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# Biochar amendment for C sequestration in a temperate agroecosystem - Implications for microbial C- and N-cycling

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# 1 Summary

Climate warming will have great impact on terrestrial ecosystems. Different soil properties such as temperature and moisture will be altered, thereby influencing C- and N-cycles, soil microbial activity, abundances and community composition as well as plant growth. This may contribute to the observed increase in soil greenhouse gas (GHG) emissions under climate change. Therefore, new soil management options are needed to mitigate theses projected consequences. Biochar is primarily suggested to be effective in long-term C sequestration in agricultural soils due to its long-term stability. In addition, it could be applied to improve physical, chemical and biological soil properties, plant growth and to reduce soil GHG emissions. To date, knowledge about such beneficial biochar effects in soil under predicted warming climate is extremely scarce. This thesis presents novel results on the interactive effects of biochar and soil warming on soil properties, microorganisms, crop growth and GHG emissions on field scale to evaluate biochar's future potential for long-term C sequestration in temperate agricultural soil.

This thesis is composed of three studies. In the first study, a slow-pyrolysis biochar from *Miscanthus* x *giganteus* feedstock (600 °C, 30 Min.) was incubated for short time (37d) under controlled laboratory conditions in agricultural soil in the presence of earthworms and N-rich litter (*Phacelia tanacetifolia* Benth.). It was aimed to investigate the potential of biochar to reduce the emissions of $CO_2$ and $N_2O$ from soil. In addition, it was examined whether possible interactions between biochar and earthworms could affect soil GHG emissions and microbial abundances. The field experiment, investigated in the second and third study, focused on the stability and long-term soil C sequestration potential of comparable *Miscanthus* biochar (850 °C, 30 Min.). Related effects on soil GHG emissions, physical, chemical and microbiological soil properties as well as plant growth were determined in an agroecosystem at year-round elevated soil temperature (+2.5 °C, since 2008).

Biochars produced from the C4 crop *Miscanthus x giganteus* differed in their isotopic ($^{13}C$) signature from investigated C3 soil. This made it possible to follow the fate of applied biochar-C into different compartments such as evolved $CO_2$, earthworm or microbial biomass. Soil microbial abundances and community composition were analyzed by using extraction methods for phospholipid fatty acids (PLFA) and microbial biomass C (chloroform-fumigation-extraction, CFE).

In the first study, biochar increased microbial abundances and the fungal-to-bacterial PLFA ratio after 37 days of incubation in arable soil applied with litter. It was suggested that biochar improved living conditions for soil microorganisms. Fungi may benefit most from newly created habitats due to colonizable biochar pores and surfaces. Additionally, fungi could have also co-metabolized small amounts of recalcitrant biochar-C during plant litter decomposition. Without litter, biochar led to interactions between earthworms and soil microorganisms resulting in enhanced bacterial and fungal abundances. This indicates better growth habitats for soil microbes in earthworm casts containing biochar. However, earthworms did not incorporate biochar-C, thus not directly influencing biochar stability. Soil respiration and metabolic quotients ($qCO_2$) were decreased after biochar application. Concurrently, biochar reduced $N_2O$ emissions in litter treatments suggesting a more efficient microbial community and underscoring the GHG mitigation potential of the used biochar.

The second study investigated the short-term effects of a similar biochar on microbial abundances and growth of winter rapeseed during the first year after field application to a warmed temperate arable soil. It was found that fungal biomass and the fungal-to-bacterial ratio were increased in the warmed biochar plots only after three months when spring barley litter from the previous growing season was present in soil. This short-term fungal response was interpreted as a limited mineralization of recalcitrant biochar-C during litter degradation at elevated soil temperature. The disappearance of this effect points to an overall high stability of the investigated biochar. Moreover, biochar proved to be effective in mitigating negative effects of seasonal dryness on microbial abundances and early plant growth in the dry spring period in 2014. However, biochar had no effect on final aboveground biomass of winter rapeseed at harvest in the first growing season.

As shown in the third study, in the second vegetation period, aboveground biomass of spring wheat was enhanced by warming, but only slightly increased with biochar. After two vegetation periods, this confirms the assumption that plant productivity in already fertile temperate arable soils is unlikely to be further enhanced with biochar amendment. Total $CO_2$ emissions after two years were not reduced by biochar and remained unchanged even under warming suggesting a high degradation stability of the used biochar. In addition, biochar had no effect on the temperature sensitivity of soil respiration and only initially on the efficiency of microbial metabolism, further emphasizing the limited impact of biochar on microbial functions in soil C cycling. $N_2O$ emissions were increased in

biochar-amended soil at elevated soil temperature, presumably due to enhanced water and fertilizer retention with biochar. Thus biochar amendment could induce unintended higher $N_2O$ emissions from agricultural soils in the future. The investigated arable soil served as a minimal sink for $CH_4$, while total $CH_4$ uptake was not significantly influenced by biochar or soil warming. The global warming potential ($GWP_{100}$) of total soil GHG emissions was enhanced by 28 % with warming, but not changed with biochar. However, the storage of biochar-C in soil was estimated to compensate warming-induced elevated soil GHG emissions for 20 years.

To conclude, this thesis revealed that biochar may have only minor influence on soil microorganisms and crop growth in temperate, fertile arable field soils. It was shown that biochar could be a valuable tool for C sequestration in temperate arable soils, thus potentially offsetting a warming-induced increase in GHG emissions. In order to face climate change impacts, more long-term studies on microbiological effects and the C sequestration potential of biochar in cultivated soil are urgently needed. Further research on biochar should also consider environmental factors affected by global warming such as elevated soil temperature and moisture variability, which particularly regulate C and N cycling in soils.

# 2 Zusammenfassung

Die Klimaerwärmung wird einen erheblichen Einfluss auf terrestrische Ökosysteme haben. Bodeneigenschaften wie Temperatur und Feuchte werden sich verändern und dabei C- und N-Stoffkreisläufe, die Aktivität, Abundanzen und Zusammensetzung der mikrobiellen Gemeinschaft im Boden sowie das Pflanzenwachstum beeinträchtigen. Dies könnte zur Erhöhung von Treibhausgas-(THG) Emissionen durch den Klimawandel beitragen. Daher werden neue Möglichkeiten für ein Bodenmanagement zur Verringerung dieser prognostizierten Folgen benötigt. Pflanzenkohle wird aufgrund ihrer Langzeitstabilität primär als ein effektives Mittel für die langfristige C-Sequestrierung in Ackerböden betrachtet. Zudem könnte diese ebenfalls für die Verbesserung von physikalischen, chemischen und biologischen Bodeneigenschaften, zur Erhöhung von Pflanzenwachstum und Verminderung von THG Emissionen angewendet werden. Bisher ist der Kenntnisstand über solche begünstigenden Pflanzenkohle-Effekte im Boden unter zukünftig erwärmtem Klima sehr gering. Die vorliegende Arbeit präsentiert neue Ergebnisse über interaktive Effekte von Pflanzenkohle und Bodenerwärmung auf Bodeneigenschaften, Mikroorganismen, Pflanzenwachstum und THG Emissionen auf Feldmaßstab, um das zukünftige Potential von Pflanzenkohle für eine langfristige C-Festlegung in einem temperaten Ackerboden zu bewerten.

Diese Doktorarbeit umfasst drei Studien. In der ersten Studie wurde *Miscanthus* x *giganteus* Pflanzenkohle aus langsamer Pyrolyse (600 °C, 30 Min.) mit Regenwürmern und stickstoffreicher Pflanzenstreu (*Phacelia tanacetifolia* Benth.) über einen kurzen Zeitraum (37 Tage) in einem Ackerboden unter kontrollierten Laborbedingungen inkubiert. Dabei sollte das Potential von Pflanzenkohle zur Reduktion von $CO_2$ und $N_2O$ Emissionen geprüft werden. Zudem wurde untersucht, ob Interaktionen zwischen Kohle und Regenwürmen die THG Emissionen oder die Abundanz von mikrobiellen Gruppen im Boden beeinflussen. Im Fokus des Feldexperiments der zweiten und dritten Studie stand die Stabilität und das langfristige C-Sequestrierungspotential von vergleichbarer *Miscanthus* Kohle (850 °C, 30 Min.). Die damit verknüpften Effekte von Kohle auf THG Emissionen, physikalische, chemische und mikrobiologische Bodeneigenschaften sowie das Pflanzenwachstum wurden in einem Agrarökosystem unter permanent erhöhter Bodentemperatur (+2,5 °C, seit 2008) untersucht.

Die Pflanzenkohlen aus der C4 Pflanze *Miscanthus x giganteus* unterschieden sich in ihrer isotopischen ($^{13}C$) Signatur gegenüber dem untersuchten C3 Boden. Dies ermöglichte

es, den Verbleib von Kohle-C in verschiedenen Kompartimenten wie emittiertem $CO_2$, Regenwurm- oder mikrobieller Biomasse zu verfolgen. Mikrobielle Abundanzen und die Gemeinschaftszusammensetzung wurden mittels der Extraktionsmethoden für Phospholipid-Fettsäuren (PLFA) und mikrobielle Biomasse-C (Chloroform-Fumigation-Extraktion) analysiert.

In der ersten Studie erhöhte Pflanzenkohle die Abundanz der Mikroorganismen und das Pilze/Bakterien-Verhältnis nach 37 Tagen Inkubation in einem Ackerboden unter Zugabe von Streu. Dies deutet auf verbesserte Lebensbedingungen für Bodenmikroorganismen mit Pflanzenkohle hin und das Pilze am stärksten von neugeschaffenen Habitaten durch kolonisierbare Poren und Oberflächen der Kohle profitieren konnten. Pilze könnten zudem geringe Mengen des rekalzitranten Kohle-C während des Streuabbaus durch Kometabolismus mineralisiert haben. Ohne Streuzugabe hat Pflanzenkohle zu interaktiven Effekten zwischen Regenwürmern und Mikroorganismen und damit erhöhten bakteriellen und pilzlichen Abundanzen geführt. Dies lässt verbesserte Wachstumsbedingungen für die Bodenmikroorganismen in Kohle enthaltenen Regenwurmgängen vermuten. Allerdings haben die Regenwürmer kein Kohle-C in ihre Biomasse eingebaut und somit den Abbau der Pflanzenkohle nicht direkt beeinflusst. Die Bodenatmung und metabolischen Quotienten wurden nach Kohle-Zugabe reduziert. Gleichzeitig verminderte Pflanzenkohle die $N_2O$ Emissionen in den Behandlungen mit Streu, was auf eine effizientere mikrobielle Gemeinschaft hindeutet und das Potential von Pflanzenkohle zur Reduktion von THG unterstreicht.

Die zweite Studie untersuchte die Kurzzeiteffekte einer vergleichbaren Pflanzenkohle auf mikrobielle Abundanzen und das Wachstum von Winterraps während des ersten Jahres nach Kohle-Applikation zu einem erwärmten temperaten Agrarökosystem. Die pilzliche Biomasse und das Pilz/Bakterien-Verhältnis wurden in den erwärmten Kohleflächen lediglich nach drei Monaten erhöht, als Streu von der Vorfrucht Sommergerste noch im Boden vorhanden war. Diese kurzzeitige Reaktion der Pilze wurde als eine geringfügige Mineralisierung von rekalzitrantem Kohle-C während des Streuabbaus unter erwärmter Bodentemperatur interpretiert. Das Verschwinden dieses Effektes deutet auf eine allgemein hohe Stabilität der untersuchten Pflanzenkohle hin. Darüber hinaus verminderte die Kohle die negativen Effekte von saisonaler Trockenheit auf die Abundanz von Mikroorganismen und das Frühwachstum von Winterraps im trockenen Frühling 2014.

Allerdings hatte die Pflanzenkohle keinen Effekt auf die oberirdische Biomasse von Winterraps in der ersten Vegetationsperiode.

Wie in der dritten Studie gezeigt, wurde die oberirdische Biomasse von Sommerweizen im zweiten Jahr durch Erwärmung erhöht, aber nur geringfügig durch Kohle gesteigert. Nach zwei Vegetationsperioden wird damit die Annahme bestätigt, dass eine zusätzliche Erhöhung der Pflanzenproduktion in bereits fruchtbaren Ackerböden gemäßigter Breiten mit Pflanzenkohle unwahrscheinlich ist. Die Gesamtemission von $CO_2$ nach zwei Jahren wurde nicht durch Kohle reduziert und blieb unverändert unter Erwärmung, was auf eine hohe Abbaustabilität der untersuchten Kohle hindeutet. Außerdem hatte Kohle keinen Effekt auf die Temperatursensitivität der Bodenatmung und nur anfangs auf die mikrobielle Effizienz, was die begrenzte Wirkung von Pflanzenkohle auf mikrobielle Funktionen im C-Kreislauf im Boden betont. Die $N_2O$ Emissionen wurden in den erwärmten Kohle-behandelten Böden erhöht, was vermutlich auf eine erhöhte Wasser- und Nährstoffretention durch Kohle zurückzuführen ist. Kohle-Anwendung könnte deshalb in Zukunft zu einer unbeabsichtigten Steigerung von $N_2O$ Emissionen von Ackerböden führen. Der untersuchte Ackerboden diente als geringfügige Methansenke, aber die Gesamtaufnahme von Methan wurde nicht signifikant durch Kohle oder Erwärmung beeinflusst. Das globale Treibhausgaspotential (GTP) der Gesamt-THG-Emissionen wurde über zwei Jahre um 28 % durch Erwärmung erhöht, aber nicht von Pflanzenkohle verändert. Es wurde jedoch geschätzt, dass die Festlegung von Pflanzenkohle-C im Boden, die durch Erwärmung in 20 Jahren erhöhte produzierte Menge an THG Emissionen kompensieren könnte.

Abschließend zeigte diese Arbeit, dass Pflanzenkohle einen eher geringen Einfluss auf Bodenmikroorganismen und das Pflanzenwachstum in temperaten, fruchtbaren Ackerböden unter Freilandbedingungen haben könnte. Es konnte festgestellt werden, dass Pflanzenkohle ein sinnvolles Mittel für die C-Sequestrierung in temperaten Ackerböden sein kann und damit das Potential besitzt die erhöhten THG Emissionen in einem erwärmten Klima auszugleichen. Um dem Klimawandel zu begegnen, werden dringend weitere Studien zu mikrobiologischen Effekten und zum Potential von Pflanzenkohle zur C-Sequestrierung in kultivierten Böden benötigt. Darüber hinaus sollten die von der globalen Erwärmung beeinflussten Umweltfaktoren wie erhöhte Bodentemperatur und Variabilität in der Bodenfeuchte berücksichtigt werden, die insbesondere die C- und N-Kreisläufe in Böden steuern.

# 3 General Introduction

## 3.1 C cycle and climate change

Soils are major reservoirs for terrestrial carbon (C) having about three times more C than the atmosphere or living plant biomass (Schmidt et al., 2011). Soil organic carbon (SOC) stocks are estimated to be 1500 Pg (Lal, 2004) from which more than 10 % are stored in agricultural soils (Paustian et al., 2000). All dead material in soil including plant litter, roots or animals containing organic C is termed as soil organic matter (SOM) and the balance between C input and output regulates the SOM pool (Sollins et al., 1996). Plant biomass (leaves and roots) and root exudates mainly constitute C inputs to soil. C loss occurs via heterotrophic or autotrophic respiration as carbon dioxide ($CO_2$), by anaerobic microbial respiration releasing methane ($CH_4$) or by leaching of dissolved carbon (Bardgett et al., 2005; Davidson & Janssens, 2006).

Soil microorganisms play an essential role in ecosystem functioning, as they are driving SOM decomposition and C- and N-mineralization which controls C cycling, nutrient availability, microbial and plant community diversity as well as plant growth (Hättenschwiler et al., 2005; van der Heijden et al., 2008). The quantity and quality of plant litter and root exudates, competition between plants and soil microorganisms for available N and other nutrients as well as further abiotic soil properties such as temperature and moisture are limiting factors for both plant biomass production and microbial activity thus influencing SOM formation (Horwath, 2007; Robertson & Groffmann, 2007; van der Heijden et al., 2008).

Increasing the SOM pool is desirable due to its multiple functions in improving soil structure, water and nutrient retention, enhancing soil biodiversity and reducing risks of soil degradation, e.g. by erosion (Lal, 2009). The protection of SOM against microbial mineralization or leaching is known as stabilization (Sollins et al., 1996). Responsible mechanisms of SOM stabilization are the chemical recalcitrance of SOM molecules (plant litter, rhizodeposits, humic substances or charred OM) as well as spatial inaccessibility of SOC to microorganisms, e.g. occlusion of SOM by aggregation or the formation of organo-mineral interactions between SOC and clay minerals (Sollins et al., 1996; von Lützow et al., 2006). Indeed, there is incomprehensive understanding on these individual processes and its relevance for SOM stabilization (von Lützow et al., 2006; Marschner et al., 2008; Dungait et al., 2012). Earthworms are known to stabilize SOC in soil aggregates, but also

mobilize soil carbon and nitrogen (N), thus interacting with soil microorganisms and promoting SOM decomposition by their feeding, burrowing and casting activities (Marhan & Scheu, 2005; Lubbers et al., 2013).

As a consequence of human activities in the past 250 years, the atmospheric concentrations of the greenhouse gases (GHG) $CO_2$, $N_2O$ and $CH_4$ increased considerably. For example, $CO_2$ concentrations have risen from 278 ppm to 391 ppm in this timespan due to the combustion of fossil fuels and emissions from land use changes (Ciais et al., 2013). $N_2O$ and $CH_4$ are 265 and 28 times more potent greenhouse gases than $CO_2$ over a period of 100 years (Myhre et al., 2013). This means that only small changes in their atmospheric concentrations have major impact on global climate (Robertson & Grace, 2004). The enhanced abundances of $CO_2$, $N_2O$ and $CH_4$ in the atmosphere cause energy uptake by the climate system through absorption of solar radiation which drives the global climate change and results in the observed changes in earth surface temperatures, precipitation patterns and extreme weather events (Hartmann et al., 2013). Global average surface temperature increased by 0.85 °C in the past 130 years and further increase is expected for the future (IPCC, 2013). Regional climate models predict a temperature elevation of 2 to 3 °C for Germany until 2100 (Umweltbundesamt, 2006).

Elevated temperature will likely affect the global carbon cycle and alter the function of soils to act as sink or source for $CO_2$ (Rustad et al., 2001; Lal, 2013). Microbial respiration and the degradation of SOM is highly dependent on soil temperature and increases in $CO_2$ emissions could have a positive feedback on global climate change (Bardgett et al., 2008, see Chapter 3.2).

Soil C sequestration is the conversion of atmospheric $CO_2$ into the SOM pool where it is stabilized and conserved for long time. Hence, management of the SOM pool in soils is considered as an effective climate change mitigation strategy (Lal, 2013). Intensively managed agricultural soils are mostly depleted in SOC and therefore have a high potential for C sequestration (Paustian et al., 2000). Adaption to climate change through C sequestration in agroecosystems can be achieved by increasing the stable SOM pool through conservation agriculture (no or reduced tillage) to preserve soil aggregates, increase of plant production and recycling of crop residues, cover cropping, an integrated nutrient management or the amendment of OM as manure, composts or biochar (Franzluebbers, 2010; Lal, 2013).

## 3.2 Effects of climate change on soil microorganisms

The driving factors of climate change such as elevated atmospheric $CO_2$ concentrations, changed precipitation patterns and global warming are expected to have major impacts on C- and N cycling in terrestrial ecosystems and involved soil microbial communities (Castro et al., 2010; Gray et al., 2011; Allison et al., 2013; De Vries & Shade, 2013; Zhou et al., 2016). Alterations in abiotic factors such as litter quality, C inputs by root exudation, nutrient availability, soil moisture and temperature under changing climate are regulating soil microbial activity, abundances as well as community composition (Frey et al., 2008; Castro et al., 2010; Lu et al., 2013).

Soil microorganisms are sensitive to changes in temperature and respond to soil warming with changed activity and community composition (Zogg et al., 1997; Pietikäinen et al., 2005). Most research was addressed on warming effects on soil respiration (e.g. Lu et al., 2013), but potential alterations in microbial physiology and the relationship between microbial activity and abundances/community composition have been scarcely investigated. Elevated temperatures are expected to increase C inputs to soil by higher plant production, directly caused by enhanced photosynthesis rates and longer growing periods or indirectly by increased N mineralization and availability (Rustad et al., 2001). Plant growth could also be limited by warming-induced decreases of soil moisture (Ciais et al., 2005). However, it is still uncertain how soil warming affects microbial communities and their functioning in litter decomposition and nutrient cycling and whether SOC stocks will be affected over time (Knorr et al., 2005; Frey et al., 2008). SOM decomposition and $CO_2$ emissions were often found to be initially enhanced under soil warming in many studies, but diminished after a few years (Luo et al., 2001; Melillo et al., 2002; Bradford et al., 2008). This short-lived warming effect on soil respiration has been attributed to the adaption of soil microorganisms to temperature increase (Bradford et al., 2008) and to depletion and limitation of readily available substrates as well as water limitation under warming, thus controlling enzyme activity, microbial respiration and growth (Bradford, 2013; Fissore et al., 2013). Moreover, it is still debated whether there are differences in the temperature sensitivity of the mineralization of substrates with varying quality, which could also explain the suggested microbial adaption to warming (Conant et al., 2011; Frey et al., 2013).

In addition to soil respiration, information on microbial abundances and community composition could help to explain how soil microorganisms control the decomposition of

SOM under warming (Bradford et al., 2008; Allison et al., 2010; Chen et al., 2015b). Primary decomposers such as bacteria and fungi likely differ in their response to changing environmental factors under warming. Several studies reported that fungi are more adapted to cold temperatures than bacteria while this behavior is reversed at high temperatures (e.g. Pietikäinen et al., 2005). Soil warming is usually accompanied by reduced soil moisture (Xu et al., 2013) and microorganisms respond sensitive to fluctuations in soil water (Schimel et al., 2007; Allison & Treseder, 2008). For example, Gram-positive bacteria and fungi are characterized by higher tolerance against drought than Gram-negative bacteria based on their physiology and acclimation strategies (Schimel et al., 2007). Moreover, bacteria may respond more quickly to soil warming than fungi due to their differences in substrate utilization (Cregger et al., 2014). In general, bacteria are known to primarily use labile C and to be fast growing, while fungi are slowly growing and mainly responsible to decompose recalcitrant SOM (De Boer et al., 2005).

Across various biomes, microbial abundances were inconsistently influenced by elevated soil temperature showing increases, decreases or no effect depending on ecosystem and plant types, experimental setup and duration as well as determination methods (Pold & DeAngelis, 2013). However, increased microbial abundances and $CO_2$ emissions under warming are mostly highest in C-rich soils in higher latitudes with cold climate (e.g. boreal regions, Antarctic) due to the abolition of substrate and water limitations (Melillo et al., 2002; Yergeau et al., 2011). Some short- to mid-term studies in temperate climate reported increased abundances and alterations in microbial community composition under warming (Zhang et al., 2005; Gray et al., 2011), whereas other did not observe a substantial effect in different ecosystems (Schindlbacher et al., 2011; Zhang et al., 2013b; Reinsch et al., 2014). By contrast, long-term experiments (> 10 years) revealed reductions in total microbial abundances and relative decrease in fungal abundances suggesting community shifts toward bacteria only after several years (Frey et al., 2008; Rinnan et al., 2007; DeAngelis et al., 2015). The lag in microbial response may be connected to slow changes in quantity and quality of substrates, alteration of plant communities or changing microbial niches under long-term warming (Rinnan et al., 2007; DeAngelis et al., 2015). An increase in the bacterial-to-fungal ratio was also found in the meta-analysis of Chen et al. (2015b) and suggests that such shifts within the microbial community toward more bacteria could have crucial impact on C dynamics and endanger long-term C sequestration potential in warmed soil (Bardgett et al., 2005).

Vice versa, fungal-dominated microbial communities could be beneficial in terms of C sequestration, especially in agricultural soils, due to their expected higher C use efficiency in comparison to bacteria (Six et al., 2006). However, shifts within fungal communities due to warming could also increase the mineralization of recalcitrant OM (Treseder et al., 2016). Compared to forest and grassland ecosystems, less attention was paid to warming impacts on microbial activity and abundances in agricultural soils. The practice of no-till land management, which leads to the provision of easily available C inputs by crop residues on soil surface and promoted growth of root biomass, could induce prolonged stimulation of $CO_2$ emissions from arable soils under warming and this needs to be further investigated (Hou et al., 2014, 2016). Otherwise, studies also showed that reduced soil moisture resulting from increased temperature hampered both soil respiration and microbial growth (Poll et al., 2013; Liu et al., 2015). This implies that a set of different factors will likely influence soil microorganisms and C-cycling in intensively used agricultural systems under climate change.

Soil warming likely also affects the fluxes of non-$CO_2$ greenhouse gases such as $N_2O$ and $CH_4$ (Dijkstra et al., 2013). Enhanced mineralization and bioavailability of N, reductions in soil moisture, and shifts in N-cycling microbial communities under warming could have influence on soil $N_2O$ emissions (Cantarel, et al., 2012; Bai et al., 2013). $N_2O$ is produced as an intermediate product of several complex microbial processes in soil such as nitrification, nitrifier denitrification, denitrification or co-denitrification (Baggs, 2011). Agricultural soils are major sources of $N_2O$ due to the application of fertilizers, manures, and crop residues (Baggs et al., 2002; Reay et al., 2012). Enhanced $N_2O$ emissions in warmed soil may be directly induced by increased soil respiration and the coupling between C- and N-cycles as well as indirectly by an increase of anaerobic zones in soil due to stimulated respiration, thus favoring denitrification (Butterbach-Bahl et al., 2013). However, substrate and water limitations could also weaken a stimulatory effect on $N_2O$ emissions by elevated soil temperature (Butterbach-Bahl & Dannenmann, 2011).

Methane is produced in soil by the microbial decomposition of OM by methanogens under anaerobic conditions and low redox potential. It can also be consumed by aerobic bacteria (methanotrophs) through oxidation (Smith et al., 2003). The role of soils to act as a source or sink for $CH_4$, i.e. the difference between production and consumption, is mainly controlled by moisture level and aeration (Dijkstra et al., 2011). Therefore, wetlands such as peatlands or paddy soils are important emitters of $CH_4$, while aerobic upland soils serve

as methane sink (Le Mer & Roger, 2001). Generally, agricultural soils are small sources or sinks for $CH_4$ (Mosier et al., 2005). Warming may either directly stimulate the activity of methanogens or indirectly favor methanotrophs by decreased soil moisture and enhanced oxygen diffusion into soil (Smith et al., 2003; Dijkstra et al., 2011).

## 3.3 Biochar – A tool for C sequestration in soil?

The idea to sequester C in soil by biochar amendment is based on the anthropogenic *Terra Preta* soils occurring in small patches in Central Amazonia in Brazil (Glaser et al., 2001). In this tropical climate, researchers found dark colored soils enriched with ancient charcoal and aromatic humic substances (termed as black carbon), thereby exhibiting higher amounts of SOM than the adjacent Oxisols with same mineralogy. The accumulation of black carbon in the *Terra Preta* soils is likely because of repeated slash-and-burn and gardening practices by the indigenous population (Glaser & Birk, 2012). Radiocarbon ages of hundreds to thousands of years documented the long-term persistence of the pyrogenic OM in soil due its inherent recalcitrant nature and organo-mineral stabilization (Glaser et al., 2001; Liang et al., 2008). In addition, the enhanced fertility by amelioration of various physical and chemical soil properties such as water and nutrient retention, soil pH or cation exchange capacity (CEC), as well as higher microbial abundances and diversity in these soils was partly attributed to the high amount of black carbon (Glaser et al., 2002; Kim et al., 2007; Grossman et al., 2010). Similar charcoal enrichments in soil due to fire-derived thermal alteration of plant residues are ubiquitous and were described in different ecosystems worldwide, for example in Germany, the Netherlands, Australia, Russia, the USA and Belgium (Schmidt et al., 1999; Hammes et al., 2008; Cheng et al., 2008; Downie et al., 2011; Vasilyeva et al., 2011; Mao et al., 2012; Hernandez-Soriano et al., 2016).

