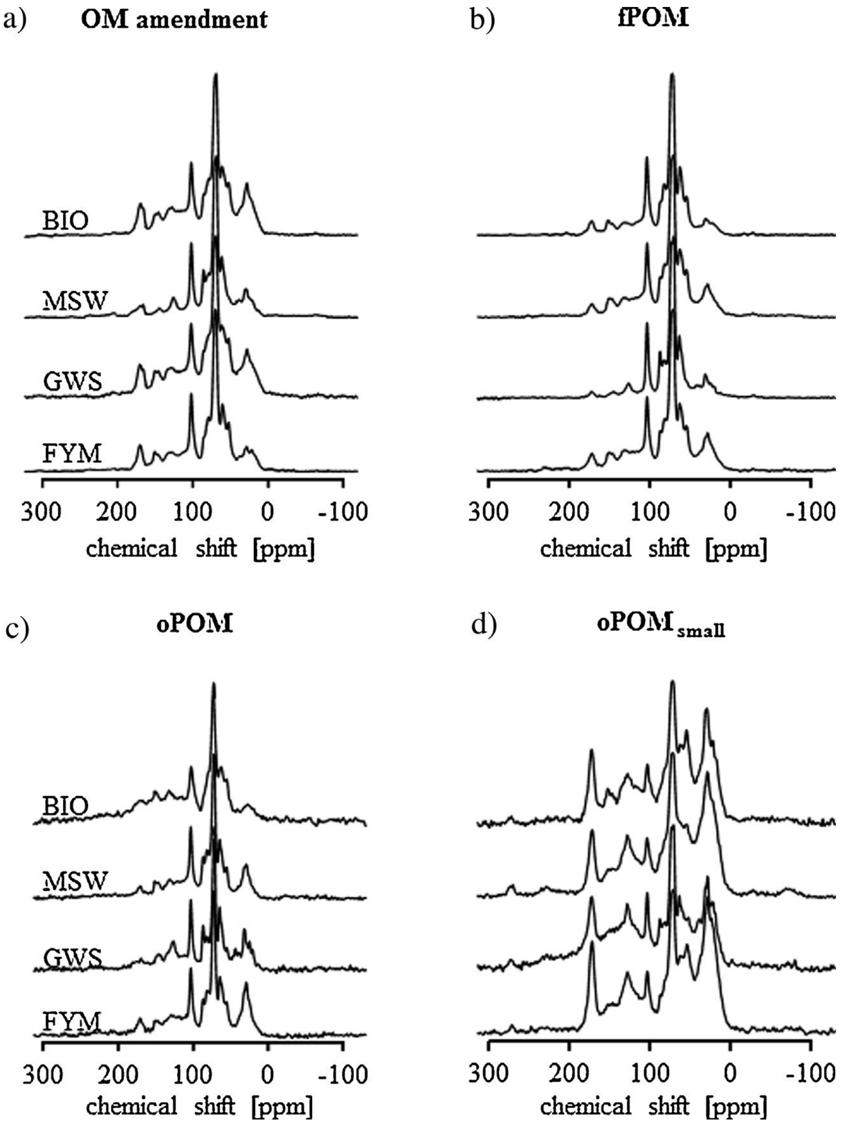

Fig. 1. Solid-state ^{13}C NMR spectra of OM amendments (a) and their fractions (b–d). BIO = biowaste compost, MSW = municipal solid waste compost, GWS = green waste and sewage sludge compost, and FYM = farmyard manure. Occluded POM = POM > 20 μm, oPOM$_{small}$ = POM < 20 μm.

2.6. Chemical composition

The solid-state ^{13}C NMR spectra of the OM amendments, fPOM, oPOM and oPOM$_{small}$ as well as HF treated clay fractions were obtained on a Bruker AvanceIII 200 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). Cross-polarization magic angle spinning (CPMAS) was applied with a ^{13}C-resonance frequency of 50.32 MHz and a spinning speed of 5 kHz. Contact time was 1 ms and recycle delay was 0.4 s for clay and 1 s for the POM fractions. Depending on the C content of the samples, between 2000 and 250,000 scans were accumulated and a line broadening between 0 and 50 Hz was applied. Spectra were integrated by using the integration routine of the spectrometer. Integrated chemical shift regions were 0–45, 45–110, 110–140, 140–160 and 160–220 ppm and were assigned to alkyl-C, O/N-alkyl-C, aromatic (aryl) C, phenolic (O-aryl) C and carboxyl-C (aldehyde and ketone), respectively (Wilson, 1987). Signals derived from lignin are assigned to 56, 72, 128, 150 to 155 ppm. Signals attributed to polysaccharides are at 62 ppm and the shoulder at 82 ppm. Typically for lipids and proteins are signals at 21 and 32 ppm and 56 ppm, respectively (Wilson, 1987; Kögel-Knabner, 2002). For calibration of the ^{13}C chemical shifts tetramethylsilane was used and set to 0 ppm.

2.7. Calculations and statistics

Total SOC and N stocks [kg m^{-2}] of the control soil were calculated by a multiplication of SOC content with bulk density (data provided by Qualiagro) and were normalized to 10 cm depth (factor 10^{-3}) (reference soil mass). Decreased bulk soil densities were considered for SOC stock calculation using 'equivalent soil mass approach' (Ellert and Bettany, 1995; Kätterer et al., 2011) which normalizes the soil volume change due to OM increase. Accordingly, the SOC and N stocks of the physical fractions were normalized to the reference soil mass.

A hydrophobicity index (H) of OM using NMR data was calculated according to Kögel-Knabner (1997) by dividing aromatic and alkyl C by the sum of O-N-alky and carboxyl C. For statistical analyses we used R Studio Version 0.98.1062 for windows (R Core Team, 2013). Significant differences between the different OM amendments were tested with a one-way analysis of variance (ANOVA). The Tukey test was performed post-hoc and all significance levels were $p < 0.05$.

3. Results

3.1. Chemical and physical characteristics of OM amendments and bulk soils

Information about production procedure and physicochemical parameters of OM amendments from 2013 are given in Table 1. Carbon contents of the OM amendments ranged from 210.5 g kg^{-1} (BIO) to 308.8 g kg^{-1} (FYM) and N content ranged from 17.4 g kg^{-1} (BIO) to 23.4 g kg^{-1} (GWS). C/N ratio ranged between 10.2 for GWS and 19.6 for MSW. The lowest pH value was recorded for GWS and the highest for FYM with 7.7 and 8.8, respectively (Table 1).

The functional group distribution as shown by ^{13}C NMR spectra of the OM amendments and their POM fractions are very similar (Fig. 1a–d). However, MSW compost and FYM had lower contribution of alkyl C, aryl C and carboxyl C and thus higher intensities in the O/N-alkyl region compared to BIO and GWS. All substrates had low alkyl-C/O-N-alky-C ratios between 0.2 (FYM, MSW) and 0.3 (BIO, GWS).

The chemical and physical characteristics of bulk soils are presented in Table 2. The bulk soil density significantly decreased for all OM amended soils in comparison to the control. Moreover, increased pH values were noted for amended soils. Carbon and N contents of the bulk soils were lowest for the control with 11.3 g C kg^{-1} and 1.2 g N kg^{-1}. The long-term OM amendments increased the C and N contents of bulk soils up to 14.9 g C kg^{-1} and 1.6 g N kg^{-1} (GWS and BIO). The C/N ratios ranged between 8.3 for the control to 9.7 for FYM amended soil (Fig. 2). Due to the OM amendments, SOC and N stocks significantly increased compared to the control (1.59 kg OC m^{-2}, 0.17 kg N m^{-2}) (Fig. 3a and b) and ranged from 1.71 kg OC m^{-2} (MSW) to 2.11 kg OC m^{-2} (BIO) and from 0.18 kg N m^{-2} (MSW) to 0.23 kg N m^{-2} (BIO).

The bulk soils of all OM amended samples and the control samples showed no difference in hydrophobicity as indicated by water drop penetration tests. The water droplets infiltrated in less that <5 s and therefore all samples were classified as hydrophilic.

3.2. Chemical and physical characteristics of the POM fractions

The mass proportion of the physical fractions after density and particle size fractionation is presented in the supplementary material Fig. A1. Carbon and N contents increased for the POM fractions in the order fPOM < oPOM$_{small}$ < oPOM and ranged on average between 150.7 g C kg^{-1} and 452.0 g C kg^{-1} (Table 3). The control had the lowest C contents compared to the amended soils

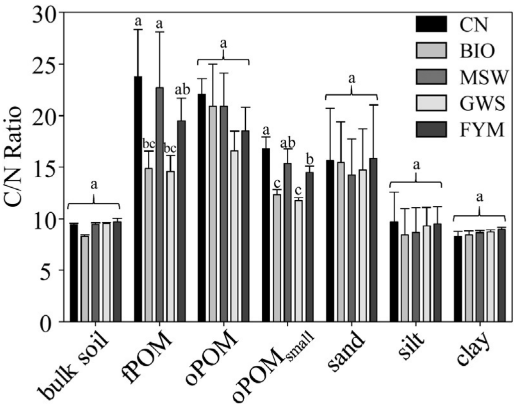

Fig. 2. C/N ratio of bulk soils and fractions (n = 4). Grouped bars with different letters are significantly different (Tukey-HSD, $p < 0.05$). CN = control, BIO = biowaste compost additions, MSW = municipal solid waste compost additions, GWS = green waste and sewage sludge compost additions, and FYM = farmyard manure additions.

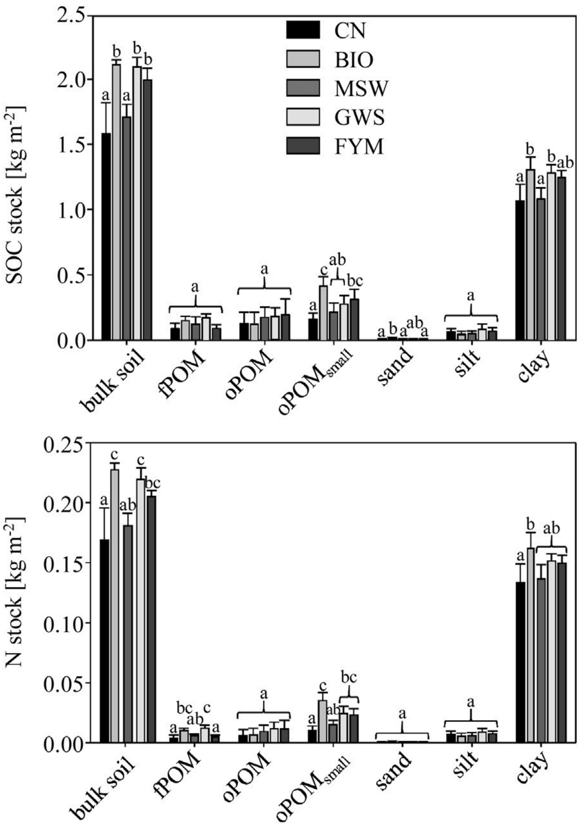

Fig. 3. Soil organic carbon stocks (a) and total nitrogen stocks (b) of bulk soils and physical fractions ($n = 4$). Grouped bars with different letters are significantly different (Tukey-HSD, $p < 0.05$). CN = control, BIO = biowaste compost additions, MSW = municipal solid waste compost additions, GWS = green waste and sewage sludge compost additions, and FYM = farmyard manure additions.

in the fPOM and oPOM fraction (137.2 g kg^{-1} and 435.7 g kg^{-1}) but in the $oPOM_{small}$ fraction C contents were similar to BIO and MSW (316.62 g kg^{-1}, 317.41 g kg^{-1}, 318.14 g kg^{-1}, respectively). The $oPOM_{small}$ fractions isolated from FYM and GWS amended soils had the lowest C contents (295.91 g kg^{-1}, 280.31 kg^{-1}). The highest C contents of the fPOM were recorded for MSW amended soils, which also had the highest standard deviations (177.55 g kg^{-1} ± 61.18). For the oPOM fraction, BIO amended soils showed the highest C contents (481.78 g kg^{-1}).

In all POM fractions, N contents were lowest for the control (fPOM: 4.24 g kg^{-1}, oPOM: 18.63 g kg^{-1}, $oPOM_{small}$: 19.45 g kg^{-1}). The POM fractions of OM amended soils had increased N contents in the fPOM and oPOM, highest for GWS (14.82 g kg^{-1} and 25.36 g kg^{-1}, respectively) and in the $oPOM_{small}$ highest for BIO (25.21 g kg^{-1}). C/N ratios of the control were 23.8 (fPOM), 22.1 (oPOM) and 16.8 ($oPOM_{small}$), while the C/N ratio of POM fractions of OM amended soils ranged from 14.6 (GWS) to 22.7 (MSW) in the fPOM, 16.6 (GWS) to 20.9 (BIO and MSW) in the oPOM and 11.7 (GWS) to 15.4 (MSW) in the $oPOM_{small}$ fraction (Fig. 2).

Soil organic C stocks in the fPOM ranged from 0.09 kg m^{-2} (FYM and control) to 0.17 kg m^{-2} (GWS) and in the oPOM between 0.12 kg m^{-2} (BIO) and 0.19 kg m^{-2} (FYM) (Fig. 3a). SOC stocks of the $oPOM_{small}$ were significantly higher for soil amended with BIO (0.41 kg m^{-2}) and FYM (0.31 kg m^{-2}) compared to the control (0.16 kg m^{-2}). Total N stocks of the fPOM ranged from 0.004 kg m^{-2} (control) to 0.012 kg m^{-2} (GWS) and from 0.006 kg m^{-2} (control) to 0.012 kg m^{-2} (FYM and GWS) in the oPOM (Fig. 3b). Total N stocks in the $oPOM_{small}$ fraction significantly increased for soils amended with BIO (0.035 kg m^{-2}), GWS (0.024 kg m^{-2}) and FYM (0.023 kg m^{-2}) compared to the control (0.010 kg m^{-2}) (Fig. 3b).

Within physical fractions, POM fractions comprised between 0.86% (control) up to 1.55% (GWS) of the total soil mass (Fig. A1) but due to their high C content, these fractions accounted for about 30% of total soil C and about 20% of total soil N (Fig. 4a and b). As 87–92% of C was comprised in the POM and clay fractions (Fig. 4a), ^{13}C NMR analysis were only performed on these fractions. In Fig. 5 the ^{13}C NMR spectra of the physical fractions of the OM amended soils and the control soils are shown. Quantitative information on the contribution of the different chemical shift regions to the ^{13}C NMR spectra are presented in Table 3. All spectra of the physical fractions were dominated by O/N-alkyl C centered at 72 ppm (range from 44% to 56%) which is commonly assigned to polysaccharides (Kögel-Knabner, 2002).

Spectra of free POM fractions showed clear signals attributable to lignin and polysaccharides (Fig. 5). The fPOM fraction of soils subject to long-term MSW amendments showed a distinctive aromatic peak at 128 ppm, whereas fPOM fractions isolated from soil under all other treatments did not show an increased aromatic signal at this particular region. The peaks assigned to lipids at 30 ppm had a lower contribution in the oPOM fraction of MSW and FYM amended soils, as well as the control soils compared to BIO and GWS amended soils. In the fPOM fraction, there was a clear trend towards higher intensities in the alkyl C region for all OM amended soils (15.4% ± 2.9 to 17.8% ± 0.6) compared to the control (10.6% ± 7.8). O-N-alkyl C ranged between 47.7% ± 3.7 (BIO) and 55.6% ± 5.6 (control).

Aryl and carboxyl C intensities of the fPOM fractions were lowest for FYM (20.5% ± 3.6 and 7.9% ± 0.8) (Table 3). The highest aryl C intensities showed MSW amended soil (25.2% ± 7.7) and the highest carboxyl C intensities were recorded for BIO amended soil

Table 3
Chemical composition of the physical fractions and relative contents of alkyl C, O/N-alkyl C, aryl C, carboxyl C and alkyl/O/N-alkyl C ratio of the fractions. Values within a column with different letters are significantly different (Tukey-HSD, $p < 0.05$). CN = control, BIO = biowaste compost, MSW = municipal solid waste compost, GWS = green waste and sewage sludge compost and FYM = farmyard manure amended soils. Occluded POM = POM > 20 μm, oPOM$_{small}$ = POM < 20 μm and *H* is the hydrophobicity index. Not analyzed samples are marked as N.A.

Soil	Sample	C content (g kg^{-1})	N content	Alkyl C Proportion (%)	O-alkyl C	Aryl C	Carboxyl C	Alkyl-C/ O-N-alkyl C (–)	H index
CN	fPOM	$137.20^{a} \pm 12.18$	$5.96^{a} \pm 1.41$	$10.6^{a} \pm 7.8$	$55.6^{a} \pm 5.6$	$23.7^{a} \pm 5.7$	$9.1^{a} \pm 2.2$	$0.19^{a} \pm 0.14$	0.40 ± 0.14
	oPOM	$435.68^{a} \pm 37.22$	$19.74^{ab} \pm 1.23$	$9.0^{a} \pm 3.2$	$46.8^{a} \pm 8.1$	$24.2^{a} \pm 8.3$	$11.2^{a} \pm 5.4$	$0.19^{a} \pm 0.07$	0.46 ± 0.04
	oPOM$_{small}$	$316.62^{a} \pm 23.27$	$19.00^{a} \pm 2.60$	$16.3^{a} \pm 2.8$	$46.5^{a} \pm 1.6$	$25.6^{a} \pm 2.2$	$10.6^{a} \pm 1.7$	$0.35^{a} \pm 0.05$	0.63 ± 0.06
	Sand	$4.35^{a} \pm 3.22$	$0.33^{a} \pm 0.27$	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	Silt	$1.00^{a} \pm 0.47$	$0.10^{a} \pm 0.04$	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	Clay	$36.08^{a} \pm 3.51$	$4.36^{a} \pm 0.56$	$20.7^{a} \pm 1.5$	$49.7^{a} \pm 0.3$	$17.6^{a} \pm 0.5$	$11.5^{a} \pm 0.9$	$0.42^{a} \pm 0.03$	0.55 ± 0.02
BIO	fPOM	$151.95^{a} \pm 36.09$	$10.44^{ab} \pm 2.87$	$17.8^{a} \pm 0.6$	$47.7^{a} \pm 3.7$	$23.6^{a} \pm 1.8$	$10.2^{a} \pm 5.2$	$0.37^{a} \pm 0.04$	0.60 ± 0.05
	oPOM	$481.78^{a} \pm 30.63$	$23.38^{bc} \pm 2.67$	$17.4^{b} \pm 2.5$	$45.5^{a} \pm 1.3$	$27.1^{a} \pm 1.2$	$8.8^{a} \pm 2.0$	$0.38^{b} \pm 0.05$	0.71 ± 0.08
	oPOM$_{small}$	$317.41^{a} \pm 6.63$	$25.73^{b} \pm 0.68$	$18.8^{a} \pm 0.7$	$44.6^{a} \pm 2.1$	$25.3^{a} \pm 0.8$	$10.6^{a} \pm 1.8$	$0.42^{a} \pm 0.02$	0.68 ± 0.01
	Sand	$6.88^{a} \pm 5.07$	$0.49^{a} \pm 0.38$	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	Silt	$0.97^{a} \pm 0.52$	$0.10^{a} \pm 0.05$	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	Clay	$46.53^{b} \pm 3.33$	$5.50^{b} \pm 0.35$	$23.7^{b} \pm 1.6$	$46.4^{b} \pm 0.7$	$18.5^{ab} \pm 0.9$	$11.1^{a} \pm 1.3$	$0.51^{b} \pm 0.03$	0.64 ± 0.03
MSW	fPOM	$177.55^{a} \pm 61.18$	$7.65^{ab} \pm 1.77$	$16.2^{a} \pm 3.7$	$49.2^{a} \pm 6.2$	$25.2^{a} \pm 7.7$	$8.1^{a} \pm 1.6$	$0.33^{a} \pm 0.03$	0.65 ± 0.12
	oPOM	$446.13^{a} \pm 44.55$	$21.61^{ab} \pm 3.12$	$14.5^{ab} \pm 1.2$	$46.8^{a} \pm 4.6$	$26.7^{a} \pm 1.9$	$10.9^{a} \pm 3.5$	$0.31^{ab} \pm 0.00$	0.60 ± 0.01
	oPOM$_{small}$	$318.14^{a} \pm 12.87$	$20.85^{ab} \pm 2.54$	$17.5^{a} \pm 1.6$	$45.6^{a} \pm 3.8$	$25.9^{a} \pm 2.2$	$10.1^{a} \pm 1.5$	$0.39^{a} \pm 0.05$	0.68 ± 0.06
	Sand	$4.98^{a} \pm 3.71$	$0.39^{a} \pm 0.28$	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	Silt	$0.85^{a} \pm 0.44$	$0.10^{a} \pm 0.04$	N.A.	N.A.	N.A.	N.A.	N.A.	N.A.
	Clay	$42.71^{ab} \pm 3.60$	$4.93^{ab} \pm 0.19$	$22.0^{ab} \pm 0.7$	$48.3^{ab} \pm 0.3$	$18.1^{ab} \pm 0.8$	$11.2^{a} \pm 0.3$	$0.46^{ab} \pm 0.01$	0.40 ± 0.14

(10.2% ± 5.2). Alkyl C to O-N-alkyl C ratio ranged between 0.19 (control) and 0.37 (BIO).

Minor differences of contribution of signal intensities were found for fPOM and oPOM. The control and FYM amended soils showed the strongest changes between fPOM and oPOM. Alkyl C was lower for oPOM as compared to fPOM and ranged between 9.0% ± 3.2 (control) and 17.4% ± 2.5 (BIO). O-N-alkyl C and carboxyl C contributions to oPOM fraction were also lower and ranged between 45.5% ± 1.3 (BIO) and 48.3% ± 0.8 (FYM) and 8.8% ± 2.0 (BIO) and 11.2% ± 5.4 (control). Aryl C increased by up to 26% to intensities between 24.2% ± 8.3 (control) and 27.1% ± 1.2 (BIO) compared to the control.

Compared to the oPOM fractions, oPOM$_{small}$ had slightly higher alkyl C intensities. Greatest alkyl C contribution was noted for oPOM$_{small}$ isolated from the control (9.0% ± 3.2 to 16.3% ± 3.2), mainly due to a dominant peak at 55 ppm, which may be attributed to lignin or proteins (Spaccini and Piccolo, 2007). No differences in chemical composition were evident for oPOM$_{small}$ isolated from soils treated by different organic amendments.

3.3. Chemical and physical characteristics of the mineral fractions

Carbon and N contents of the clay fraction significantly increased for GWS, FYM and BIO amendments compared to the control (48.6 g kg^{-1}, 47.5 g kg^{-1}, 46.5 g kg^{-1} and 36.1 g kg^{-1}, respectively) (Table 3). The C/N ratio of the clay fraction showed low variation with values between 8.3 (control) and 9.0 (FYM).

Most soil C was stored in the clay fraction (between 61% for GWS and 68% for the control). N stocks of the clay fractions were lowest for the control (0.13 kg m^{-2}) and highest for BIO (0.16 kg m^{-2}) (Fig. 3).

The HF treated clay fractions showed broad alkyl C signals ranging between 20.7% ± 1.5 (control and FYM) to 23.7% ± 1.6 (BIO) of the total signal intensity and an enrichment of carboxyl C (11.3% ± 0.9) (Fig. 5). However, main characteristic of the NMR spectra of the clay fraction is their similarity. For clay fractions isolated from all soils, differences within the chemical regions were at most 3%. There are significant differences in the aryl C region of NMR spectra recorded for the clay fraction isolated from the control and FYM amended soils, though they are in range with uncertainties of the NMR.

Sand and silt had low C contents ranging between 5.35 g C kg^{-1} (control) and 7.84 g C kg^{-1} (BIO) and between 0.43 g N kg^{-1} (control) and 0.59 g N kg^{-1} (MSW). C/N ratio of sand fractions was on average 15.3 and C/N of the silt fractions was 9.3 with low variations between those isolated from amended soils and the control. SOC and N stocks for the sand and silt fractions together ranged between 0.06 kg C m^{-2} (MSW and BIO) to 0.09 kg C m^{-2} (GWS) and between 0.007 kg N m^{-2} (BIO and MSW) and 0.01 kg N m^{-2} (GWS).

4. Discussion

4.1. Organic C and total N contents, stocks and distribution within SOM fractions

All soils in the QualiAgro experiment received an annual OC input from crop residues of approx. 0.4 kg m^{-2} y^{-1} (Peltre et al., 2012) in addition to biannual application of different organic amendments (0.4 kg m^{-2}). After 15 years, we found significant increases in OC and N contents for bulk soils (Table 2) as well as for physical fractions compared to the control soil, except for MSW. Increases of bulk soil OC contents were low for MSW (8%) but remarkably high for soils treated with the other OM amendments: BIO (19%), GWS (32%) and FYM (26%) (Table 2). These results are in a similar range as those reported two and three years after urban waste compost amendments by Weber et al. (2007) (MSW increases SOC content between 8 and 13%) and Giusquiani et al. (1995) (26% OC content increase but with 3 kg m^{-2}), respectively. Generally, long-term amendments with conventional composts or manure increased OC contents of bulk soils in a similar range (Kirchmann et al., 1994; Diacono and Montemurro, 2010; Yu et al., 2015) as the urban composts used in our study.

Regarding SOC stocks, our results indicated significantly higher SOC stock values for soils amended with BIO (33%), GWS (32%) and FYM (26%), whereas no significant difference was noted for soils amended with MSW compared to the control (Fig. 3a). Total N was similar distributed within the physical fractions than SOC (Fig. 3b).

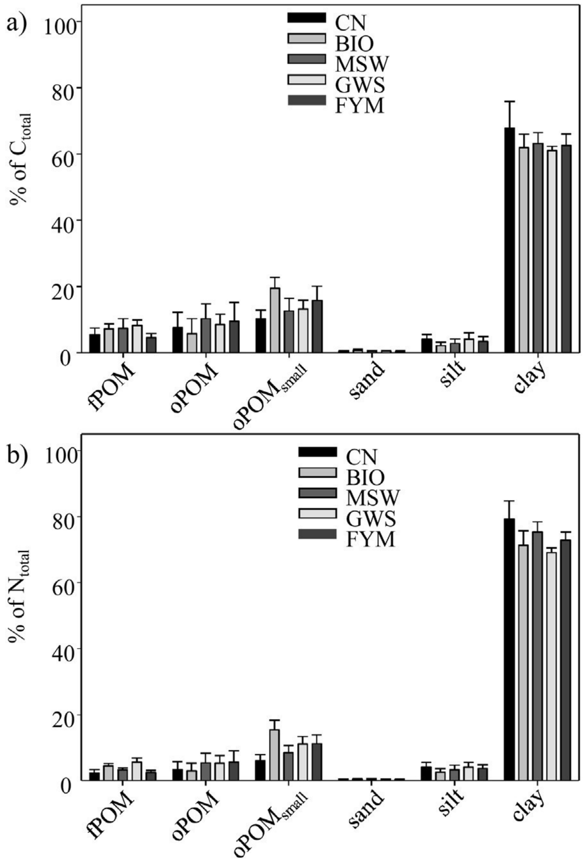

Fig. 4. Mean proportion of C (a) and N (b) with standard deviations in the physical fractions ($n = 4$). CN = control, BIO = biowaste compost additions, MSW = municipal solid waste compost additions, GWS = green waste and sewage sludge compost additions, and FYM = farmyard manure additions.

Consequently, the amendments had no effect on the bulk soil C/N ratios (Fig. 2).

Soil amendment with urban composts BIO and GWS resulted in similar SOC stock gains as the conventional FYM treatment. Peltre et al. (2012) attributed the low OC stock increase in the MSW-treated soil to higher contents of fast degradable polysaccharides and lower contents of lignin in the MSW amendment compared to BIO, GWS and FYM. This would suggest introduction of recalcitrant lignin through the BIO, GWS and FYM amendments, which might be reflected by the chemical composition of the amendments itself and physical fractions isolated from amended soils. However, the overall chemical composition of the amendments as seen by ^{13}C CPMAS NMR showed only slight differences. We found neither in the POM fractions nor in the clay fractions of BIO or GWS amended soils distinctive accumulations of lignin (Fig. 5). The lower quality of MSW (as indicated by its high C/N ratio and contribution of aromatic compounds) compared to the other amendments and a lower overall SOC sequestration support the hypothesis of microbial use efficiency as control of C-stabilization in soil (Cotrufo et al., 2013; Rumpel et al., 2015).

Physical fractionation allows following SOC formation by isolating free and occluded particulate as well as mineral-associated OM. Whereas POM fractions store up to one third of the total OC, the major proportion of OC was stored in the clay fraction (Fig. 5). The clay fraction also stored the majority of total N (up to 79% for control). This is in agreement with the literature, indicating that the clay fraction often represents the dominating SOC and N pool in cropland soils (Christensen, 2001; von Lützow et al., 2007; Jagadamma and Lal, 2010).

POM fractions store fresh OC, and hence, first changes due to OM amendments are expected within these fractions (Janzen et al., 1992; Sollins et al., 1996; von Lützow et al., 2008). Fast changes in the fPOM were found after two applications of OM amendments on cropland soils (Carter et al., 2003; Leroy et al., 2008). While Ngo et al. (2011) observed enhanced aggregation and higher amounts of POM in water stable aggregates already after one conventional compost application, Leroy et al. (2008) concluded that the duration of their experiment of less than one year was too short to show effects on the oPOM and mineral-associated fractions of temperate soils.

In this study, repeated OM amendments (8 times) did not significantly increase the SOC stocks in the fPOM and oPOM fraction. Our results fully agree with literature data for conventional amendments, reporting a fast decomposition of labile OM from conventional amendments. We assume, that fPOM and oPOM originating from the urban waste composts were mainly decomposed two years after the last amendment and that at the sampling time these fractions originate mainly from crop residues inputs. However, significantly higher SOC stocks were found for oPOM$_{small}$ (BIO and FYM) and clay fractions (BIO and GWS). Increases of these fractions accounted for 55% (MSW) to 94% (BIO) of the total SOC stock increases. Whereas BIO, GWS and FYM showed increased SOC stocks in the clay fraction ranging between 42 and 46%, no differences to the control were noted for MSW. These data show that amendment of agricultural soil with the urban waste composts BIO and GWS allows building a mineral associated SOM pool, considered relevant to reduce the carbon release to the atmosphere (Scholes and Scholes, 2013).

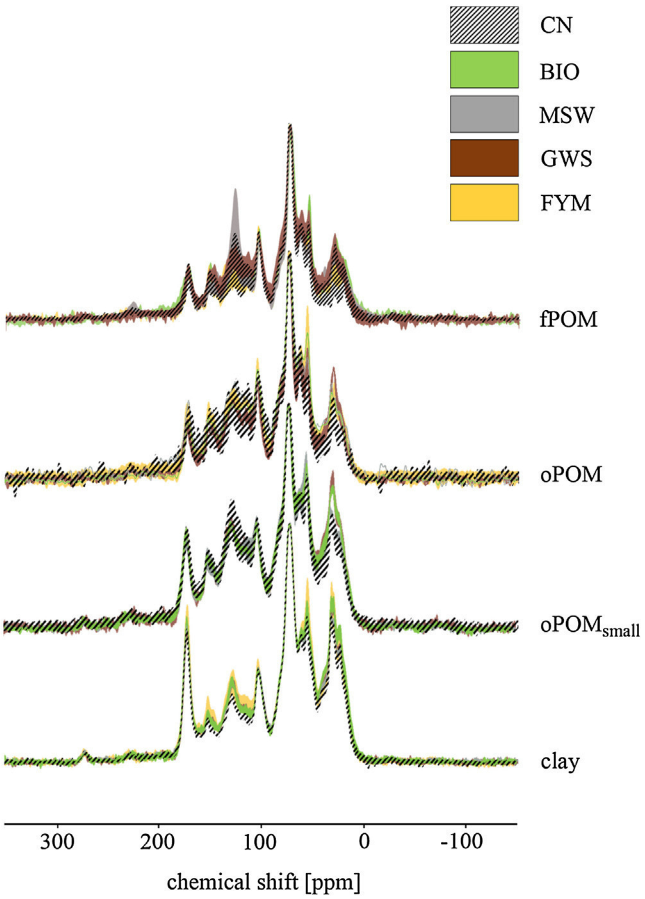

Fig. 5. Solid-state ^{13}C NMR spectra of the physical fractions ($n = 4$). The spectra are normalized to the O-N-alky peak at 72 ppm. CN = control, BIO = biowaste compost additions, MSW = municipal solid waste compost additions, GWS = green waste and sewage sludge compost additions, and FYM = farmyard manure additions.

C/N ratios of the clay fractions were similar for all treatments. Obviously, the tight coupling of C and N sequestration in the clay fraction was neither affected by an increase of OM input, nor by the different amendment types. Our data show that urban waste compost amendments (BIO and GWS) were even more effective than conventional amendments (FYM) in maintain the high mineral-associated SOC pool.

4.2. Chemical composition of amendments and SOM fractions

With our fractionation method we were able to obtain specific data on the long-term effects of urban compost amendments on the OM composition of differently stabilized SOM fractions.

The solid-state ^{13}C NMR spectra of the amendments (Fig. 1a) are strikingly similar. They are dominated by polysaccharides, with a high proportion of cellulose as indicated by the high resolution for the crystalline C at 62 and 89 ppm (Earl and VanderHart, 1981; Newman and Hemmingson, 1995). The alkyl/O-N-alkyl ratios of the amendments are generally low, indicating a low degree of transformation during composting (Table 1). It should be noted that MSW and FYM inputs have slightly lower alkyl C contribution than BIO and GWS. The C/N ratios of the OM amendments range from 12.0 to 19.6 (Table 1). Physical fractionation of OM amendments showed that the C/N ratios between the fractions vary widely (Table A1).

The data indicate high variability, as well as significant differences, for the C/N ratio of the POM fractions (Fig. 2) despite similar crop residue and root inputs (Peltre et al., 2012). The high variability of the C/N ratio of the POM fractions may be explained by the heterogeneous composition of the different input materials (Table A1). FYM, for example, is composed of straw with a high C/N ratio (Douglas et al., 1980; Eiland et al., 2001) and manure which has a low C/N ratio (Achmad et al., 2011). These results were underlined by our findings from OM amendment fractionation (Table A1). Interestingly, in the case of BIO and GWS, addition of organic materials seemed to decrease the variability of fPOM and oPOM$_{small}$ compared to the control soil (Fig. 2). These substrates seem to be more homogenized input materials compared to MSW and FYM (Table 1).

The C/N ratios of the fPOM fractions, which may indicate their degradability, were lowest for BIO and GWS (Fig. 2). Similar results were obtained for the oPOM$_{small}$ fractions while oPOM showed no significant differences. Obviously the POM fractions do not reflect the narrow C/N ratios (12.0–19.6) of the OM amendments. Instead the POM fractions seem to be dominated by the residues from the wheat and maize crops, whereas the amendment N is already processed by the microbial biomass and at least partly transferred to the fine fraction. That is similar to the findings of Pan et al. (2009), who concluded that the bulk soil OC increases of their long-term field experiment on paddy soils mainly resulted from crop residues and not from conventional manure.

Higher C/N ratios were noted for MSW and control soil, which may be explained by the low degree of transformation during the composting procedure for MSW and the input of high C/N crop residues in the case of the control soil (Table 1) (Peltre et al., 2012).

Similar to the C/N ratio, the NMR spectra of all POM fractions were characterized by a higher variability compared to those of the clay fractions (Fig. 5,Table 3). The fPOM and oPOM fractions isolated from amended soils are only slightly altered compared to the OM amendments themselves. Again it should be noted that the composition of these fractions is affected by the intrinsic contribution of crop residues from wheat and maize, which are dominated by polysaccharides (Bolinder et al., 1997; Kögel-Knabner, 2002). The alkyl/O-N-alkyl ratio of the physical fractions does not vary significantly among soils treated with different amendments (Table 3). For all soils we noted increasing ratios from fPOM to $oPOM_{small}$ fractions indicating increasing decomposition degrees (Baldock et al., 1997). The *H* index (Tables 1 and 3) is a measure for the hydrophobic OM components. The *H* indices of our OM amendments are lower with higher differences between the amendments than those used by Eldridge et al. (2013) (FYM, 0.53, GWS 0.58, MSW 0.51). The slightly higher hydrophobicity of the amendments BIO (0.46) and GWS (0.44) compared to FYM (0.31) and MSW (0.29), was even increased in the POM fractions ($CN < FYM < MSW < GWS < BIO$) with hydrophobicity increasing in the more decomposed POM fractions reaching *H* values around 0.6 and 0.7 (Table 3). The conventional amendments (pig manure and crop straw) used by Zhou et al. (2010) also resulted in more hydrophobic POM fractions ($H \sim 0.79$). Our data thus indicate that the POM fraction derived from waste compost amendments shows enhanced hydrophobicity, leading to a quantitative increase of POM fraction. However, with regard to the clay fraction, we found that amended soils do not show enhanced hydrophobicity and MSW amended soils showed even lower hydrophobicity than the control (Table 3). The higher OM contents in the clay fractions of the amended soils do not affect hydrophobicity, as the composition of the amended soil clay fraction OM is similar to the control soil. The increase of hydrophobic POM compounds did not change wettability of bulk soils as indicated by the results of the WDPT test (Table 2). Obviously the generally low proportion of POM does not have an impact on the bulk soil wettability. This is in agreement with results of Annabi et al. (2007) and Doerr et al. (2006).