By definition, biochar is chemically similar to charcoal, but intentionally produced for C sequestration in soil (Lehmann & Joseph, 2009). Biochar is produced by pyrolysis, the incomplete combustion of organic material (e.g. crop residues) under limited oxygen supply and at a wide temperature range between 300 and ~1000 °C for varying time periods (Spokas, 2010; EBC, 2012). During pyrolysis, the plant polymers cellulose, hemicellulose and lignin are thermochemically decomposed thereby forming gaseous, liquid and solid (biochar) products (Brown, 2009; Laird et al., 2010; Libra et al., 2011). The yielded biochar is composed of highly polycondensed aromatic C structures, volatile

(labile) matter and ash (Keiluweit et al., 2010; Lehmann et al., 2011). Typically, biochars can be characterized by high C content, large surface area, high micro- and macroporosity as well as CEC and alkaline pH (Downie et al., 2009; Amonette and Joseph, 2009; Brewer et al., 2011). However, biochars from distinct feedstock or pyrolysis conditions (temperature and duration) can significantly vary in their physical and chemical characteristics and suitability for soil application (Brewer et al., 2011; Schimmelpfennig & Glaser, 2012). First guidelines and certificates were introduced to ensure sustainable biochar production and to define quality standards in order to prevent negative effects of biochar field application on soil functions (EBC, 2012; IBI, 2015).

Biochar research has gained increasing attention in the last years and the *Terra Preta* model is aimed to be transferred from tropical to temperate climate regions (Jeffery et al., 2015). Many researchers emphasize the multifaceted potential of biochar to simultaneously sequester C in soil and increase SOM pools, to improve several soil properties and agronomic yields and to mitigate soil greenhouse gas emissions (Atkinson et al., 2010; Spokas et al., 2012; Lorenz & Lal, 2014; Ameloot et al., 2016). This made biochar also interesting for the application in temperate agricultural field soils (e.g. Ameloot et al., 2014; Domene et al., 2014). The feasibility of biochar for C sequestration in soils could be evaluated from its stability *in situ* and possible influences of biochar on the preservation of native SOM and promotion of C inputs to soil by enhanced plant production (Lorenz & Lal, 2014).

Only few studies exist on the decomposability of biochar in the field (Gurwick et al., 2013; Wang et al., 2016a). In addition, the *in situ* stability of biochar was rarely evaluated by stable isotope techniques (Major et al., 2010; Knoblauch et al., 2011; Ventura et al., 2015). Biochar degradation is mostly derived from incubation studies with durations between days and years (Kammann et al., 2012; Singh et al., 2012; Ameloot et al., 2013a; Kuzyakov et al., 2014). From these studies and observations from historical charcoal sites (see above) it can be concluded that most biochars are relatively stable in soil due to their chemical recalcitrance, although not inert (Schneider et al., 2011; Vasilyeva et al., 2011). Though, it seems to be biochar-, site- and soil-specific whether the mineralization of native SOM is suppressed or increased (negative or positive priming effect) with biochar. This could question its usefulness to increase SOM stocks (Wardle et al., 2008; Zimmerman et al., 2011). Generally, plant-derived biochars produced by slow pyrolysis at higher temperatures (≥500 °C) with high proportion of aromatic C and low volatile matter content

should be preferred over more labile lower-temperature biochars for purposes of C sequestration in soil (Spokas, 2010; Brewer et al., 2011; Wang et al., 2016a). Results of short-to mid-term field studies (1-3 years) in agricultural sites indicate that biochar could help to sequester C by stabilizing SOM and preventing breakup of soil aggregates or by being physically protected against degradation through aggregate occlusion (Zhang et al., 2015; Dong et al., 2016; Ma et al., 2016). An improved soil aggregation through biochar amendment would reduce the risk of SOC losses by erosion (Jien & Wang, 2013).

Biochar can also indirectly increase SOM by promoting above- and belowground plant growth and rhizodeposits (Lorenz & Lal, 2014). Increases in crop growth after biochar addition to soil was observed in a number of studies which could be attributed to increased soil pH, enhanced nutrient retention in soil as well as plant availability of P and K, and increased water holding capacity in soil (Jeffery et al., 2011; Biederman & Harpole, 2013). Yield benefits could be rather small in temperate fertile agricultural soils (Crane-Droesch et al., 2013; Jay et al., 2015). However, leaching of mineral N from fertilized soils is of major concern for the productivity of agricultural systems and groundwater quality (Stavi & Lal, 2013). The affinity of biochar to adsorb nutrients due to its large surface area and high CEC could reduce $NO_3^-$ leaching and increase the N-use efficiency of plants in fertilized agricultural soils (Steiner et al., 2008b; Jones et al., 2012; Zheng et al., 2013). Many researchers argue that the application of unweathered biochar initially reduces plant-available N (Nelissen et al., 2014; Schmidt et al., 2014), though this effect became weaker during ageing of biochar in the field (Gronwald et al., 2015). Therefore, biochar is also often mixed with compost, or co-composted to charge it with nutrients before soil application to prevent such temporal negative impacts on plant growth (Schulz & Glaser, 2012; Schmidt et al., 2014; Kammann et al., 2015).

Long-term field research is definitely needed to evaluate biochar stability and possible priming of native SOM decomposition *in situ* as well as biochar effects on plant productivity over several growing periods. Another challenge will be to elucidate the role of soil microorganisms in affecting biochar`s C sequestration potential under changing climate and soil warming.

## 3.4 Effects of biochar on soil microorganisms

Some studies demonstrated the preferential colonization of biochars by microorganisms, particularly by AMF (Warnock et al., 2007; Jin, 2010; Luo et al., 2013). Microhabitats in biochar pores may also protect microorganisms against their predators and extreme conditions such as heat and water stress (Pietikäinen et al., 2000; Thies & Rillig, 2009).

Biochar has the potential to ameliorate several physical and chemical soil properties and thus change soil habitat conditions, which could positively affect soil microorganisms (Lehmann et al., 2011; Gul et al., 2015). For example, such improvements in properties of biochar-amended soil include a decrease in soil bulk density (Major et al., 2010; Downie et al., 2009), increased CEC (Laird et al., 2010), enhanced water holding capacity (Karhu et al., 2011; Omondi et al., 2016), improved nutrient retention (Zheng et al., 2013), increases of soil pH (van Zwieten et al., 2010) or adsorption of toxic compounds (Chen & Yuan, 2011). In addition, biochar sometimes promoted aboveground and belowground plant growth, which could also have direct impact on plant-associated microbial communities or indirectly influence microorganisms by changed substrate and nutrient availability in soil (Jones et al., 2012; Biederman & Harpole, 2013). However, chemical and physical properties of biochars and thus its soil amelioration potential vary with used feedstock and pyrolysis conditions (Ronsse et al., 2013; Gai et al., 2014). The magnitude of biochar effects in improving soil properties and, in turn, to alter soil microbial communities as well as increase plant growth relies on initial fertility status of soil. Therefore, degraded, acidic and coarse-textured soils with high drainage and leaching potential for nutrients may benefit most from biochar amendment (Atkinson et al., 2010; Gul et al., 2015; Omondi et al., 2016).

Interactions between biochar and earthworms are likely also relevant for soil microbial habitats. Earthworms were commonly used to assess the biotoxicity of different biochars in soil (e.g. Busch et al., 2012). Negative effects of biochars on earthworm survival rates or its avoidance could arise from physical and chemical alteration of soil properties or toxic substances of biochar itself (Liesch et al., 2010; Li et al., 2011). Biochar particles may also be ingested by earthworms without direct benefit, but mixed with SOM during passage through their gut, which could create more profitable habitats for microorganisms in biochar-amended soils (Augustenborg et al., 2012). In addition, earthworms may either mobilize biochar by grinding and breakup of soil aggregates or stabilize biochar in cast

aggregates, therefore changing substrate availability for soil microorganisms (Domene, 2016).

Increased microbial abundances and alterations in community composition in soils containing black carbon were reported from *Terra Preta* soils (Kim et al., 2007; Grossman et al., 2010. In addition, biochar application positively affected abundances and diversity of soil microorganisms in incubation studies (Khodadad et al., 2011; Gomez et al., 2014; Prayogo et al., 2014; Ameloot et al., 2015) and in short- or medium-term (< 4 years) field experiments in agroecosystems (Jones et al., 2012; Domene et al., 2014; Zhang et al., 2014). Otherwise, no effect or even decreases in microbial abundances were observed in other studies (Dempster et al., 2012; Rutigliano et al., 2014). This reflects that biochar effects on soil microorganisms strongly depend on biochar types (feedstock, pyrolysis conditions), application rates and soil/ecosystem (Liu et al., 2016). Increased microbial biomass in biochar-amended soils may be mainly explained with the above mentioned improvements of habitat conditions due to alterations in physical and chemical soil properties after biochar amendment (Gul et al., 2015). Until now, no long-term study exists showing whether positive effects of biochar on soil microbial abundances will be persistent. The application of biochar to agricultural soils led also to shifts in the soil microbial community composition and increased bacterial-to-fungi ratios (Jones et al., 2012; Chen et al., 2013). Such changes in the microbial community suggest differences in the response of bacteria and fungi to biochar application and also alterations in the function of microorganisms in C- and N-cycling (Lehmann et al., 2011).

Several studies showed an initial increase in $CO_2$ emissions after the application of fresh biochar (e.g. Smith et al., 2010). This is mainly correlated to the amount of easily available C of the biochar, which is rapidly consumed by soil microorganisms (Spokas, 2010). On longer terms, biochar may not serve as substrate for microbial growth in soil (Ameloot et al., 2013b), although fungi are able to decompose recalcitrant materials such as biochar (Ascough et al., 2010). The incorporation of labeled biochar-C into microbial biomass measured by chloroform fumigation extraction (CFE) or phospholipid fatty acid analysis (PLFA) was observed to be quite low (Kuzyakov et al., 2009; Farrell et al., 2013; Luo et al., 2013; Watzinger et al., 2014). After short-term stimulation of soil respiration, biochar is expected to be only slowly mineralized (Kuzyakov et al., 2009, 2014). In addition, in many studies biochar suppressed total $CO_2$ emissions (Liu et al., 2016). Possible mechanisms for the reduction in soil respiration are (i) the stability of biochar due

to its high chemical aromaticity, (ii) stabilization of labile C through sorption on the surface of biochar preventing it from being mineralized, (iii) N immobilization due to sorption on biochar or by enhanced plant-uptake thereby inducing nutrient limitation for soil microorganisms, (iv) precipitation of $CO_2$ as carbonate on biochar surfaces or (v) biochar-derived toxic substances inhibiting microbial activity (Lehmann et al., 2011; Case et al., 2012; Saarnio et al., 2013). However, reduced $CO_2$ emissions were frequently observed together with enhanced microbial biomass and community shifts resulting in decreased metabolic quotients ($qCO_2$) and enhanced microbial efficiency (Jin, 2010; Domene et al., 2014). This points to a limited relevance of the above mentioned mechanisms for reduced soil respiration. Lehmann et al. (2011) hypothesized that biochar offers microhabitats where the co-location of substrates, nutrients and microorganisms supports increased microbial efficiency. This would legitimate the use of biochar as soil amendment for soil C sequestration and amelioration of soil properties. However, further research on the influence of biochar on the mineralization of native SOM is of particular importance. Contradicting results were reported from experiments using varying biochars and soils showing both enhanced and reduced mineralization of SOM, also termed as positive or negative priming effects (Kuzyakov et al., 2009; Zimmerman et al., 2011).

Changes in soil abiotic factors after biochar addition may also affect the emissions of $N_2O$ and $CH_4$. Biochar likely has distinct effects on nitrifier and denitrifier communities in soil and related $N_2O$-genic processes depending on biochar and soil properties (Prommer et al., 2014; Harter et al., 2016). Several studies demonstrated reductions in $N_2O$ emissions following biochar application (Taghizadeh-Toosi et al., 2011; Harter et al., 2013; Ameloot et al., 2016). Some suggested explanations are the suppression of denitrification through enhanced soil aeration, reduced availability of labile C or nitrate due to adsorption on biochar or by higher plant N-uptake, as well as increases in soil pH (Clough et al., 2013, 2014). In addition, many authors showed reduced $N_2O$ emissions by further reduction of $N_2O$ to $N_2$. This process may be facilitated by the creation of denitrification hotspots in biochar pores, where soil water, substrate availability as well as pH are locally favorable and electrons may be shuttled faster to denitrifiers (Cayuela et al., 2013; Harter et al., 2013; Ameloot et al., 2016). On the other hand, $N_2O$ fluxes were also increased with low-temperature biochars from N-rich litter, after rewetting soils and application of N-fertilizer (Yanai et al., 2007; Singh et al., 2010; Saarnio et al., 2013; Sánchez-García et al., 2014; Chen et al., 2015a). This requires further clarification to

determine possible reasons in order to adjust biochar production and selection of fertilizers for applications in agricultural land management.

Biochar was also recently applied to decrease $CH_4$ emissions from rice paddy soils, but with varying success (Yu et al., 2013; Zhang et al., 2013a). Yu et al. (2013) found that biochar increased methane oxidation at low moisture levels, whereas increasing $CH_4$ production at higher water-filled spore space (WFPS) above 60 %. By contrast, Karhu et al. (2011) reported short-term increased $CH_4$ uptake in a field agricultural soil and hypothesized that enhanced aeration and $CH_4$ diffusion into soil are possible reasons. No consistent results were obtained in other experiments in the laboratory or field using different arable soils (Spokas & Reicovsky, 2009; Castaldi et al., 2011; Kammann et al., 2012). This underlines that biochar may differently affect methanogenic and methanotrophic microorganisms depending on changes in abiotic soil properties (Feng et al., 2012). However, the effects of biochar on soil $CH_4$ fluxes are strongly determined by soil moisture levels and aeration as well as by soil and biochar properties and are therefore difficult to predict.

The effects of biochar on terrestrial ecosystems affected by climate warming are mostly unexplored. It is largely unknown whether climate warming intensifies biochar mineralization in soil, thus influencing its long-term stability and GHG budgets. Only little research was done on the temperature sensitivity of biochar decomposition in incubation studies (Nguyen et al., 2010; Fang et al., 2014, 2015) and biochar effects on $N_2O$ emissions and $CH_4$ emissions at elevated soil temperature (Case et al., 2012; Han et al., 2016). Until now, no study focused on interactive effects of biochar and elevated soil temperature on microbial abundances and community composition under field conditions.

# 4 Objectives

New management options are required to mitigate the expected climate change effect on soil temperature and moisture likely influencing soil microbial communities involved in C- and N-cycling and further indirect effects on abiotic factors, thus leading to stimulated decomposition of SOM and enhanced greenhouse gas (GHG) emissions (Bardgett et al., 2008; Dijkstra et al., 2013; Chen et al., 2015b). Biochar is proposed as a promising tool to sequester C in agricultural soils, to increase SOC stocks and thereby may have high potential to mitigate soil GHG emissions and to improve overall soil fertility (Lorenz & Lal, 2014). However, it is uncertain whether the proposed beneficial effects of biochar can be achieved under soil warming. This thesis aims to evaluate the potential of C4 plant-derived *Miscanthus* x *giganteus* biochar to be a valuable soil management strategy to attenuate projected climate change effects in a temperate agroecosystem in south-west, Germany. The use of an isotopically labeled ($^{13}C$) biochar allowed to trace the fate of biochar-C in different C pools such as $CO_2$ and microbial or earthworm biomass.

The present thesis is structured in three studies. The first study, a short-term laboratory incubation (37d), examined the potential of a slow-pyrolysis biochar produced from *Miscanthus* x giganteus feedstock (pyrolysis at 600 °C for 30 Min.) to reduce the emissions of $CO_2$ and $N_2O$ in the presence of earthworms and N-rich *Phacelia tanacetifolia* Benth. litter in agricultural soil under controlled conditions. It was aimed to investigate whether endogeic earthworms of the species *Aporrectodea caliginosa*, the dominating earthworms in temperate arable soils (Marhan et al., 2015), could contribute to biochar mineralization by mobilizing and incorporating biochar-C into their biomass. In addition, it was studied whether single or interactive effects of biochar and earthworms affect soil microbial abundances and community composition measured by phospholipid fatty acid analysis (PLFA).

For this pre-experiment soil was taken from the same agroecosystem which was later investigated in study two and three. Hence, the results of the first study should give useful information on possible toxic effects of *Miscanthus* biochar on soil fauna, as represented by earthworms, the biological stability of the used biochar and its potential to mitigate soil greenhouse gas emissions from the used arable soil.

The second and third study focused on the feasibility of long-term soil C sequestration with *Miscanthus* biochar and its biological effects on an agroecosystem under predicted

climate warming. A two-factorial field experiment, the Biochar Hohenheim Climate Change Experiment (BC-HoCC) was established in August 2013 as a part of the already existing HoCC-experiment (Poll et al., 2013). A naturally labeled ($^{13}C$) *Miscanthus* biochar produced at high-temperature (850 °C) by slow pyrolysis (30 Min.) was applied to selected plots at a rate of 30 t $ha^{-1}$ and incorporated into 0-20 cm soil depth. Half of the plots were warmed by 2.5 °C against ambient soil temperature by heating cables since July 2008.

In the second study of this thesis, short-term (after one year) effects of biochar and soil warming on physical and chemical soil properties as well as on microbial abundances and community composition (PLFA) were investigated at three sampling dates (November 2013, March and September 2014) and in two depths (0-5 and 5-15 cm). In addition, the growth of winter rapeseed (*Brassica napus* L.) was determined by canopy height and aboveground biomass at maturity. The first hypothesis was that the used *Miscanthus* biochar is stable against microbial decomposition even under predicted soil warming, which was examined by PLFA abundances and the quantification of incorporated labeled biochar-C in microbial biomass ($^{13}C_{mic}$). Secondly, it was hypothesized that the positive effect of biochar on soil moisture regime, especially in dry periods, would compensate for water loss in warmed soil, thus positively influencing soil microbial abundances and growth of winter rapeseed.

In the third study, the effects of biochar and soil warming on microbial activity and greenhouse gas fluxes of $CO_2$, $N_2O$ and $CH_4$ were monitored over two years under winter rapeseed and spring wheat growing seasons. It was hypothesized that the used biochar will form a persistent constituent of OM in soil, thus not increasing $CO_2$ emissions, microbial metabolic efficiency and temperature sensitivity of soil respiration on the medium-term even under warming. In addition, it was aimed to study how biochar effects on physical and chemical soil properties would influence $N_2O$ and $CH_4$ fluxes under changing weather conditions, agricultural land management (ploughing, cropping and fertilization) and soil warming.

# 5 Effects of biochar, earthworms, and litter addition on soil microbial activity and abundance in a temperate agricultural soil

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**Abstract**

Biochar application to arable soils could be effective for soil C sequestration and mitigation of greenhouse gas (GHG) emissions. Soil microorganisms and fauna are the major contributors to GHG emissions from soil, but their interactions with biochar are poorly understood. We investigated the effects of biochar and its interaction with earthworms on soil microbial activity, abundance, and community composition in an incubation experiment with an arable soil with and without N-rich litter addition. After 37 days of incubation, biochar significantly reduced $CO_2$ (up to 43 %) and $N_2O$ (up to 42 %), as well as $NH_4^+$-N and $NO_3^-$-N concentrations, compared to the control soils. Concurrently, in the treatments with litter, biochar increased microbial biomass and the soil microbial community composition shifted to higher fungal-to-bacterial ratios. Without litter, all microbial groups were positively affected by biochar × earthworm interactions suggesting better living conditions for soil microorganisms in biochar-containing cast aggregates after the earthworm gut passage. However, assimilation of biochar-C by earthworms was negligible, indicating no direct benefit for the earthworms from biochar uptake. Biochar strongly reduced the metabolic quotient $qCO_2$ and suppressed the degradation of native SOC, resulting in large negative priming effects (up to 68 %). We conclude that the biochar amendment altered microbial activity, abundance, and community composition, inducing a more efficient microbial community with reduced emissions of $CO_2$ and $N_2O$. Earthworms affected soil microorganisms only in the presence of biochar, highlighting the need for further research on the interactions of biochar with soil fauna.

**Keywords:** Biochar, Respiratory efficiency, Soil microbial community composition, *Aporrectodea caliginosa*

## 5.1 Introduction

The addition of biochar to arable soils has been often shown to increase soil fertility and crop yield (Jeffery et al., 2011; Spokas et al., 2012). Another beneficial effect of biochar could be the reduction of greenhouse gas emissions from soils (Case et al., 2012; Kammann et al., 2012). However, reported effects of biochar on carbon dioxide ($CO_2$) emissions from soil have been variable, ranging from a short-term increase to a decrease in $CO_2$ emissions (Jones et al., 2011; Kammann et al., 2012; Ameloot et al., 2013a). Differences in C mineralization can be explained by different biochar and soil characteristics as well as various underlying processes, such as abiotic C-release from biochar, soil organic carbon (SOC) adsorption, and positive or negative priming effects (Zimmerman, 2010; Jones et al., 2011; Bamminger et al., 2014a). In addition, the emission of nitrous oxide ($N_2O$), which is 265 times more potent as greenhouse gas than $CO_2$ over a time period of 100 years (IPCC, 2013), was found to be significantly reduced by biochar (e.g., Taghizadeh-Toosi et al., 2011; Kammann et al., 2012), while only a few studies have also shown increased $N_2O$ emissions (Saarnio et al., 2013). Possible reasons for these inconsistent biochar effects on $N_2O$ emissions could be related to different biochar and soil characteristics showing divergent effects of biochar on soil aeration and moisture conditions, nutrient availability, or soil microbial community structure (Clough & Condron, 2010).

Biochar-related changes in micro-environmental conditions have been suggested to be responsible for observed modifications in soil microbial community composition (Khodadad et al., 2011) and abundances of different bacterial families (Anderson et al., 2011). Moreover, shifts to bacteria-dominated communities and decreases in fungal abundances have been observed in fields after biochar application (Jones et al., 2012; Chen et al., 2013). This emphasizes that there is a preferential microbial response to biochar addition, which may differ between fungi and bacteria, but the reasons for this are not well understood (Lehmann et al., 2011).

The pyrogenic C in biochar is more recalcitrant than other organic matter pools in soils (Vasilyeva et al., 2011), but it is not inert and can be slowly decomposed by abiotic and biologically mediated oxidation (Zimmerman, 2010). Indeed, microbial biomass increased in biochar-amended soil (Jin, 2010), but direct microbial consumption of labile fractions of biochar was observed mainly within the first 3 days and declined afterwards (Farrell et al., 2013). This suggests that the major parts of biochar are stable against microbial

decomposition and that direct uptake of biochar-C is of minor importance for the activity and abundance of soil microorganisms. Finally, the enhanced soil microbial biomass and reduced $CO_2$ respiration in the presence of biochar indicate a more efficient microbial community (Jin, 2010), which may be caused by shifts in the community composition and changed substrate use patterns (Lehmann et al., 2011).

Beside soil microorganisms, which are most responsible for C and N mineralization in soils, earthworms have also been shown to increase emissions of $CO_2$ and $N_2O$ (Lubbers et al., 2013) and to affect the mobilization as well as the stabilization of soil C and N (Marhan & Scheu, 2005). Burrows and casts of earthworms provide substrates and nutrients for soil microorganisms, enhancing the decomposition and C-mineralization of plant residues. In addition, low oxygen availability in combination with high nutrient content in the gut of earthworms and their cast material provide ideal conditions for denitrifying bacteria and concomitant high $N_2O$ emissions (Drake & Horn, 2007).

In comparison to the effects of biochar on soil microorganisms, even less is known about biochar effects on earthworms. The few existing studies have detected weight loss and mortality of earthworms after 28 days of incubation (Li et al., 2011), especially in soils with high doses of biochar (67.5 and 90 Mg $ha^{-1}$) (Liesch et al., 2010). Negative effects on earthworm activity and biomass could arise from physical or chemical effects of biochar amendments, i.e., insufficient soil moisture due to the dry biochar (Li et al., 2011) or toxicity/salinity (Liesch et al., 2010). Furthermore, biochar may interact with earthworms, modifying greenhouse gas emissions from soils. In a pot experiment with endogeic earthworms of the species *Aporrectodea icterica*, Augustenborg et al. (2012) observed a reduction of the earthworm-induced $N_2O$ emissions by 20 to 95 % in the presence of biochar, while biochar reduced $CO_2$ emissions only in the absence of earthworms. This illustrates the potential of biochar to mitigate the $N_2O$-emission stimulating earthworm effect. However, the stability of biochar against decomposition might be also affected by endogeic earthworms, which have been suspected of increasing the mobilization of old and possibly stable C resources in soils (Marhan et al., 2007).

We performed a factorial incubation experiment based on the following research questions: (1) Are endogeic earthworms able to mobilize and incorporate stable biochar-C, leading to increased decomposition of biochar? and (2) Will there be effects only of the single factors, earthworms, and biochar on C and N turnover, i.e., $CO_2$ and $N_2O$ emissions or will there be interactions between both factors? In addition to the second question, we

investigated whether the effects and interactions between biochar and earthworms will change when litter, as an additional C and N resource, is present in the soil and to which extent analyses of soil microbial abundance and community composition could help to explain the results? To address these questions, we mixed pyrolysis biochar (*Miscanthus*) with an arable soil and added specimens of *Aporrectodea caliginosa*, a common endogeic earthworm in temperate arable soils. Biochar derived from a C4 plant and showing another $^{13}C$-signature than the soil made it possible to quantify the earthworm effect on biochar-C mobilization. To one half of the experiment, we added N-rich plant litter, reflecting the incorporation of a green manure into arable soil, which is typically accompanied by high $N_2O$ emissions (Baggs et al., 2002). The effects of biochar and earthworms on C and N turnover were investigated by measuring $CO_2$ and $N_2O$ emissions, microbial abundance and community composition were quantified by phospholipid fatty acid analyses (PLFA).

## 5.2 Materials and methods

### Experimental setup

The experiment was conducted in vessels consisting of airtight Perspex tubes (height 150 mm, Ø 45 mm) fixed on water saturated ceramic plates. The vessels were closed at the top with a lid and a rubber stopper with a three-way stopcock, enabling gas sampling for $CO_2$ and $N_2O$ measurements with a syringe from the head space. At the bottom of the lid, a small vial was attached, which was filled with NaOH to trap $CO_2$ for determination of isotopic signature of $CO_2$ produced inside the vessel (Marhan et al., 2007). The following treatments were established: soil only (Ctrl), soil with biochar (BC), soil with one juvenile *A. caliginosa* (EW), soil with biochar, and one juvenile *A. caliginosa* (BC + EW). Half of the vessels were set-up without litter (‘no litter’ treatments), the other half with *Phacelia* litter (‘with litter’ treatments). In total, 46 vessels were established (Ctrl, treatments n=5; all others, n=6), all soil mixtures were initially rewetted to 60 % of water holding capacity (WHC) of the control and incubated in darkness in a climate chamber at 20 °C for 37 days.

## Materials

### *Soil*

Soil was taken from the Ap-horizon (0–10 cm) of an arable field at the agricultural experimental station 'Heidfeldhof' (University Hohenheim, Germany). The soil is a slightly stagnic luvisol with a silty texture of 9 % sand, 69 % silt, and 22 % clay (Table 5.1). The soil was sieved (<2 mm) to remove stones, plant residues, earthworms, and their cocoons and stored at 4 °C for a few days until the experiment was set up. Each vessel was filled with fresh soil equivalent to 100 g dry weight (DW) and compacted to a bulk density of 1.2 g $cm^3$.

### *Biochar*

The biochar was produced by slow pyrolysis (approximately 600 °C; production rate up to 40 kg biochar $h^{-1}$) in a continuous reactor from *Miscanthus x giganteus* and was provided by Pyreg GmbH (Dörth, Germany) (Table 5.1). There was a low toxicity potential of PAHs, dioxins, heavy metals, or other persistent organic pollutants in the biochar (see further details in Table S5.1). The low toxicity of the biochar was revealed in a grassland field experiment showing no negative effects on plant growth (Schimmelpfennig et al., 2014). The biochar was sieved and particles <2 mm were homogenously mixed with soil (2 % w/w) to obtain an application rate of 30 Mg $ha^{-1}$, assuming biochar incorporation into ploughing depth of 30 cm in the field. This is the typical application rate in several biochar experiments (Augustenborg et al., 2012).

### *Litter*

Litter material was taken from *Phacelia tanacetifolia* Benth. plants grown in the same soil in a greenhouse for 10 weeks. Aboveground biomass was harvested, fragmented into <10 mm size pieces, and dried at 40 °C to constant weight. The green litter material had a low C/N ratio of 17 (350.2 g C $kg^{-1}$, 20.6 g N $kg^{-1}$). Litter was shredded to 5 mm size and homogenously mixed into the soil of the litter treatments at a rate of 1.54 % w/w of soil, which is equivalent to 23.1 Mg $ha^{-1}$; this represents the amount of *Phacelia* litter ploughed into the soil as green manure from an arable field at the 'Heidfeldhof' field station.

**Table 5.1.** Characteristics of soil, biochar and litter.

| Parameter | Soil | Biochar | Litter |
|---|---|---|---|
| $C_{org}$ [g $kg^{-1}$] | 12.1 | 671.7 | 350.2 |
| $N_t$ [g $kg^{-1}$] | 1.3 | 2.3 | 20.6 |
| C/N ratio | 9.3 | 292 | 17 |
| $\delta^{13}C$ [‰] | -27.28 | -13.82 | -29.82 |
| pH [0.01 M $CaCl_2$] | 6.8 | 8.8 | n.d. |
| Sand [%] | 9 | n.d. | n.d. |
| Silt [%] | 69 | n.d. | n.d. |
| Clay [%] | 22 | n.d. | n.d. |

n.d. = not determined.

*Earthworms*

Juvenile endogeic earthworms (*A. caliginosa* Savigny) were extracted from grassland adjacent to the arable field by hand sorting. The use of juvenile specimens enabled the detection of earthworm biomass decrease as well as increase (Marhan & Scheu, 2005). The earthworms were kept in the experimental soil for 6 days until the incubation experiment was set up. Before the earthworms were placed into the vessels, they were kept on wet filter paper for 1 day to void their guts. Afterwards, they were washed with water, dabbed dry, and weighed, giving the initial live weight. The mean body mass of *A. caliginosa* specimens was 138 mg fresh weight with a range of 90 to 192 mg. Smaller and larger specimens were homogenously distributed over the earthworm containing treatments. After incubation, the soil was carefully removed from the vessels to avoid injuring the earthworms and earthworm body mass was determined in the same way as described above in order to calculate changes in individual body mass.

## Analyses

*C, $\delta^{13}C$, N analyses, and pH values*

Initial soil, litter, and biochar C and N concentrations and their isotopic signatures ($\delta^{13}C$) were measured using an elemental analyzer (EA, Euro EA 3000, Euro Vector, Milan, Italy) coupled with an isotope mass spectrometer (IRMS, DeltaXP Plus, Thermo Finnigan,Waltham, USA). For this analysis, the sieved and dried soil, litter, and biochar were finely ground. Earthworm $\delta^{13}C$ signatures were determined by analyzing tissue

material from the anterior part of specimens, which contained no soil particles. For this, earthworms were killed by freezing and the anterior part of the frozen earthworms was cut off and dried at 60 °C. About 1.3 to 3.98 mg dry tissue material from each individual earthworm was analyzed. The earthworm $\delta^{13}C$ signatures were determined for three specimens per treatment. After the incubation, soil pH values were measured in 0.01 M $CaCl_2$ solution (1:4 w/v).

*Extractable organic C (EOC), ammonium ($NH_4^+$-N), and nitrate ($NO_3^-$-N)*

At the end of the incubation EOC, $NH_4^+$-N and $NO_3^-$-N concentrations in soil were determined by extracting the soil with 0.5 M $K_2SO_4$ (1:4 w/v). Soil suspensions were shaken on a horizontal shaker (30 min at 250 rpm) and centrifuged (30 min at 4400 × g). Concentrations of EOC in the supernatant were then analyzed with a DOC analyzer (multi N/C 2100 S, Analytik Jena AG, Jena, Germany). $NH_4^+$-N and $NO_3^-$-N concentrations in the supernatant were determined colorimetrically with a continuous flow analyzer (Bran + Luebbe Autoanalyzer 3, SEAL Analytical, Hamburg, Germany).

*$CO_2$, $N_2O$, and $^{13}CO_2$ emission*

To measure $CO_2$ and $N_2O$ emissions, vessels were tightly closed and 15 ml of the headspace volume was sampled immediately and 60 min after closure. Gas samples were taken with 20 ml syringes via three-way stopcocks and injected into pre-evacuated 5.9 ml exetainers (Labco Ltd., UK). $CO_2$ and $N_2O$ concentrations in the headspace samples were determined on an Agilent 7890 gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a methanizer and a FID for $CO_2$ and an ECD for $N_2O$ measurements. Three external standards per gas were used for calibration by linear regression (0.304, 1.487, and 2.966 mmol $mol^{-1}$ $CO_2$; 0.568, 1.099, and 2.056 µmol $mol^{-1}$ $N_2O$; Westfalengas, Germany). Gas samples were taken at days 1, 2, 3, 6, 8, 10, 13, 16, 21, 27, 30, and 34 of incubation. Cumulative $CO_2$ and $N_2O$ fluxes were calculated by linear interpolation between two consecutive measurements. $^{13}C$ in produced $CO_2$ was determined according to the method of Marhan et al. (2008) by capturing emitted $CO_2$ in NaOH solution (1 M) in the headspace of each vessel and measuring $^{13}C$ in the precipitated $SrCO_3$ at days 1, 4, 7, 10, 15, 18, 22, 29, 32, and 37 of incubation. Between gas sampling dates, lids or holes were left open to ensure free gas exchange.

*Calculation of biochar-derived C in $CO_2$ and priming effects*

The biochar derived from the C4 plant *Miscanthus* has a $\delta^{13}C$ value of −13.82 ‰, different from that of the soil (−27.28 ‰). Determination of biochar-C and SOC mineralization was possible for the 'no litter', but not for the 'with litter' treatments, due to the different $^{13}C$ signature of the litter-C in comparison to soil-C, which served as a third, not quantifiable $CO_2$ source. For the calculation of the relative amounts of biochar-C and native SOC in $CO_2$ at specific dates of the incubation, a simple two-pool mixing model was used (Gregorich et al., 1995). Priming effects (PEs) were calculated for 'no litter' treatments based on the $^{13}C$ data by determining the difference in the native SOC mineralization between biochar-amended samples (BC and BC + EW) and respective controls (Ctrl and EW) (Bamminger et al., 2014a) as shown in Eq. 1:

$$PE\ [\%] = (\text{mineralized } SOC_{treatment} - \text{mineralized } SOC_{control}) / \text{mineralized } SOC_{control} \times 100 \quad (1)$$

*Phospholipid fatty acid analysis*

The PLFAs of 4 g incubated soil (fresh weight) from each vessel were extracted according to Frostegård et al. (1993) with Bligh & Dyer solution (chloroform, methanol, citrate buffer; pH=4; 1:2:0.8 v/v/v) and separated into glycolipid, neutral lipids, and phospholipid fatty acids with silica acid columns (0.5 g silicic acid, 3 ml; Varian Medical Systems, Palo Alto, California). Only the PLFA-fraction was analyzed. The branched fatty acids i15:0, a15:0, i16:0, and i17:0 were summed as Gram-positive and the cy17:0 and cy19:0 as Gram-negative bacteria (Zelles, 1999). In addition to these biomarkers, 16:1ω7 was included for total bacteria calculation (Frostegård & Bååth, 1996). The biomarker 18:2ω6,9c was considered as fungal PLFA (Frostegård & Bååth, 1996; Kaiser et al., 2010). Total microbial PLFA ($PLFA_{mic}$) consists of total bacterial and fungal PLFA. Metabolic efficiency ($qCO_2$) of the soil microbial community was calculated by the ratio between $CO_2$-C and microbial PLFA.

**Statistical analysis**

Data on cumulative $CO_2$-C and $N_2O$-N production, contents of EOC, $NH_4^+$-N, $NO_3^-$-N, soil pH, and microbial PLFA data were analyzed by two-way analysis of variance

(ANOVA) separately for the 'no litter' and 'with litter' treatments due to the strong effect of the litter, which led to inhomogeneity of the variances. Factors for two-way ANOVA were 'biochar' (BC; without and with BC) and 'earthworm' (EW; without and with EW). For the two-way ANOVA of earthworm biomass changes, the litter treatments were not separated and factors were 'biochar' and 'litter' (no and with litter). Prior to analysis, data were log-transformed in the case of non-normal distribution and inhomogeneity of variance (Levene test). A statistical probability of $P<0.05$ was considered significant. The STATISTICA 6.0 software package (Statsoft, Tulsa, OK, USA) was used for statistical analyses.

## 5.3 Results

### Earthworm biomass

All earthworms survived the incubation in the 'no litter' treatments and only one earthworm died in the 'with litter' treatments. This replicate was excluded from further analyses. Litter significantly affected earthworm biomass; in the 'no litter' treatments, earthworms reduced their biomass by 19 and 23 %, whereas in the 'with litter' treatments earthworms gained biomass by 42 and 32 % in the absence and presence of biochar, respectively (data not shown). Earthworm biomass showed no significant effects from biochar addition. After 37 days, the $\delta^{13}C$ signatures of the earthworms' biomass seemed to be more depleted in the 'with litter' than in the 'no litter' treatments ($P=0.08$), showing that litter derived-C was assimilated (Fig. S5.1), but no significant differences in earthworm $\delta^{13}C$ signatures were found between treatments with and without biochar.

### $CO_2$ emissions

$CO_2$ emission rates declined slightly in the 'no litter' treatments, whereas in the 'with litter' treatments decomposition of the litter was highest during the first 4 days (Fig. S5.2a). Basal respiration ($R_B$), which was the $CO_2$ production rate at the end of the experiment, was 36-fold higher in the 'with litter' than in the 'no litter' treatments. In both litter treatments, $R_B$ was significantly reduced by biochar, but not affected by earthworms (Tables 5.2 and 5.3). Cumulative $CO_2$ production over the incubation period ranged between 0.05 and 0.11 mg $CO_2$-C $g^{-1}$ dws in the 'no litter' treatments and between 0.91 and 1.30 mg $CO_2$-C $g^{-1}$ dws in the 'with litter' treatments (Fig. 5.1a). Biochar significantly

($P$<0.05) reduced cumulative $CO_2$ emissions in the 'no litter' and 'with litter' treatments by 43 and 27 %, respectively (Fig. 5.1a, Table 5.3). Earthworms had no significant effect on the $CO_2$ efflux.

**Table 5.2.** Physicochemical and microbial soil properties within the 'no litter' and 'with litter' treatments after 37 days of incubation.

| Parameter | No litter | | | | With litter | | | |
|---|---|---|---|---|---|---|---|---|
| | Ctrl | BC | EW | BC+ EW | Ctrl | BC | EW | BC+ EW |
| $R_B$ (µg $CO_2$-C $g^{-1}$ dws $d^{-1}$) | 1.24 ± 0.44 | 0.12 ± 0.05 | 1.25 ± 0.28 | 0.27 ± 0.10 | 2.87 ± 0.42 | 2.13 ± 0.33 | 3.37 ± 0.32 | 1.81 ± 0.23 |
| pH | 6.69 ± 0.18 | 7.04 ± 0.06 | 6.82 ± 0.04 | 6.99 ± 0.05 | 7.17 ± 0.03 | 7.16 ± 0.06 | 7.21 ± 0.03 | 7.12 ± 0.12 |
| EOC (µg C $g^{-1}$ dws) | 47.9 ± 4.2 | 48.4 ± 3.4 | 45.5 ± 3.7 | 57.1 ± 2.1 | 107.7 ± 6.0 | 106.7 ± 4.0 | 95.3 ± 2.5 | 114.8 ± 8.9 |
| Total bacterial PLFA (nmol $g^{-1}$ dws) | 14.1 ± 0.37 | 14.9 ± 1.1 | 13.8 ±0.55 | 19.6 ± 0.54 | 36.4 ± 2.5 | 41.7 ± 1.2 | 35.9 ± 0.69 | 39.3 ± 0.54 |
| $PLFA_{mic}$ (nmol $g^{-1}$ dws) | 15.0 ± 0.40 | 15.7 ± 1.2 | 14.7 ± 0.62 | 20.7 ± 0.55 | 43.5 ± 3.9 | 52.6 ± 2.9 | 42.5 ± 1.2 | 49.2 ± 1.3 |
| Gram-positive/Gram-negative ratio | 4.23 ± 0.09 | 4.48 ± 0.14 | 4.22 ± 0.06 | 4.52 ± 0.15 | 3.52 ± 0.05 | 3.87 ± 0.13 | 3.52 ± 0.06 | 3.48 ± 0.04 |
| Fungal/Bacterial ratio | 0.06 ± 0.00 | 0.06 ± 0.00 | 0.06 ± 0.00 | 0.06 ± 0.00 | 0.19 ± 0.02 | 0.26 ± 0.04 | 0.18 ± 0.01 | 0.25 ± 0.02 |
| $qCO_2$ (µg $CO_2$-C µmol$^{-1}$ $PLFA_{mic}$ $d^{-1}$) | 82.3 ± 31.7 | 10.0 ± 3.4 | 67.7 ± 5.3 | 14.1 ± 5.9 | 70.7 ± 14.6 | 42.6 ± 7.9 | 79.3 ± 7.6 | 37.4 ± 5.7 |

Ctrl = only soil, BC = soil with biochar, EW = soil including one endogeic earthworm, BC+EW= soil with biochar and one earthworm. Means ± SE.

$R_B$ (basal respiration), $qCO_2$ (metabolic efficiency)

**Table 5.3.** Two-Way ANOVA results for physicochemical and microbial soil properties within the 'no litter' and 'with litter' treatments. The table shows F-values for the effects of biochar (BC) and earthworm (EW) and their interaction (BC × EW). Significant effects are bold and indicated by asterisks (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

| Parameter | No litter ($n$ = 23) | | | With litter ($n$ = 22) | | |
|---|---|---|---|---|---|---|
| | BC | EW | BC × EW | BC | EW | BC × EW |
| Cumulative $CO_2$ | **12.8**** | 0.21 | 0.05 | **64.8 ***** | < 0.01 | 1.00 |
| Cumulative $N_2O$ [#] | 0.33 | 1.27 | 0.02 | **22.7***** | 0.01 | 0.90 |
| $R_B$ [#] | **25.4***** | 2.08 | 1.72 | **12.5**** | 0.08 | 1.56 |
| pH | **8.46**** | 0.19 | 1.0 | 0.46 | 0.01 | 0.21 |
| EOC | 3.21 | 0.87 | 2.71 | 2.30 | 0.13 | 2.82 |
| $NH_4^+$-N | 0.02 | 1.44 | 0.11 | **6.49*** | **26.9***** | **6.36*** |
| $NO_3^-$-N | **99.6***** | 0.53 | 0.25 | **8.83**** | 0.02 | 0.02 |
| Gram-positive bacteria | **17.7***** | **7.17*** | **9.00**** | **14.9***** | 2.72 | 1.45 |
| Gram-negative bacteria | **18.4***** | **14.0**** | **17.4***** | 4.22 | 0.01 | 0.09 |
| Total bacteria | **18.9***** | **8.55**** | **11.0**** | **9.99**** | 1.10 | 0.51 |
| Fungi | **6.29*** | **8.92**** | **5.87*** | **6.97*** | 0.25 | 0.03 |
| $PLFA_{mic}$ | **18.7***** | **8.96**** | **11.1**** | **9.61**** | 0.69 | 0.22 |
| Gram- positive/ Gram- negative ratio | **5.27*** | 0.01 | 0.04 | 3.47 | **5.42*** | **5.56*** |
| Fungal/bacterial ratio | 1.41 | 1.29 | 0.06 | **6.32*** | 0.022 | <0.001 |
| $qCO_2$ | **20.7***** | 0.72 | 0.97 | **14.5***** | 0.04 | 0.57 |

[#] Data for $N_2O$ ('with litter'), $R_B$ (basal respiration) and $qCO_2$ (metabolic efficiency) (both 'no litter') were log-transformed prior ANOVA.

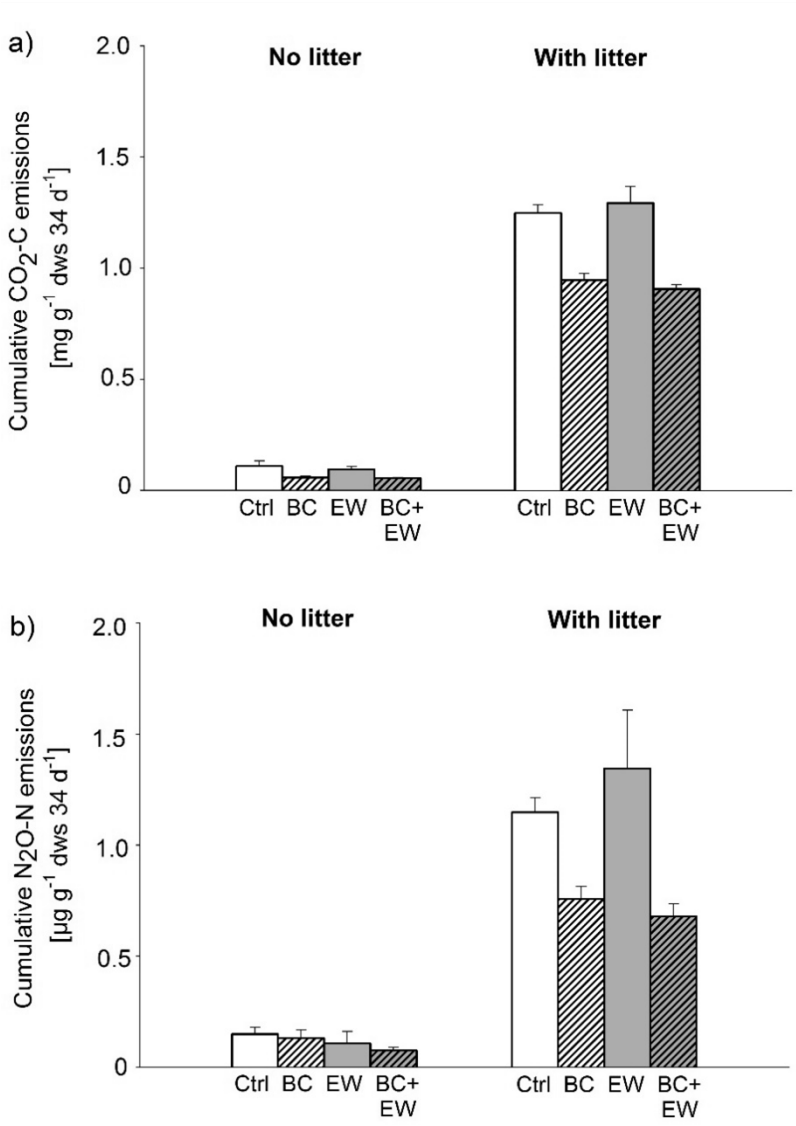


**Fig. 5.1.** Cumulative emissions of a) $CO_2$ and b) $N_2O$ in the 'no litter' and 'with litter' treatments at the end of the experiment (after 37 days). Ctrl soil only, BC soil with biochar, EW soil including one endogeic earthworm, BC + EW soil with biochar and one earthworm. Means±SE.

## Mineralization of biochar-C and SOC

Calculations of biochar-C derived $CO_2$ were not possible for the first half of the experiment due to strong variations in $^{13}CO_2$ values, likely caused in part by initial release of inorganic C from the biochar (Bruun et al., 2008). During the second half of the experiment (days 18, 22, 29, and 32), the contribution of mineralized biochar-C to total $CO_2$ emissions was on average 6.3 % in the 'no litter' treatment without earthworms (BC) and showed no increasing or decreasing trend (data not shown). In combination with the reduced total $CO_2$ emissions in this treatment, we calculated a negative priming effect of biochar, reducing soil-C mineralization by 56 % on an average. Biochar-C mineralization

seemed to be higher, by an average of 9.5 %, in the presence of earthworms in the 'no litter' treatment (BC + EW), but this difference was not significant ($P$=0.097). However, as total $CO_2$ production was similar to the treatment without earthworms, the negative priming effect of biochar increased due to earthworm activity up to 68 %.

**$N_2O$ emissions**

$N_2O$ emission rates were much lower in the 'no litter' treatments than in the 'with litter' treatments, but were generally highest during the first 2 days of incubation and declined afterwards rapidly until the end of the incubation (Fig. S5.2b). Cumulative $N_2O$ production in the 'no litter' treatments ranged from 0.08 to 0.15 μg $N_2O$-N $g^{-1}$ while in the 'with litter' treatments $N_2O$ production ranged from 0.68 to 1.35 μg $N_2O$-N $g^{-1}$ dry soil after 37 days (Fig. 5.1b). While biochar did not significantly reduce $N_2O$ emissions in the 'no litter' treatments, cumulative $N_2O$ production was significantly reduced by 42 % in the 'with litter' treatments (Fig. 5.1b, Table 5.3). Earthworms did not exhibit a significant effect on $N_2O$ emissions neither in the 'no litter' nor in the 'with litter' treatments.

**pH, EOC, $NH_4^+$-N, and $NO_3^-$-N**

Litter amendment increased pH values consistently above 7. Overall, biochar slightly increased pH values, by 0.1 to 0.3 units (Table 5.2), which was significant in the 'no litter', but not in the 'with litter', treatments (Table 5.3). EOC content in the 'with litter' treatments was almost twofold higher than in the 'no litter' treatments (Table 5.2). Neither biochar nor earthworms affected EOC contents significantly (Table 5.3). $NH_4^+$-N concentrations were between four to sixfold higher in the 'with litter' than in the 'no litter' treatments (Fig. 5.2a). In the 'with litter' treatments only, both biochar and earthworms reduced $NH_4^+$-N concentrations, but the earthworm effect was less pronounced in the presence of biochar (BC × EW interaction, Table 5.3). Concentrations of $NO_3^-$-N strongly exceeded those of $NH_4^+$-N and were almost twofold higher in the 'with litter' than in the 'no litter' treatments (Fig. 5.2). Biochar significantly ($P$<0.05) reduced $NO_3^-$-N by 40 % in the 'no litter' and by 16 % in the 'with litter' treatments (Fig. 5.2b, Table 5.3). Earthworms did not affect the amount of extractable $NO_3^-$-N in the present experiment.

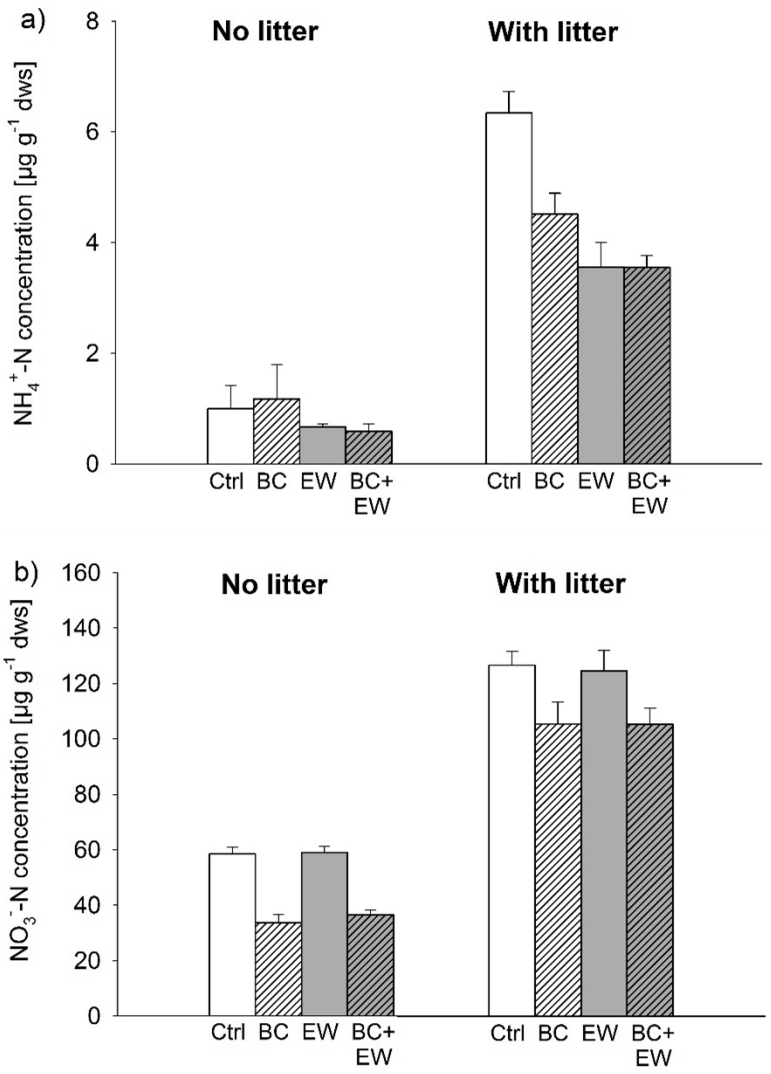


**Fig. 5.2.** Soil extractable a) $NH_4^+$-N and b) $NO_3^-$-N in the 'no litter' and 'with litter' treatments at the end of the experiment (after 37 days). Ctrl soil only, BC soil with biochar, EW soil including one endogeic earthworm, BC + EW soil with biochar a biochar and one earthworm. Means±SE.

## Microbial PLFA content

Total microbial and bacterial PLFA abundances (Table 5.2) and PLFA abundances of Gram-positive bacteria, Gram-negative bacteria, and fungi were higher in the 'with litter' than the 'no litter' treatments (Fig. 5.3a–c). While a two to threefold increase was observed for bacterial PLFAs, fungal PLFA abundance was 8 to 13-fold higher in the 'with litter' compared to the 'no litter' treatments. In the 'no litter' treatments, abundances of total bacteria, Gram-positive, Gram-negative, and fungi were significantly increased by both biochar and earthworms, but generally highest abundances were found in the BC + EW treatment (significant BC × EW interaction, $P<0.05$, Tables 5.2 and 5.3, Fig. 5.3a–c). In

the ‘with litter’ treatments, biochar significantly ($P$<0.05) increased total microbial PLFA abundance ($PLFA_{mic}$) by 16 % and that of total bacteria by 11 % (Tables 5.2 and 5.3). This biochar effect was mostly derived from a significant increase in abundances of Gram-positive bacteria (Fig. 5.3a, b; Tables 5.2 and 5.3). Moreover, biochar significantly ($P$<0.05) increased the abundance of fungi by 34 % (Fig. 5.3c, Tables 5.2 and 5.3). The ratio of Gram-positive to Gram-negative bacteria was significantly ($P$<0.05) increased by biochar only in the ‘no litter’ treatments (Tables 5.2 and 5.3). In the ‘with litter’ treatments, earthworms decreased the ratio of Gram-positive to Gram-negative bacteria only in the presence of biochar (BC × EW interaction). Biochar also significantly ($P$<0.05) increased the fungal to bacterial ratio, but only in the ‘with litter’ treatments (Tables 5.2 and 5.3). At the end of the experiment, the metabolic quotient ($qCO_2$) was significantly ($P$<0.05) decreased by biochar in the ‘no litter’ (86 %) and in the ‘with litter’ (37 %) treatments, but remained unaffected by earthworms (Tables 5.2 and 5.3).