Solid-state ^{13}C NMR spectra of POM fractions isolated from soil amended with MSW show a more pronounced aromatic signal at 128 ppm (Fig. 1b), which likely originated from black C or soot (Kögel-Knabner, 1997; Pichler et al., 2000; Mueller and Kögel-Knabner, 2009). The accumulation of recalcitrant aromatic constituents could be the consequence of repeated MSW amendments (González-Vila et al., 2002; Almendros et al., 2003; De la Rosa et al., 2008). However, the contribution of these compounds may not be systematic, as there was only one field replicate out of four, which showed such a contribution. Also in the more altered $oPOM_{small}$ and clay fractions, we did not record higher aromatic peaks for MSW amended soils compared to soils treated with other amendments or the control.

The OM composition of the clay fractions is dominated by high contributions of polysaccharides and alkyl C (Fig. 5 and Table 3). The ^{13}C NMR spectra are generally similar to other clay fractions isolated from loamy arable topsoils (Mueller and Kögel-Knabner, 2009), even when amended with solid waste (Leifeld et al., 2002). We found highly similar OM compositions for the more altered $oPOM_{small}$ and clay fractions of all treatments and the control soil, resulting in low standard deviations for the NMR signals recorded in these two fractions. The OM amendments were shown to be variable with respect to chemical OM composition and C/N ratio. But this is not reflected any more by OM composition and C/N ratios of $oPOM_{small}$ and clay fractions. We suggest that the decomposition process starting from variably composed OM amendments leads to the accumulation of a uniform residual fine OM material isolated as $oPOM_{small}$ fraction. The OM in the clay fraction is formed during plant residue and OM amendment decomposition by association of microbial-derived OM with soil minerals (Simpson et al., 2007; Cotrufo et al., 2013). Our data show that this leads to a highly uniform OM composition of the organo-mineral associations. Their OM composition in a soil with its site-specific mineral assemblage is not affected by the chemical nature of OM amendments. There is increasing evidence that microbial involvement in the decomposition process leads to the direct deposition of microbial cell wall remains and extracellular microbial polymers on mineral surfaces in soils (Kelleher and Simpson, 2006; Miltner et al., 2012; Schurig et al., 2013; Kleber et al., 2015). Our data are in agreement with this concept and we suggest that the OM in the clay fraction is composed of microbial residues which are independent in their composition from the input material. As we found higher C storage in the clay fraction isolated from soil amended with high quality amendments BIO, GWS and FYM as compared to MSW (indicated by their C/N ratio and chemical composition), our data support the hypothesis of microbial use efficiency as control of C-stabilization in soil (Cotrufo et al., 2013; Rumpel et al., 2015).

5. Conclusion

This study provides detailed information about quantitative and qualitative effects of long-term (15 y) biannual urban waste compost and manure amendments on OM composition and stocks in bulk soil and physical fractions of a loamy agricultural soil under temperate climate. Long-term application of BIO and GWS composts enhances OC and N stocks in bulk soils and especially the fine mineral fraction in a magnitude similar to conventional FYM application. No such effect was observed for MSW compost.

These SOC stocks changes were recorded despite normalized biannual OC inputs ($0.4\,kg\,m^{-2}$). Farmyard manure and urban waste composts with longer transformation times (BIO, GWS) increased SOC storage in bulk soils, $oPOM_{small}$ and clay fractions. The highly variable composition of the amendments and POM fractions was not reflected by the uniform composition of the mineral-associated OM. It seems that mainly microbial residues are present in the clay fraction, leading to a similar clay OM composition in soil of all treatments despite the contrasting input material. We suggest that higher C storage in the clay fraction may be due to a better microbial use efficiency leading to C sequestration as microbial compounds. Consequently, use of OM amendments should aim to stimulate microbial uptake in order to achieve efficient C sequestration. Our results further showed that urban waste compost amendment was able to enhance OM storage in the fine fraction and thereby most probably improving soil structure. At the same time all soils remained hydrophilic even after long-term biannual inputs of $0.4\,kg\,m^{-2}$. With regard to management practice our results suggest that it could be tested if even higher or annual OM amendments are possible to further increase OC sequestration of this soil.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.agee.2016.03.008.

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The results of study II are summarized and published in the article:

A multi-technique approach to assess the fate of biochar in soil and to quantify its effect on soil organic matter composition

by Lydia Paetsch, Carsten W. Mueller, Alexandra C. Wiesheu, Šárka Cepáková, Cyril Girardin, Cornelia Rumpel, Natalia P. Ivleva, Reinhard Niessner, Ingrid Kögel-Knabner

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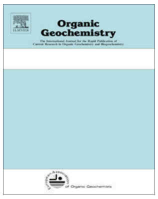

A multi-technique approach to assess the fate of biochar in soil and to quantify its effect on soil organic matter composition

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ABSTRACT

Differentiation of biochar and native soil organic matter (SOM) is required to assess the effect of biochar amendment on *in-situ* changes of SOM. Therefore, we used C_4 biochar produced at high temperature (1200 °C) by gasification (BC_{GS}) and measured the ^{13}C abundance of density and particle size fractions. We quantified the BC_{GS} effects on distinct native C_3-SOM pools of a grassland topsoil one year after BC_{GS} amendment. The chemical composition was analyzed with solid-state ^{13}C CPMAS NMR, whereas information on the nanostructure of BC_{GS} were obtained by Raman microspectroscopy measurements. Our aim was to assess BC_{GS} induced chemical changes of SOM and physical fractions and to validate the accuracy of BC_{GS} detection by ^{13}C NMR spectroscopy. Quantification by isotopic measurements and ^{13}C NMR spectroscopy for aromatic C yielded similar estimates of BC in soils. Of the total BC_{GS}, 52% were recovered as free particulate organic matter (POM) and 33% were located in aggregated soil structures isolated as occluded POM particles. Around 4% of the total BC_{GS} was detected in the clay fraction. After one year of field exposure, the surface of the BC_{GS} particles decreased in unordered graphitic-like structures. The higher ordered BC residue is supposed to be more recalcitrant. The native SOC stock increase ($p = 0.06$, $n = 4$) in the clay fraction indicated increased sequestration of organic matter as mineral-bound SOM due to BC_{GS} amendment. With respect to soil functionality, the BC_{GS} amendment induced a tremendous shift from a soil system dominated by organo-mineral associations to POM–dominated OC storage, resulting in increased soil air capacity.

1. Introduction

Over the past few years, pyrolysis of biomass with no or limited air supply has been used to produce biochar (BC), a new soil amendment with considerable potential for climate change mitigation strategies (Sohi et al., 2010; Woolf et al., 2010) as negative emission technology (Woolf et al., 2016), waste recycling (Laird, 2008) and soil improvement. In this context, BC obtained at high temperatures (> 700 °C) by pyrolysis or gasification (BC_{GS}) is produced as an industrial waste product. High-temperature BCs are assumed to have a long residence time in soils due to their highly condensed aromatic structure, which implies high biochemical recalcitrance (Cimò et al., 2014; Mukome et al., 2014). Recent work showed that high-temperature BC has a significant potential for long-term C storage in soils, in contrast to BC produced at lower temperatures (Naisse et al., 2015). Thus, BC_{GS} is potentially suited to enhance C sequestration (Swift, 2001), while improving various other soil functions. Specifically, soil water retention and water that is available to plants can be improved (Abel et al., 2013), due to the highly porous nature of BC, up to a certain production temperature threshold. But contrasting results have been obtained on the effect of BC on aggregation as well as hydraulic properties (Ma et al., 2016), and that seems to be strongly dependent on the molecular structural and 3D composition of the added BC.

Information about the effects of BC on soil organic matter (SOM) pools are mainly derived from laboratory incubation experiments (Maestrini et al., 2015) using finely ground model BC rather than

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commercial scale BC (Zhang et al., 2016). Field studies are scarce but their importance for the validation of results obtained under controlled conditions in the lab has been recently underlined (Bruun and EL-Zehery, 2012; Yin et al., 2014; Yang et al., 2016; Chen et al., 2017). Laboratory studies show that low temperature BC is mainly found as free particulate organic matter (fPOM) or within microaggregates (> 53–250 μm) after several month of incubation (Dharmakeerthi et al., 2015; Kerré et al., 2016).

Few results from field experiments indicate that low temperature BC inclusion into soil aggregates already occurs within a few years after application (Kimetu and Lehmann, 2010; Plaza et al., 2016). Chemical changes of BC were suggested to occur even more rapidly under field conditions (Mia et al., 2017). An important process affecting the fate of BC after field exposure is physical weathering, which leads to its physical disintegration (Spokas et al., 2014) and modifications of physico-chemical properties (Sorrenti et al., 2016). However, observing these changes occurring in the first years after application requires the capacity to trace BC particles in the field. Moreover, a detailed chemical investigation of molecular – as well as nano-structure of BC enables us to study the BC allocation to specific SOM fractions assignable to mechanistic pools. The combination of physical fractionation and detailed chemical characterization may enable a more complete assessment of the fate of BC_{GS} amendments in soils.

A prerequisite for such an approach is the quantification of amended BC-C versus native SOC. We made use of ^{13}C isotopic signatures differing naturally between native C_3-SOC and C_4-BC_{GS}, which enables a complete C balance through an isotopic mixing model (IMM).

In the present study, this approach was combined with analyses of molecular structural changes of BC_{GS} and SOM using ^{13}C CPMAS NMR spectroscopy and Raman microspectroscopy (RM) – a combination of Raman spectroscopy with an optical microscope. Applying a molecular mixing model (MMM) (Nelson and Baldock, 2005) to the solid-state ^{13}C CPMAS NMR spectroscopic data was suggested to provide quantitative information on the amount of aromatic C in different soil fractions. However, the use of solid-state ^{13}C CPMAS NMR spectroscopy to quantify BC in soil has been questioned. While pyrogenic C from vegetation fires can be quantified by CPMAS spectroscopy (Knicker et al., 2005), difficulties were encountered for highly condensed polyaromatic networks of kerogen (Smernik et al., 2006) and chars produced at high temperatures (Freitas et al., 1999). Moreover, NMR spectroscopy, as a bulk method may not be sensitive enough to assess small structural changes occurring during the first years of environmental exposure. Therefore, we additionally used RM, capable of providing more detailed structural information. In general, the Raman spectra of (unordered) carbonaceous substances contain two main peaks. The G ("graphitic")-peak (E_{2g}-symmetry) around 1580 cm^{-1}, and the D ("defect")-peak around 1350 cm^{-1} (A_{1g}-symmetry). The relative intensity of the D peak (I(D)/I(G) ratio) is a measure for the structural order of the sample. In carbonaceous substances with crystallite size below 2 nm (e.g. biochar and soot samples), the relative intensity is directly proportional to the order of the nanostructure (Ferrari and Robertson, 2000).

We applied these techniques to samples from a field experiment, where BC_{GS} from maize pellets was used to increase soil C sequestration. The BC_{GS} is an industrial waste product, and was studied as part of a realistic BC supply chain as a soil amendment (Kaiser et al., 2014). This unique approach allowed us to (1) determine the accuracy of quantification of aromatic C from BC_{GS} in soil by solid-state ^{13}C NMR spectroscopy, (2) assess its fate, (3) investigate its effect on native SOM and (4) its consequences for soil physico-chemical properties. This information allows elucidating the structural alteration of the BC_{GS} in soils, which in turn is crucial for its effect on fundamental soil properties such as aeration and water holding capacity.

2. Material and methods

2.1. BC production

The BC_{GS} was produced by gasification of maize (*Zea mays* L.) (C_4 plant) silage (10 mm). The feedstock pellets were pyrolized for 40 min at 1200 °C in a commercial reactor (©A.G.T. – Advanced Gasification Technology s.r.l., Cremona, Italy). The main physico-chemical characteristics of the fresh and aged BC are given in Table 1. The pH values of the BCs were measured in 0.01 *M* $CaCl_2$ solution (1:2.5 w:vol). Biochar C and N contents were determined by dry combustion using the elemental analyzer EA3000 (Hekatech, Wegberg, Germany). To determine the ash content of the fresh BC, we combusted the BC at 550 °C for 20 h.

2.2. Study site

The BC field experiment is located at the site of the long-term field experiment "SOERE ACBB", which is managed by INRA (National Institute of Agricultural Research) and located in Lusignan (46°25″12.91″N; 0°07″29.35″E), France. Mean annual rainfall is 800 mm and the mean annual temperature is 11 °C. The soil at the site was classified as Dystric Cambisol (IUSS Working Group WRB, 2014) with loamy texture (11% sand, 72% silt and 17% clay). The organic carbon (OC) and total N content was 1.4% and 0.16%, respectively (Sanaullah et al., 2014). At the study site, no carbonate was detected in the soil and there are no indications for fire-derived OC input.

The field experiment was established in May 2012, prior to which the field was a temporary grassland (C_3 plants). The establishment of the experiment included hand application of 3 kg m^{-2} (30 T ha^{-1}) BC_{GS} with incorporation at 10 cm depth by rotary hoeing followed by grassland cultivation with *Festuca arundinacea* and *Dactylis glomerata*. Grass yields were determined in 2013 (A.1). The experiment was comprised of 8 plots: 4 BC_{GS} amended plots and 4 control plots, each covering 16 m^2. Soil pH changed from 6.0 (control) to 6.4 (BC) one year after BC_{GS} amendment.

2.3. Sampling and sample pretreatment

The soil was sampled in October 2013. Per plot, three undisturbed (100 cm^3 sampling rings) and disturbed soil samples were randomly taken from the top 10 cm of the soil. The disturbed soil samples were taken with a shovel and composited together to form a representative sample from each plot. Bulk soils for fractionation were air dried and manually broken. To avoid loss of BC_{GS} pellets, which were mainly > 2 mm, we did not sieve the soils.

To determine chemical changes of the BC_{GS} before and one year after field exposure, we manually sampled BC pellets ('weathered' BC) from 50 g of dried bulk soil. Mineral residues attached to the

Table 1
Chemical characteristics of non-weathered (n-w) and weathered (w) pure biochar. Analysis are done in triplicates and standard deviations of weathered BC are conducted from 4 field replicates with *n* = 3 each (not analyzed = N.A.).

Parameter	Unit	BC_{pure} n-w	BC_{pure} w
pH (0.01 M $CaCl_2$)	–	9.7	N.A.
Salinity	mS cm^{-1}	9.6	N.A.
OC content	mg g^{-1}	633.3	707.6 ± 18.1
N content	mg g^{-1}	13.5	16.5 ± 0.9
C/N ratio	–	46.9	43.0 ± 2.2
Ash content (550 °C)	% DM	24.6 ± 1.7	N.A.

BC_{GS} surface were removed carefully with deionized water and a silicon spatula. Non-weathered and handpicked weathered BC_{GS} were then dried at 60 °C and ground for further analysis.

2.4. Physical fractionation

We used a combined density and particle-size fractionation protocol to separate SOM into particulate OM (POM) and mineral-associated OM (Amelung and Zech, 1999; Schmidt et al., 1999b; Mueller et al., 2009). Briefly, 20 g of unsieved soil was capillary-saturated with 250 ml sodium polytungstate solution (SPT) (TC Tungsten compounds, Grub am Forst, Germany) with a density of 1.8 g cm^{-3}. After settling overnight, roots were removed and the floating free particulate organic matter (fPOM) was siphoned from the surface using a vacuum pump. The fPOM fraction was rinsed with deionized water and sieved to remove mineral particles (< 20 μm) sticking on the fPOM fraction. Due to the C and N contents of this mineral fraction, we assigned it to the clay fraction. The remaining sample was dispersed ultrasonically (Bandelin, Sonopuls HD 2200, and Berlin, Germany) with an energy input of 400 J ml^{-1} to release occluded particulate organic matter (oPOM). Thereafter, the samples were centrifuged (~3700*g* for 30 min) and the floating material (oPOM) was siphoned off and sieved to separate oPOM < 20 μm ($oPOM_{small}$) and > 20 μm (oPOM). To remove SPT, fPOM and oPOM fractions were rinsed with deionized water using a pressure filtration device until the electrical conductivity of the solution was below 1.5 μS cm^{-1}.

In order to remove SPT (conductivity < 50 μS) from the mineral residues, several centrifugation-decantation steps (~4000*g* for 30 min) followed. Afterwards, the sand fraction (> 63 μm) and the coarse silt fraction (20–63 μm) were separated by wet sieving, and the medium silt fraction (> 6.3 μm) and the combined fine silt and clay fraction (< 6.3 μm) were separated by sedimentation. This fine fraction (< 6.3 μm) is in the following called the clay fraction. All fractions were freeze-dried. The particle-size and density fractionation resulted in recovery rates of 99 ± 1.1% of the initial sample mass and 88 ± 6.7% recovery of OC.

To reduce noise and enhance the solid-state ^{13}C NMR spectral signals, the clay fractions were treated with 10% hydrofluoric acid (HF) (Schmidt et al., 1997). Briefly, 20 ml of HF was added to 2 g of soil, which was shaken for 2 h, centrifuged (~2500*g* for 10 min) and the supernatant was decanted. This procedure was repeated five times. Afterwards, the samples were rinsed with deionized water until the pH was buffered to pH 4 and then the samples were freeze-dried. Carbon recoveries after HF treatment ranged between 73% (control) and 80% (BC_{GS} plots).

2.5. Soil hydraulic measurements

To determine the soil water retention characteristics, we saturated duplicates of undisturbed soil samples overnight and determined water contents at pressure heads (h) of −1, −10, −32, −63, −100, −159, −316, −500, −1000 and −15,848 cm. For pressure head levels of up to −500 cm, ceramic tension plates were used (pF pressure step module; ecoTech, Bonn, Germany). For pressure head levels of −1000 and −15,848 cm, pressure plate extractors (SOILMOISTURE equipment, Santa Barbara, USA) were used.