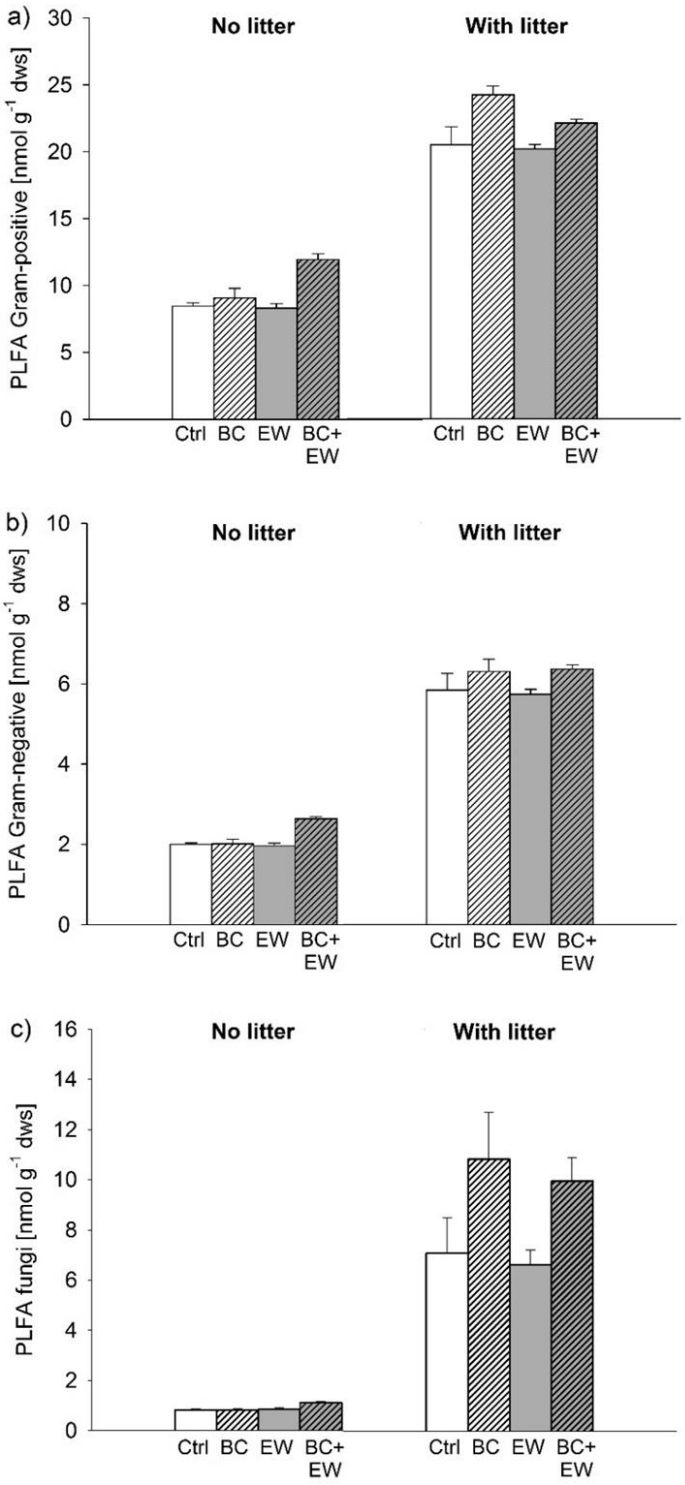


**Fig. 5.3.** Concentrations of phospholipid fatty acids (PLFA) for a) Gram-positive, b) Gram-negative, and c) fungi in the 'no litter' and 'with litter' treatments at the end of the experiment (after 37 days). Ctrl soil only, BC soil with biochar, EW soil soil including one endogeic earthworm, BC + EW soil with biochar and one earthworm. Means±SE.

## 5.4 Discussion

The first aim of the present study was to answer the question whether endogeic earthworms are able to mobilize and incorporate stable biochar C, likely leading to increased decomposition of biochar. Biochar had no effect on earthworm biomass and stable isotope technique ($^{13}C$) revealed that $^{13}C$-signatures of earthworm tissues did not change when biochar was present in soil. This indicates that endogeic earthworms did not assimilate biochar-C in relevant amounts. A selective avoidance of biochar by *A. caliginosa*, as suggested by Tammeorg et al. (2014), can be excluded from the present study as biochar particles were visible in produced earthworm casts. However, earthworms may ingest biochar particles due to its detoxifying and liming effects rather than for nutrient supply (Topoliantz & Ponge, 2003). Although the assimilation of biochar-C by earthworms seems to be negligible, the presence of earthworms led to a slightly higher mineralization of biochar-C in the 'no litter' treatments. As stated above, the quantification of biochar-C mineralization was only possible for the 'no litter' treatments due to the non-quantifiable litter derived-C contribution to total $CO_2$ emission. Measurements of $\delta^{13}CO_2$ revealed that only a small contribution (6.3 %) of the respired $CO_2$ was biochar-derived in the treatment without earthworms. This indicates that the *Miscanthus* biochar was not inert, but rather stable, resisting decomposition by soil microorganisms. This is comparable to other studies with plant-derived biochars produced at 500–600 °C (Zimmerman, 2010; Singh et al., 2012). In the presence of earthworms, the proportion of biochar-C in evolved $CO_2$ was increased slightly (9.5 vs. 6.3 %). However, mineralization of earthworm biomass derived-C could influence $^{13}C$-signatures of emitted $CO_2$ from soil (Marhan et al., 2007). During the present incubation, earthworm biomass decreased in the 'no litter' treatment and it could be that this earthworm derived-C, which has a $\delta^{13}C$ signature between biochar and SOC might have contributed to the produced $CO_2$. This could lead to an overestimation of the earthworm effect on biochar-C mineralization.

The second aim of our study was to determine whether only the single factors, earthworms and biochar, affect C and N turnover, i.e., $CO_2$ and $N_2O$ emissions or whether they interact. We found no significant earthworm effect on $CO_2$ and $N_2O$ emissions. This is in contrast to a recent meta-analysis by Lubbers et al. (2013), which has shown that earthworms alone often increase $CO_2$ and $N_2O$ emissions from soil. The lack of an earthworm effect on $CO_2$ and $N_2O$ emission was unexpected because increased $CO_2$ production was found for the same soil and earthworm species by Marhan et al. (2010). In

addition, no interactive effect of earthworms and biochar on $CO_2$ and $N_2O$ emissions was observed in the present study. This is in contrast to Augustenborg et al. (2012) who found that endogeic earthworms increased GHG emissions and that this was mitigated by biochar.

However, biochar alone strongly reduced $CO_2$ (by 43 %) in the 'no litter' and reduced both $CO_2$ (by 27 %) and $N_2O$ (by 42 %) emissions in the 'with litter' treatments. These strong reductions of GHGs from soil by biochar are comparable to the results from other short-term laboratory studies using biochars from plant feedstock produced at 500–600 °C (Augustenborg et al., 2012; Cayuela et al., 2014). One of the most likely explanations for the reductions of $CO_2$ and $N_2O$ emissions by biochar could be a decrease in soil microbial abundance. In contrast, analyses of PLFAs showed an increasing effect of biochar on microbial abundances in the 'with litter' treatments and at least no negative effect in the 'no litter' treatments in our study. It is still unclear whether biochar could interfere with extraction-based microbial analyses like PLFA, probably stabilizing dead microbial PLFA onto biochar particles, which may result in an overestimation of living microbial abundance (Lehmann et al., 2011). Although we cannot finally exclude these biochar effects on PLFA stabilization in soils, we assume that this could not explain the strong discrepancy between concurrent reduction of $CO_2$ and $N_2O$ emissions and increment of PLFA abundances in the 'with litter' treatments.

Other potential reasons to explain the reductions of $CO_2$ and $N_2O$ emissions by biochar have been also discussed in the literature (Ameloot et al., 2013a; Augustenborg et al., 2012; Cayuela et al., 2013, 2014; Lehmann et al., 2011): (1) Changes of soil moisture conditions, e.g., a biochar induced decrease of soil moisture could lead to water limitation for soil microorganisms, thus decreasing $CO_2$ as well as $N_2O$ emissions; (2) Reduced C and N resource availability due to the adsorption onto biochar surfaces, protecting it from microbial decomposition; (3) Immobilization of N by soil microorganisms for building up their biomass thereby reducing mineral-N as substrates for $N_2O$ production by nitrification and denitrification; and (4) Changes in the microbial community composition resulting in a modified activity, e.g., due to biochar effects on soil pH. In the following paragraphs, these potential reasons will be discussed in the context of the results of our study:

We analyzed the potential effect of biochar on soil moisture in an additional laboratory analysis (see supplemental text, Fig. S5.3). This analysis revealed that at 60 % WHC, which was the adjusted water content for the incubation experiment, no changes due to

biochar addition were found. We, therefore, excluded different soil moisture conditions as a possible explanation for the observed biochar effect on $CO_2$ and $N_2O$ emissions.

Adsorption or diffusion of C and N substrates into biochar micropores could result in reduced SOC mineralization or $N_2O$ emission in biochar-amended soil by preventing C and N from being used by microorganisms (Ameloot et al., 2013a). The unavailability of SOC would induce a negative priming effect (Kuzyakov et al., 2009), which was found in the 'no litter' treatments, where biochar alone induced a negative priming effect, thus decreasing the mineralization of native soil organic matter by 56 %. Similar negative SOC priming effects of 38 % (Bamminger et al., 2014a) and 52 % (Zimmerman et al., 2011) have been reported in other studies with similar biochars. In contrast, the amount of EOC, which is considered as readily available substrate for microorganisms and which serves as electron donor during the denitrification process, was not decreased by biochar in the present study (Table 5.2). Therefore, we assume that adsorption of C to biochar particles in larger quantities is unlikely. Biochar can also reduce the availability of $NH_4^+$ and $NO_3^-$ (Clough et al., 2013), which serve as substrates for the $N_2O$ producing processes of nitrification and denitrification, respectively. Biochar reduced extractable $NO_3^-$-N in both litter treatments and reduced $NH_4^+$-N in the 'with litter' treatments after 37 days (Fig. 5.2). In both cases, adsorption of inorganic-N on biochar particles could be one explanation for the observed reduction of $N_2O$ emissions.

Immobilization of N by soil microorganisms could be another explanation for a reduction of $N_2O$ emissions by biochar. Microbial abundance was increased by biochar in the 'with litter' treatments, enhancing microbial PLFAs by 16 % (Table 5.2). Biochar is known to alter physicochemical soil properties and, therefore, the living conditions for soil microorganisms thereby often promoting microbial growth (Ameloot et al., 2013a). In the biochar 'with litter' treatments higher $PLFA_{mic}$ contents corresponded to lower extractable $NO_3^-$-N and $NH_4^+$-N (for $NH_4^+$-N only in the treatment without earthworms). This indicates that N-mineralization was decreased in the presence of biochar (Prayogo et al., 2014), i.e., organic N from the litter was assimilated by soil microorganisms and, therefore, immobilized rather than being mineralized. The reduction in the availability of $NO_3^-$-N and $NH_4^+$-N could be responsible for the observed reduction of $N_2O$ emissions in the biochar treatments when litter was present. The reductive effect of biochar on $N_2O$ emission is lacking in the 'no litter' treatments and here $PLFA_{mic}$ is only enhanced in

combination with earthworms. The reason for the different effects of biochar might be the lack of C and N resources, which limited microbial growth in the 'no litter' treatments.

Soil pH is known to affect soil microorganisms and biochar often enhances pH, thus possibly influencing soil microbial abundance and activity due to a liming effect (Lehmann et al., 2011). In our study, biochar increased pH by on average 0.26 units only in the 'no litter' treatments (Table 5.2). This increase of pH might be the reason for the observed increase of the Gram-positive/Gram-negative ratio. The question is now, whether this significant but rather small shift in bacterial community composition could explain the pronounced reductions of total $CO_2$ emissions by 43 % and the strong decrease of the metabolic quotient ($qCO_2$) by 86 %. In the 'with litter' treatments, where no effect of biochar on pH was observed, $CO_2$ emissions (by 27 %) and the metabolic quotient (37 %) were also reduced. The non-existence of a biochar effect on pH in the 'with litter' treatments indicates that additional factors influence microbial community composition and activity. Biochar may provide ideal conditions for soil microorganisms by improving nutrient retention and, therefore, higher N bioavailability at the surface of the biochar particles (e.g., Zheng et al., 2013). This biochar-supported co-location of substrates and soil microorganisms on their surfaces could reduce the stress potential leading to a more efficient metabolic C use (Lehmann et al., 2011).

Biochar increased the fungal-to-bacterial ratio and the Gram-positive/Gram-negative ratio (only in the treatments without earthworms) in the 'with litter' treatments, and similarly the Gram-positive/gram-negative ratio in the 'no litter' treatments. This indicates that biochar selectively influences microbial abundances of some microbial groups, leading to a different microbial community composition (Farrell et al., 2013) with enhanced microbial C use efficiency (Jin, 2010). Gram-positive bacteria seem to benefit more from biochar addition than Gram-negative bacteria. Gram-positive bacteria are known to preferentially degrade aromatic C in soil and they may, therefore, better utilize biochar-C, thus profiting more from the presence of the aromatic structure of the biochar (Farrell et al., 2013). Conversely, the relative decrease in the abundance of Gram-negative bacteria could be related to low quantities of easily available substrates in high temperature biochars (Ameloot et al., 2013a).

In the 'with litter' treatments, biochar generally increased fungal abundance more than bacterial abundance, resulting in increased fungal-to-bacterial ratios (Table 5.2), which was also found in the study of Prayogo et al. (2014). In our study, the presence of N-rich

and easily degradable litter could have promoted higher growth rates in fungi, which indicates that the fungal community may serve as major litter decomposers, mobilizing nutrients from dead plant material. In addition, it is possible that fungi are able to grow into biochar pores using additional resources or habitats better than bacteria (Lehmann et al., 2011). Our results stand in contrast to field studies, including litter incorporation into soil, which found decreased fungal abundances and shifts towards more bacterial-dominated soil microbial communities when biochar was present in soil (Chen et al., 2013; Jones et al., 2012). The mechanisms behind the observed suppressed SOC mineralization, improved metabolic efficiency, and change in the soil microbial community composition clearly warrant further investigations to clarify causes and effects.

In addition, the observed shifts in soil microbial community composition after biochar addition probably also influenced nitrification and denitrification processes (Clough & Condron, 2010). In the N-rich plant litter containing 'with litter' treatments, biochar reduced $N_2O$ emissions by 42 %. A mitigating effect of biochar on $N_2O$ had been observed in other short-term studies (e.g., Kammann et al., 2012). In addition to the above mentioned possible reason, that reduced availability of N due to adsorption onto biochar surfaces or immobilization by soil microorganisms is responsible for the observed reduction of $N_2O$ emissions, other studies found out that biochar promotes the last step of denitrification (Cayuela et al., 2013; Harter et al., 2013). Acceleration of the last step of the denitrification, the reduction of $N_2O$ to $N_2$, could decrease $N_2O$ emission from soil. Harter et al. (2013) showed that biochar increased the abundance of denitrifying bacteria performing this last step of the denitrification. However, as this was not measured in the present study, we cannot confirm this proposed mechanism as a reason for the biochar induced reduction of $N_2O$ emissions for the present experiment.

Overall, our results confirm those of the study of Prayogo et al. (2014), who also showed that the presence of litter influences the effects of biochar on the abundance and activity of soil microorganisms. In addition, earthworms, as an additional factor, partly influenced these effects of biochar in the present study as well. Although earthworms alone and in interaction with biochar showed no effects on greenhouse gas emissions, we found positive interactive effects of earthworms and biochar on Gram-positive, Gram-negative bacteria, and fungi in the 'no litter' BC + EW treatment (Fig. 5.3). As biochar particles were found in earthworm casts, we expect that pure biochar particles were mixed with soil organic matter during passage through the earthworm gut. This would lead to a closer association of soil and earthworm gut microorganisms with biochar (Ameloot et al.,

2013b). We conclude that the formation of these was more suitable habitats for soil microorganisms in earthworm worked biochar amended soils will increase microbial abundance. However, the mechanisms behind the increased microbial abundance in the 'no litter' treatment with biochar and earthworms cannot yet be identified, as neither pH, available EOC, nor mineral N was similarly affected in the combined treatment.

## 5.5 Conclusion

A major result of this short-term study was that biochar reduced $CO_2$ and $N_2O$ emissions, while it simultaneously increased microbial abundance in soil. This resulted in a more efficient metabolic C use, emphasizing the potential beneficial effect of biochar on soil microbial activity. In addition, the effect of reduced $N_2O$ emissions was especially pronounced when litter with a low C/N ratio was applied to the soil, suggesting that biochar amendments may mitigate the typical high $N_2O$ emissions from arable fields after green manure is ploughed into the soils. The mechanisms for increased respiratory C-use efficiency, negative priming effects, and reduced $N_2O$ emissions may be interrelated and are likely connected to the observed changes in the soil microbial community composition, warranting more detailed investigation. Moreover, the observed interactive effects of earthworms with biochar on soil microbial abundance highlight the importance for additional research including the different kinds of soil organisms as well as biochar, under natural field conditions in the medium to long term.

## 5.6 Acknowledgments

We thank Kathleen Regan and Kathleen A. Mackie for English correction and Wolfgang Armbruster for isotopic analyses. In addition, we kindly thank the editor and the two anonymous reviewers for their helpful comments on the manuscript. The first author was funded by a PhD scholarship awarded by the faculty of Agricultural Sciences at the University of Hohenheim.

## 5.7 Supplementary material

Supplemental text

Determination of soil hydraulic properties of all treatments

Before the microcosm experiment was set up, the effects of biochar and litter on WHC, soil hydraulic properties (saturated hydraulic conductivity and water retention curve) were separately measured for all treatments. Briefly, 280 g of oven dry soil and mixtures with biochar with and without litter were packed into 250 $cm^3$ soil cores yielding a bulk density of 1.12 g $cm^{-3}$. The cores were slowly saturated from the bottom up with degased and deionized water. The saturated hydraulic conductivity (Ksat) was measured by means of the falling-head experiment following the standard DIN 19683-9 using the UMS Ksat system (UMS, Munich, Germany). For each soil core, we repeated the Ksat-measurement five times.

The water retention curve was determined by the evaporation method for each soil core using the HYPROP-system (UMS, Munich, Germany) (Fig. S5.3). In our study, the tensiometers failed at tensions around 1 bar. To gain additional information in the drier range of the retention curve, we determined water retained at 15 MPa tension (pF 4.2) by the pressure plate method using a Ceramic Plate Extractor (Soil Moisture Equipment Corp., Santa Barbara, CA, USA). Rubber rings (n=4) were filled with soil suspension and placed on the ceramic plate. After 10 days of pressure, the soil was weighed, oven dried at 105 °C for 48 hours, and reweighed to calculate the water content. Plant available water capacity of each soil sample was determined by calculating the difference in volumetric water content held at tensions of 0.063 MPa (pF 1.8) and 15 MPa (pF 4.2). Soils of all treatments exhibited almost the same water content at 60 % WHC without an effect of biochar or litter on the available water capacity. Thus, all soil mixtures were rewetted to 60 % of water holding capacity (WHC) of the control treatment at the start of the microcosm experiment.

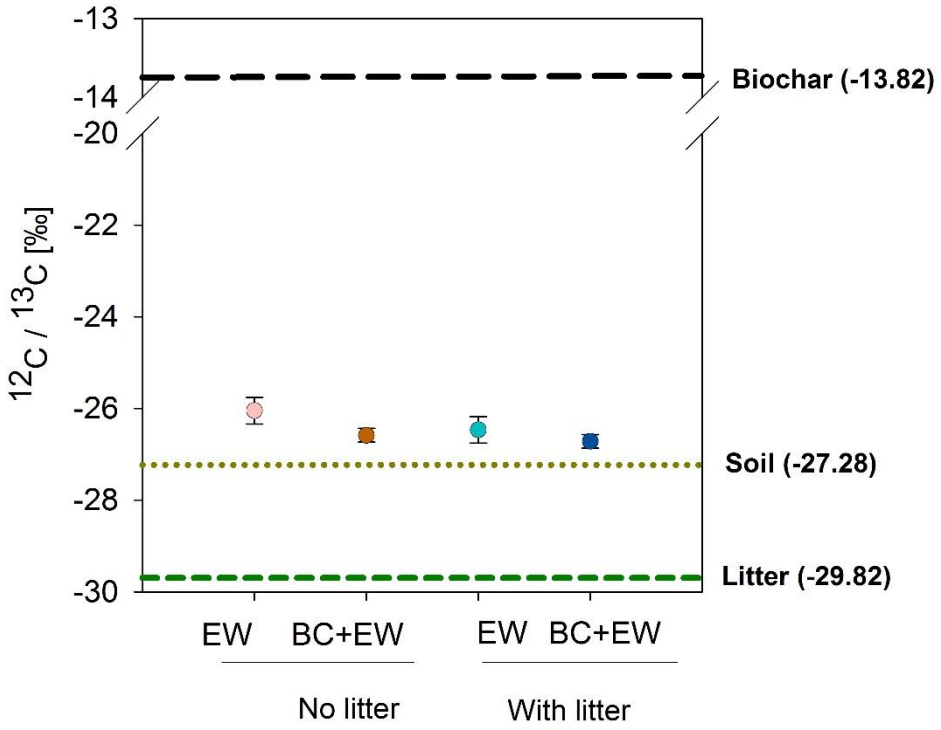


**Fig. S5.1.** $\delta^{13}C$-values of earthworm biomass in soils without (EW) and with biochar (BC+EW) in the 'No litter' and 'With litter' treatments.

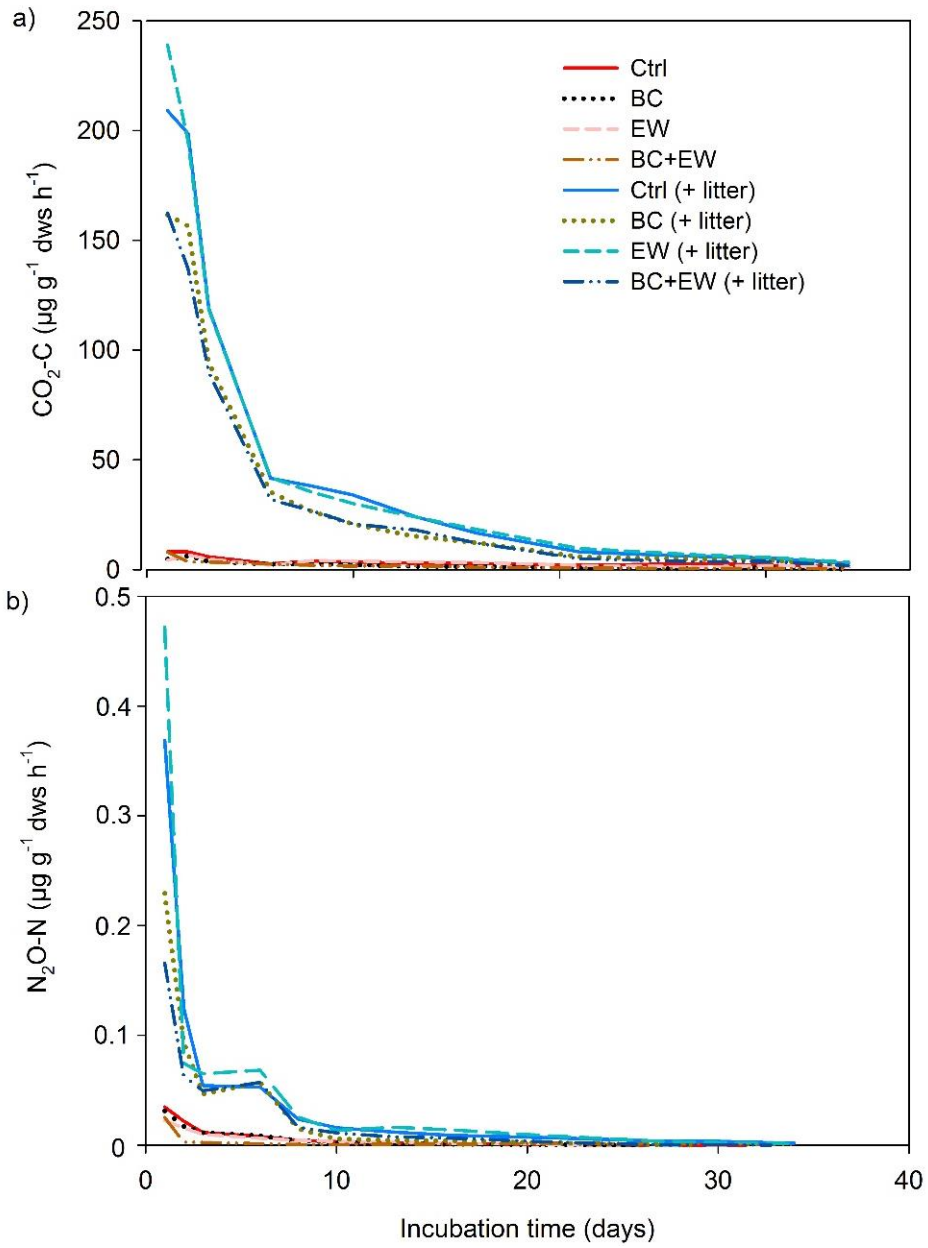


**Fig. S5.2.** Rates of a) $CO_2$ and b) $N_2O$ fluxes in the 'No litter' and 'With litter' treatments during the 37 days of incubation. Ctrl = only soil, BC = soil with biochar, EW = soil including one endogeic earthworm, BC+EW = soil with biochar and one earthworm.

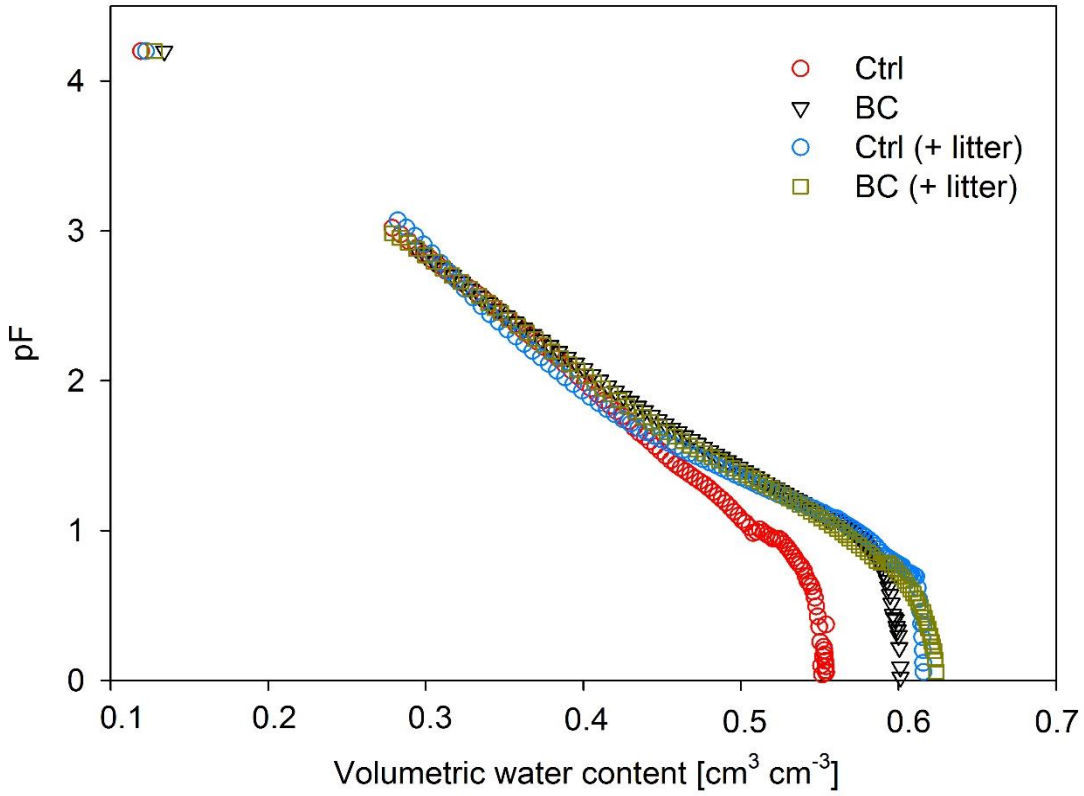


**Fig. S5.3.** Water retention curve (pF curve) of soil without (Ctrl) and with biochar (BC) and without and with litter (+ litter).

**Table S5.1.** Chemical characteristics of the used biochar from *Miscanthus x giganteus* (Pyreg GmbH, Dörth, Germany).

| **Parameter** | **Value** |
|---|---|
| Bulk density (1.5 mm ground) [g 100 $ml^{-1}$] | 15.9 |
| Water holding capacity [g $H_2O$ $g^{-1}$ Biochar] | 3.7 |
| BET surface [$m^2$ $g^{-1}$] | 864 ± 26.7 |
| Ash content [%] | >20 |
| *Nutrient concentration [g $kg^{-1}$]* | |
| Ca | 12.6 |
| K | 29.8 |
| Mg | 5.3 |
| P | 3.0 |
| S | 0.55 |
| *Micronutrient / heavy metal concentration [mg $kg^{-1}$]* | |
| Al | 2380 |
| As | 1.2 |
| Cd | 0.07 |
| Cr | 20.4 |
| Cu | 16.2 |
| Fe | 2520 |
| Hg | < 0.01 |
| Ni | 17.4 |
| Pb | 1.23 |
| Zn | 97.1 |
| ∑PAH (EPA)[1] | 2.2 |
| PCB | n.d. |

n.d. = not detectable. [1] Sum of 16 Polycyclic aromatic carbohydrates (PAH) after EPA classification

**Table S5.2.** Soil hydraulic properties in soil without (Ctrl) and with biochar (BC), without and with litter addition.

| Properties | No litter | | With litter | |
|---|---|---|---|---|
| | Ctrl | BC | Ctrl | BC |
| $\theta_s$ [$cm^3$ $cm^{-3}$] | 0.55 | 0.60 | 0.62 | 0.63 |
| $K_s$ [cm $d^{-1}$] | 414±2.0 | 139±2.8 | 270±3.2 | 203±3.7 |
| $\Theta_{(pF\ 1.8)}$ [$cm^3$ $cm^{-3}$] | 0.42 | 0.44 | 0.42 | 0.43 |
| $\Theta_{(pF\ 4.2)}$ [$cm^3$ $cm^{-3}$] | 0.12 | 0.13 | 0.12 | 0.13 |
| AWC [$cm^3$ $cm^{-3}$] | 0.30 | 0.31 | 0.30 | 0.30 |

$K_s$ = saturated hydraulic conductivity; AWC = plant available water content ($\Theta_{(pF\ 1.8)} - \Theta_{(pF\ 4.2)}$)

# 6 Short-term response of soil microorganisms to biochar addition in a temperate agroecosystem under soil warming

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## Abstract

The amendment of biochar to agricultural soils is presumed to mitigate global warming through long-term carbon (C) sequestration. In addition, biochar may support microbial biomass and diversity as well as plant growth by the improvement of soil properties. So far, no information is available on the stability of biochar and the effects of biochar on soil microbial and plant properties under predicted soil warming at the field scale. We investigated the impacts of biochar addition (*Miscanthus* pyrochar, 30 t $ha^{-1}$, August 2013) and long-term soil warming (+2.5 °C, since July 2008) and their interactive effects on microbial biochar-C utilization and physical, chemical and microbial soil properties of a silty-loamy stagnic Luvisol in a temperate agroecosystem (Stuttgart, Germany) over one year under winter rapeseed (*Brassica napus*). Three months after biochar application (November 2013), microbial abundances remained unaffected, indicating that readily available C from fresh biochar had been consumed before sampling. However, we found evidence for initial decomposition of more recalcitrant biochar-C by fungi under soil warming after three months. We suggest that the added biochar was very stable, since increased biochar degradation by fungi could not be detected after seven and twelve months. Nevertheless, during spring 2014, biochar reduced water loss in warmed soil by 16 % and decreased negative effects of soil dryness on microbial abundances by up to 80 %. In addition, the positive effect of biochar on soil moisture affected canopy height of winter rapeseed in the non-warmed plots in the early growth stages, although it did not change the final aboveground biomass in the first year after biochar application. Overall, biochar could be an appropriate tool for C sequestration by improving or maintaining soil fertility and productivity in temperate agroecosystems under future elevated temperatures.

**Keywords:** Pyrogenic carbon, Soil warming, Soil microbial community composition, *Brassica napus*, Agroecosystem

**Highlights:**

- Biochar improved soil properties without triggering strong microbial responses.
- Fungi may decomposed biochar initially under soil warming.
- Biochar mitigated drought effects on soil microorganisms under soil warming.
- Biochar supported the growth of winter rapeseed under drought conditions.

## 6.1 Introduction

The application of biochar to soil organic carbon-(SOC) depleted agricultural soils is thought to be an effective means of carbon (C) sequestration as well as for mitigation of the impacts of global warming on C cycling (Lal, 2011). The concept of using charcoal for C sequestration in soils originates from its low degradability due to a high percentage of polycondensed aromatic C structures, which was shown in the Amazonian 'Terra Preta' soils (Glaser & Birk, 2012). In these tropical soils enriched with ancient charcoal particles, various soil physical and chemical properties were found to have improved (Glaser et al., 2002) and microbial abundance and diversity was enhanced compared to adjacent soils (Grossman et al., 2010). However, there is limited knowledge about the interactive effects of biochar with future climate warming, although this knowledge is essential in order to evaluate the potential of biochar as a climate change mitigation tool.

Biochar may interact with soil microorganisms either directly, by being degraded and utilized, or indirectly, by improving soil properties and habitat conditions (Ameloot et al., 2013b). Certainly, the stability of biochar against microbial degradation is a prerequisite for its use in C sequestration and the longevity of expected beneficial effects on soil organisms. Feedstock type and particularly pyrolysis conditions are determining the properties and stability of biochar (Zimmerman, 2010). For example, increasing pyrolysis temperature and duration generally leads to higher pH and surface area and decreased cation exchange capacity as well as degradability of biochar (Ronsse et al., 2013; Gai et al., 2014). Laboratory experiments using slow-pyrolysis biochars produced at moderate temperatures (400–525 °C) indicated mostly low values of biochar-C of about 2 % in microbial biomass after 100 days (Watzinger et al., 2014) and 624 days (Kuzyakov et al., 2009). Even for high-temperature biochar (700 °C), Luo et al. (2013) found that after 90 days, less than 2 % of microbial biomass carbon was biochar-derived in an alkaline soil. Unfortunately, to date, microbial biochar-C uptake has not been quantified under both field conditions and soil warming.

As the previous results of Luo et al. (2013) and Watzinger et al. (2014) indicate, due to the resistance of biochar to microbial consumption, biochar may indirectly alter soil microbial communities. As proposed by Lehmann et al. (2011), biochar could positively affect soil microbial abundance and community composition by i) serving as a refuge habitat, which protects microbes against grazers and predators, ii) improving physical soil properties, e.g. water holding capacity, bulk density and aeration, and iii) modifying

chemical soil properties, e.g. pH, cation exchange capacity (CEC), nutrient retention and sorption of soil organic matter. A more abundant and diverse soil microbial community under added biochar can improve the fertility and productivity of agricultural soils by, e.g., promoting nutrient cycling and suppression of plant diseases such as pathogens, possibly leading to increased crop yields (Jeffery et al., 2011; Bonanomi et al., 2016).

There is some evidence that biochar may have positive effects on soil microorganisms in agroecosystems. Domene et al. (2014) reported an increase in microbial biomass by nearly 100 % after three years, attributing this primarily to increased soil moisture due to biochar application. Similarly, two years after biochar application, enhanced microbial growth rates were observed by Jones et al. (2012). In contrast, other studies have found no or only slight impact of biochar on microbial abundances (Quilliam et al., 2012; Rutigliano et al., 2014; Imparato et al., 2016). However, recent studies have reported not only changes in the structure of the soil microbial community and shifts to bacterial-dominated communities (Jones et al., 2012; Chen et al., 2013), but also higher fungal-to-bacterial ratios in biochar-amended soils (Bamminger et al., 2014b). These quite different effects of biochar on soil microbial abundances could be explained by variations in soil fertility, biochar or crop type and climate conditions. In addition, divergent responses of bacteria and fungi to biochar may be due to differences in their abilities to cope with biochar in the soil environment; a faster response of bacteria compared to fungi to changed substrate availability; differences between bacteria and fungi in their mobility and colonization of biochar pores, and the fact that some fungal species can decompose biochar (Thies et al., 2015).

Global warming will unequivocally impact C cycling in terrestrial ecosystems; therefore, adequate mitigation and adaptation strategies, such as biochar addition to soils, are required (Lal, 2011). Soil temperature is an important regulator of microbial activity and community structure, as some organisms may be better suited to substrate utilization under elevated temperatures compared to others (Zogg et al., 1997). However, the response of microorganisms to soil warming is not only directly dependent on increased temperature, but also driven by environmental constraints such as soil moisture (Allison & Treseder, 2008), quantity of easily available C pools (Frey et al., 2008), and changes in aboveground and belowground plant growth, factors which affect soil fungi and bacteria differently (De Vries & Shade, 2013). These observations are restricted to forest and grassland soils, but the microbial response to soil warming in agricultural sites has been

tested in only a limited number of field experiments. During five years of soil warming, soil respiration was not enhanced in an agricultural soil in China, due most probably to soil-drying (Liu et al., 2015). Likewise, at the same field site where we conducted our study, Poll et al. (2013) found reductions in microbial biomass and respiration in summer 2009 when soil warming negatively affected moisture. This underscores the claim that soil moisture affected by warming may be one of the most important drivers of microbial activity and abundance in agroecosystems in a changing climate. Warming-induced alterations of environmental conditions (e.g. soil moisture) and their effects on the activity of plant-associated microbial communities may also affect crop growth in agricultural ecosystems (Compant et al., 2010). However, especially high-temperature biochar is able to increase soil water content due to its porous structure and hydrophilic functional groups, which makes it capable of retaining water on its surfaces (Gray et al., 2014). This could reduce the water loss in warmed soil, thus positively affecting soil microorganisms and plant growth (Liang et al., 2014; Kammann et al., 2011).

Very little is known to date about the stability of biochar against microbial decomposition, or biochar effects on soil microorganisms and plants under predicted soil warming in a changing climate. We aimed to close these gaps in knowledge in order to evaluate the potential of biochar as an appropriate climate change mitigation tool in agricultural soils. Biochar has already been shown to be highly resistant to microbial decomposition and we hypothesized that our high-temperature biochar from slow-pyrolysis would be stable in soil even under elevated soil temperature. Soil microbial biomass and community composition will likely change under soil warming and soil moisture especially will play a crucial role in this context (Poll et al., 2013). We hypothesized that biochar will alter the response of soil microorganisms to elevated soil temperature, i.e. the enhanced water retention in soil with biochar may reduce the limiting conditions for microorganisms induced by lower soil moisture content. In addition, we expected that the positive effect of biochar on the water regime in warmed soil, especially in dry periods, will be responsible for enhanced growth of crops such as winter rapeseed.

In this study, we added *Miscanthus* (C4) biochar to a temperate agroecosystem which has been exposed to soil warming for the last five years. We were interested in the potential interactions between biochar and soil microorganisms, especially under soil warming, followed over a period of one year after biochar application under winter rapeseed. The

use of an isotopically labeled ($^{13}C$) biochar made it possible to quantify the incorporation of biochar derived-C into microbial biomass.

## 6.2 Materials and methods

### Field site and experimental setup

The Hohenheim Climate Change (HoCC) experiment, in which both soil temperature and precipitation amount and patterns are manipulated (Poll et al., 2013), was established on an arable site at the experimental field station Heidfeldhof of the University of Hohenheim (Stuttgart, Germany) in July 2008. The experiment of the present study, the Biochar Hohenheim Climate Change experiment (BC-HoCC), is part of the HoCC experiment and was established in August 2013 in order to investigate biochar stability as well as biochar effects on soil microbial properties and crop growth after long-term soil warming (five years).

The HoCC experiment consists of four blocks with four plots (4 m × 1 m) each. Each plot is subdivided into four subplots (1 m × 1 m) of which two were used in the present experiment. According to the predicted temperature increase of 2.5 °C by 2100 (Umweltbundesamt, 2006), half of the plots (Te: elevated temperature) are warmed using heating cables at the soil surface (RS 611–7918, RS Components GmbH) since 2008, while the other non-heated plots are covered with dummy cables as experimental controls (Ta: ambient temperature). Additional information on the HoCC experimental setup can be found in Poll et al. (2013).

The commercially available biochar used was produced from C4 *Miscanthus* x *giganteus* litter feedstock by slow pyrolysis for 30 min at 850 °C ($\delta^{13}C$ = -14.63 ‰, PYREG GmbH, Dörth, Germany, Table 6.1). At the start of the BC-HoCC experiment (August 21st, 2013), the biochar (30 t $ha^{-1}$) was first put on top of the subplots ($BC_{Ta}$: soil with biochar at ambient temperature, $BC_{Te}$: soil with biochar at elevated temperature) together with spring barley litter (80 g $m^{-2}$) from the previous growing season. Both amendments were then manually ploughed into 0–20 cm soil depth. Control subplots ($Ctrl_{Ta}$: control soil at ambient temperature, $Ctrl_{Te}$: control soil at elevated temperature) were not amended with biochar, but with litter, while incorporation and ploughing was done in the same way as in the biochar plots. In total, 16 subplots (four replicates per treatment) were investigated in the present study.

The BC-HoCC experiment is equipped with a wireless sensor network consisting of wireless sensors (12 cm rods, CWS655) connected to a control data logger (CR800, all devices from Campbell Scientific Ltd., Logan, Utah, USA) to measure surface-near soil temperature (0–2 cm) and soil moisture (0–12 cm depth) in each subplot. We decided to measure soil temperature only in the upper 2 cm, where heating cables should have the highest impact on soil temperature, and to check whether the soil heating treatment was working correctly. In addition, a possible greater warming in biochar-amended plots by the decreased albedo of the soil due to its darker color (Verheijen et al., 2013), could most accurately be determined near the soil surface.

Data were recorded every 20 min. Mean annual temperature and precipitation in this area (1981–2010) is 9.4 °C and 718.7 mm, respectively (DWD, 2016). In 2013 and 2014, mean annual air temperature was 9.5 °C and 11.0 °C (Fig. 6.1a) and annual precipitation was 790.1 mm and 654.1 mm, respectively (Fig. 6.1b; weather station ‘Hohenheim’, LTZ Augustenberg, 2016). The C3 arable soil ($\delta^{13}C$ = -27.94 ‰) is a loess-derived stagnic Luvisol with silty loam-texture and neutral pH (Table 6.1).

**Table 6.1.** Main characteristics of arable soil and biochar.

| | C [%] | $\delta^{13}C$ [‰] | $N_t$ [%] | H [%] | S [%] | O [%] | C/N ratio | H/C ratio | O/C ratio | Ash content [%] | Volatile matter [%] | Fixed C [%] | pH [$CaCl_2$] | Sand [%] | Silt [%] | Clay [%] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Soil | 1.14 | -27.94 | 1.3 | n.d | n.d. | n.d. | 9.3 | n.d. | n.d. | n.d | n.d | n.d | 6.8 | 9 | 69 | 22 |
| Biochar | 77.97 | -14.63 | 0.7 | 1.0 | 0.2 | 7.2 | 113 | 0.16 | 0.07 | 14.70 | 8.59 | 75.90 | 9.1 | n.d. | n.d. | n.d. |

n.d. = not determined.

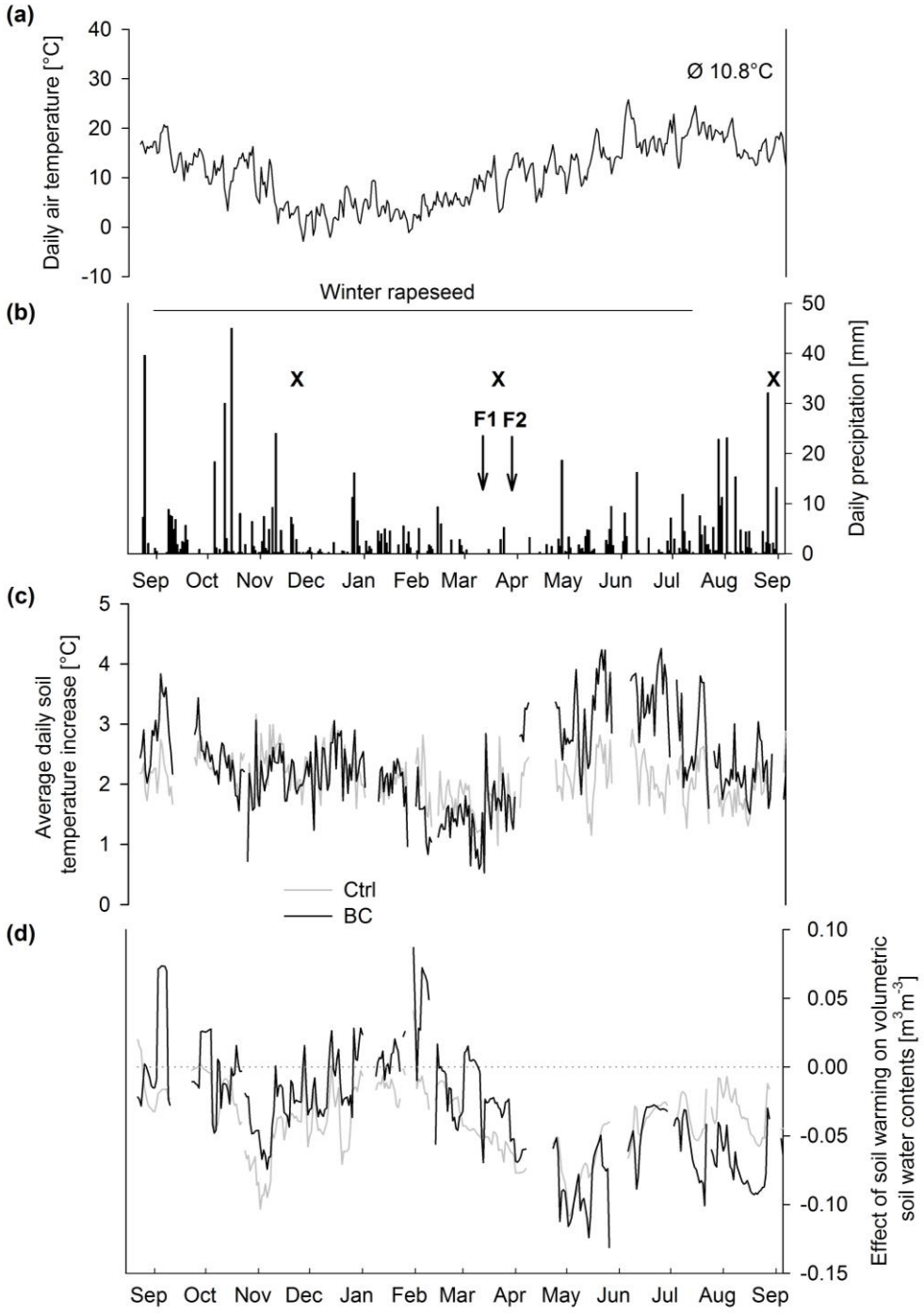


**Fig. 6.1.** (a) Daily air temperature (2 m) and (b) daily precipitation amount for the weather station "Hohenheim". Data provided by the agricultural technology center in Baden-Württemberg, Germany (LTZ Augustenberg). F1, F2 = fertilization events, X = soil sampling. (c) Increase of average daily soil temperature due to soil warming and (d) effect of soil warming on average daily volumetric soil water contents (VWC) in control (Ctrl) and biochar (BC) plots.

**Crop management, plant and soil sampling**

On September 3rd, 2013, 13 days after biochar application, winter rapeseed (Brassica napus L., 60 plants $m^{-2}$, ≈ 20 cm row spacing) was sown manually on all subplots. In the same month, slug pellets were applied to avoid seedling damage by slug grazing. On March 13th, 2014, plant numbers were adjusted to achieve usual plant density for rapeseed (30 plants $m^{-2}$). On the same day, the plants were fertilized with calcium ammonium nitrate at 70 kg nitrogen (N) $ha^{-1}$. On March 28th, 2014, the insecticide Trebon 30 EC (200 mL $ha^{-1}$) was applied to protect the plants from rape beetles (*Meligethes aeneus*). At the second fertilization event (March 31st, 2014), ammonium thiosulfate (17.2 kg N $ha^{-1}$ and 37.2 kg S $ha^{-1}$) was added to the plots. Canopy height was recorded weekly between April 9th and July 16th, 2014, always from the same five plants per subplot which were selected at the beginning of the experiment. Plants were harvested at maturity on July 17th, 2014, by cutting the aboveground biomass by hand. Total aboveground biomass was determined by weighing the oven-dried plant material (3 days at 37 °C).

Soil samples for physical, chemical and microbial analyses were taken with a soil auger from the control and biochar subplots under ambient and elevated temperature at two soil depths (0–5 and 5–15 cm) during the growing period of winter rapeseed in November 2013, March 2014, and after final harvest in September 2014.

**Physical and chemical analyses**

To determine soil bulk density, undisturbed soil cores (100 $cm^3$) were taken in duplicate at 0–5 and 5–15 cm soil depth from uncropped subplots in August 2014 after harvest of winter rapeseed. Soil water content (SWC) was determined gravimetrically at two depths on three occasions (November 2013, March 2014 and September 2014) after drying at 65 °C for three days. In addition, we obtained volumetric soil water content (VWC) by continuous time-domain reflectometry (TDR)-based measurements. By converting SWC into VWC, we calculated that TDR-based VWC in biochar-amended soil was likely overestimated by on average 37 %. This phenomenon has also been described in other studies and can be explained by the high electrical conductivity of high-temperature (>800 °C) biochar, which was like that used in our study, interfering with the TDR technique (Kameyama et al., 2014). We therefore only considered soil warming as a fixed effect in the statistical analysis for VWC.

The pH of initial biochar and collected soil samples was measured in 0.01 M $CaCl_2$ solution using a 1:50 w/v and 1:4 w/v ratio, respectively. Concentrations of ammonium-N ($NH_4^+$-N) and nitrate-N ($NO_3^-$-N) in soil were determined by extracting field moist soil with 0.5 M $K_2SO_4$ (1:4 w/v ratio). Soil suspensions were shaken on a horizontal shaker (30 min at 250 rev. $min^{-1}$) and centrifuged (30 min at 4400 × g). $NH_4^+$-N and $NO_3^-$-N concentrations in the supernatant were determined colorimetrically with a continuous flow analyzer (Bran + Luebbe Autoanalyzer 3, SEAL Analytical, Hamburg, Germany).

For elemental analysis, the sieved and dried soil was ground using a ball mill. The biochar was ground using a swing mill (CryoMill, Retsch GmbH, Haan, Germany), which was cooled with liquid nitrogen (-196 °C) to avoid heat development and gaseous carbon loss. Total C and N content, as well as C isotopic signatures ($\delta^{13}C$) of the initial biochar and of control soils at the three sampling dates were measured using an elemental analyzer (EA, Euro EA 3000, Euro Vector, Milan, Italy) coupled with an isotope mass spectrometer (IRMS, DeltaXP Plus, Thermo Finnigan, Waltham, USA). Acetanilide ($C_8H_9NO$, Merck, Darmstadt) was used as a laboratory reference material for internal calibration. $\delta^{13}C$ values are expressed relative to the international reference standard Vienna Pee Dee Belemnite (V-PDB). In addition, H and S contents of the biochar were determined using a Euro EA – CHNSO Elemental Analyzer (Hekatech, Wegberg, Germany). H content was corrected by the residual moisture of the biochar sample. Due to an instrumental defect, O content of the biochar was calculated by difference (%O = 100 - %C - %N - %H - %S - %ash).

Fourier transform infrared (FTIR) spectrum (Fig. S6.4) of the biochar was recorded on a VARIAN 660-IR spectrophotometer (Agilent Technologies Inc., CA) using the pellet technique by mixing 2 mg of dried biochar with 200 mg of pre-dried and pulverized spectroscopic-grade KBr (from Merck and Co., Whitehouse Station, NJ) and pressing at compressive force of 10 tons.

Thermogravimetric analysis (TGA) and derivative (DTG) profiles of the biochar (Fig. S6.5) were obtained using a simultaneous thermogravimetric analyzer (STA 449 F3 Jupiter, Netzsch, Germany) and performed under inert nitrogen atmosphere at a constant flow of (120 mL $min^{-1}$) and heating rate of 10 K $min^{-1}$ in a temperature range from ambient temperature to 1000 °C. Volatile matter (VM) associated with the biochar was assessed from weight loss after heating for 7 min at 900(±10) °C according to the standard procedure DIN 51720. Ash content of the biochar was determined by treating the sample at 815(±25) °C until weight constancy was reached (DIN 51719). The content of fixed C

of the biochar was calculated by the difference between 100 % and the sum of measured residual moisture (from TGA), VM and ash content. Key characteristics of soil and biochar are displayed in Table 6.1.

**Microbial biomass carbon and quantification of biochar-C incorporation**

Microbial biomass C ($C_{mic}$) was estimated by chloroform-fumigation-extraction (CFE) according to Vance et al. (1987). Field moist soil was fumigated under vacuum with ethanol-free chloroform in a desiccator for 24 h. After removing the chloroform, samples were extracted with 0.5 M $K_2SO_4$ solution (1:4 w/v ratio), then shaken and centrifuged as described for $NH_4^+$-N and $NO_3^-$-N. Further subsamples were non-fumigated, but similarly extracted. Concentrations of organic C in the supernatant of fumigated (f) and non-fumigated (nf) samples (EOC) were analyzed with a total organic C analyzer (multi N/C 2100 S, Analytik Jena AG, Jena, Germany). $C_{mic}$ was calculated by the difference in organic C content between f and nf samples divided by a $k_{EC}$ factor of 0.45 (Joergensen, 1996).

Biochar may influence the extraction efficiency of microbial C due to its potential to adsorb organic C compounds onto its surfaces, especially in sandy soils (Liang et al., 2010; Gomez et al., 2014). In soils with finer texture (like our silty-loam soil), clay minerals may have similar sorption characteristics to biochar (Gomez et al., 2014). Hence, we assumed that biochar effects on the determination of microbial biomass were negligible in our soil, but this cannot be entirely excluded.

For analysis of $\delta^{13}C$ values of $C_{mic}$, an additional CFE was performed with 0.025 M $K_2SO_4$ solution. Ten ml aliquots of the supernatants of both f and nf samples were dried in a vacuum rotary evaporator (RVC 2–25, Martin Christ, Osterode am Harz, Germany) at 60 °C. Subsequently, the remnant was ground and weighed into tin capsules (7–20 mg) to guarantee a minimum concentration of 5 mg C per capsule. Samples were measured by an elemental analyzer (Euro EA 3000, EuroVector, Milan, Italy) coupled with an isotope ratio mass spectrometer (IRMS, DeltaXP Plus, Thermo Finnigan, Waltham, USA).

$\delta^{13}C_{mic}$ was calculated by using the following equation:

$$\delta^{13}C_{mic} = (c_{nf} \times d_{nf}) - (c_f \times d_f) / (c_{nf} - c_f) \quad (1)$$

where $c_{nf}$ and $c_f$ are the extracted organic C content (mg C $g^{-1}$ soil) of the nf and f sample and $\delta_{nf}$ and $\delta_f$ are the corresponding $\delta^{13}C$ values (‰).

Finally, the proportion of biochar-derived C under Ta or Te at each sampling date was calculated as follows:

$$\% \text{ Biochar-derived C} = (\delta^{13}C_{mic} (BC) - \delta^{13}C_{mic} (Ctrl)) / (\delta_{BC} - \delta_{Soil}) \times 100 \quad (2)$$

where $\delta^{13}C_{mic}$ (BC) and $\delta^{13}C_{mic}$ (Ctrl) are the microbial biomass $\delta^{13}C$ (‰) of biochar and control obtained from Eq. (1), respectively, and $\delta_{BC}$ and $\delta_{Soil}$ are the $\delta^{13}C$ values (‰) of initial biochar and control soil collected in November 2013, March, and September 2014.

**Phospholipid fatty acid (PLFA) analysis**

We used PLFAs as biomarkers for soil microorganisms to investigate the microbial community structure in soil by separation of fungal and bacterial PLFAs. PLFAs of 4 g soil (fresh weight) were extracted according to Frostegård et al. (1993) with Bligh and Dyer solution (chloroform, methanol, citrate buffer; pH = 4; 1:2:0.8; v/v/v) and separated into glyco- and neutral lipids as well as phospholipid fatty acids. We analyzed only the PLFA-fractions as described in Kramer et al. (2013).

To group the data, the branched fatty acids i15:0, a15:0, i16:0 and i17:0 were summed as Gram-positive and the cy17:0 and cy19:0 as Gram-negative bacteria (Zelles, 1999). Total bacteria were calculated by adding the phospholipid fatty acid 16:1v7 to the sum of Gram-positive and Gram-negative bacterial PLFAs (Frostegård & Bååth, 1996). The biomarker 18:2ω6,9c was regarded as fungal PLFA (Frostegård & Bååth, 1996; Kaiser et al., 2010).