2.6. Chemical analysis and isotopic composition

The pH values of the bulk soils were measured in 0.01 *M* $CaCl_2$ solution (1:2.5 w:vol). The OC and N concentrations of bulk soils and BCs were measured in duplicate by dry combustion with a Vario EL elemental analyzer (Elementar Analysensysteme, Hanau, Germany). The OC and N concentrations of the physical fractions were determined by dry combustion using the elemental analyzer EA3000 (Hekatech, Wegberg, Germany). Stable isotope analyses were carried out using an elemental analyzer (Fisons Instruments EA/NA, Italy) coupled to an isotopic ratio mass spectrometer (VG IOSOGAS, England). All $\delta^{13}C$ values (‰) are expressed relative to the Pee Dee Belemnite (PDB) international isotope standard.

The solid-state ^{13}C NMR spectra of the BC, POM fractions as well as HF treated clay fractions were obtained on a Bruker AvanceIII 200 spectrometer (Bruker BioSpin GmbH, Karlsruhe, Germany). Cross-polarization magic angle spinning (CPMAS) was applied with a ^{13}C-resonance frequency of 50.32 MHz and a spinning speed of 5 kHz. Contact time was 1 ms and recycle delay was 1 s for all fractions. Depending on the OC content of the samples, between 2000 and 250,000 scans were accumulated and a line broadening between 0 and 50 Hz was applied. For calibration of the ^{13}C chemical shifts, tetramethylsilane was used and set to 0 ppm. Spectral analysis was performed using the spectrometer software. Integrated chemical shift regions were 0–45, 45–110, 110–160 and 160–220 ppm and were assigned to alkyl-C, O-N-alkyl-C, aryl-C and carboxyl-C, respectively (Wilson, 1987). We applied a spinning side band correction by subtracting the spinning sidebands from carboxyl-C and O-N-alkyl-C region and adding the proportion to the aryl-C. Signals at 56, 72, 128, 150–155 ppm are assigned to lignin. Signals centered at 128 ppm and 150 ppm are commonly attributed to C—C and C—O bonds in aromatic compounds (Kögel-Knabner, 2002). Polysaccharides were assigned to signals at 72 ppm and 103 ppm as well as to the shoulders at 65 and 80–90 ppm (Kögel-Knabner, 1997; Preston et al., 1998). Lipids and proteins are responsible for signals at 21 and 32 ppm and 56 ppm, respectively (Wilson, 1987; Kögel-Knabner, 2002). To estimate the relative content of aryl-C derived from BC_{GS} in physical fractions of amended soil, we used the molecular mixing model (MMM) described by Nelson and Baldock (2005). The MMM was applied using seven spectral integration regions: carbonyl-C (210–165 ppm), O-aromatic-C (165–145 ppm), aryl-C (145–110 ppm), O_2-alkyl-C (110–95 ppm), O-alkyl-C (95–60 ppm), N-alkyl/methoxyl-C (60–45 ppm) and alkyl-C (45–10 ppm). The aryl-C contribution was corrected for native aryl-C by using MMM data of the corresponding physical fractions of the control soil. The accuracy of determination of BC-derived aryl-C was tested by comparison with quantitative BC contribution obtained from the isotopic mixing model (IMM) (see below).

Raman spectra were recorded using a LabRAM HR Raman microscope system (Horiba Jobin Yvon, Japan) with a HeNe-laser (633 nm, 0.14 mW at the sample) and an integrated Olympus BXFM microscope with a 50× objective (Olympus MPlan N, NA = 0.75). The control soil fractions and the pure BCs were randomly measured at 5 spots for 100 s, whereas we optically detected and analyzed five black spots in the BC amended soil fractions. As the fPOM fraction of the BC amended soils contained 97% BC, RM analyses of the pure weathered BC_{GS} represent the fPOM fraction.

The spectra were baseline-corrected and normalized, then mean spectra for BC_{GS} and the physical fractions were calculated. The G (“graphitic”)-peak (E_{2g}-symmetry) was assigned 1580 cm^{-1}, whereas the D (“defect”)-peak was assigned to 1350 cm^{-1} (A_{1g}-symmetry). To obtain information about the nanostructure of the BC and the SOM, the ratio of the relative intensities of the peaks (I(D)/I(G)) was used. For graphites or graphitic carbon the Tuinstra Koenig relation is valid, means the I(D)/I(G) ratio is inverse proportional to the structural order. In contrast, for amorphous carbonaceous materials e.g. soot samples, the I(D)/I(G) ratio is proportional to the probability of finding a sixfold ring in the cluster that is proportional to the cluster area, and hence, to the structural order. Bear in mind that this means that in amorphous carbons the development of a *D* peak indicates ordering, exactly opposite from the case of graphite (Ferrari and

Robertson, 2000). The penetration depth of the analysis is in the range of some micrometers.

2.7. Calculations and statistics

The BC_{GS} amendment leads to an increasing soil volume resulting in lower bulk density, which needs to be considered for SOC stock and volumetric water content calculations. Therefore, we corrected the stocks by using the 'equivalent soil mass' approach (Ellert and Bettany, 1995; Kätterer et al., 2011). The total SOC and N stocks (kg m^{-2}) of the control soils were calculated by multiplying SOC and N content with bulk density (BD) (g cm^{-3}) and the depth of 10 cm (factor 10^{-3}). Accordingly, the SOC and N stocks of the BC_{GS} amended soils and its physical fractions were normalized to the reference soil mass of the control soils. In order to obtain a volumetric water content correction, we used the same approach as above. We considered the increased soil volume by BC_{GS} amendments by using the equivalent soil volume (ESV):

$$\mathrm{ESV} = \frac{(BD_{control} - BD_{BC})}{BD_{BC}} \tag{1}$$

where m_{BC} (g) is the mass of the BC_{GS} amended soil per unit soil volume. With the expanded soil volume (cm^3) and the volumetric water content ($cm^3\ cm^{-3}$), we calculated the effective soil water storage and converted this to the standard unit (L m^{-2}).

By using the software SHIPFIT2.0 (Durner and Peters, 2009) we fitted the water retention characteristics to the data by using the unimodal Kosugi retention function (Kosugi, 1996) given by:

$$\Gamma(h) = \frac{1}{2} erfc\left[\frac{\ln\left(\frac{h}{h_m}\right)}{\sqrt{2}\sigma}\right] \tag{2}$$

where h_m (L) is the pressure head corresponding to the median pore radius, σ (–) is the standard deviation of the log-transformed pore-size distribution density function, and erfc() is the complementary error function. For unsaturated soils h (L) is defined as positive.

The proportion of native SOC (bC_3) was calculated by the isotopic mixing model (IMM):

$$bC_3 = \frac{\delta C_{\mathrm{mixture}} - \delta C_4}{\delta C_3 - \delta C_4} \tag{3}$$

where δC_4 is the $\delta^{13}C$ signature of the pure BC_{GS} and δC_3 of the native SOC of the control (‰). $\delta C_{\mathrm{mixture}}$ is the $\delta^{13}C$ signal of the bulk soils and the specific values of each physical fraction.

For statistical analyses we used R Studio Version 0.98.1062 for Windows (RStudio Team, 2016). Significant differences between BC amendments and control were tested with a one-way analysis of variance (ANOVA).

3. Results

3.1. Biochar properties

Carbon content of BC_{GS} increased from 633.3 mg g^{-1} (non-weathered) to 707.6 ± 18.1 mg g^{-1} one year after field exposure (Table 1). Nitrogen contents of BC_{GS} increased from 13.5 mg g^{-1} (non-weathered) to 16.5 ± 0.9 mg g^{-1} (weathered). The changes of elemental contents of BC_{GS} after field exposure are further reflected in a lower C to N ratio of weathered BC_{GS} compared to non-weathered BC. The $\delta^{13}C$ signatures of the non-weathered and handpicked weathered BC_{GS} were identical (−13.7‰) (Table 2).

Biochar showed no alteration of chemical composition one year after field exposure as seen by ^{13}C CPMAS NMR spectroscopy (A.2 and Table 2). The spectra of non-weathered and weathered BC_{GS} were dominated by aryl-C, which accounted for around 75% of the signal of BC_{GS} samples. O-N-alkyl-C was the second main contributor to NMR spectra, accounting for around 16% of the signal. Alkyl-C and carboxyl-C contributed only 2% to the NMR spectra. Raman microspectroscopic analysis showed higher I(D)/I(G) ratios of the weathered BC_{GS} compared to non-weathered BC_{GS}, indicating a higher structural order in the weathered BC_{GS} (Fig. 1a and Table 2). These results suggest that the nanostructure of the BC_{GS} changes during weathering. The unordered and amorphous parts of the BC_{GS} are decreasing and the more graphitic-like, ordered portions of BC_{GS} remain.

3.2. Bulk soil and physical fractions

3.2.1. SOM contents, stocks and distribution

One year after the BC_{GS} amendment, we recovered 2.5 ± 0.6 kg m^{-2} BC_{GS} in the top 10 cm of soil. Chemical parameters of bulk soils and physical fractions are listed in Table 3. The mass of the fPOM fraction increased significantly by a factor of 10, and the mass of the $oPOM_{small}$ by a factor of 3. In addition, the mass of the silt fraction decreased significantly by 3.6% compared with the control (Table 3).

Soil OC contents were significantly higher in BC_{GS} amended bulk soils as well as in all POM fractions compared to the control (Table 3). The changes were greatest in the fPOM fraction followed by oPOM and $oPOM_{small}$. There were no significant differences of OC content in the mineral fractions between control and BC_{GS} amended soils.

Soil OC stocks were over twice as high in the top 10 cm of BC_{GS} amended soils, mainly due to higher OC stocks allocated to fPOM and oPOM fractions (Table 3 and Fig. 2). Additionally, there were also smaller but still significant stock increases in the $oPOM_{small}$ and sand fraction, though not in the other mineral fractions.

The control soil showed a $\delta^{13}C$ value of −27.3‰. All physical fractions had similar $\delta^{13}C$ ranging from 27.0 to 28.4‰ (Table 2). In contrast, the $\delta^{13}C$ signature of BC_{GS} amended soils strongly decreased with decreasing particle size, with the fPOM signature (−14.5‰ ± 0.2) similar to pure BC_{GS} and the clay fraction (−26.3‰ ± 0.3) similar to native SOC. In the BC_{GS} amended soils, 55% of SOC was BC_{GS} derived (Fig. 2) and the proportion of BC_{GS} within the fractions strongly decreased with decreasing particle size: whereas BC_{GS} derived OC accounted for 96.8% ± 1.2 of the fPOM fraction, it only accounted for 5.2% ± 2.1 of the clay fraction (Fig. 2). The contribution of OC to physical fractions strongly changed in BC_{GS} amended soils. While OC associated to clay contributed to 71% to OC stocks in the control soil, it contributed only 34% in BC_{GS} amended soils (Fig. 2).

Despite significantly higher N contents in the fPOM fraction of the BC_{GS} amended soil of 13.2 ± 0.9 mg g^{-1} soil compared to 5.6 ± 2.2 mg g^{-1} soil in the control, the bulk soil N content did not significantly increase due to BC_{GS} amendment, as the N contents of the other fractions decreased in BC_{GS} amended soil (Table 3). Accordingly, bulk soil N stocks did not significantly increase, although there was a shift to significantly higher N stocks in the fPOM and oPOM fractions of the BC_{GS} amended soils compared to those of the control soil (Table 3). In both treatments, the highest N stocks were found in the clay fractions without any difference between them.

Except for the silt fraction, all C to N ratios of BC_{GS} amended bulk soils and physical fractions were higher compared to the control (Table 3). The highest C to N ratios of 42 and 31 were observed in the fPOM fraction and oPOM fraction of BC_{GS} amended soils compared to about 20 in the control soil.

Table 2

Chemical composition of pure non-weathered (n-w) and weathered (w) pure biochar (BC$_{pure}$) and physical fractions of gasification biochar amended soils (BC) and not biochar amended control soils. The mean relative contents of C compounds of the fractions are determined on field replicates ($n = 4$). (Occluded POM = POM > 20 μm, oPOM$_{small}$ = -POM < 20 μm, ratio of graphitic C (G-peak) and defects of graphitic-like structures (D-peak) = I(D)/I(G), not analyzed = N.A). Values within a column with different letters are significantly different ($p < 0.05$).

Soil	Fraction	^{13}C signature ‰	I(D)/I(G)	Alkyl-C %	O-N-alkyl-C %	Aryl-C %	Carboxyl-C %	Alkyl-C/O-N-alkyl-C
BC$_{pure}$ n-w	–	−13.7	1.02 ± 0.02	1.2 ± ‡	15.8 ± ‡	76.1 ± ‡	6.9 ± ‡	0.1 ± ‡
BC$_{pure}$ w	–	−13.7 ± 0.2	1.11 ± 0.03	3.2 ± 1.7	17.5 ± 3.1	73.3 ± 4.8	6.1 ± 2.6	0.2 ± 0.1
Control	fPOM	−28.4 ± 1.0	†	12.5 ± ‡	46.4 ± ‡	30.1 ± ‡	11.0 ± ‡	0.3 ± ‡
	oPOM	−28.4 ± 0.3		16.5 ± 0.5	52.3 ± 3.4	22.8 ± 1.9	8.4 ± 1.4	0.3 ± 0.0
	oPOM$_{small}$	−27.7 ± 0.2		21.6 ± 3.2	39.9 ± 6.5	28.6 ± 10.2	9.9 ± 0.9	0.5 ± 0.0
	clay	−27.0 ± 0.1		23.0 ± 1.7	49.1 ± 1.4	15.4 ± 2.5	12.4 ± 0.6	0.5 ± 0.0
BC	fPOM	−14.5 ± 0.2*	1.11 ± 0.03	1.3 ± 1.4*	17.3 ± 2.2*	74.7 ± 2.4*	6.6 ± 1.4*	0.1 ± 0.1
	oPOM	−17.8 ± 9.0*	0.98 ± 0.12	9.8 ± 1.8*	27.5 ± 4.2*	55.7 ± 4.7*	7.1 ± 1.4	0.4 ± 0.0*
	oPOM$_{small}$	−21.5 ± 4.5*	0.99 ± 0.08	15.6 ± 1.3	30.5 ± 1.8*	45.0 ± 3.1*	8.9 ± 0.8	0.5 ± 0.0
	clay	−26.3 ± 2.1*	1.02 ± 0.12	23.2 ± 0.6	48.0 ± 0.8	16.5 ± 1.2	12.2 ± 0.2	0.5 ± 0.0

‡ $n = 1$, no standard deviation; † no C bands detectable.

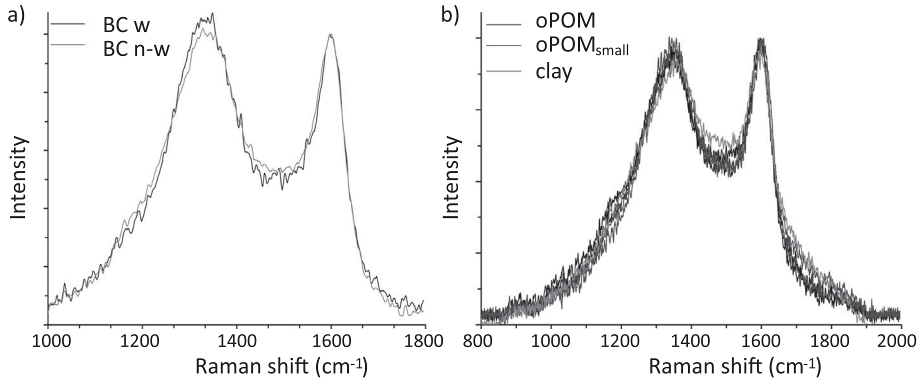

Fig. 1. (a) Raman spectra of weathered (BC w) and non-weathered biochar (BC n-w). (b) Raman spectra of the BC amended soil physical fractions (occluded particulate organic matter fractions = oPOM > 20 μm = oPOM, oPOM < 20 μm = oPOM$_{small}$).

Table 3

Physical and chemical characteristics of bulk soils and physical fractions of gasification biochar (BC) amended soils and unamended control soils ($n = 4$) (soil organic Carbon = SOC, free and occluded particulate organic matter fractions = fPOM and oPOM, respectively, oPOM > 20 μm = oPOM, oPOM < 20 μm = oPOM$_{small}$, not analyzed = N.A.). Values within a column with asterisks are significantly different between corresponding fractions of the control and the gasification BC amended soils ($p < 0.05$).

	Bulk density g cm^{-3}	Mass proportion of fraction mg g soil^{-1}	OC content mg g^{-1}	N content mg g^{-1}	C/N ratio	SOC stock kg m^{-2}	N stock kg m^{-2}	Native SOC stock kg m^{-2}	BC induced SOC stock mg g^{-1}
Bulk soil control	1.4 ± 0.1	–	14.7 ± 1.1	1.5 ± 0.1	9.6 ± 0.3	1.99 ± 0.15	0.208 ± 0.014	1.99 ± 0.15	–
fPOM	N.A.	1.3 ± 0.5	130.9 ± 62.5	5.6 ± 2.2	23.1 ± 5.5	0.02 ± 0.01	0.001 ± 0.000	0.02 ± 0.01	
oPOM	N.A.	2.6 ± 0.3	426.8 ± 3.7	21.2 ± 0.7	20.1 ± 0.7	0.17 ± 0.02	0.007 ± 0.001	0.17 ± 0.02	
oPOM$_{small}$	N.A.	4.4 ± 0.4	237.4 ± 44.2	18.1 ± 2.2	13.1 ± 0.9	0.16 ± 0.02	0.010 ± 0.001	0.16 ± 0.02	
Sand	N.A.	121.4 ± 7.3	2.7 ± 0.2	0.2 ± 0.0	11.0 ± 0.5	0.05 ± 0.01	0.008 ± 0.001	0.05 ± 0.01	
Silt	N.A.	608.8 ± 12.3	1.9 ± 0.3	0.2 ± 0.0	10.3 ± 0.4	0.18 ± 0.03	0.036 ± 0.002	0.18 ± 0.03	
Clay	N.A.	249.8 ± 10.1	36.5 ± 2.2	4.2 ± 0.3	8.7 ± 0.2	1.41 ± 0.11	0.146 ± 0.012	1.41 ± 0.11	
Bulk soil BC	1.3 ± 0.1	–	33.0 ± 11.8*	1.9 ± 0.5	17.9 ± 6.3*	4.48 ± 0.92*	0.260 ± 0.061	2.00 ± 0.49	2.48 ± 0.58
fPOM$_{BC}$	N.A.	13.7 ± 4.6*	560.5 ± 33.7*	13.2 ± 0.9*	42.4 ± 1.3*	1.34 ± 0.31*	0.028 ± 0.012*	0.05 ± 0.02	1.30 ± 0.29
oPOM$_{BC}$	N.A.	8.1 ± 2.8	611.1 ± 35.2*	19.6 ± 1.2*	31.4 ± 2.9*	0.89 ± 0.29*	0.023 ± 0.008*	0.23 ± 0.12	0.66 ± 0.23
oPOM$_{smallBC}$	N.A.	5.2 ± 1.5*	346.3 ± 55.3*	17.7 ± 2.1	19.6 ± 2.7*	0.33 ± 0.11*	0.014 ± 0.005	0.18 ± 0.05	0.15 ± 0.05
Sand	N.A.	120.0 ± 4.6	4.1 ± 1.2	0.3 ± 0.0	15.6 ± 2.5*	0.09 ± 0.03*	0.009 ± 0.002	N.A.	N.A.
Silt	N.A.	586.8 ± 9.8*	2.1 ± 0.9	0.2 ± 0.0	12.1 ± 2.2	0.26 ± 0.22	0.038 ± 0.008	N.A.	N.A.
Clay	N.A.	256.1 ± 12.1	32.8 ± 3.5	3.6 ± 0.4	9.1 ± 0.1*	1.57 ± 0.58	0.147 ± 0.036	1.5 ± 0.5	0.1 ± 0.1

‡ no field replicates.