**Statistical analyses**

Field data on average daily soil temperature and volumetric water content (VWC), physical and chemical soil analyses, microbial and PLFA abundances (separately for each soil depth) and weekly measured canopy height of winter rapeseed were analyzed by linear mixed-effects (lme) models (lme function from the nlme package of R 3.2.1; R Core Team, 2015). Fixed-factors were ‘biochar’ (BC; without and with BC), ‘soil warming’ (W; Ta and Te) while time dependency was investigated by including ‘season’ for soil temperature and VWC analysis (S; autumn: 22.08.2013 to 30.11.2013; winter: 01.12.2013 to

28.02.2014; spring: 01.03.2014 to 31.05.2014 and summer: 01.06.2014 to 05.09.2014), 'date of collection' (DC) for physical, chemical and microbial soil properties or 'day' (D) for weekly measurement of canopy height of winter rapeseed. Block, plot and subplot were nested random effects in the lme models used.

In addition, soil temperature and VWC data were separately analyzed for each seasonal average, soil properties separately at each collection date, canopy height one day before harvest and aboveground biomass of winter rapeseed at maturity by using the same lme models excluding the time factor. In all VWC analyses, the fixed-factor BC was not considered due to its potential overestimation in the BC plots (see physical and chemical analyses). Differences in the incorporation of biochar-C into microbial biomass between ambient temperature ($BC_{Ta}$) and elevated temperature ($BC_{Te}$) biochar plots were tested for each sampling date and soil depth separately (lme model, fixed-factor W). In addition, we analyzed whether the proportion of biochar-C in microbial biomass was significantly higher than zero by T-test.

Prior to analyses, data were log- or square-root transformed if non-normally distributed and inhomogeneity of variance (Levene test) was found. A statistical probability of $P \leq 0.05$ was considered to be significant.

## 6.3 Results

### Soil temperature and moisture

Average daily soil temperature was significantly increased by soil warming ($P \leq 0.05$) by 2.25 °C in control (Ctrl), and by 2.40 °C in biochar (BC) plots during the experimental period of 380 d (Fig. 6.1c). Biochar had no significant effect on soil temperature, although we found a higher temperature increase (2.71 °C vs. 2.01 °C) in warmed biochar plots ($BC_{Te}$) than in the control plots during summer. Depending on the season, soil warming decreased VWC on average (W × S, $P \leq 0.001$) (Figs. 6.1d, S6.1, S6.2b). In the very dry spring season (March–May 2014), the warming-induced water loss was on average 16 % lower in biochar-amended than in control plots. In contrast, in summer (June–September 2014), biochar intensified soil water loss under warming by 71 % (Figs. 6.1d and S6.2b). Biochar increased SWC across all sampling dates, which was statistically significant at 0–5 cm ($P \leq 0.05$), but not at 5–15 cm (Table 6.2). In contrast, soil warming significantly decreased SWC at 0–5 cm ($P \leq 0.05$), but this effect was dependent on sampling date (W ×

D, $P \leq 0.01$), showing a negative impact of soil warming on SWC only in March (-23 %, $P \leq 0.01$; Table 6.2). The same pattern was observed at 5–15 cm.

**Table 6.2.** Physical and chemical soil properties in 0-5 and 5-15 cm soil depth in November 2013, March 2014, August 2014 and September 2014 (mean ± SE). Significant results of linear mixed-effects models for the effects of biochar addition (BC) and soil warming (W) and their interactions separately for each sampling date and soil depth as indicated by asterisks (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

| Parameter | | 0-5 cm | | | | | 5-15 cm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $Ctrl_{Ta}$ | $Ctrl_{Te}$ | $BC_{Ta}$ | $BC_{Te}$ | **Effects** | $Ctrl_{Ta}$ | $Ctrl_{Te}$ | $BC_{Ta}$ | $BC_{Te}$ | **Effects** |
| BD [g $cm^{-3}$] | Aug | 1.39±0.02 | 1.27±0.02 | 1.21±0.03 | 1.13±0.04 | **BC↓**** | 1.24±0.06 | 1.16±0.01 | 1.13±0.06 | 1.10±0.02 | **BC↓**** |
| SWC [%] | Nov | 24.9±0.4 | 25.0±0.5 | 27.3±0.7 | 26.9±1.2 | **BC↑*** | 24.0±0.7 | 23.9±0.8 | 24.8±1.4 | 26.4±1.1 | |
| | Mar | 17.5±0.5 | 13.8±0.7 | 18.2±0.3 | 13.9±0.6 | **W↓**** | 17.6±0.3 | 14.7±0.7 | 20.2±0.3 | 16.2±0.4 | **BC↑**, W↓*** |
| | Sep | 19.5±1.0 | 18.0±1.2 | 20.7±0.4 | 19.7±0.6 | | 19.1±0.3 | 19.2±0.6 | 20.8±0.1 | 21.1±0.3 | **BC↑**** |
| Soil pH | Nov | 6.74±0.06 | 6.72±0.03 | 6.90±0.01 | 6.97±0.03 | **BC↑**** | 6.82±0.04 | 6.78±0.03 | 6.93±0.05 | 7.06±0.03 | **BC↑**** |
| | Mar | 6.67±0.08 | 6.68±0.08 | 6.80±0.06 | 6.95±0.04 | **BC***, BC×W*** | 6.78±0.08 | 6.75±0.06 | 6.97±0.03 | 6.96±0.04 | **BC↑**** |
| | Sep | 6.74±0.12 | 6.79±0.09 | 6.86±0.08 | 6.91±0.06 | **BC↑**** | 6.82±0.03 | 6.82±0.06 | 6.91±0.02 | 7.02±0.03 | **BC↑**** |
| EOC [µg C $g^{-1}$ dw] | Nov | 49.7±0.7 | 51.0±8.7 | 55.1±3.1 | 67.1±4.8 | | 59.7±2.3 | 69.0±3.2 | 62.7±6.0 | 78.9±5.6 | |
| | Mar | 53.8±2.4 | 67.8±9.5 | 59.6±4.8 | 71.1±5.6 | | 47.9±1.7 | 53.6±5.5 | 46.4±4.5 | 57.2±8.5 | |
| | Sep | 58.0±4.4 | 69.8±7.5 | 71.5±7.1 | 69.9±5.7 | | 51.1±2.7 | 71.54±7.0 | 65.6±8.1 | 69.4±4.3 | |
| $NH_4^+$ [µg $NH_4^+$-N $g^{-1}$ w] | Nov | 1.21±0.09 | 1.07±0.13 | 1.00±0.03 | 1.59±0.27 | **BC×W*** | 0.97±0.08 | 1.21±0.05 | 1.58±0.46 | 1.60±0.42 | |
| | Mar | 49.8±12.9 | 56.8±7.3 | 57.3±10.0 | 88.0±17.3 | | 0.71±0.12 | 0.62±0.03 | 0.97±0.19 | 0.82±0.31 | |
| | Sep | 1.20±0.24 | 1.27±0.20 | 1.92±0.49 | 1.70±0.43 | | 1.39±0.32 | 0.94±0.07 | 1.61±0.44 | 1.05±0.21 | |
| $NO_3^-$ [µg $NO_3^-$-N $g^{-1}$ dw] | Nov | 0.07±0.04 | 0.20±0.10 | 0.00±0.00 | 0.03±0.03 | **BC↓*** | 0.68±0.02 | 0.65±0.11 | 0.30±0.08 | 0.19±0.03 | **BC↓*** |
| | Mar | 18.5±5.3 | 20.9±3.5 | 15.0±3.4 | 25.3±5.8 | | 3.49±0.06 | 2.87±0.19 | 1.51±0.26 | 1.94±0.54 | **BC↓*** |
| | Sep | 0.80±0.03 | 1.34±0.13 | 0.50±0.09 | 0.85±0.14 | **BC↓**** | 1.30±0.15 | 2.08±0.60 | 0.86±0.16 | 1.77±0.59 | |

Arrows show the direction of single effects. $Ctrl_{Ta}$ = control under ambient soil temperature, $Ctrl_{Te}$ = control under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature. Please note that soil bulk density (BD) was determined only once (August 2014).

**Plant growth**

Canopy height of winter rapeseed tended to increase with biochar ($P$=0.089), mainly due to short-term enhanced growth under ambient soil temperature ($BC_{Ta}$) in the early phase of the growing period in spring 2014 (BC × D, $P \leq 0.001$; Fig. 6.2a). Soil warming significantly increased canopy height throughout the experiment, but the large differences between Te and Ta plots in April diminished afterwards (W × D, $P \leq 0.05$). One day before final harvest, canopy height of rapeseed tended to be higher in warmed than in ambient plots ($P$=0.06), while biochar amendment had no effect (Fig. 6.2a). Total aboveground biomass at maturity was increased by soil warming only in the control plots, but not in the biochar plots (Fig. 6.2b; BC × W, $P \leq 0.05$).

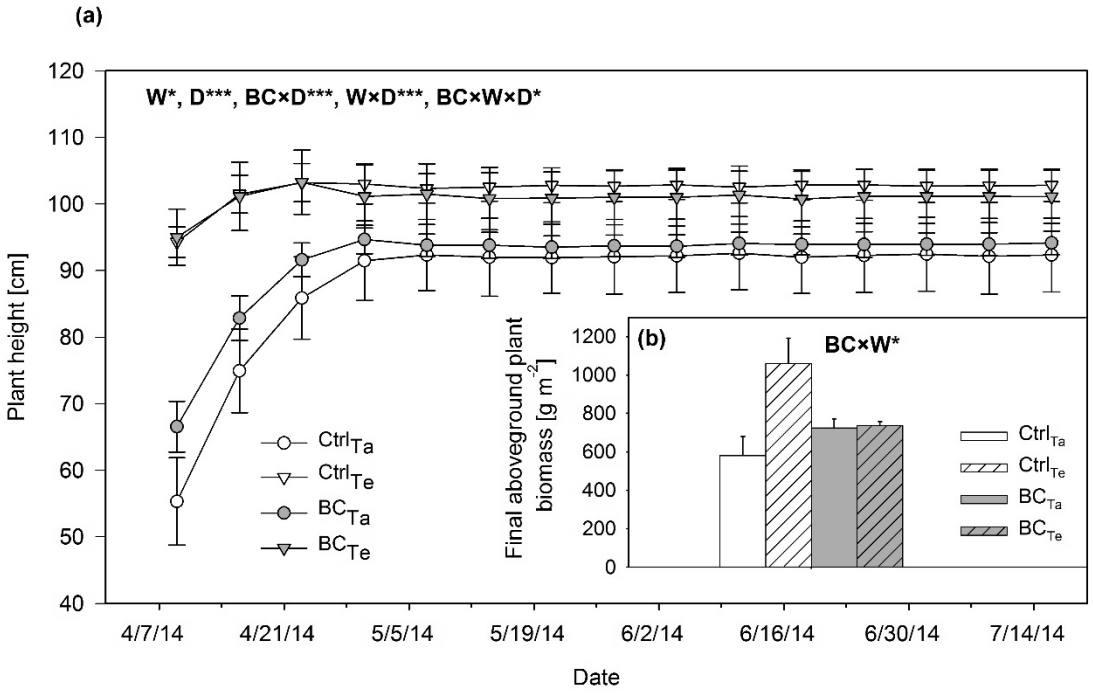


**Fig. 6.2.** (a) Canopy height between April and July 2014 (mean ± SE). Significant results of linear mixed-effects model for the effects of biochar addition (BC), soil warming (W) and date (D) and their interactions as indicated by asterisks (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$). (b) Final aboveground biomass of winter rapeseed at crop maturity (07/15/2014) (mean ± SE). Results of linear mixed-effects model for the effects of biochar addition (BC) and soil warming (W) and their interactions are shown when significant. $Ctrl_{Ta}$ = control under ambient soil temperature, $Ctrl_{Te}$ = control under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature.

**Physical and chemical soil properties**

Soil bulk density (BD) was significantly decreased in biochar-amended plots by 12 % and 7 % at 0–5 cm and 5–15 cm depth, respectively (Table 6.2, $P \leq 0.01$). Biochar increased soil pH at each sampling date and at both soil depths by 0.1 to 0.3 pH units ($P \leq 0.001$, Table 6.2). In addition, soil pH was enhanced by warming in the biochar, but not in the control plots in March (BC × W, $P \leq 0.05$). EOC was not affected by biochar or soil warming alone over the entire experiment, but tended to increase at 0–5 cm under biochar in November ($P = 0.095$) and under soil warming in March ($P = 0.086$) (Table 6.2). Concentrations of $NH_4^+$-N and $NO_3^-$-N at 0–5 cm soil depth were considerably higher in March compared to November and September (Table 6.2). In November, $NH_4^+$-N was slightly reduced by warming at 0–5 cm, but enhanced with biochar (BC × W, $P \leq 0.05$). Biochar reduced $NO_3^-$-N at 0–5 cm in November (by 89 %, $P \leq 0.05$) and in September (by 37 %, $P \leq 0.01$) as well as in the deeper soil layer (5–15 cm) by 63 % ($P \leq 0.05$) and 46 % ($P \leq 0.05$) in November and March, respectively.

**Microbial biomass carbon and biochar-C incorporation**

At each sampling date, $C_{mic}$ was higher at 0–5 cm than at 5–15 cm soil depth (Fig. 6.3a). There were no significant effects of biochar or soil warming alone, but the interaction between the two factors affected $C_{mic}$ in November (0–5 cm, BC × W, $P \leq 0.05$), with no effect of warming in the biochar plots, but increased microbial abundance in the $Ctrl_{Te}$ treatment (Fig. 6.3a).

The biochar-derived C in microbial biomass appeared to decrease at 0–5 cm, while it increased at 5–15 cm during our one-year study (Fig. 6.4). Microbial incorporation of biochar-C was highly variable (up to 15 % and 12 % on average at 0–5 cm and 5– 15 cm soil depths, respectively), irrespective of the soil warming treatment (Fig. 6.4, $P > 0.05$). Although microbial incorporation of BC-derived C was significant in September 2014 (5–15 cm, $P \leq 0.05$), the data were highly variable and at the other dates microbial BC-C assimilation was not significantly higher than zero.

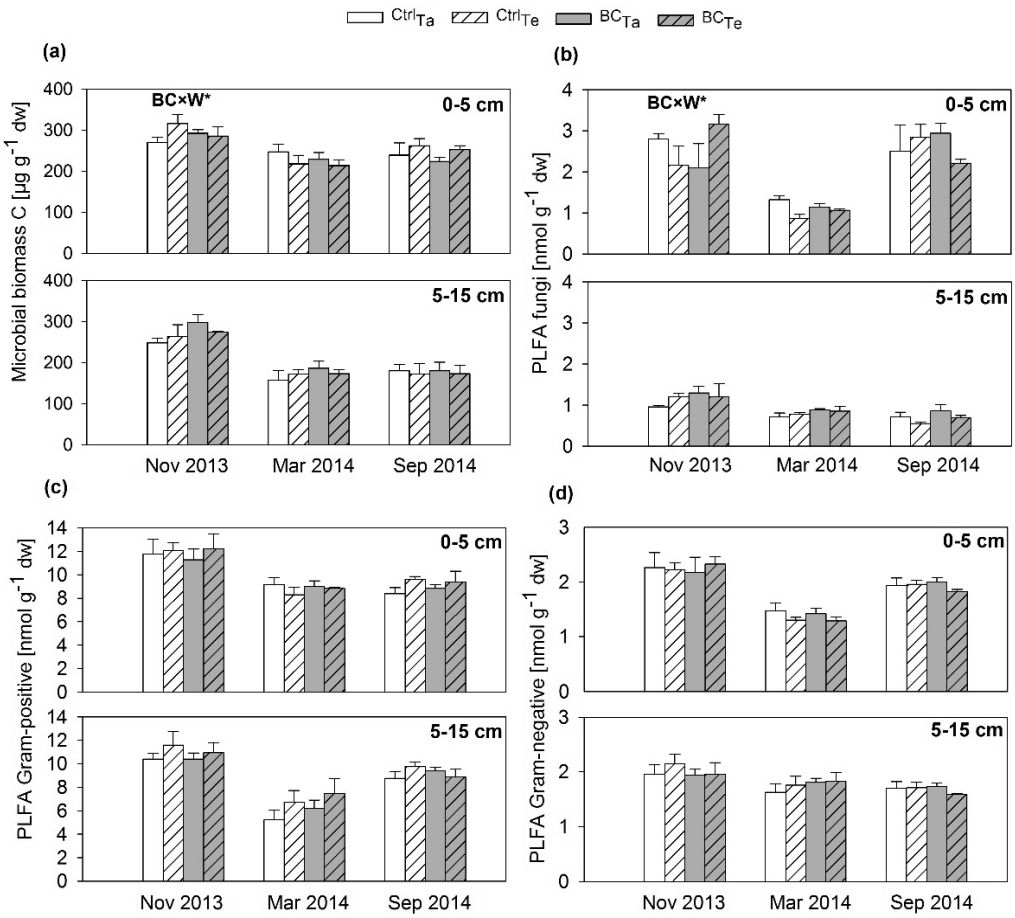


**Fig. 6.3.** Concentrations of (a) microbial biomass carbon ($C_{mic}$) as well as phospholipid fatty acids (PLFA) for (b) fungi, (c) Gram-positive bacteria and (d) Gram-negative bacteria at 0–5 and 5–15 cm soil depth in November 2013, March 2014 and September 2014 (mean ± SE). Significant results of linear mixed-effects models for the effects of biochar addition (BC) and soil warming (W) and their interactions separately for each sampling date and soil depth as indicated by asterisks (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$). $Ctrl_{Ta}$ = control under ambient soil temperature, $Ctrl_{Te}$ = control under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature.

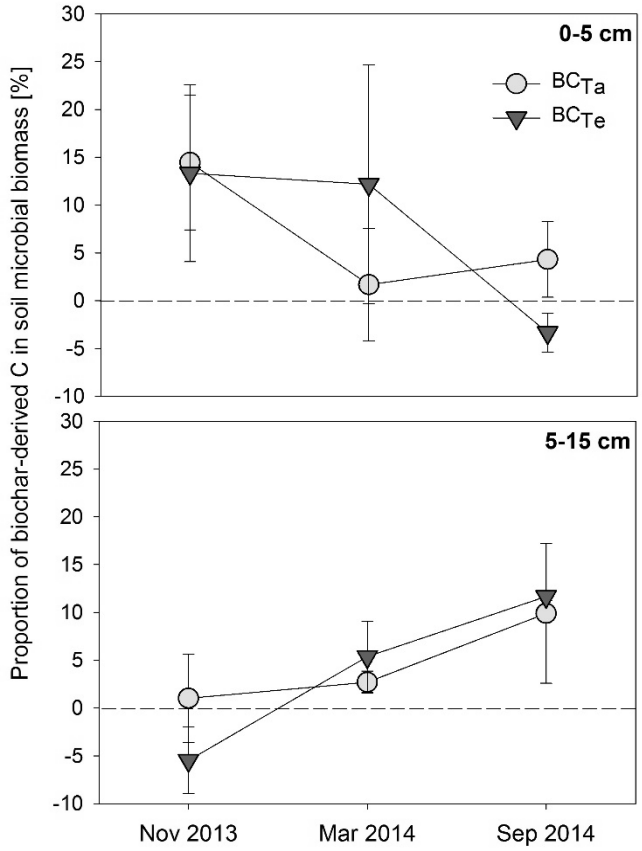


**Fig. 6.4.** Proportion (%) of biochar-derived C in soil microbial biomass at 0–5 and 5–15 cm soil depth in November 2013, March 2014 and September 2014 (mean ± SE). $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature.

## Microbial PLFA content

Changes in soil microbial community composition under biochar or soil warming treatment were statistically tested by using abundances of single PLFA biomarkers and microbial groups of Gram-positive and Gram-negative bacteria as well as fungi. At a depth of 0–5 cm, biochar had no significant effect on the microbial community during our study, while soil warming generally increased PLFA abundances ($P \leq 0.001$) and specifically the abundance of the i16:0 biomarker, representing Gram-positive bacteria ($P \leq 0.05$, Fig. S6.3). However, in November (0–5 cm), fungal abundance and fungal-to-bacterial ratio increased under soil warming in the biochar plots, whereas warming slightly decreased fungi in the control plots (BC × W, $P \leq 0.05$; Fig. 6.3b and Table 6.3). In March (0–5 cm), fungal biomass decreased under soil warming, while this reduction was up to 80 % lower in plots with biochar addition (BC × W interaction, $P = 0.06$, Fig. 6.3b). Concurrently, biochar and soil warming had an interactive effect on the fungal-to-bacterial ratio at 0–5

cm (BC × W, $P \leq 0.05$), showing almost no change in the biochar plots, whereas warming slightly decreased this ratio in plots without biochar addition (Table 6.3).

At a depth of 5–15 cm, soil warming had a similar effect on the microbial community during the experiment ($P \leq 0.001$), but this was dependent on sampling date without showing a clear pattern (W × DC, $P \leq 0.05$; Fig. S6.3). At this depth, biochar had no overall effect on PLFA abundances, but contents of the fungal PLFA-marker 18:2ω6:9c were significantly increased by biochar ($P \leq 0.05$, Fig. S6.3).

**Table 6.3.** Microbiological soil properties in 0-5 and 5-15 cm soil depth in November 2013, March 2014 and September 2014 (mean ± SE). Significant results of linear mixed-effects models for the effects of biochar addition (BC) and soil warming (W) and their interactions separately for each sampling date and soil depth as indicated by asterisks (* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$).

| Parameter | | 0-5 cm | | | | | 5-15 cm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $Ctrl_{Ta}$ | $Ctrl_{Te}$ | $BC_{Ta}$ | $BC_{Te}$ | **Effects** | $Ctrl_{Ta}$ | $Ctrl_{Te}$ | $BC_{Ta}$ | $BC_{Te}$ | **Effects** |
| Total bacteria [nmol $g^{-1}$ dw] | Nov | 19.7±1.9 | 19.6±1.2 | 18.9±1.7 | 20.2±1.9 | | 16.4±0.8 | 18.4±1.8 | 16.5±0.9 | 17.2±1.6 | |
| | Mar | 16.4±1.1 | 14.0±1.3 | 16.0±0.9 | 14.8±0.3 | | 11.4±1.7 | 13.4±1.7 | 14.2±1.4 | 14.9±2.5 | |
| | Sep | 15.4±0.9 | 17.1±0.2 | 16.3±0.6 | 16.5±1.4 | | 14.3±1.1 | 15.4±0.7 | 15.3±0.6 | 14.3±0.8 | |
| Gram-pos./ Gram-neg. ratio | Nov | 5.22±0.09 | 5.45± 0.06 | 5.28±0.24 | 5.24±0.30 | | 5.36±0.39 | 5.38±0.18 | 5.37±0.17 | 5.60±0.16 | |
| | Mar | 6.27±0.27 | 6.35± 0.31 | 6.39±0.27 | 6.90±0.32 | **BC↑*** | 3.16±0.22 | 3.80±0.38 | 3.45±0.40 | 4.05±0.30 | |
| | Sep | 4.35±0.29 | 4.93± 0.29 | 4.41±0.12 | 5.14±0.42 | | 5.18±0.16 | 5.72±0.11 | 5.44±0.06 | 5.61±0.48 | |
| Fungal/ Bacterial ratio | Nov | 0.15±0.02 | 0.11±0.02 | 0.11±0.02 | 0.16±0.02 | **BC×W*** | 0.06±0.00 | 0.07±0.01 | 0.08±0.01 | 0.07±0.02 | |
| | Mar | 0.08±0.08 | 0.06±0.00 | 0.07±0.00 | 0.07±0.00 | **BC×W*** | 0.06±0.00 | 0.06±0.00 | 0.06±0.00 | 0.06±0.00 | |
| | Sep | 0.16±0.03 | 0.17±0.02 | 0.18±0.01 | 0.14±0.02 | | 0.05±0.00 | 0.04±0.00 | 0.05±0.01 | 0.05±0.00 | **BC*, BC×W*** |

Arrows show the direction of single effects. $Ctrl_{Ta}$ = control under ambient soil temperature, $Ctrl_{Te}$ = control under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature.

## 6.4 Discussion

### Biochar utilization by soil microorganisms

The aim of this study was to investigate the effect of soil warming on the utilization and incorporation of biochar-C by microorganisms as a measure of biochar stability in soil. During one year after biochar addition, we found only slight initial degradation of biochar and assimilation by soil microorganisms under ambient and elevated soil temperature. This is in accordance with previous studies showing that biochar initially triggers microbial activity by providing limited amounts of labile C, but that long-term stability is determined by slowly decomposable aromatic C structures (Ameloot et al., 2013b). Some experiments, using different biochars and soils, indicated low microbial assimilation of biochar-C under controlled conditions (Kuzyakov et al., 2009; Luo et al., 2013; Watzinger et al., 2014). Similar to our study, Luo et al. (2013) incubated a high-temperature biochar from Miscanthus (700 °C) in alkaline arable soil confirming our results of low microbial degradation of such biochar types. From the physical-chemical point of view, enhancing pyrolysis temperature to 850 °C lead to a great disappearance of aliphatic CH, $CH_2$ and $CH_3$ groups, i.e. the bio-degradable carbon fraction, due to the decomposition and de-volatilization of mainly hemicellulose and cellulose (bands between 1200 and 1000 $cm^{-1}$) as well as partly lignin, while the appearance of aromatic C-H (850–780 $cm^{-1}$) and C=C (around 1160–1580 $cm^{-1}$ and 1430–1400 $cm^{-1}$) groups increased, as shown by FTIR spectrum (Fig. S6.4) and confirmed by TGA (Fig. S6.5). In addition, the low O/C molar ratio (0.07) of the used biochar can be seen as predictor of high degradation stability (Spokas, 2010).

From our $^{13}C_{mic}$ data we cannot definitely estimate biochar stability in our field experiment. The results on BC incorporation by soil microorganisms (up to 15 %) were highly variable and not affected by soil warming. One year after biochar amendment we determined the vertical distribution of biochar at 0–15 cm soil depth and found highly variable results (personal communication D. Grunwald). In the BC-HoCC experiment we applied unsieved and large-sized biochar particles of 4–20 mm length to simulate a realistic scenario for biochar amendment in agriculture. This resulted in a highly heterogeneous distribution of the biochar particles at the small-scale, which made it difficult to detect changes in biochar content, i.e. the persistence or fate of the biochar in the field over time. In conclusion, our results should be interpreted with caution, but it appears that microbial assimilation at 0–5 cm was highest at the beginning and decreased over time, while at 5–

15 cm depth it increased until the last sampling in September 2014. As the arable field was not ploughed between sampling dates, this may indicate a vertical transport of biochar particles by fragmentation and bioturbation as shown in other soils (Topoliantz & Ponge, 2003), or in the form of dissolved organic carbon (DOC; Major et al., 2010).

Our data on microbial abundances three months after biochar application (November) indicated that the labile C pools of the biochar were utilized by soil microorganisms before sampling, leaving poorly degradable (recalcitrant) biochar in soil. This is supported by data on $CO_2$ emissions (unpublished data) which were initially enhanced in both BC treatments after biochar incorporation into soil, but leveled off after a few weeks, indicating a rapid utilization of mostly labile biochar-C. The used *Miscanthus* biochar exhibits limited volatile matter (<10 %), which is lower compared to other high-temperature biochars from different feedstock (Enders et al., 2012), but partly forms a short-term biodegradable fraction mainly consisting of aliphatic functional groups (Zimmerman, 2010). However, fungal abundance as well as the fungal-to-bacterial ratio increased under warming in the biochar-amended soil ($BC_{Te}$ vs. $BC_{Ta}$), together with the highest EOC and $NH_4^+$-N contents in $BC_{Te}$ in November in the uppermost soil depth. Fungi are known to degrade recalcitrant material such as biochar in soil, and to use it as a growth substrate (Ascough et al., 2010). Further, stable C pools are considered to be more sensitive to increased soil temperature than less stable C (Conant et al., 2011) and different incubation studies have shown that the mineralization of recalcitrant biochar-C was temporarily enhanced with increasing soil temperature (e.g. Fang et al., 2014). In our experiment, the increased fungal biomass and EOC concentrations in the $BC_{Te}$ treatment after three months may indicate that soil warming triggered the decomposition of more stable biochar components by fungi, leading to the enhanced fungal biomass. In addition, spring barley litter was ploughed into the soil together with biochar at the beginning of the experiment in August and was still present in November. This likely stimulated the growth of soil fungi and perhaps also led to a utilization of biochar-derived C due to co-metabolic pathways (Ascough et al., 2010). Budai et al. (2016) showed that high-temperature biochars, containing low volatile matter and high fixed C like the biochar we used in our study, had considerable impacts on the soil microbial community composition, although fungi and bacteria responded similarly to biochar amendment. In contrast, the shift in the fungal-to-bacterial ratio in our experiment indicates that soil fungi benefited most from the potential biochar mineralization in the early phase of the experiment. In a short-term incubation study (37 days) with *Miscanthus* biochar added to the same soil as that

investigated in the present study, biochar increased microbial abundances by 16 % and shifted the microbial community toward a higher fungal-to-bacterial PLFA ratio when litter was added to the soil (Bamminger et al., 2014b). Certainly, the response of fungi to biochar in our field experiment may be transient or strongly connected to the presence of litter as the effect of enhanced fungal biomass could no longer be observed in March or September.

A further potential reason for the short-term effect on fungal biomass under warming may be a biochar-induced modification of soil properties and microhabitat conditions (e.g. bulk density, soil moisture, pH) (Lehmann et al., 2011). However, changes in soil properties and microhabitat conditions were not pronounced under soil warming and therefore not expected to be primarily responsible for the observed fungal response.

Our results point to an initial mineralization and utilization of biochar-C by fungi in warmed soil; however, this study examined only the short-term effects of biochar on soil microorganisms. Assuming that the biochar will not be degraded by soil microorganisms in relevant amounts in the future, an outcome suggested by our results, biochar turnover may be a negligible factor in long-term C sequestration.

**Biochar effects on soil microorganisms and plants under drought and soil warming**

We were interested in the potential interactive effects of biochar together with predicted soil warming on soil microbial abundances related to seasonal changes in soil moisture. During the dry spring season in 2014, biochar reduced warming-induced water loss in comparison to the control plots. Likewise, biochar led to maximum 80 % lower reduction of fungal biomass in warmed soil in March. We observed the same patterns for Gram-positive bacteria and the fungal-to-bacterial ratio, but these interactive effects of biochar and soil warming were only tendencies. Nevertheless, according to our hypothesis it seems likely that the compensatory effect of biochar on water content in warmed soil positively influenced soil microbial abundances. Similarly, Liang et al. (2014) observed increased drought tolerance of the microbial community in a tropical soil amended with biochar, likely due to beneficial effects of biochar on habitat conditions and water retention. It is well known that Gram–positive bacteria and fungi are more resistant to water stress than Gram-negative bacteria due to their physiologies and their acclimation capacities (Schimel et al., 2007). In the present study, Gram-negative bacteria did not profit

from the enhanced water retention by biochar, emphasizing their high sensitivity to soil dryness compared to Gram-positive bacteria and fungi. Another important driver of microbial processes and abundances in soil is pH, and the liming potential of many biochars may have a major impact on the soil microbial community (Lehmann et al., 2011). We found highest soil pH in biochar-amended soil under warming in March, which could have influenced microbial abundances as well. However, we do not expect that pH played an important role in our experiment as our soil has nearly neutral pH and it was increased by only a maximum of 0.3 pH units with biochar. This would suggest, in contrast to our results, an increased bacterial abundance rather than higher fungal abundance, as it has been shown that bacterial abundance increases with rising pH up to 7, while fungal biomass decreases or shows no effect (Rousk et al., 2009).

In September, soil moisture was generally higher than in March due to extensive precipitation during July and August; SWC was increased by biochar, but not reduced by warming. At the same time, microbial abundances were rarely affected by single effects or the interaction of biochar and soil warming. We suggest that below a specific threshold even small differences in soil moisture affect the competition between differently adapted microbial groups, whereas in September the positive effect of biochar on soil moisture was not sufficient to alter the soil microbial community.

We expected that crop growth is mainly determined by soil moisture in spring and summer, which is reduced by soil warming, but enhanced with biochar. Soil warming enhanced canopy height during the experiment and aboveground biomass at crop maturity in the control plots. Similarly, Siebold & Tiedemann (2012) have shown that soil warming accelerated the phenology of rapeseed. Enhanced aboveground biomass production under soil warming may be caused by fewer frost events, longer growing seasons, higher nutrient availability and enhanced photosynthesis rates up to a species-specific temperature optimum (Rustad et al., 2001). However, warming effects on crops seemed to be species-specific since aboveground biomass production of barley was not affected by elevated soil temperature in a previous study (Högy et al., 2013).

Biochar has sometimes been shown to increase crop growth by increasing soil pH as well as water and nutrient retention in soil, but this is assumed to be less likely in temperate fertile soils with almost neutral pH (Biederman & Harpole, 2013; Jay et al., 2015). In our study, biochar did not increase canopy height in warmed, but instead in the non-warmed plots in the early growth stages in the dry spring season (April-May). This was likely

caused by the vertical distribution of biochar in the soil profile and subsequent differences in soil moisture. Due to accelerated phenology under soil warming, the rapeseed plants were bigger in the warmed than in the non-warmed plots and we assume that their rooting systems reached deeper soil horizons where biochar was not incorporated (>20 cm depth). In contrast, the plants in the non-warmed plots may have met their water demand in the (drier) upper soil layers where biochar was incorporated and in this case biochar may have been effective in countering water limitation for the plants. However, at harvest, the effect of biochar on aboveground crop biomass at ambient temperature disappeared. This could be attributed to decreased availability of nutrients to plants due to initial strong adsorption of nutrients (e.g. nitrate) on the positive as well negative adsorption sites due to functional groups on fresh biochars' surfaces (Schmidt et al., 2014). Some studies have shown that crop yield after biochar application increases over time (Crane-Droesch et al., 2013) suggesting the need for field weathering of fresh biochar to reduce negative effects on plant nutrient uptake (Schmidt et al., 2014). In addition, a further partitioning of the different potential biochar effects on aboveground and belowground crop growth under soil warming in future studies would help to better understand biochar-plant-soil nutrient interactions in agroecosystems.

## 6.5 Conclusion

Biochar is increasingly discussed as a climate change mitigation tool, but there is limited knowledge about its interactive effects with future soil warming, possibly influencing its stability as well as its beneficial effects on soil microorganisms and plants. In this field experiment, the high-temperature biochar used was considered to be stable against microbial utilization during the first year after application, despite evidence of initial degradation by fungi under soil warming. This indicates that biochar could persist in soil and may be suitable for C sequestration in soils even under elevated temperature. In addition, we have shown that biochar could mitigate seasonal effects of climate change (e.g. drought) on microorganisms and plants depending on weather conditions, thus increasing or maintaining the fertility and productivity of agricultural soils. However, the interrelationships between biochar, soil warming, plants and soil microorganisms have to be investigated in more detail and over additional vegetation periods to identify the impact of biochar on the soil environment, on microorganisms, and under different crops. Its long-term stability must be better characterized as well.

## 6.6 Acknowledgements

We thank Wolfgang Armbruster for isotopic analyses and Michael Kaiser and Dennis Grunwald (Institute of Organic Agricultural Sciences, University of Kassel, Germany) for providing additional information on soil bulk densities and distribution of biochar in soil. In addition, we thank Kathleen A. Mackie and Kathleen Regan for English corrections. C. Bamminger was funded by a PhD scholarship awarded by the faculty of Agricultural Sciences at the University of Hohenheim.

## 6.7 Supplementary material

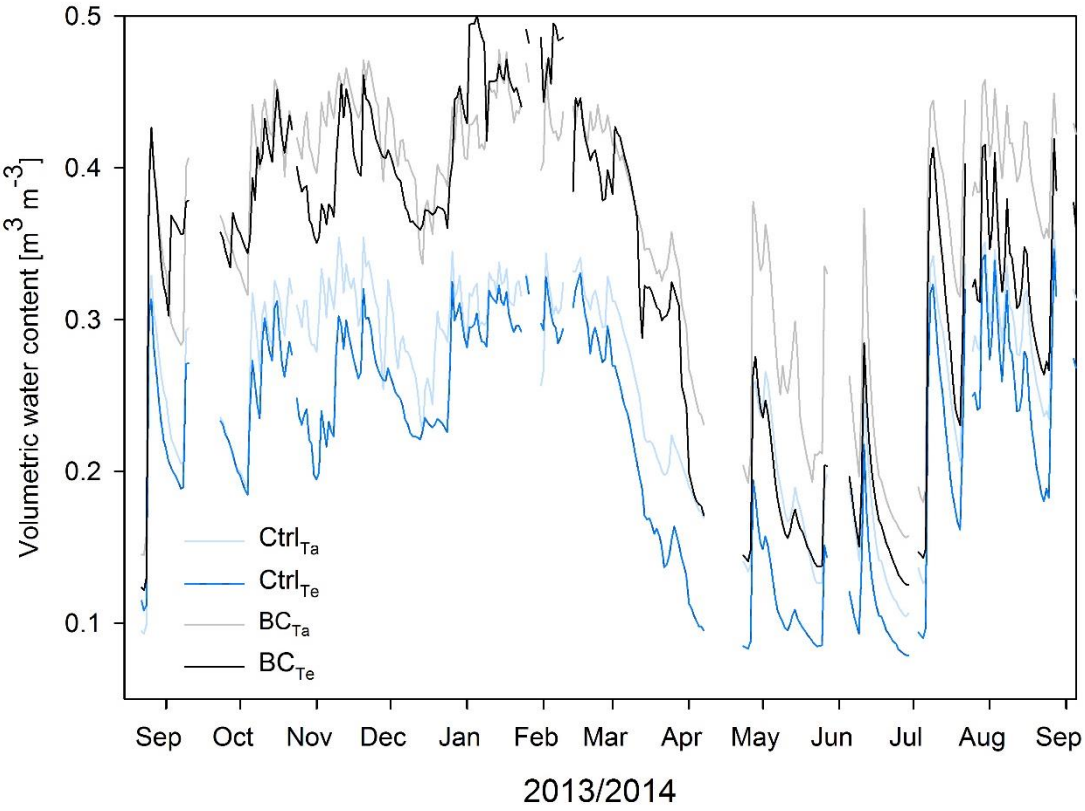


**Fig. S6.1.** Daily average volumetric water contents (VWC) of soil during the experiment. $Ctrl_{Ta}$ = control under ambient soil temperature, $Ctrl_{Te}$ = control under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature.

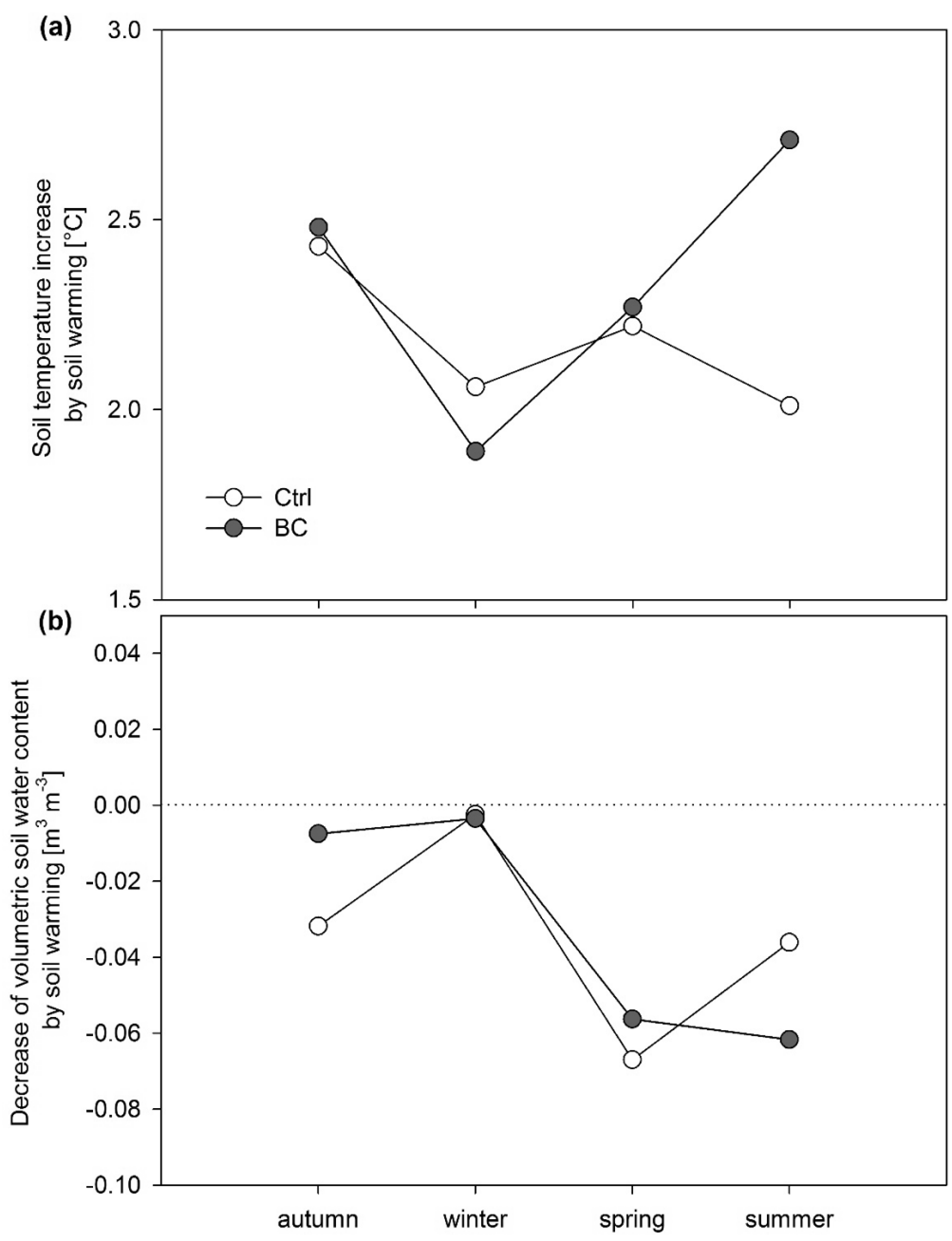


**Fig. S6.2.** (a) Average seasonal soil temperature increase due to soil warming and (b) Average seasonal decrease of volumetric water content (VWC) in soil due to warming in control (Ctrl) and biochar (BC) plots.

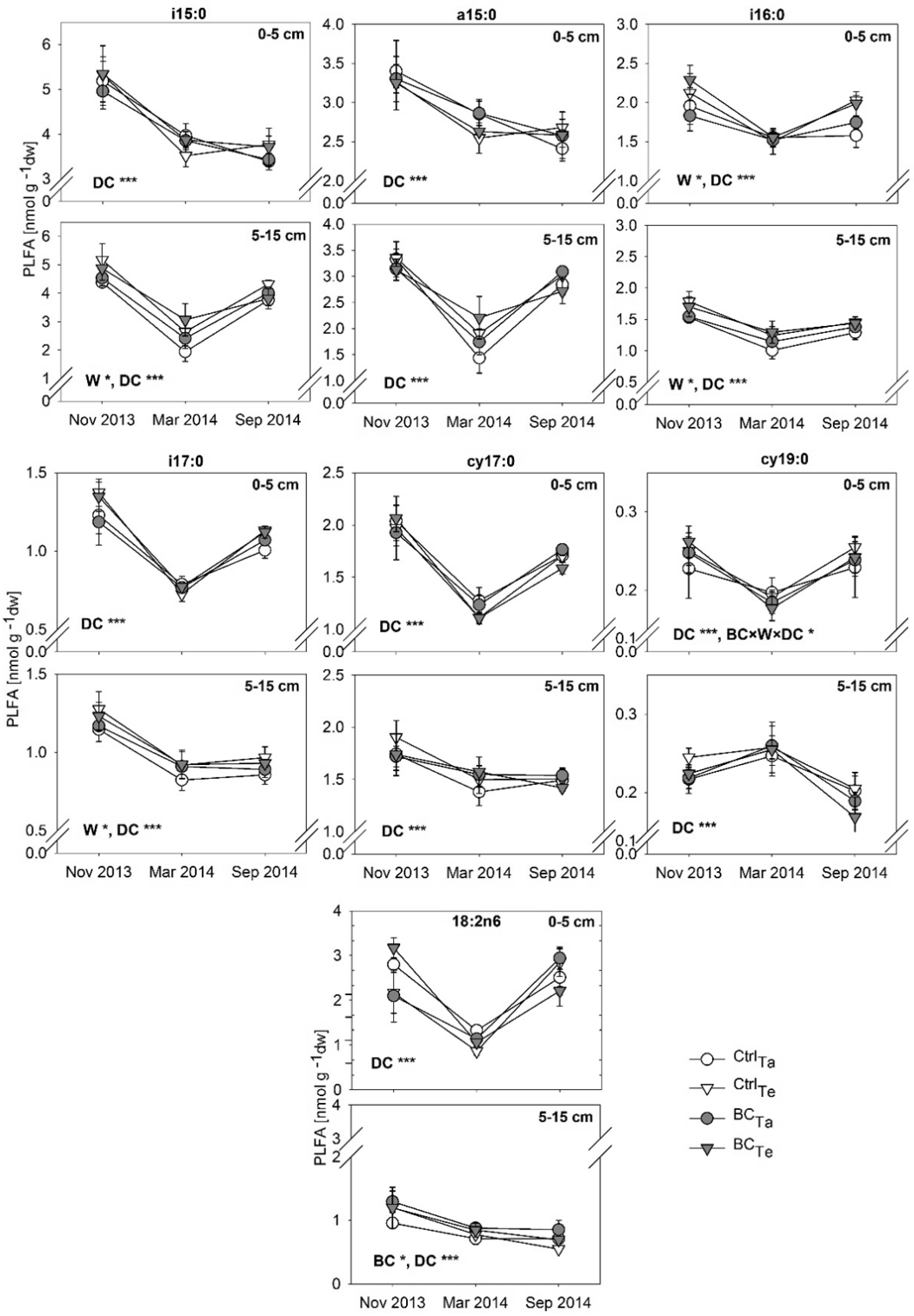


**Fig. S6.3.** Concentrations of the phospholipid fatty acid (PLFA) biomarkers i15:0, a15:0, i16:0 and i17:0 (Gram-positive bacteria); cy17:0 and cy19:0 (Gram-negative bacteria) and 18:2ω6:9c (fungi) at 0-5 and 5-15 cm soil depth in November 2013, March 2014 and September 2014 (mean ± SE). Significant results of linear mixed-effects models for the effects of biochar addition (BC), soil warming (W) and date of collection (DC) and their interactions separately for each soil depth as indicated by asterisks (* P≤0.05, ** *P*≤0.01, *** *P*≤0.001). $Ctrl_{Ta}$ = control under ambient soil temperature, $Ctrl_{Te}$ = control under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature.

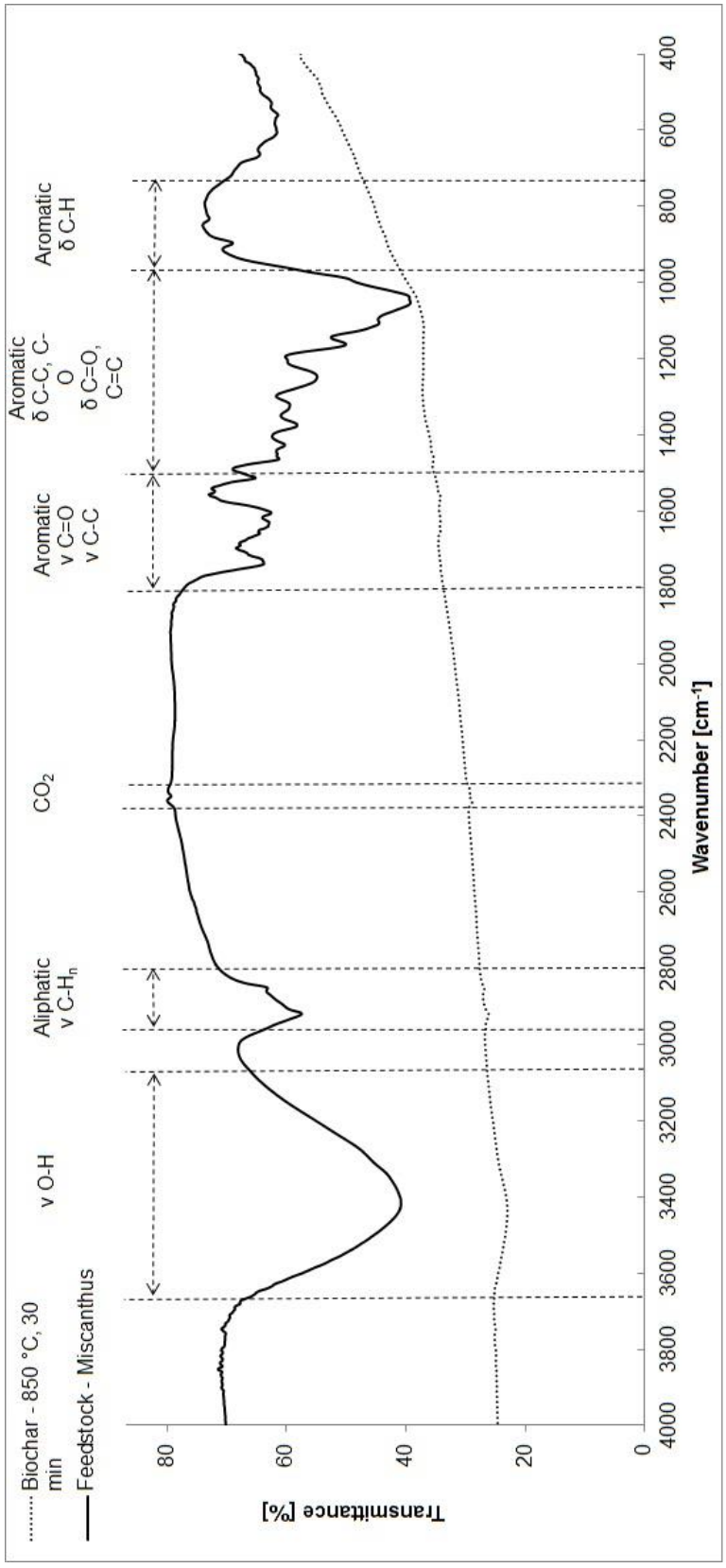


**Fig. S6.4.** Fourier transform infrared (FTIR) spectra of the used 850 °C *Miscanthus* biochar and *Miscanthus* feedstock as reference material.

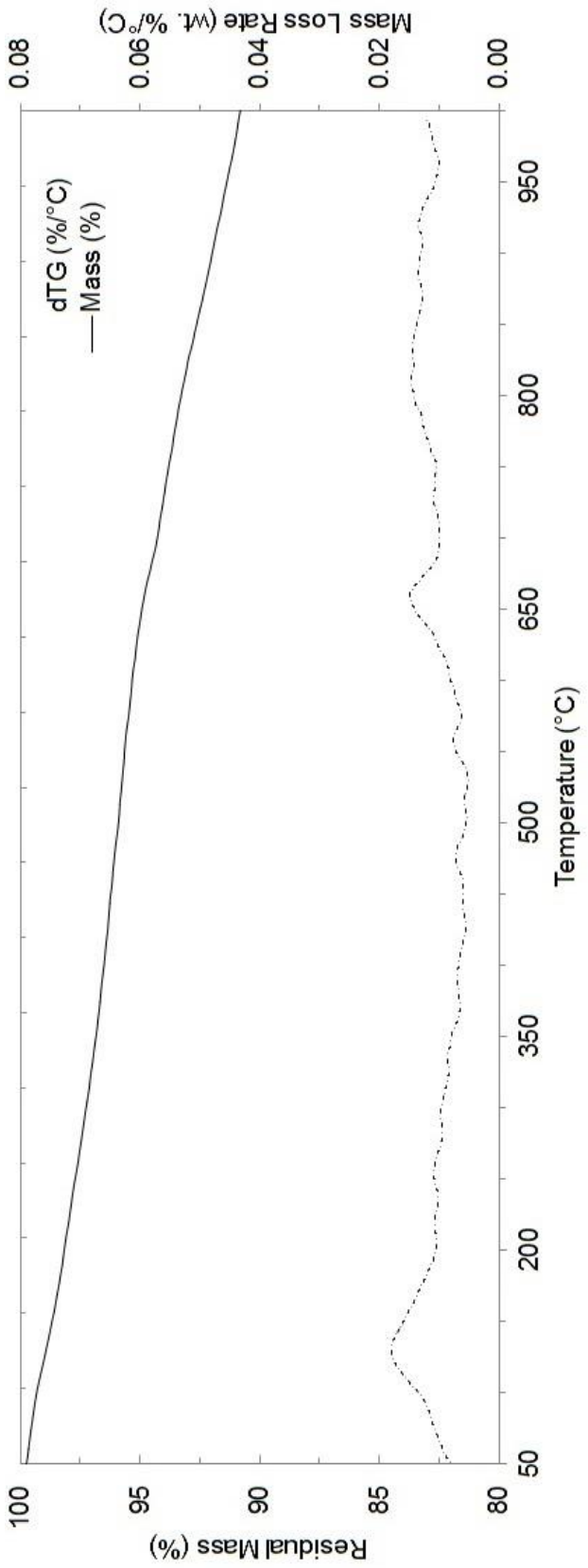


**Fig. S6.5.** Thermogravimetric (TGA) and derivative (DTG) profile of the used 850 °C *Miscanthus* biochar.

# 7 Offsetting global warming-induced elevated greenhouse gas emissions from an arable soil by biochar application

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## Abstract

Global warming will likely enhance greenhouse gas (GHG) emissions from soils. Due to its slow decomposability, biochar is widely recognized as effective in long-term soil carbon (C) sequestration and in mitigation of soil GHG emissions. In a long-term soil warming experiment (+2.5 °C, since July 2008) we studied the effect of applying high-temperature *Miscanthus* biochar (0, 30 t $ha^{-1}$, since August 2013) on GHG emissions and their global warming potential (GWP) during two years in a temperate agroecosystem. Crop growth, physical and chemical soil properties, temperature sensitivity of soil respiration ($R_s$) and metabolic quotient ($qCO_2$) were investigated to yield further information about single effects of soil warming and biochar as well as on their interactions. Soil warming increased total $CO_2$ emissions by 28 % over two years. The effect of warming on soil respiration did not level off as has often been observed in less intensively managed ecosystems. However, the temperature sensitivity of soil respiration was not affected by warming. Overall, biochar had no effect on most of the measured parameters, suggesting its high degradation stability and its low influence on microbial C cycling even under elevated soil temperatures. In contrast, biochar × warming interactions led to highest total $N_2O$ emissions, possibly due to accelerated N-cycling at elevated soil temperature and to biochar-induced changes in soil properties and environmental conditions. Methane uptake was not affected by soil warming or biochar. The incorporation of biochar-C into soil was estimated to offset warming-induced elevated GHG emissions for 20 years. This highlights the suitability of biochar for C sequestration and GHG mitigation in cultivated temperate agricultural soil under a future elevated temperature.

**Keywords:** soil warming, biochar, agroecosystem, carbon dioxide, nitrous oxide, methane, temperature sensitivity, carbon sequestration

## 7.1 Introduction

Global warming has been shown to increase the emission of greenhouse gases (GHG) from soils, potentially creating a positive feedback and increasing the rate of global climate change (Davidson & Janssens, 2006; Dijkstra et al., 2012). Biochar (BC) amendment to agricultural soils has been proposed as a mitigation strategy to offset global climate change effects through long-term carbon (C) sequestration, reduction of GHG emissions, and by maintaining or improving soil fertility and crop productivity (Ippolito et al., 2012; Lorenz & Lal, 2014; Zhang et al., 2016). Although biochar is considered a future climate change mitigation option, its C sequestration potential and effects on GHG emissions have rarely been evaluated under predicted elevated soil temperatures in field experiments to date.