3.2.2. *Effects of BC$_{GS}$ on chemical composition of OC pools*

The functional group distribution as shown by ^{13}C NMR spectra of the physical fractions is given in Fig. 3. Quantitative information on the contribution of the different chemical shift regions to the ^{13}C NMR spectra are presented in Table 2. As 88% (control) to 92% (BC$_{GS}$ amended) of OC was found in the POM and clay fractions (Fig. 2), ^{13}C NMR analysis was only performed on these fractions. The fPOM fraction of the control was well decomposed since only

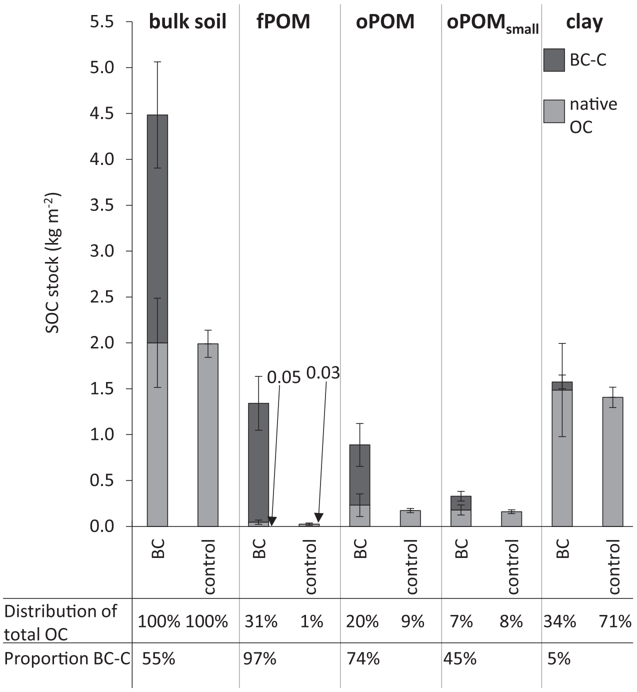

Fig. 2. Total soil organic Carbon (SOC) stocks, distribution total organic per fraction and proportion of gasification biochar induced C (BC-C) versus native organic carbon (native OC) in bulk soils and physical fractions. The mean SOC stock is calculated from field replicates ($n = 4$) (free and occluded particulate organic matter fractions = fPOM and oPOM, respectively, oPOM > 20 μm = oPOM, oPOM < 20 μm = oPOM$_{small}$).

1 of the 4 field replicates had sufficient material that could be collected for ^{13}C NMR measurements (Fig. 3). The spectrum of the fPOM of the control was dominated by the O-N alkyl signal (46%) followed by the aryl-C signal (30%) (Table 2). Carboxyl-C and alkyl-C contributed with 11% and 13% of total C intensities, respectively. In the control, aryl-C contribution was higher in the POM fractions than in the clay fraction, whereas the alkyl-C was higher in the clay fraction than the POM fractions.

The ^{13}C NMR spectra of all POM fractions of BC$_{GS}$ amended soils were dominated by aryl-C, and the spectra of the fPOM fractions were nearly identically to the spectra of the pure weathered and non-weathered BCs. The contribution of aromatic compounds decreased with decreasing particle size (fPOM > oPOM > oPOM$_{small}$ > clay) (Table 2). No effect of BC$_{GS}$ addition on the chemical composition of the clay fraction was observed. After BC$_{GS}$ amendment, signals of the clay fraction were dominated by O-N-alkyl-C (48 ± 1%), whereas carboxyl-C, alkyl-C and aryl-C contributed to similar proportions of 12 ± 1%, 23 ± 1% and 17 ± 1%, respectively (Table 2). Alkyl-C to O-N-alkyl-C ratio was only significantly increased in the oPOM fraction and increased from fPOM to clay fractions.

The RM analysis of the oPOM and clay fractions of the BC$_{GS}$ amended soils showed similar I(D)/I(G) ratios, which indicates that the nanostructures of these fractions are rather similar (Fig. 1b and Table 2). Moreover, the Raman spectra of the unamended control fractions exhibit no signals of graphitic-like substances, in agreement with the absence of BC$_{GS}$ amendment.

We found a strong correlation between aryl-C and BC-derived C (IMM) ($n = 16$, $R^2 = 0.96$) showing the lowest proportions for the clay fraction and the highest for the fPOM fraction (Fig. 4a). However, this approach may fail due to the fact that the BC$_{GS}$ amended plots have a significant proportion of native aryl-C (Table 2). Consequently, the clay fraction – consisting of 5% BC$_{GS}$ – had around 17% aryl-C. The deviation of the results from the MMM compared to the results of the IMM varies between 8% (fPOM) to 35% (oPOM$_{small}$) ($n = 16$, $R^2 = 0.98$) (Fig. 4b) and we found no BC$_{GS}$ contribution to the clay fraction with the MMM.

3.3. Effects of BC$_{GS}$ on water retention

There were no significant differences in water holding capacity between BC$_{GS}$ amended and control soils (Fig. 5a). However, we found a clear trend to increased coarse pore volume in the BC$_{GS}$ amended soils below −100 hPa, thus a trend to better aeration (Fig. 5b).

4. Discussion

4.1. Effect of BC$_{GS}$ on soil OC and N balances

The OC content of the control soil and the distribution of OC stocks within physical fractions is in a range typical for grassland topsoils (Wiesmeier et al., 2014), with more than 70% of the total OC stocks in the mineral-associated fraction (Fig. 2). The application of BC$_{GS}$ leads to significantly increased SOC sequestration due to content and stock increases of the POM fractions. After BC$_{GS}$ amendment, 58% of the total OC is stored in the POM fractions in contrast to 18% in the control soil. The SOC stock increase in the POM fractions is fully explained by BC-derived C as no significant changes in native SOC stocks were found (Table 3). The detection of 33 % of the BC$_{GS}$ in aggregated structures isolated as oPOM and oPOM$_{small}$ one year after application indicates, similarly to former studies with low temperature BC (Kimetu and Lehmann, 2010), a rapid rate of incorporation of BC$_{GS}$ into aggregates. However, in our study we applied less BC$_{GS}$ in a single application, compared with the higher dosages and repeated BC amendments of Kimetu and Lehmann (2010). Taking advantage of the high sensitivity of the C$_4$-BC approach in our study, we were even able to detect the contribution of 4% of the total C$_4$ carbon from BC$_{GS}$ (as calculated with the IMM) associated with minerals of the clay fraction. To the best of our knowledge, such a rapid accumulation of BC derived C, either from low- or high-temperature BC, in mineral-associated OM has not been reported before.

Our data reveal that after one year of field exposure, the BC$_{GS}$ C$_4$ from a single amendment found its way into all soil fractions. It induced a shift from mineral-associated to POM dominated OC storage. This indicates that the BC$_{GS}$ underwent substantial comminution and incorporation into aggregates, where the fine BC$_{GS}$ particles may serve as binding agent, while being protected from degradation (Brodowski et al., 2006; Yang et al., 2016). Moreover, microbial use of BC$_{GS}$ and protection of C$_4$ containing microbial products by mineral interactions cannot be excluded. Even when we found no significant effect of BC$_{GS}$ on the soil physical fractions, the trend to increased native SOC storage in the clay fraction after only one year of field exposure is quite remarkable and needs to be studied in longer terms. Future studies should consider if these changes occur due to altered inputs to the clay fraction (Farrell et al., 2013), or altered mineralization (Cross and Sohi, 2011; Kerré et al., 2016).

We found no significant effects of BC$_{GS}$ amendments on bulk soil N or mineral associated N, but significant increases of N content and stock in the fPOM fraction (Table 3). Most likely, this increase is mainly derived from the BC$_{GS}$ itself. However, as the weathered BC$_{GS}$ has higher N contents than the non-weathered BC$_{GS}$, we assume additionally a sorption of N to weathered BC (Table 1). This would support the results from a pot experiment of Anderson et al. (2011), who concluded low temperature BC

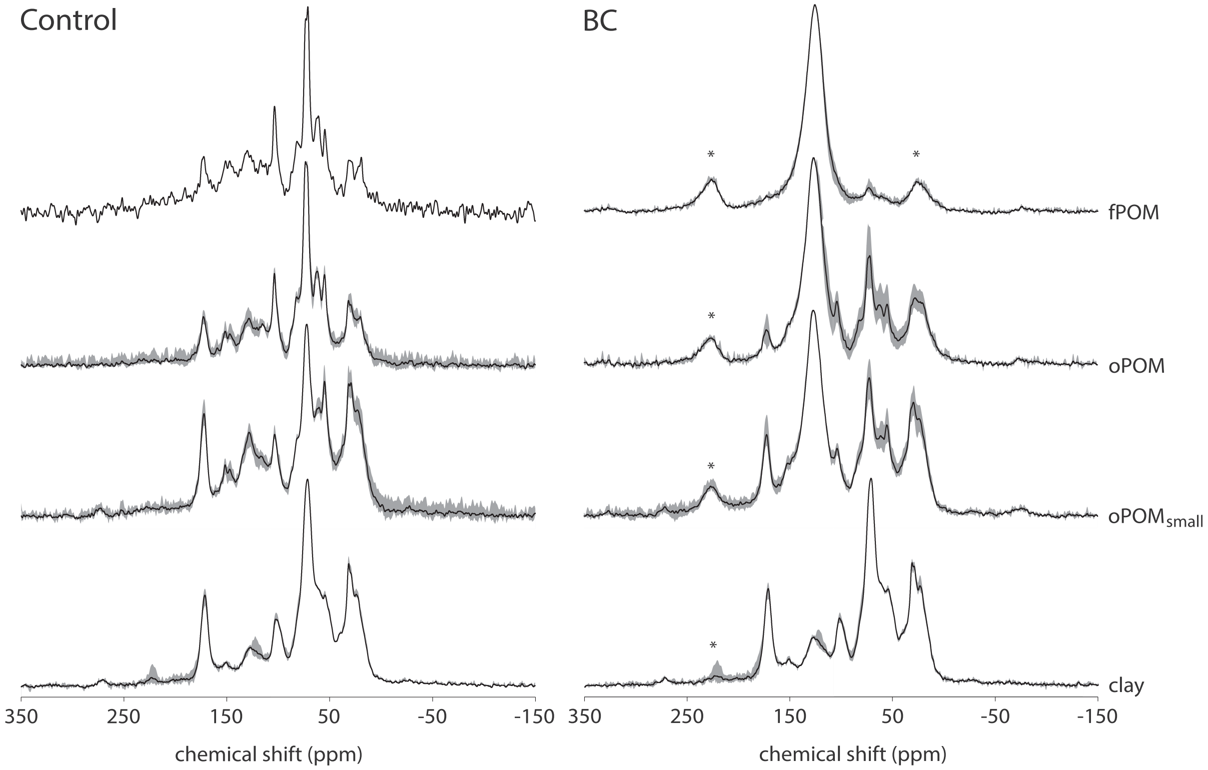

Fig. 3. ^{13}C Cross polarization magic angle nuclear magnetic resonance (CPMAS NMR) mean spectra of soil physical fractions (black) of gasification biochar amended soils (BC) and non-amended control soils (four field replicates). Gray shade indicates minimum and maximum values. In the free occluded particulate organic matter (fPOM) fraction of the control is $n = 1$ because only one sample had sufficient mass for ^{13}C NMR analyses. The asterisks mark spinning side bands of aryl-C (free and occluded particulate organic matter fractions = fPOM and oPOM, respectively, oPOM > 20 μm = oPOM, oPOM < 20 μm = oPOM$_{small}$).

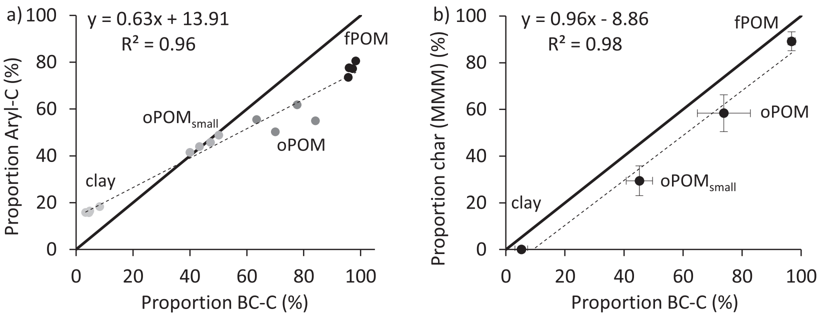

Fig. 4. (a) Correlation of aryl-C and gasification biochar (BC) induced C (isotopic mixing model = IMM) on BC amended soils. Every field replicate per fraction is shown by a single dot (BC) ($n = 4$). (b) Correlation of char content (molecular mixing model = MMM) and BC induced C (IMM). Each dot is a mean of four field replicates (free and occluded particulate organic matter fractions = fPOM and oPOM, respectively, oPOM > 20 μm = oPOM, oPOM < 20 μm = oPOM$_{small}$).

would act as a transient ‘nitrogen island’ in the soil. Our results show that also single BC$_{GS}$ particles may be considered as ‘N islands’ after 1 year of field exposure due to preferential accumulation of N and may be important with respect to N availability. In addition, BC may also reduce ammonia (NH_3), nitrous oxide (N_2O) and nitrate (NO_3^-) losses (Anderson et al., 2011) and results in an overall attenuation of N cycling dynamics directly after the amendment. This has implications for N management, as recently shown by (Criscuoli et al., 2016), who observed N deficiency in the first growing cycle after low temperature BC amendment. As in the present study similar plant growth was observed on BC$_{GS}$ amended plots and control plots (A.1), we assume that BC$_{GS}$ amendment does not cause any risk concerning N availability.

4.2. Changes in chemical composition of BC$_{GS}$ during field exposure

The solid-state ^{13}C NMR spectra of the pure non-weathered and weathered BC$_{GS}$ (A.2) are strikingly similar, dominated by an aryl-C peak with minor contributions of O-N-alkyl-C and carboxyl-C. This indicates that the weathered BC$_{GS}$ residues were not altered in their bulk chemical composition as revealed by NMR spectroscopy after one year of field exposure, in line with the findings of dos Anjos Leal et al. (2015) and McBeath et al. (2013) for low temperature BC. In order to detect structural changes occurring in response to environmental exposure, we used Raman microspectroscopy (RM), a more sensitive method with respect to alterations of graphitic-like structures. Previously, RM was successfully used

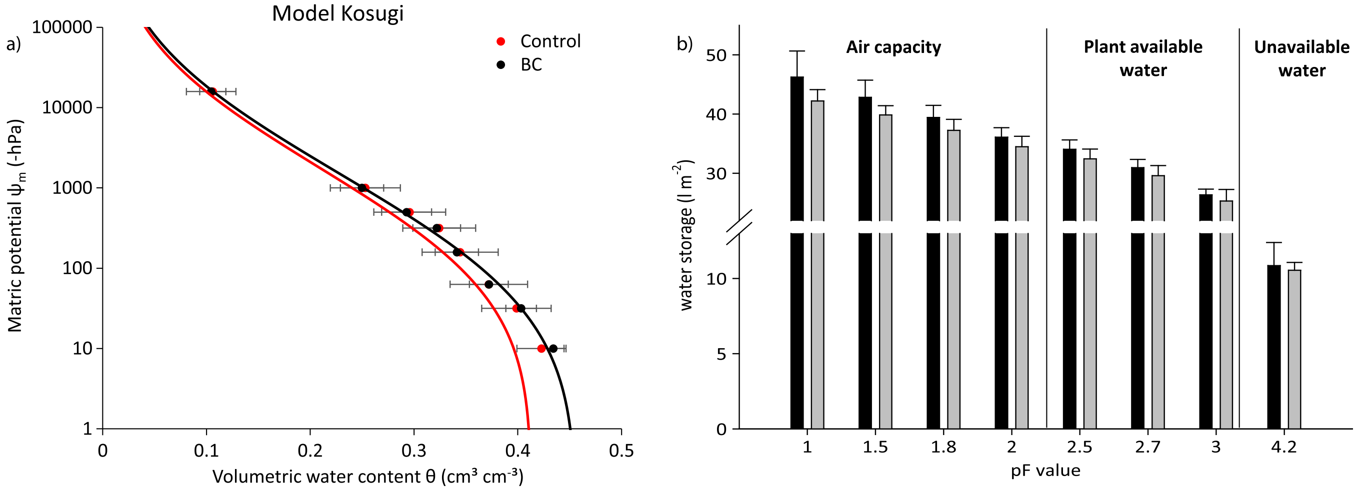

Fig. 5. (a) Water retention curves for the undisturbed control and biochar amended (BC) soil samples of four field replicates ($n = 3$). (b) Water storage on different pressure heads (permanent wilting point = PWP). The blacks bars are BC amended soils, the gray bars are control soil samples.

to study the structure of pyrogenic compounds in the Amazonian Terra preta soils (Ribeiro-Soares et al., 2013). The authors could show that the ratio of sp^2- and sp^3-C as well as the crystallite size of the compounds is in the limit range between nanographite and amorphous carbons. Moreover, a differentiation of the ancient BC and freshly produced BC was possible. Two recent studies dealt with the application of RM for a direct characterization of SOM. The obtained spectra, however, contained a high fluorescence background, which hampered a useful interpretation (Xing et al., 2016a, 2016b). In our study, RM clearly detected changes in the nanostructure of the BC$_{GS}$ due to weathering and/or degradation occurring during one year field exposure (Fig. 1b).

Analysis of the oPOM and clay fractions of the BC amended soils showed no significant differences in the nanostructures, indicating only a physical fragmentation and protection of the BC from weathering within the soil structure, with structural order as found in the pure unweathered BC. To our knowledge, this study is the first combination of CPMAS-NMR and RM for the analysis of SOM. Due to the different nature of information gained, RM and CPMAS can be used as complementary methods.

A preferential loss of less ordered and amorphous structures takes place, resulting in residual higher ordered structures detected in the handpicked BC$_{GS}$. The effect of field exposure is also found by ^{13}C CPMAS NMR spectroscopy in the soil fractions with larger proportions of BC$_{GS}$, i.e. fPOM, oPOM and oPOM$_{small}$. In the soil fractions with low proportions of BC$_{GS}$, such as the clay fraction, only marginal changes due to BC amendment can be detected (not shown). An artificial weathering experiment performed with the same BC$_{GS}$ by Naisse et al. (2015) showed a strong increase in the biological stability and thus half-life in soil of the residual material. The two-phase degradation of low temperature BC observed in laboratory incubation studies is generally associated with a labile, aliphatic components of BC versus a more recalcitrant aromatic component (Mukome et al., 2014). Our data indicate that two fractions of BC with different accessibility for weathering or degradation are also present in the BC$_{GS}$, containing condensed aromatic C, presumably resulting in increased recalcitrance of the residual highly ordered graphitic-like structures.

4.3. Is solid-state ^{13}C CPMAS NMR spectroscopy suitable to quantify the contribution of BC$_{GS}$ to soil?

Although solid-state ^{13}C CPMAS NMR spectroscopy is intensively used to characterize the contribution of charred OM to SOM composition (Schmidt et al., 1999a; Smernik et al., 2000; Nguyen and Lehmann, 2009), problems may arise for the quantification of this material with the cross polarization (CP) technique. These arise from the low degree of protonation (i.e. high degree of condensation), the rapid relaxation times (specifically the T1ρH proton spin–lattice relaxation time) (Smernik et al., 2002) and the interference with other aromatic OM present (Hammes et al., 2008). The detection problems usually increase with increasing degree of aromatic condensation (McBeath et al., 2011). Thus, the BC$_{GS}$ used in this study may be regarded as a worst case for quantitative ^{13}C NMR spectroscopic determination.

Taking advantage of the quantitative analysis of the BC$_{GS}$ in bulk soil and its fractions via stable isotope measurements (IMM), we could compare these data with compositional changes of SOM in the solid-state ^{13}C NMR spectra (Fig. 3) to evaluate the accuracy of quantitative assessment of BC$_{GS}$ derived condensed aromatic structures in soil. Data from these two independent methods match very well in an almost 1:1 line ($n = 16$, $R^2 = 0.98$). Only slight underestimation of the contribution of BC$_{GS}$ derived from the ^{13}C NMR spectroscopic analysis was observed (Fig. 4b). We attribute this to the differing chemical composition of the char used in the MMM of Nelson and Baldock (2005) (65% aryl-C) versus the slightly higher aryl-C contents (73%) in our BC$_{GS}$. As we found indications for microbial BC$_{GS}$ degradation, it may also be possible that small proportions of the BC$_{GS}$ were transformed into non-aromatic compounds (Luo et al., 2013; Singh et al., 2014) and are not any more identifiable as BC$_{GS}$ in the ^{13}C NMR spectra. In the fPOM fraction, the underestimation accounted for 8%, indicating that BC$_{GS}$ is detected almost completely by the ^{13}C CPMAS NMR analysis. Our results indicate that ^{13}C NMR spectroscopy accurately reveals the presence of higher BC proportions. But our data also show the limits of the technique for the detection and quantification of low proportions of BC in soils. Here a combination with other methods is recommended.

4.4. BC$_{GS}$ effects on plant growth, SOM composition and soil hydraulic properties

Although a number of studies revealed a positive effect of low temperature BC amendments on plant growth and therefore higher OM inputs (Glaser et al., 2002; Amonette and Joseph, 2009; Bell and Worrall, 2011; Basso et al., 2013), we did not find significant differences in grass yields during the first year after BC$_{GS}$ amendment (A.1).

The chemical composition of the SOC fractions of the control were similar to other loamy agricultural topsoils (Paetsch et al., 2016). They were dominated by polysaccharides and aryl-C in the POM fractions, whereas the clay fraction showed higher contribution of polysaccharides and alkyl-C. Gasification BC amendments resulted in strong changes in the chemical composition of POM fractions, which were dominated by BC_{GS} derived aryl-C. Its contribution decreased with particle size. In the clay fraction, the small contribution of BC_{GS}-derived C did not affect the SOM composition. As SOM increases could be related to BC_{GS}-C itself, we can conclude that BC_{GS} amendments did not change the quality of the native SOM associated with the clay fraction after one year of field exposure in agreement with results from Plaza et al. (2016).

Generally BC_{GS} amendments are intended to increase plant available water, specifically in coarse textured soils (Burrell et al., 2016) and thus are considered an option to improve moisture storage and thus water use efficiency of plants growing on such soils (Omondi et al., 2016). A better water supply or aeration due to BC_{GS} amendment hence may affect native as well as BC derived C degradation with consequences for biogeochemical cycles (Manzoni et al., 2012). This is not the case for the BC_{GS} amendment to the loamy topsoil investigated here, where the plant available water capacity was similar despite BC_{GS} amendment. In contrast, we found a trend to higher coarse pore volume in the BC_{GS} amended soils as compared to the control soils (Fig. 5a and b). The higher porosity of the loamy soil is selectively attributed to an increase of the coarse pore-volume, associated with a higher air-filled pore capacity. This effect may be due to the BC_{GS} itself and its allocation to the POM fractions. Loamy soils can specifically benefit from BC_{GS} amendments as the BC_{GS}, as the one used in this study, increase these soils' air capacity.

5. Conclusions

The fate of high-temperature BC industrially produced by gasification of maize pellets and applied to an agricultural soil was followed by tracing stable C isotope signatures and spectroscopic determination of aromatic C after one year of field exposure. Accounting of the BC balance in soil fractions suggests that ^{13}C CPMAS NMR spectroscopy allows for accurate quantification of condensed aromatic BC_{GS} in soil. Raman microspectroscopic analysis indicated that during the weathering, the unordered amorphous parts of the nanostructure are degraded and the more ordered graphitic-like parts remained resulting in increased recalcitrance of the residual proportion of the BC_{GS}. Moreover, BC_{GS} contributed to all physical fractions and changed the amended soil from a system dominated by organo-mineral associations to a POM dominated system. However, RM analysis suggests the same structural order of the graphitic-like structures in oPOM and clay fractions, and hence, only physical fragmentation and protection of the residual BC_{GS} in the soil structure with minor degradation. We conclude that loamy soils can benefit from BC_{GS} amendments, which increases the coarse pore volumes and can improve the soil functionality of such soils. However, results may depend strongly on the type of BC used. Our data show that BC_{GS} addition also affects native SOM through stimulation of C sequestration in associations with the fine-sized minerals.

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Appendix A. Supplementary material

Grass yields after harvesting in 2013 (A.1); solid-state ^{13}C NMR spectra of aged and non-weathered biochar. BC1-BC4 are field replicates of biochar amended soils (A.2). Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.orggeochem.2017.06.012.

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Manuscript III

The results of study III are summarized in the manuscript:

Effect of in-situ aged and fresh biochar on soil water holding capacity and microbial C use under drought conditions

by Lydia Paetsch, Carsten W. Mueller, Ingrid Kögel-Knabner, Margit von Lützow, Cyril Girardin, Cornelia Rumpel

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Effect of in-situ aged and fresh biochar on soil water holding capacity and microbial C use under drought conditions

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Abstract

Biochar (BC) amendments may be suitable to increase the ecosystems resistance to drought due to their positive effects on soil water retention and availability. We investigated the effect of BC *in situ* ageing on water availability and microbial parameters of a grassland soil. We used soil containing ^{13}C labeled BC and determined its water holding capacity, microbial biomass and activity during a 3 months incubation under optimum and drought conditions. Our incubation experiment comprised three treatments: soil without BC (Control), soil containing aged BC (BC_{aged}) and soil containing fresh BC (BC_{fresh}), under optimum soil water (pF 1.8) and drought conditions (pF 3.5).

Under optimum water as well as drought conditions, soils containing BC showed higher SOC mineralization as compared to control soil. Moreover, BC effects on soil water regime increase upon *in situ* aging. Native SOC mineralization increased most for soils containing BC_{aged}. The BC_{aged} led to improved C use under drought as compared to the other treatments. We conclude that BC addition to soils can ameliorate their water regime, especially under drought conditions. This beneficial effect of BC increases upon its aging, which also improved native substrate availability.

Keywords

Mineralization, microbial biomass, microbial activity, Biochar degradation, metabolic quotient (qCO_2), grassland, agricultural soil, carbon sequestration, water stress, gasification

Introduction

A major challenge of climate change is the increasing frequency of extreme hydrological events such as droughts (IPCC 2007), which will have strong impacts on terrestrial ecosystems and the biogeochemical C cycle [1]. In particular, droughts can affect the quantity and quality of organic matter (OM) retained in soil, as soil moisture is one of the most important factors driving microbial processes. In mineral soils, drought reduces microbial activity [2] due to physiological stress, but also due to limited substrate supply to microbial cells [3]. Microorganisms can react to this physiological stress by physiology changes or by a shift of their communities towards microbes with higher water stress resistance [4]. Highly stressed, microbes will then use substrate for maintenance and not for growth [5], which ultimately affects their metabolic efficiency.

Microbial activity response to drought was found to be dependent on soil organic matter quality [6] and soil properties [7]. Recently, addition of biochar (BC) to soil was suggested to ameliorate water retention under drought conditions [8]. Biochar is a highly aromatic material produced by thermal degradation of organic materials with limited or no air supply and is distinguished from charcoal by its use as a soil amendment [9]. Its addition to soil was found to alter the soil's physical structure and air capacity [10] and to be beneficial for water holding capacity (WHC), water retention, and plant available water [11,12]. However, depending on soil type, BC feedstock [13] and production conditions [14-16] the effects of BC on hydraulic properties strongly differ [17]. The degradation of BC itself is assumed to be mostly microbial induced, but Zimmerman [18] and Cheng, et al. [19] showed that abiotic BC-C release due to processes such as chemical oxidation, photooxidation, or solubilisation can significantly contribute to the C release. Aging after field exposure can significantly alter physico-chemical parameters of the BC [20] and therefore most probably its effects on soil parameters [21,22]. Biochar addition to soil may also change microbial community composition [23]. An indicator used to determine the microbial use of carbon is the metabolic quotient (qCO_2), considering the respiration rate CO_2-C per unit microbial biomass C [24]. The qCO_2 was found to decrease by 13% after BC amendment compared to the control indicating improved soil biophysical conditions [25]. While the microbial biomass responded with growth, the total soil CO_2 production remained unchanged after BC amendment.

Biochar properties strongly changed as a consequence of short term field exposure of less than 6 months. In particular, increasing surface area and changes in chemical properties were observed [26-28]. This resulted in increasing wettability of the BC over time, and hence, a better availability for microbial degradation [29]. Artificial weathering induced through chemical and/or physical treatments increased carbonyl and carboxylic functional groups as well as the biological stability of the residual BC [30,31].

We investigated the effects of physico-chemical changes occurring in BC amended soils with time of field exposure on the response of microbial parameters to drought stress. We hypothesized that BC aging impacts soil hydraulic properties and affects microbial activity under drought conditions. We set up an incubation experiment with soil containing ^{13}C labeled BC (1) added to soil after production (BC_{fresh}) and (2) sampled after 3 years of field exposure (BC_{aged}). Thanks to ^{13}C labeling, we were able to monitor mineralization of native SOC in addition to total SOC. Our specific objectives were to quantify the effect of fresh and aged BC (i) on soil hydraulic properties ii) microbial biomass, its activity and metabolic quotient and iii) native SOC mineralization. The soils were incubated under two different water potentials to investigate the impact of drought on these parameters.

Material and Methods

Study sites

The soil used in this study was sampled from a BC field experiment located at the site of the long-term field experiment "SOERE ACBB" managed by INRA (National Institute of Agricultural Research) in Lusignan (46°25"12.91"N; 0°07"29.35" E), France. Mean annual rainfall is 800 mm and the mean annual temperature is 11°C. The soil is classified as a Dystric Cambisol (IUSS Working Group WRB, 2014) with loamy texture (11% sand, 72% silt and 17% clay). We consider that total C concentrations represent OC because we did not find any evidence of inorganic C contribution to our soils using acid treatment. The organic carbon (OC) and total N content was 1.4 % and 0.16 %, respectively [32]. A single addition of 3 kg m^{-2} ^{13}C labeled BC to the upper 10 cm took place in May 2012 by a rotary hoeing. Before 2012, the field was a temporary grassland (C_3 plants) and is now cultivated with *Festuca arundinacea* and *Dactylis glomerata*. The BC was produced by gasification of maize (*Zea mays* L.) (C_4 plant) silage for 40 min at 1200°C in a commercial reactor (©A.G.T. – Advanced Gasification Technology s.r.l., Cremona, Italy). General parameters of the gazification BC used for the experiment are listed in Table 1.

Table 1: General parameters of A.G.T. biochar.

Parameter	**Unit**	
pH (0.01 *M* $CaCl_2$)		10.1 ± 0.2
salinity	mS cm^{-1}	9.6
Bulk density	g cm^{-3}	1.41
Ash (550°C)	% DM	10.3 ± 1.7
Carbon	%	69.5 ± 1.3
Nitrogen	%	1.6 ± 0.1
C/N		41
H/C		0.4
O/C		2.9
$\delta^{13}C$	‰	-13.7 ± 0.1

The field experiment comprised 8 plots of a size of 16 m²: 4 BC amended plots and 4 control plots. After three years, BC amendment led to pH increase from 6.0 (control) to 6.4 (BC). An aliquot of the BC used for the field experiment was stored in the laboratory for three years for reasons of comparison.

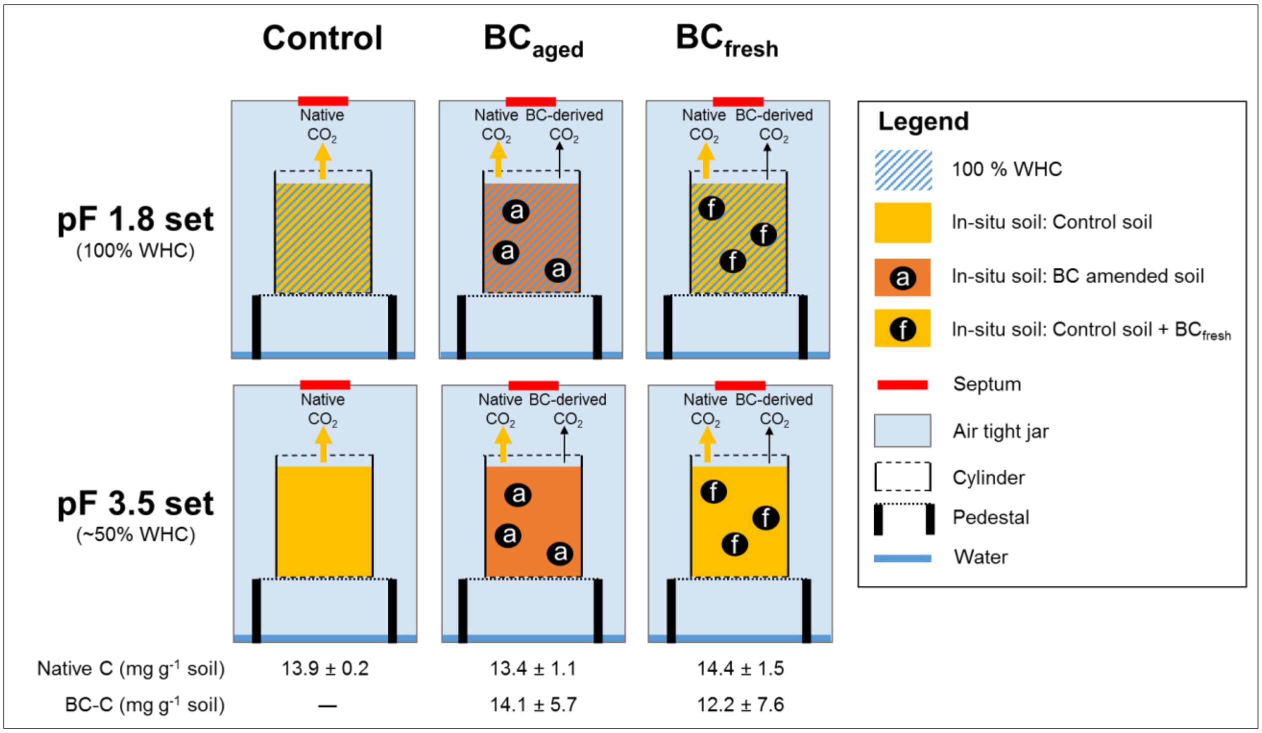
Sampling and Pre-treatment

For the incubation experiment disturbed soil samples were randomly taken from the top 10 cm from the eight soil plots in September 2015. Disturbed samples were stored in plastic bags and transported to the laboratory. We did not sieve the soils to retain BC pellets >2 mm. Plant residues and roots were manually removed. In addition, we collected three undisturbed (100 cm³ sampling rings) soil samples from each of the eight plots for determining soil water retentions curves of control soil and BC amended soils.

Incubation Experimental Design

The experimental design is presented in Fig. 1.

Fig. 1: Experimental set-up: Each amendment and set had three replicates, analyzed at five time points (n=30). Control soil was soil from the field experiment without BC addition. BC_{aged} was disturbed BC amended soil of the field experiment 3 years after field exposure. BC_{fresh} was control soil from the field experiment mixed with fresh BC.



Incubations were carried out in triplicates with three different treatments: control soil from the field experiment, BC containing soil from the field experiment sampled three years after the amendment (BC_{aged}) and control soil from the field experiment amended with fresh BC (BC_{fresh}). BC_{fresh} was the same BC as used for the field experiment but stored for three years in the laboratory. The amounts added were similar to those present in the field, which had been determined before using the C content and $\delta\,^{13}C$ ratio of BC amended soils from the field experiment.

We filled 160 g of the sample into plasic cylinders and compressed it to a bulk density of 1.4 g cm^{-3} (according to the *in-situ* bulk density). Afterwards, these samples were drained in pressure plate extractors at pF 1.8 and 3.5 to set up the experimental water conditions, representing a water holding capacity (WHC) of 100% and about 50%, respectivly.

The cylinders were then placed in 1 L glass jars and 10 ml distilled water were added to the jars to maintain soil humidity. In total the experment consisted of 90 jars. All jars were flushed for 20 min with moistened CO_2 free-air to remove CO_2 from the jars atmosphere and capped with air-tight lids. The jars were incubated in the dark at a constant temperature of 20°C to minimize abiotic degradation by photooxidation. Decomposition was measured by monitoring the CO_2 release at days 2, 7, 15, 28 and 90 using a MICROGC (Agilent, Santa Clara, USA). In addition, we determined the stable isotope signature of the mineralised CO_2 using a isotopic mass spectrometer (Microgas, Hawlett 500P). This allowed to distinguish C_4-BC mineralisation from native C_3-SOC mineralisation using the isotopic mass balance. After gas measurement, the jars were flushed and sealed as discribed above. At the measuring days whole sample sets were removed to determine water content, microbial biomass and contents of C and N. To avoid anaerobic conditions, we additionally measured gases and flushed the jars at least every 14 days throughout the incubation period.

The C and N concentrations of bulk soils and fresh BC were measured by dry combustion with an elemental CNS analyser (elementar vario MAX CUBE, Hanau, Germany). The $\delta^{13}C$ signature of the pure fresh BC was $\delta^{13}C = -13.7$ ‰ and of the C_3-SOM $\delta^{13}C = -27.3$ ‰.

Microbial biomass

Extraction of microbial biomass was carried out after modification of the chloroform fumigation extraction method [33]. Each sample was divided into two sub-samples of 10 g: a non-fumigated reference sample and a sample fumigated with chloroform. The fumigated samples were incubated under ethanol-free chloroform ($CHCl_3$) vapor in a desiccator for 16 h, followed by 6 vacuum-purge cycles to remove the $CHCl_3$. Both sets were extracted with 40 ml of 0.03 *M* K_2SO_4, shaken in an overhead shaker for 30 min and centrifuged for 10 min with 10,000 RPM. The supernatants were removed, filtered, frozen to -20°C and freeze dried. Organic C and N content as well as $\delta^{13}C$ signature of the K_2SO_4-extractable C were analyzed using an isotope ratio mass spectrometer (Delta V Advantage Thermo Fisher Scientific, Bremen, Germany) coupled with an elemental analyzer (Flash 2000, Thermo Fisher Scientific, Bremen, Germany). All $\delta^{13}C$ values were expressed relative to the Pee Dee Belemnite (PDB) international isotope standard.

Calculations and statistics

The proportion of native C in the microbial biomass (bC_3) was calculated by the two component stable isotopic mixing model approach (IMM) after Balesdent and Balabane [34]:

$$bC_3 = \frac{\delta C_{mixture} - \delta C_4}{\delta C_3 - \delta C_4} \qquad (1)$$

where δC_4 is the $\delta^{13}C$ isotope signature of the pure BC and δC_3 of the native SOC of the control soil. $\delta C_{mixture}$ is the $\delta^{13}C$ signal of the soil sample extracts. The same model was used to partition the BC-C contribution to the CO_2 efflux. For the determination of respired BC-C, the BC-C proportion were multiplied by accumulated CO_2–C. Correspondingly, remaining BC-C in the soil was calculated by subtracting mineralized BC-C from total BC-C added at the beginning of the experiment. The extent of the priming effect of the biochars on native SOC mineralization were calculated as the difference between the CO_2 efflux from SOC in the control compared to the native CO_2 efflux of native SOC from soil-biochar mixtures, estimated with the two-component isotopic mixing model.

Microbial biomass was calculated by dividing the measured OC concentrations per g dry soil by the factor k_{EC} =0.45. The extractable C of the non-fumigated set were used as approximated values for salt-extractable organic carbon (DOC).

The microbial biomass based metabolic quotient (qCO_2) was calculated to evaluate the microbial C use efficiency and the substrate availability. A high metabolic quotient may indicate a low efficiency of C mineralization and a higher substrate availability [35]. The qCO_2 was calculated by using the equation

$$qCO_2 = \left(\frac{CO_2-C}{C_{mic}}\right) \qquad (2)$$

where CO_2–C is the cumulative respired CO_2-C in mg kg^{-1} soil h^{-1} and C_{mic} the corresponding microbial biomass C in g kg^{-1} soil.

By using the software SHIPFIT2.0 [36], we fitted the water retention characteristics to the data by using the unimodal Kosugi retention function [37] given by:

$$\Gamma(h) = \frac{1}{2}\, erfc\left[\frac{ln\left(\frac{h}{h_m}\right)}{\sqrt{2}\sigma}\right] \qquad (3)$$

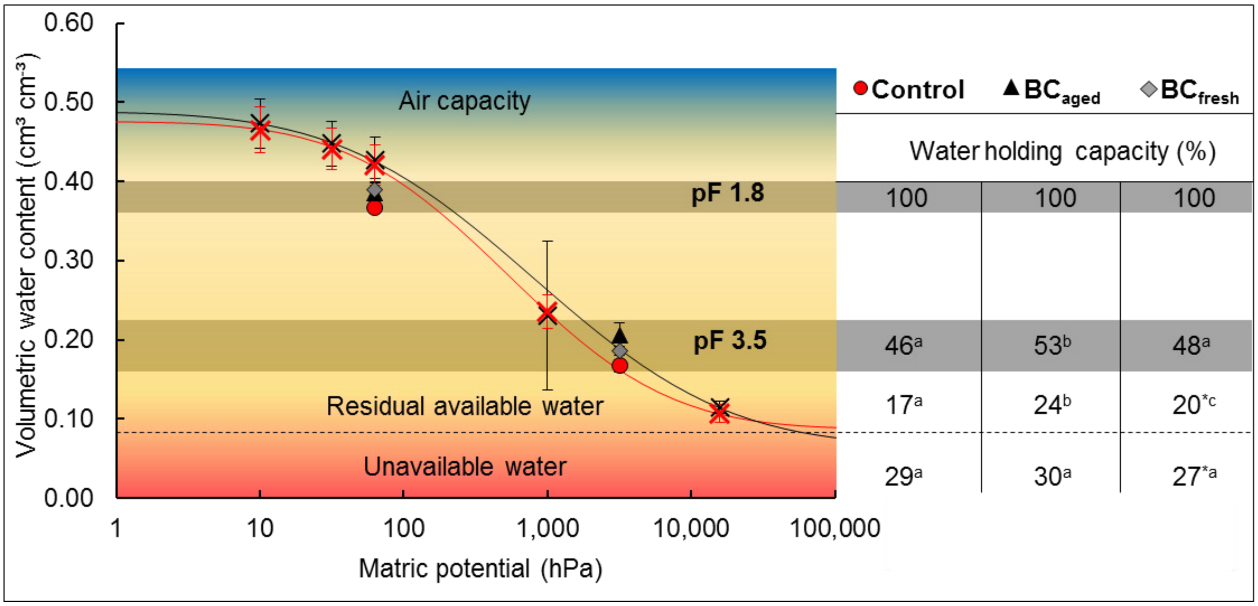
where h_m (L) is the pressure head corresponding to the median pore radius, σ (–) is the standard deviation of the log-transformed pore-size distribution density function, and erfc() is the complementary error function. For unsaturated soils h (L) is defined as positive.

For statistical analyses we used the software RStudio, version 3.3.1 for Windows [38]. Significant differences between the amendments and control were tested with a one-way analysis of variance (ANOVA).

Results

While the water retention curves of undisturbed field samples showed little differences for plots with and without BC, in the incubation experiment, volumetric water contents of BC containing soils at pF 3.5 were significantly increased compared to the control (Fig. 2).

Fig. 2: Volumetric water contents of the undisturbed soil samples (red (control) and the black crosses (BC$_{aged}$) (n=12) fitted with the model of Kosugi [37] (lines)). The water holding capacities (WHC) of the pF 1.8 and 3.5 sets (n=30) were determined after the incubation. Data within one row with different letters are significantly different ($p<0.05$).



Moreover, after incubation, BC$_{aged}$ showed higher volumetric water contents compared to BC$_{fresh}$ at pF 3.5. Air capacity as well as plant unavailable water (> pF 4.2) was not affected by the amendments. However, the presence of BC$_{aged}$ increased remaining plant available water under drought conditions (pF 3.5 to 4.2) compared to control soil and soils containing BC$_{fresh}$.

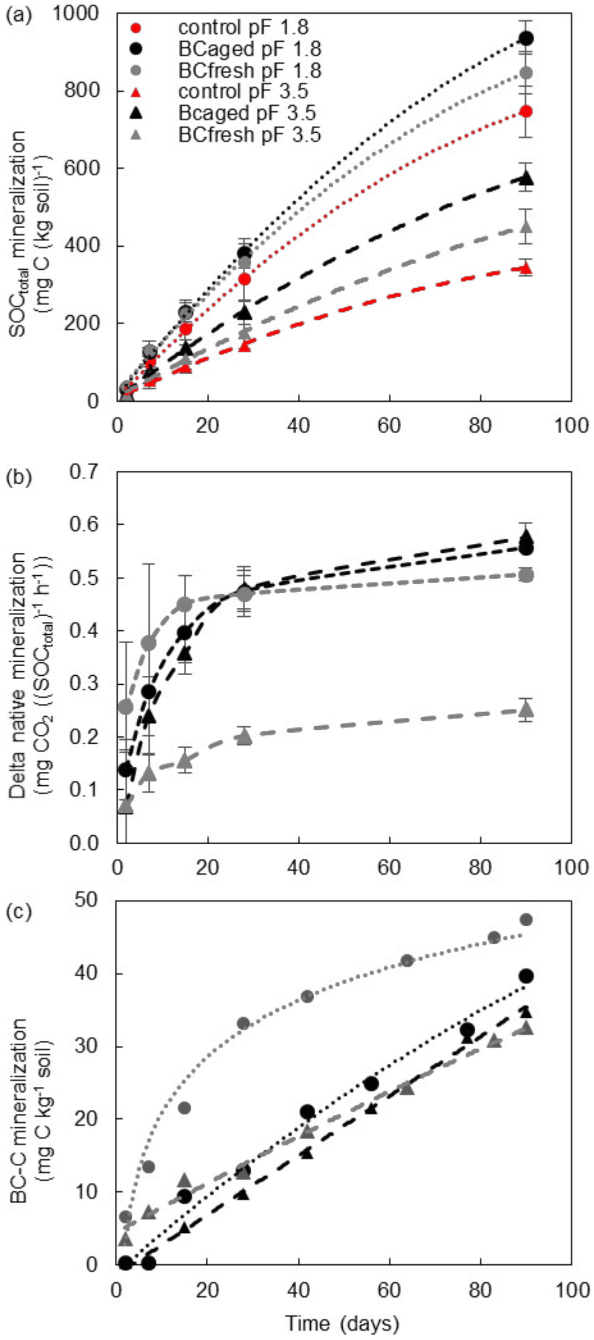
Carbon and N contents are given in Table 2. Following BC addition (aged or fresh), the SOC contents doubled, whereas SON contents increased only by about 16 %. Consequently, C to N ratio increased from 9.8 for control soil to 16.5 or 15.7 for soils containing BC$_{aged}$ or BC$_{fresh}$, respectively.

Table 2: Mean C contents, N contents and C to N ratios of bulk soils (n=18) from 0 days to 90 days of incubation with standard deviations. Different letters within one column mark significant different values ($p<0.05$). Control soil was soil from the field experiment without BC addition. BC$_{aged}$ was disturbed BC amended soil of the field experiment 3 years after field exposure. BC$_{fresh}$ was control soil from the field experiment mixed with fresh BC.

	pF value	C content	N content	C to N ratio
		mg g^{-1}	mg g^{-1}	
Control	1.8	$13.88^a \pm 0.41$	$1.44^a \pm 0.03$	$9.62^a \pm 0.17$
	3.5	$14.01^a \pm 0.33$	$1.41^a \pm 0.03$	$9.91^a \pm 0.03$
BC$_{aged}$	1.8	$28.27^b \pm 4.36$	$1.65^b \pm 0.08$	$16.83^b \pm 1.54$
	3.5	$26.87^b \pm 2.29$	$1.65^b \pm 0.04$	$16.09^b \pm 0.92$
BC$_{fresh}$	1.8	$27.52^b \pm 5.82$	$1.69^b \pm 0.09$	$15.90^b \pm 0.84$
	3.5	$26.38^b \pm 3.21$	$1.69^b \pm 0.06$	$15.55^b \pm 0.59$

The cumulative total C mineralization, native SOC and BC-C emissions during the 90 days of incubation are presented in Fig. 3a-c.

Fig. 3 a-c: a) Cumulative CO_2 emissions, b) additional native mineralization (mg per kg SOC) in BC amended soil and c) cumulative BC-C mineralization (mg per kg) during 90 days of the experiment. Control soil was soil from the field experiment without BC addition. BC_{aged} was disturbed BC amended soil of the field experiment 3 years after field exposure. BC_{fresh} was control soil from the field experiment mixed with fresh BC.



Our results show two separated groups with regard to the OC mineralization according to the soil water potential (Fig. 3a). The total respired C at pF 1.8 was higher than at pF 3.5 for all three treatments. Under optimum water conditions (pF 1.8), the highest C mineralization was observed for BC_{aged} (935.5 mg C kg^{-1} soil) followed by BC_{fresh} (846.5 mg C kg^{-1} soil) and control (734.0 mg C kg^{-1} soil). Under drought conditions (pF 3.5), these values decreased by 54 % for the control soil and by 38 % and 47 % for BC amended soils (BC_{aged} or BC_{fresh}, respectively).

Both amendments increased native SOC mineralization (Fig. 3b). The highest native SOC losses were found at optimum water conditions for BC_{aged} (896.3 mg C g^{-1} soil). This corresponds to a positive priming effect leading to 21 % increased native SOC mineralization with regards to the control. The addition of BC_{fresh} induced a lower priming effect corresponding to 8 % increase of native SOC mineralization as compared to the control. Under drought conditions, the increase of native SOC mineralization was even higher, with BC_{aged} leading to 59 % and BC_{fresh} to 22 % more native C loss compared to the control.

The BC-C contributions to the respired CO_2-C were higher under drought than under optimum conditions (Fig. 3c). Biochar derived C in BC_{aged} soils accounted for 3.5 % (pF 1.8) and 5.9 % (pF 3.5) and in BC_{fresh} soils for 5.6 % (pF 1.8) and 8.8 % (pF 3.5) of the total mineralized C, over the 90 days of incubation period. However, low amounts of BC-C were mineralized during the 90 days of incubation. Less than 0.5 % of BC-C was respired. Higher BC-C proportions were respired in soils containing BC_{fresh} (0.3 % of BC-C) than in soils containing BC_{aged} (0.2 % of BC-C). Despite a 'BC-C flush' at pF 1.8 after BC_{fresh} addition at the beginning of the experiment (Fig. 3c), the mineralization of both BC types differed only marginal after 90 days.

Under optimum water conditions, microbial biomass ranged between 155.4 ± 12.4 mg kg^{-1} soil to 171.2 ± 13.7 mg kg^{-1} soil at the beginning of the experiment. After 90 days of incubation, microbial biomass decreased in all treatments and ranged between 98.2 ± 6.2 mg kg^{-1} soil and 121.7 ± 3.3 mg kg^{-1} soil. Similar values were recorded for drought conditions (Table 3).

Table 3: Microbial biomass C (MBC) of all treatments during the 90 days of incubation. Control soil was soil from the field experiment without BC addition. BC$_{\text{aged}}$ was disturbed BC amended soil of the field experiment 3 years after field exposure. BC$_{\text{fresh}}$ was control soil from the field experiment mixed with fresh BC.

Set		Unit	Treatment	Incubation time (days)				
				2	7	15	28	90
pF 1.8	MBC	mg C (kg soil)$^{-1}$	Control	158 *	142.9 ± 7.9	149 ± 4.2	108.4 ± 4.0	98.2 ± 6.2
			BC$_{\text{aged}}$	171.2 ± 13.7	162 ± 1.0	154.7 ± 33.8	168.7 ± 34.0	111.2 ± 11.4
			BC$_{\text{fresh}}$	155.4 ± 12.4	156.5 ± 16.6	160.9 ± 4.3	155.1 ± 3.7	121.7 ± 3.3
	Proportion BC-C	%	BC$_{\text{aged}}$	-8.1 ± 0.4	-7.1 ± 1.8	-0.4 ± 1.4	-1 ± 1.0	-1.4 ± 1.6
			BC$_{\text{fresh}}$	-4.6 ± 0.6	-2.3 ± 1.5	-0.7 ± 0.4	2.9 ± 1.0	**3.4 ± 1.7**
pF 3.5	MBC	mg C (kg soil)$^{-1}$	Control	130.3 ± 9.2	138.6 ± 3.1	137.2 ± 12.3	136.1 ± 16.4	118.3 ± 13.9
			BC$_{\text{aged}}$	137 ± 9.1	157.7 ± 37.4	145.7 ± 16.8	157 ± 42.1	132.8 ± 1.5
			BC$_{\text{fresh}}$	151.7 ± 0.8	162.6 ± 5.5	153.1 ± 5.8	156.7 ± 4.2	162.2 ± 18.7
	Proportion BC-C	%	BC$_{\text{aged}}$	-4.4 ± 1.2	-5.8 ±8.9	-0.8 ± 1.8	-2 ± 1.7	-4.3 ± 1.1
			BC$_{\text{fresh}}$	1.2 ± 2.7	0.2 ± 0.9	0.5 ± 1.4	2.5 ± 0.7	-1.2 ± 2.5
pF 1.8	MBC	mg C (kg SOC)$^{-1}$	Control	10.9 *	10.4 ± 0.3	11 ± 0.4	7.5 ± 0.4	7.3 ± 0.1
			BC$_{\text{aged}}$	5.7 ± 2.2	7 ± 3.3	6.4 ± 1.4	6.7 ± 1.4	3.6 ± 1.2
			BC$_{\text{fresh}}$	6.2 ± 3.7	4.4 ± 1.3	6.2 ± 1.1	7.4 ± 2.4	5.3 ± 1.5
pF 3.5		mg C (kg SOC)$^{-1}$	Control	9.2 ± 0.6	9.9 ± 0.2	10.1 ± 0.5	9.5 ± 1.0	8.5 ± 0.6
			BC$_{\text{aged}}$	4.8 ± 1.6	6.4 ± 3.5	5.5 ± 1.0	6.3 ± 3.1	5.3 ± 1.2
			BC$_{\text{fresh}}$	4.6 ± 1.1	6 ± 0.9	5.8 ± 0.1	7.5 ± 0.7	5.9 ± 1.2

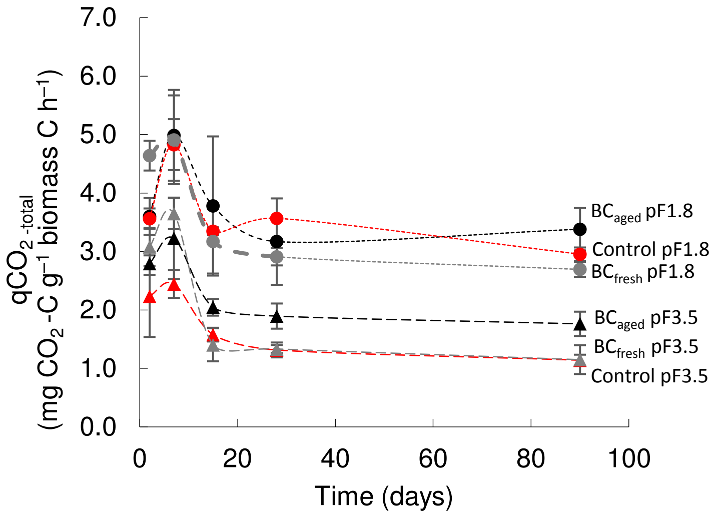
* no replicates

Between the start and the end of the experiment, the microbial biomass of BC amended soils decreased by 35 % and 38 % at pF 1.8 (aged and fresh), whereas it remained rather constant at pF 3.5. The δ^{13}C abundance in the fumigation extracts from control, BC$_{\text{aged}}$ and BC$_{\text{fresh}}$ were very similar resulting in high uncertainties for the calculation of the BC-C proportion (Table 3).

Within the first week of the incubation experiment, microbial biomass from all samples showed a depletion of ^{13}C to differing degrees (up to 8.1 ± 0.4 % for BC$_{\text{aged}}$ at pF 1.8). This effect leveled out with progressing time but microbial biomass in the treatments BC$_{\text{aged}}$ remained depleted in ^{13}C at both water potentials compared to the control after 90 days of incubation. BC$_{\text{fresh}}$ showed 3.4 ± 1.7 % incorporation of BC derived C into microbial biomass under optimum conditions, whereas under drought, BC-C was not incorporated into the soil microbial biomass.

The metabolic quotient (qCO_2) of the control soils decreased during incubation from 3.56 to 2.95 mg CO_2 -C g^{-1} biomass C h^{-1} under optimum water conditions and from 2.24 to 1.14 mg CO_2 -C g^{-1} biomass C h^{-1} under drought conditions (Fig. 4).

Fig. 4: Metabolic quotient (qCO_2) during the incubation experiment. Every data point represents a mean value of three replicates with standard deviation. Control soil was soil from the field experiment without BC addition. BC_{aged} was disturbed BC amended soil of the field experiment 3 years after field exposure. BC_{fresh} was control soil from the field experiment mixed with fresh BC.



Due to the CO_2 flush and consistent microbial biomass C in the first week, the qCO_2 increased in all treatments. In drought-affected soils, only BC_{aged} addition had an effect on qCO_2. The qCO_2 of soils containing BC_{fresh} remained similar to the qCO_2 of the control (1.15 and 1.14 mg CO_2 -C g^{-1} biomass C h^{-1}, respectively) after 90 days of incubation. In contrast, BC_{aged} showed a significantly higher qCO_2 (1.76 mg CO_2 -C g^{-1} biomass C h^{-1}).

Discussion

Biochar effects on water conditions

We found a positive effect of BC on the water holding capacity, which was more pronounced in the BC_{aged} than in the BC_{fresh} amended soil (Fig. 2). BC_{aged} treatments showed at the end of the incubation experiment increased volumetric water content under drought conditions compared to the other treatments. This additional water can be assigned to plant available water because the unavailable water (> pF 4.2) was not affected by BC addition (Fig. 2). We assume that fragmentation and mechanical stresses of freeze-thaw cycles during field exposure form new cracks and fractures and thus increase the pore connectivity of BC$_{\text{aged}}$ compared to BC$_{\text{fresh}}$ particles [21]. The additionally retained plant available water could reduce water stress and hence retard drought effects to plants [39].

Biochar effects on microbial activity

As found in previous studies e.g. [2,7], total mineralization decreased with decreasing water contents in all treatments (Fig. 3a). Nevertheless, SOC mineralization decreased less in BC containing soils and was strongly dependent on the nature of BC (fresh or aged; Fig. 3a). In agreement with the literature, under optimum water conditions, we found a flush of SOC mineralization at the beginning of the experiment for treatments with BC$_{\text{fresh}}$ (pF 1.8). This flush was observed in many studies after addition of fresh BC to soil [40-43], and may be related to mineralization of labile C compounds of the BC and stimulation of native SOC mineralization [44,]e.g. [45]. It may indicate an immediate adaption of microorganisms to BC$_{\text{fresh}}$ usage [46] but is most likely a short-term effect on SOM e.g. [47]. Responsible for this fast response might be so-called ‘r-strategist’ microbes, which are adapted to respond quickly to newly available C sources, which may be present in BC in form of volatile organic matter [48,49]. These organisms re-mineralize soil nutrients and co-metabolize more refractory OM in the process (Kuzyakov et al., 2000; Kuzyakov, 2010).

The absence of a mineralization flush in the beginning of the experiment for BC$_{\text{aged}}$ treatments may be explained by depletion of labile C compounds leaving behind a recalcitrant BC residue [10,44,50]. After degradation of labile BC components, it was even found that BC lowered native SOC mineralization under the level of control samples, likely by toxic compounds of the BC or mineral adsorptive protection [51,52]. Higher native C mineralization in treatments with BC$_{\text{aged}}$ as compared to BC$_{\text{fresh}}$ may be explained by sorption of potential inhibitors in the SOM. Extracellular reactions could consequently increase the breakdown of native SOM [53]. Increased drought intensified the effect of BC aging on native SOC mineralization (Fig. 3b).

Microbial biomass growth and incorporation of Biochar-C

Microorganisms benefit from improved water supply during droughts due to the presence of BC as indicated by increased microbial biomass and microbial activity as compared to the control (Table 2 and 3). The values for incorporation of BC-C into microbial biomass presented here reflect the maximal possible values as chloroform can contribute to a dissolution of BC and extraction with K_2SO_4 may overestimate BC-C incorporation into microbial biomass [47,54]. For all amendments, we found a general trend of decreasing microbial biomass at pF 1.8 (22 % in BC_{fresh} and 38 % in the control), whereas it remained rather constant at pF 3.5 (-7 % in BC_{fresh} and 9% in the control) during the 90 days of incubation (Table 3). The dynamics of microbial biomass at pF 1.8 may be related to a fast consumption of C, followed by a depletion of easy available substrate. In contrast, at pF 3.5 the development of microbial biomass suffers from water stress as indicated by stagnating values.

We found no significant differences in microbial biomass between the three treatments under optimal water conditions (Table 3). However, the BC_{fresh} addition affected the dynamics of microbial biomass during the experiment, maintaining higher total biomass amounts as compared to soils containing BC_{aged} or control soils. Zhou, et al. [25] found in BC amended soils an overall moderate increase in microbial biomass by 26%, which tended to decrease with increasing duration of the experiment. The increase in microbial biomass may be explained by microbial use of the labile or extractable carbon pool [55] BC, and the decreasing effect with time to its exhaustion [47,56]. The porous structure of BC can be a suitable habitat for microbes (Lehmann et al., 2014), offering favorable microsites and protection from predators [57]. This aspect, however, tend to play a minor role, as microsites and therefore microbial biomass should increase with aging. The BC_{aged}, however, showed no significant effects on microbial biomass compared to the control.

The effect of BC properties are underlined by Ameloot, et al. [58] and Durenkamp, et al. [59], who found even lower microbial biomass in BC amended soils than in the control and related this to BC production conditions or / and feedstocks. Wood derived BC, as used in their studies may be unfavorable for microbial colonization [51,60].

Under drought conditions, we observed stable microbial biomass in the control soil during the 90 days incubation period. In BC_{aged} soils, microbial biomass tended to increase only during the first 28 days, whereas BC_{fresh} addition augmented microbial biomass by 37 % compared to the control. These observations may be explained by more favorable water conditions in BC containing soils, combined with easily decomposable compounds in the case of BC_{fresh} addition.

In contrast to the significant BC-C mineralization (Table 2, Fig 3a), the incorporation into microbial biomass is rather low (Table 3). Incorporation of BC-C into microbial biomass strongly varies between fresh and aged BC. Incorporation of BC_{fresh} into the microbial biomass (Table 3) demonstrates that labile BC-C was utilized not only as energy source but also as a C substrate by microorganisms [47,51,61]. Microorganisms tended to incorporate BC_{fresh} at pF 1.8, at similar amounts as observed by other authors (e.g. Kuzyakov, et al. [47] (1.5 to 2.6 %). In contrast, BC_{aged} seems to be preferentially mineralized, as the ^{13}C content of the microbial biomass was depleted (Table 3).

Additionally, the BC-C proportion to OC released at pF 3.5 significantly increased compared to pF 1.8 (Table 2, Fig. 3c). The low but continuous proportions of BC-C released throughout the experiment in BC_{aged} treatments (Fig. 3c) indicate a persistent microbial use of BC-C. In contrast, Ameloot, et al. [58] found that wood derived BC was not used as substrate for microorganisms after 1 to 4 years of field exposure.

Effects of Biochar on metabolic efficiency

The qCO_2 or the specific respiration rate is used to evaluate the metabolic efficiency of the soil microbial biomass. In this experiment the measured qCO_2-values of 1.14 – 4.99 mg CO_2 -C g^{-1} biomass C h^{-1} are in range to other disturbed arable soils [62]. We found the highest qCO_2-values of 3.56 – 4.64 mg CO_2 -C g^{-1} biomass C h^{-1} (pF 1.8) and 2.24 – 3.08 mg CO_2 -C g^{-1} biomass C h^{-1} (pF 3.5) at the beginning of the experiment. Thereafter, the values decreased slightly in all treatments until the end of the incubation (Fig. 4). Only at day 7 of incubation, the qCO_2 increased by 6 – 39% (pF 1.8) and 9-19% (pF 3.5) due to the CO_2 flush. Decreasing qCO_2 in our experiment can be explained by relatively constant mineralization (per h) and decreasing microbial biomass (Figure 3a and Table 3) in contrast to many other studies, where respiration remained constant but microbial biomass increased [25]. High qCO_2 values imply relatively 'large' C losses (through respiration) and less C converted to biomass, ultimately reducing the potential for long-term C sequestration in organo-mineral complexes [63,64]. However, as changes in mineralization were not in parallel to decreases in microbial biomass, this could suggest microbial community shifts. We cannot exclude changes in microbial community composition, which additionally would affect the qCO_2 because distinct microbial groups are able to decompose and assimilate C compounds at different rates depending on their composition (Waldrop & Firestone, 2004). These have been evidenced with some taxa-specific community changes in the works by Farrell, et al. [48], [65]Chen, et al. [66] and Gomez, et al. [67]. Gomez, et al. [67] additionally found that as BC amendments became larger, the decrease of microbial biomass with time was alleviated. This suggests that the BC confers buffering on the microbial community. Another explanation for microbial biomass decreases but constant mineralization could be recycling of the dead microbial biomass as a labile C source. The high qCO_2 of all treatments at pF 1.8 could further indicate nutrient gain by overflow respiration or C excretion ('luxury consumption', 'waste metabolism'[68], 'N-mining' [69]). This is also found for nutrient-limited conditions across a wide range of soil and litter types e.g. for N by [69].

Whereas all treatments at pF 1.8 show similar qCO_2-values, water stress induced significant BC effects. At pF 3.5, a low qCO_2 of 1.14 mg CO_2 -C g^{-1} biomass C h^{-1} and 1.15 mg CO_2 -C g^{-1} biomass C h^{-1} in control and BC_{fresh} samples could be ascribed to shifts from growth to maintenance respiration or preparation for dormancy stages as water stress is an important constraint for microorganisms. This is explained by limited substrate supply due to slow diffusion rates along the increasingly tortuous paths of thin water films or a change in the physiology of microbes as they adjust to more desiccating conditions [4]. For example, intracellular solutes are accumulated, which affect microbial growth biochemically because of high costs for osmoregulation [70]. Highly stressed, microbes will then use substrate for maintenance energy requirements and not for growth [5].

In contrast, the qCO_2-values in soils containing BC_{aged} differ from the other two treatments, showing a qCO_2-value of 1.76 mg CO_2 -C g^{-1} biomass C h^{-1} under drought conditions. In general, higher qCO_2-values suggest improved biophysical conditions for microbial activity. We assume that control soil and soil containing BC_{fresh} were strongly affected by water stress as reflected by stagnating microbial biomass and lower C-mineralization than in BC_{aged} samples. This suggests that higher available water in the latter treatment might have led to improved conditions and microbes remaining longer metabolically active with retardation of their dormant stage.

Conclusion

We investigated water content, microbial biomass and activity under contrasting water conditions in temperate grassland soils containing similar amounts of fresh and aged BC produced by gasification. We conclude that aging of BC significantly increased plant available water in drought-affected soils. Both BC amendments led to considerable increases in SOC mineralization despite water stress, with

BC_{aged} showing the greatest effects. Moreover, the BC_{fresh}-treatment maintained microbial biomass, whereas the BC_{aged} treatment showed significantly increased qCO_2 values. While representing only a small fraction of the C mineralized, BC seems to be a constantly available C source. We thus conclude that BC addition to soil is beneficial for microbial biomass and activity under drought and that these effects are increasing with time after field exposure. Our results have further implications. Particularly, the results presented in this study support the assumption that BC amendment may be a viable means of mitigating current and future water shortages on drought-affected soils and due to climate change with major positive effects for available water for plant and microbial activities.

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Authors contribution

L.P. designed and conducted experiment and analyses, evaluated the data and wrote the manuscript; C.W.M. designed the experiment, evaluated the data and wrote the manuscript; I.K.K. designed the experiment and wrote the manuscript; M.vL. evaluated the data and wrote the manuscript; C.G. conducted experiments and analyses; C.R. designed the experiment and wrote the manuscript.

Competing financial interests

The authors declare no competing financial interests.

Anhang I

Eidesstattliche Erklärung

Ich erkläre an Eides statt, dass ich die bei der promotionsführenden Einrichtung

Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt, Lehrstuhl für Bodenkunde

der TUM zur Promotionsprüfung vorgelegte Arbeit mit dem Titel:

Compost and biochar amendments to agricultural soils: Alteration of soil water conditions and organic matter composition.

in Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt, Lehrstuhl für Bodenkunde

Fakultät, Institut, Lehrstuhl, Klinik, Krankenhaus, Abteilung

unter der Anleitung und Betreuung durch: Frau Univ. –Prof. Dr. Dr. h.c. Ingrid Kögel-Knabner ohne sonstige Hilfe erstellt und bei der Abfassung nur die gemäß § 6 Ab. 6 und 7 Satz 2 angebotenen Hilfsmittel benutzt habe.

☒ Ich habe keine Organisation eingeschaltet, die gegen Entgelt Betreuerinnen und Betreuer für die Anfertigung von Dissertationen sucht, oder die mir obliegenden Pflichten hinsichtlich der Prüfungsleistungen für mich ganz oder teilweise erledigt.

☒ Ich habe die Dissertation in dieser oder ähnlicher Form in keinem anderen Prüfungsverfahren als Prüfungsleistung vorgelegt.

☐ Die vollständige Dissertation wurde in ____________________ veröffentlicht. Die promotionsführende Einrichtung

____

hat der Veröffentlichung zugestimmt.

☒ Ich habe den angestrebten Doktorgrad noch nicht erworben und bin nicht in einem früheren Promotionsverfahren für den angestrebten Doktorgrad endgültig gescheitert.

☐ Ich habe bereits am __________ bei der Fakultät für ____________________

____

der Hochschule ____________________

unter Vorlage einer Dissertation mit dem Thema ____________________

____

die Zulassung zur Promotion beantragt mit dem Ergebnis: ____________________

____

Die öffentlich zugängliche Promotionsordnung der TUM ist mir bekannt, insbesondere habe ich die Bedeutung von § 28 (Nichtigkeit der Promotion) und § 29 (Entzug des Doktorgrades) zur Kenntnis genommen. Ich bin mir der Konsequenzen einer falschen Eidesstattlichen Erklärung bewusst.

Mit der Aufnahme meiner personenbezogenen Daten in die Alumni-Datei bei der TUM bin ich

☒ einverstanden, ☐ nicht einverstanden.

Freising, 21.11.2017, Unterschrift

Presentations

2016

Lydia Paetsch, Carsten W. Mueller, Cornelia Rumpel, Sabine Houot, Ingrid Kögel-Knabner, Waste additions to soils: Long-term organic carbon storage to mitigate climate change or just a flash in the pan? Second HEZagrar PhD symposium, Freising, 21. 04. 2016

2015

Lydia Paetsch, Carsten W. Mueller, Cornelia Rumpel, Sabine Houot, Ingrid Kögel-Knabner, Fate of organic carbon from different waste materials in cropland soils, General Assembly of the European Geosciences Union, Vienna, 12.-17.04.2015

Lydia Paetsch, Carsten W. Mueller, Cornelia Rumpel, Sabine Houot, Ingrid Kögel-Knabner, Stabilisierung organischer Bodensubstanz in Agrarböden anhand eines Langzeitversuchs mit Kompost- und Mistdüngung, Annual conference of the German Soil Science Society (DBG), Munich, 07.-12.09.2013

Lydia Paetsch, Carsten W. Mueller, Cornelia Rumpel, Sabine Houot, Ingrid Kögel-Knabner, Long-term urban waste compost amendment enhances OC and N stocks but does not alter organic matter composition, Assemblée Générale du SOERE PRO, Colmar, 24.-25.11.2015

2014

Lydia Paetsch, Carsten W. Mueller, Cornelia Rumpel, Ingrid Kögel-Knabner, Sicherung der landwirtschaftlichen Produktivität durch die Verbesserung der Wasserhaltekapazität- Projektvorstellung und erste Ergebnisse - Chair of soil conservation, Berlin 10.06.

2013

Lydia Paetsch, Thomas Nehls, Sarah Rokia, Christoph Schwartz, Gert Wessolek, Phosphorvorräte und -nachlieferung technogener Substrate (Phyto-P-mining), Annual conference of the German Soil Science Society (DBG), Rostock, 07.-12. 09. 2013

Freising, den 21. November 2017