Several field studies have reported short-term increases in $CO_2$ emissions under elevated soil temperature which leveled off after a few years (Luo et al., 2001; Melillo et al., 2002; Allison et al., 2010). This effect has been variously explained by depletion of easily available C substrates or by thermal adaption of microbial respiration; the latter involved changes in microbial community structure (Bradford et al., 2008; Crowther et al., 2013). However, experimental warming studies on soil GHG fluxes have been largely restricted to forest and grassland ecosystems, and limited in agricultural soils. The studies of Poll et al. (2013) and Liu et al. (2015) indicate that soil drying, a process that accompanies elevated soil temperature, may be a crucial factor limiting microbial biomass and activity in arable soils under changing climate. Conversely, enhanced plant growth and related higher belowground C input by root exudation under a warmer climate may provide additional substrates for soil microorganisms and promote soil respiration (Trumbore, 1997). These elevated soil temperature-related modifications of physical and chemical soil properties (e.g. soil moisture) can influence $N_2O$ and $CH_4$ fluxes from soil as well (Smith et al., 2003). In a meta-analysis, Bai et al. (2013) showed that N-mineralization, N pools and $N_2O$ emissions increased under experimental warming, while temperature-related reduction in soil moisture and C availability could limit microbial activity and N cycling. The uptake of $CH_4$ in upland soils was observed to be enhanced under warming through increased methane oxidation activity and reduced soil moisture, likely resulting in higher diffusivity of methane into the soil (Dijkstra et al., 2012). Although the uptake of atmospheric methane may be enhanced under soil warming, the global warming potential (GWP) of GHG emissions from temperate agricultural soils is dominated by $CO_2$ and $N_2O$ emissions (Robertson & Grace, 2004), since temperate arable

soils are small sinks for atmospheric $CH_4$ compared to grasslands and forests (Le Mer & Roger, 2001). This points to the importance of developing mitigation options to counteract $CO_2$ and $N_2O$ emissions from arable soils.

The stability of biochar is fundamental to its function as a long-term option for C sequestration, GHG reduction and soil amelioration. The turnover of biochar in soil is typically characterized by rapid depletion of labile biochar-C, followed by slow mineralization of more stable and highly polycondensed aromatic C fractions (Kuzyakov et al., 2014; Wang et al., 2016a). This suggests long-term persistence of biochar in soil (Glaser et al., 2001). However, biochar may also interact with soil organic matter (SOM), either by decreasing or enhancing SOM mineralization (priming effect) (Zimmerman et al., 2011; Ventura et al., 2015). If biochar were to trigger SOM degradation, it would challenge the concept of biochar application to soil as a strategy for long-term C storage and climate warming mitigation. Increased $CO_2$ emissions from agricultural soils after the application of low-temperature biochars (< 450 °C) (Jones et al., 2012; Ameloot et al., 2013a) may be attributed to a high proportion of labile biochar-C. In contrast, the amendment of high-temperature biochar (>500 °C) to arable soils has often resulted in either a decrease or no effect on $CO_2$ emissions in lab (e.g. Ameloot et al., 2013a, Ameloot et al., 2014; Bamminger et al., 2014a) and field experiments (Castaldi et al., 2011; Case et al., 2014). This suggests that high-temperature biochars may be suitable for long-term C sequestration in arable soils (Liu et al., 2016). Reduced $CO_2$ fluxes from biochar-amended soil have often been linked to decreased metabolic quotients ($qCO_2$) and enhanced microbial C use efficiencies (Jin, 2010; Domene et al., 2014; Bamminger et al., 2014b), even though microbial abundances often increased. Decreased $qCO_2$ in biochar-amended soil could be due to shifts in soil microbial community composition and co-location of microorganisms and substrates on biochar surfaces, increasing microbial C use efficiency (Jin, 2010; Lehmann et al., 2011).

Agricultural soils are the main sources of $N_2O$ emissions due to increasing application of both mineral N-fertilizers and manure; thus, strategies to reduce $N_2O$ emissions in the context of global climate change mitigation are required (Reay et al., 2012). Biochar-induced reduction of $N_2O$ emissions has been demonstrated in several lab and field experiments (Taghizadeh-Toosi et al., 2011; Harter et al., 2013; Ameloot et al., 2016). In contrast, other studies have found increased $N_2O$ emissions following biochar incorporation (Saarnio et al., 2013; Sánchez-García et al., 2014). Biochar may influence

different $N_2O$-genic processes by altering soil aeration, water retention, N-availability, and soil pH or by sorption of labile C, leading to shifts in the nitrifying and denitrifying soil microbial community (Clough et al., 2013; Cayuela et al., 2014). It is therefore challenging to predict the effects of biochar on $N_2O$ emissions in soil environments, especially under field conditions. Biochar could also change $CH_4$ uptake from arable soils by increasing aeration, soil moisture, pH, or by sorption of C and N depending on biochar characteristics and soil management (Jeffery et al., 2016). This would affect methane production by methanogenic archaea and methane consumption by methanotrophs, both of which depend on native soil conditions (Feng et al., 2012; Yu et al., 2013). Hence, varying results have been observed in several studies; decreased $CH_4$ uptake (Spokas & Reicovsky, 2009), no effect (Castaldi et al., 2011; Kammann et al., 2012) or increased $CH_4$ uptake from arable soils (Karhu et al., 2011).

It is not clear to date whether elevated soil temperature impacts biochar stability or how interactions between elevated temperatures and biochar could influence GHG fluxes under field conditions. Studies in ecosystems along climate gradients (Glaser & Amelung, 2003; Cheng et al., 2008) have provided no information on the actual mineralization of pyrogenic organic matter under field conditions at elevated temperature. Under controlled conditions, the proportion of mineralized biochar-C (pyrolysis at 550-600 °C) was somewhat enhanced with increasing temperature (Nguyen et al., 2010; Fang et al., 2014), but biochar's effects on the temperature sensitivity of native SOM degradation have sometimes been ambiguous, depending on soil properties, biochar properties, and incubation temperature (Fang et al., 2014, Fang et al., 2015). Likewise, there is also no clear evidence that biochar could reduce $N_2O$ and $CH_4$ fluxes from field soil at elevated temperature (Case et al., 2012; Han et al., 2016).

We investigated how biochar affects GHG emissions in warmed arable soil during two vegetation periods to assess the feasibility of biochar for future C sequestration and climate change mitigation in temperate arable soils. Based on existing knowledge about biochar stability in soil, we hypothesized that the high-temperature *Miscanthus* biochar (850 °C) used in this study will form a persistent constituent of OM in soil, thus preventing an increase in soil $CO_2$ emissions, $qCO_2$, or the temperature sensitivity of soil respiration in the medium-term even under warming. Further, we aimed to explore how potential biochar effects on soil properties (e.g. increases in soil moisture, pH and aeration), under changing

weather conditions and different stages of management (ploughing, cropping and fertilization) will affect the fluxes of $N_2O$ and $CH_4$ from soil under warming.

## 7.2 Materials and methods

### Field site

The investigated arable field is located at the experimental station Heidfeldhof of the University of Hohenheim (Stuttgart, Germany). The area is characterized by mean annual temperature and precipitation (1981–2010) of 9.4 °C and 718.7 mm, respectively (DWD, 2016). In the investigated years, 2013, 2014, and 2015, mean annual air temperatures were 9.5 °C, 11.0 °C and 10.9 °C and annual precipitation was 790.1 mm, 654.1 mm and 492.1 mm, respectively (Fig. S1c; weather station 'Hohenheim', LTZ Augustenberg, 2016). The arable soil is a loess-derived stagnic Luvisol with silty loam-texture (9 % sand, 69 % silt and 22 % clay), total C content of 11.4 g C $kg^{-1}$ soil dry weight and pH 6.8.

### Experimental design

The Biochar Hohenheim Climate Change experiment (BC-HoCC) was established in August 2013 (Bamminger et al., 2016) as part of an existing climate change experiment in a temperate agroecosystem (HoCC), where soil has been warmed since 2008 (Poll et al., 2013). The HoCC experiment has a split-plot-design, with four blocks consisting of four plots each subdivided into subplots (1×1 m). In the warmed plots (Te), heating cables were installed near the soil surface to increase soil temperature by 2.5 °C above ambient soil temperature at 4 cm depth, and non-heated plots (Ta) serve as experimental controls which are fitted with dummy cables. In August 2013, biochar from *Miscanthus x giganteus* (slow-pyrolysis for 30 min at 850 °C) was added to soil ($BC_{Ta}$: soil with biochar at ambient soil temperature, $BC_{Te}$: soil with biochar at elevated soil temperature) at a rate of 30 t $ha^{-1}$ and manually incorporated into 0-20 cm soil depth together with spring barley litter from the previous growing season. Control plots ($Ctrl_{Ta}$: control soil at ambient soil temperature, $Ctrl_{Te}$: control soil at elevated soil temperature) were not amended with biochar, but litter was incorporated in the same way. For more information on the site, establishment of the BC-HoCC experiment and soil as well as biochar properties we refer to Poll et al. (2013) and Bamminger et al. (2016).

**Soil management, plant and soil sampling**

In the first cropping season of the BC-HoCC experiment, winter rapeseed (*Brassica napus L.)* was manually sown on all subplots (3 September 2013, 60 plants $m^{-2}$, ≈ 20 cm row spacing). In spring 2014 (13 March), plant numbers were adjusted to obtain standard plant density for rapeseed in each subplot (30 plants $m^{-2}$). Fertilizer was applied twice to all subplots; first, immediately after plant number adjustment, calcium ammonium nitrate at 70 kg nitrogen (N) $ha^{-1}$ and second, on 31 March 2014, ammonium thiosulfate (17.2 kg N $ha^{-1}$ and 37.2 kg S $ha^{-1}$) was applied to the soil surface. Winter rapeseed was harvested at maturity on 17 July 2014 by cutting the aboveground biomass by hand. After drying and determination of aboveground biomass weight (see Bamminger et al., 2016), the rapeseed litter was shredded and re-applied to the respective plots on 29 August 2014. On 21 October 2014, soil was ploughed for the first time since incorporation of the biochar. Spring wheat (*Triticum aestivum*) was sown on 20 March 2015 (about 450 plants $m^{-2}$, ≈ 10 cm row spacing). Before sowing, standard fertilizer (35 kg P, 70 kg K, 13 kg Mg, 19 kg S) was applied to soil. In addition, fertilizer was added twice as calcium ammonium nitrate on 7 May 2015 (60 kg N $ha^{-1}$) and on 3 June 2015 (80 kg N $ha^{-1}$) to the subplots. Aboveground biomass of spring wheat was harvested at maturity on 31 July 2015.

Soil samples were collected using a soil auger to determine soil physical, chemical, and microbial properties from all subplots at two soil depths (0-5 cm and 5-15 cm) in November 2013, March and September 2014, and in March and August 2015.

**Physical and chemical soil analyses**

To determine soil bulk density (BD), undisturbed soil cores (100 $cm^3$) were taken in duplicate at 0-5 and 5-15 cm soil depth from non-vegetated subplots after harvest of winter rapeseed and spring wheat in August 2014 and November 2015, respectively.

Soil water content (SWC) was determined gravimetrically from the regularly taken soil samples after drying for three days at 65 °C. In addition, we obtained volumetric soil water content (VWC) by continuous time-domain reflectometry (TDR)-based measurements throughout the experiment.

pH of soil samples was measured in 0.01 M $CaCl_2$ solution using a 1:4 w/v ratio. Concentrations of ammonium ($NH_4^+$-N) and nitrate ($NO_3^-$-N) were determined by extracting field moist soil with 0.5 M $K_2SO_4$ (1:4 w/v ratio). Subsequently, soil

suspensions were shaken on a horizontal shaker (30 min at 250 rev. $min^{-1}$) and centrifuged (30 min at 4400 × g). Concentrations of $NH_4^+$-N and $NO_3^-$-N in the supernatant were determined colorimetrically with a continuous flow analyzer (Bran + Luebbe Autoanalyzer 3, SEAL Analytical, Hamburg, Germany).

**Determination of greenhouse gas fluxes**

During the two-year experimental period, greenhouse gas emissions ($CO_2$, $N_2O$ and $CH_4$) were measured weekly using closed chambers (Hutchinson & Livingston, 2002). Closed chambers were located between the crop rows, and had an inner volume of 4850 $cm^3$ covering an area of 270 $cm^2$ as described in Poll et al. (2013). Gas samples (20 ml) of the headspace volume were taken with 20 ml syringes via three-way stopcocks and injected into pre-evacuated 12 ml exetainers (Labco Ltd., UK). Samples were taken between 8:00 AM and 2:00 PM after 0, 15 and 30 min in warm periods and after 0, 30 and 60 min after closure in cold periods. In cold periods, closure time was extended to account for lower soil respiration and to ensure sufficient accumulation of GHG within the chamber. The concentrations of $CO_2$, $N_2O$ and $CH_4$ in the headspace samples were determined on an Agilent 7890 gas chromatograph (Agilent Technologies Inc., Santa Clara, CA, USA) equipped with a methanizer and FID for $CO_2$ and $CH_4$ and an ECD for $N_2O$ measurements. Three external standards per gas were used for calibration by linear regression. Cumulative fluxes of $CO_2$, $N_2O$ and $CH_4$ were calculated by linear interpolation between two successive gas samplings and expressed per $m^2$.

Due to technical problems with soil heating during winter 2014/2015, cumulative GHG fluxes were calculated separately for the period between 27 August 2013 and 11 November 2014 as well as for that between 24 March and 11 September 2015. Consequently, total GHG emissions after two years were obtained by the sum of the cumulative emissions in these two periods. GHG emissions in non-vegetated and vegetated periods were calculated by the sum of cumulative emissions during bare soil periods as well as winter rapeseed and spring wheat growing seasons. The global warming potential of soil GHG emissions over a period of 100 years ($GWP_{100}$) was calculated by summing total emissions of $CO_2$, $N_2O$ and $CH_4$, with $N_2O$ and $CH_4$ emissions converted to $CO_2$ equivalents by applying the factors 265 and 28, respectively (Myhre et al., 2013).

We estimated biochar turnover and residence time at elevated soil temperature from the difference in $CO_2$ emissions between $BC_{Te}$ and $Ctrl_{Te}$ plots and the known amounts of applied biochar (30 t $ha^{-1}$). For this calculation, we assumed that the additional release of $CO_2$ equivalents would correspond solely to mineralized biochar-C and that no priming effects occurred in the soil. In a further step, we calculated the warming-induced increase in $GWP_{100}$ in biochar plots ($BC_{Te}$ – $Bc_{Ta}$) and estimated how long C fixation through biochar sequestration would compensate for the enhanced GHG emissions in warmed arable soil. It has to be noted that we assumed a constant warming-induced increase of GHG emissions and a $CO_2$-neutral biochar production system. Possible indirect effects of biochar and warming on GHG emissions by influences on plant and root growth as well as litter input were not considered in this calculation.

**Microbial biomass carbon ($C_{mic}$) and metabolic quotient ($qCO_2$)**

Microbial biomass C was measured by chloroform-fumigation-extraction (CFE) in soil samples of March and August 2015 as described in (Bamminger et al., 2016). In addition, $C_{mic}$ data from the first year of the BC-HoCC experiment (August 2013 to September 2014) was taken from (Bamminger et al., 2016) to determine metabolic quotients ($qCO_2$) at 0-15 cm soil depth during vegetation-free periods. The metabolic quotient, as a measure of respiratory efficiency of the soil microbial community, was calculated as soil respiration per unit microbial biomass and expressed as mg $CO_2$-C $g^{-1}$ $C_{mic}$ $d^{-1}$. For calculation of $qCO_2$, area-related $CO_2$ data (per $m^2$) were converted to emitted $CO_2$ per g soil in 0-15 cm soil depth by applying plot-specific bulk densities (0-5 and 5-15 cm), which were weighted according to depth thickness. Bulk density determined in August 2014 was used to convert $CO_2$ data between November 2013 and September 2014, while bulk density from November 2015 was used for March and August 2015. For $C_{mic}$, values of 0-5 and 5-15 cm soil depth were integrated to 0-15 cm values by weighting according to depth thickness.

Soil respiration data for $qCO_2$ was calculated by the cumulative $CO_2$-C emission of two or three consecutive gas sampling events within three weeks before soil sampling and divided by the number of days of the total period between these gas samplings. In general, $qCO_2$ was determined in periods without vegetation, either shortly after sowing when plant growth was still negligible or after crop harvest. We ensured that there were no extreme influences such as heavy rainfall or fertilization events between the gas samplings or directly before soil sampling in order to avoid times of a strongly disturbed soil microbial

community. Therefore, $qCO_2$ was not calculated in March 2014 because of both fertilizer additions shortly before gas and soil sampling and advanced growth of winter rapeseed, which could have contributed to $CO_2$ emissions by root respiration.

**Temperature sensitivity of soil respiration ($R_s$)**

We aimed to investigate the effects of biochar and soil warming on the temperature response of soil respiration during the two-year study. Fitting soil respiration with an exponential temperature response function ($Q_{10}$ model) was not adequate mainly due to decreasing respiration activity above a threshold of 25 °C (see Fig. S4a). Therefore, data on soil respiration was natural log (ln) transformed to achieve normal distribution and homogeneity of variances. Based on the approach of (Carey et al., 2016), we found a log-quadratic function (Eq. 1) to best describe the temperature sensitivity of soil respiration:

$$\ln(R_s) \sim \boldsymbol{\gamma_0} + \boldsymbol{\gamma_1} T + \boldsymbol{\gamma_2} T^2 \qquad (1)$$

where $\ln(R_s)$ is the natural log of soil respiration, T is soil temperature at 2 cm soil depth, $\gamma_0$ is the y-intercept, while $\gamma_1$ and $\gamma_2$ describe the shape (slope) of the curve.

**Statistical analyses**

Field data on average daily soil temperature and volumetric water content (VWC), physical, chemical, and microbial soil properties (separately for each soil depth), GHG rates of $CO_2$, $N_2O$, and $CH_4$ as well as $qCO_2$ were analyzed by linear-mixed effects (lme) models (nlme package of R 3.2.1; R Core Team, 2015). Fixed-factors were 'soil warming' (W; Ta and Te) and 'biochar' (BC; without and with BC), while time dependency was investigated by including 'season' (S; autumn 2013: 22.08.2013 to 30.11.2013; and autumn 2014: 01.09.2014 to 11.11.2014; winter 2013/14: 01.12.2013 to 28.02.2014; spring 2014: 01.03.2014 to 31.05.2014 and spring 2015: 12.03.2015 to 31.05.2015, summer 2014: 01.06.2014 to 31.08.2014 and summer 2015: 01.06.2015 to 11.09.2015) or 'soil cover' (V, vegetated or non-vegetated) for analysis of soil temperature, VWC and GHG fluxes. For physical, chemical and microbial soil properties we added 'sampling date' (D) as time factor. Block, plot and subplot were included as nested random effects in the lme models. In case of significant single effects of the time factor or interactions with warming and/or biochar, the following analyses were done. In order to relate

environmental conditions and plant growth to soil GHG emissions, we averaged soil temperature and VWC data over seasons and the entire experimental period and calculated cumulative GHG emissions for each season and in non-vegetated and vegetated periods. These data, total GHG emissions and $GWP_{100}$ data after two years (excluding winter season 2014/2015), soil properties separately at each collection date, and total aboveground crop biomass, were analyzed using the same lme models excluding the time factor. In all VWC analyses, the fixed-factor BC was not included due to the overestimation in the BC plots (Bamminger et al., 2016). Prior to analyses, data were log- or square-root transformed if non-normally distributed and inhomogeneity of variance (Levene test) was found. In the cases of GHG rates or cumulative data, we included variance functions (varPower or varIdent, nlme package in R) into the lme models where needed to account for heteroscedasticy of the data (Zuur et al., 2009). A statistical probability of $P \leq 0.05$ was considered significant.

Temperature sensitivity of soil respiration was determined using linear models (lm) instead of lme models due to overlapping confidence intervals of the model parameter estimates ($\gamma_0$, $\gamma_1$, $\gamma_2$) between individual plots (subject-to-subject variability) which revealed that random effects did not need to be considered in our models (Pinheiro & Bates, 2000). First, we fitted data of all observations ('full model') and, in addition, included the factors 'soil warming' (W) and 'biochar' (BC) as categorical variables stepwise into the models to investigate their interactions with temperature sensitivity. Differences in the temperature response in plots with or without biochar in warmed or control plots were examined by analyzing the magnitude of the temperature response ($\gamma_0$) and by the shapes of the curves ($\gamma_1$ and $\gamma_2$) indicating temperature sensitivity. Regression curves were plotted using the xyplot function of the lattice package in R.

## 7.3 Results

### Soil temperature and moisture

Between August 2013 and September 2015, soil warming significantly increased soil temperature by on average 1.88 and 2.03 °C in control and biochar plots, respectively (Fig. S7.1a, $P \leq 0.01$). The warming effect on soil temperature depended on the season (W×S, $P \leq 0.05$) and declined with experimental duration (Fig. S7.1a, S7.2a). Soil temperature increase was considerably higher in biochar-amended than in control plots in the summer

seasons 2014 (2.72 vs. 2.01 °C) and 2015 (1.74 vs. 1.29 °C) (Fig. S7.2a), but without showing statistical significance.

Soil warming led to a reduction of VWC during the two-year experiment by on average 19 % (Fig. S1b, $P$=0.22). This reduction varied with season (W×S, $P \leq 0.001$), ranging from 4 % in winter 2013 to 41 % in summer 2015, averaged over control and biochar treatments (Fig. S7.2b). Likewise, the reducing effect of soil warming on SWC varied with sampling date at 0-5 cm and 5-15 cm (W×D, $P \leq 0.01$), but was only significant in spring 2014 (March) at both soil depths (Table 7.1). The overall effect of biochar on VWC could not be evaluated in this study due to high electrical conductivity of the biochar (Bamminger et al., 2016). However, biochar was effective in reducing water loss caused by soil warming between August 2013 and April 2014, but this effect on VWC disappeared afterwards (Fig. S7.1b, S7.2b). The most pronounced compensatory effect of biochar on soil moisture in warmed soil was present in spring 2014, showing 16 % less water loss than in the control plots (Figs. S7.1b, S7.2b).

In addition, we used SWC, which was measured in the sampled soils, to compare differences in soil moisture between control and biochar plots at different soil depths. Biochar significantly increased SWC at both soil depths across all soil sampling dates ($P \leq 0.05$). This biochar-induced increase of SWC was more pronounced at 5-15 cm soil depth than at 0-5 cm on most sampling dates (Table 7.1).

**Physical and chemical soil properties**

Soil bulk density was not affected by warming, but significantly ($P \leq 0.01$) reduced by biochar at 0-5 cm depth, in August 2014 by 12 % and in November 2015 by 7 % (Table 7.1). At 5-15 cm, biochar decreased BD by 7 % ($P \leq 0.01$) and 8 % ($P$=0.06) in August 2014 and November 2015, respectively. Soil pH was not changed by elevated temperature, but soil warming interacted with biochar in April 2014 showing the highest value in the $BC_{Te}$ treatment (Table 7.1, BC×W, $P \leq 0.05$). Biochar significantly increased soil pH throughout the study by a maximum of 0.3 pH units, but this effect declined with time (BC×D, $P \leq 0.01$) and in August 2015, two years after application, biochar no longer had an effect on soil pH. Overall, soil warming and biochar had no effect on EOC at 0-5 cm, while warming tended to increase EOC at 5-15 cm throughout the study (Table 7.1, $P$=0.06). Three months after application (November 2013), biochar appeared to increase

EOC concentrations at 0-5 cm ($P$=0.09). $NH_4^+$-N concentration in soil was inconsistently affected by biochar at both depths depending on sampling date (BC×D, $P \leq 0.05$), which was only significant at single sampling dates (Table 7.1). In November 2013, $NH_4^+$-N was enhanced in $BC_{Te}$ (BC×W, $P \leq 0.05$). While warming had no effect, biochar significantly decreased concentrations of $NO_3^-$-N at 0-5 cm and 5-15 cm soil depth between November 2013 and March 2015 (Table 7.1).

**Table 7.1.** Physical and chemical soil properties at 0-5 and 5-15 cm soil depths in 2013 (November), 2014 (March, August and September) and 2015 (March, August and November) (mean ± SE). Significant results of linear mixed-effects models for the effects of soil warming (W), biochar addition (BC) and their interactions separately for each sampling date and soil depth are indicated by asterisks (* P≤0.05, ** *P*≤0.01, *** *P*≤0.001).

| Parameter | month/ year | 0-5 cm | | | | | 5-15 cm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $Ctrl_{Ta}$ | $Ctrl_{Te}$ | $BC_{Ta}$ | $BC_{Te}$ | **Effects** | $Ctrl_{Ta}$ | $Ctrl_{Te}$ | $BC_{Ta}$ | $BC_{Te}$ | **Effects** |
| BD (g $cm^{-3}$) | Aug14 | 1.39±0.02 | 1.27±0.02 | 1.21±0.03 | 1.13±0.04 | **BC↓**** | 1.24±0.06 | 1.16±0.01 | 1.13±0.06 | 1.10±0.02 | **BC↓**** |
| | Nov15 | 1.16±0.01 | 1.17±0.02 | 1.13±0.02 | 1.04±0.03 | **BC↓*** | 1.27±0.02 | 1.19±0.06 | 1.17±0.05 | 1.10±0.01 | |
| SWC (%) | Nov13 | 24.9±0.4 | 25.0±0.5 | 27.3±0.7 | 26.9±1.2 | **BC↑*** | 24.0±0.7 | 23.9±0.8 | 24.8±1.4 | 26.4±1.1 | |
| | Mar14 | 17.5±0.5 | 13.8±0.7 | 18.2±0.3 | 13.9±0.6 | **W↓**** | 17.6±0.3 | 14.7±0.7 | 20.2±0.3 | 16.2±0.4 | **BC↑**, W↓*** |
| | Sep14 | 19.5±1.0 | 18.0±1.2 | 20.7±0.4 | 19.7±0.6 | | 19.1±0.3 | 19.2±0.6 | 20.8±0.1 | 21.1±0.3 | **BC↑**** |
| | Mar15 | 11.8±2.6 | 12.4±0.9 | 13.2±1.0 | 14.1±1.1 | | 17.9±0.9 | 19.0±1.3 | 21.2±0.1 | 20.4±0.5 | **BC↑*** |
| | Aug15 | 7.41±0.48 | 5.47±0.36 | 7.75±0.48 | 6.92±0.26 | | 12.2±0.8 | 11.6±0.3 | 12.2±0.2 | 11.1±0.6 | |
| Soil pH | Nov13 | 6.74±0.06 | 6.72±0.03 | 6.90±0.01 | 6.97±0.03 | **BC↑**** | 6.82±0.04 | 6.78±0.03 | 6.93±0.05 | 7.06±0.03 | **BC↑**** |
| | Mar14 | 6.67±0.08 | 6.68±0.08 | 6.80±0.06 | 6.95±0.04 | **BC***, BC×W*** | 6.78±0.08 | 6.75±0.06 | 6.97±0.03 | 6.96±0.04 | **BC↑**** |
| | Sep14 | 6.74±0.12 | 6.79±0.09 | 6.86±0.08 | 6.91±0.06 | **BC↑**** | 6.82±0.03 | 6.82±0.06 | 6.91±0.02 | 7.02±0.03 | **BC↑**** |
| | Mar15 | 6.79±0.08 | 6.82±0.14 | 6.81±0.06 | 6.86±0.06 | | 6.82±0.06 | 6.80±0.07 | 6.87±0.04 | 6.92±0.03 | **BC↑*** |
| | Aug15 | 6.37±0.09 | 6.32±0.06 | 6.35±0.06 | 6.42±0.05 | | 6.73±0.08 | 6.72±0.05 | 6.75±0.05 | 6.77±0.02 | |
| EOC (µg C $g^{-1}$ dw) | Nov13 | 49.7±0.7 | 51.0±8.7 | 55.1±3.1 | 67.1±4.8 | | 59.7±2.3 | 69.0±3.2 | 62.7±6.0 | 78.9±5.6 | |
| | Mar14 | 53.8±2.4 | 67.8±9.5 | 59.6±4.8 | 71.1±5.6 | | 47.9±1.7 | 53.6±5.5 | 46.4±4.5 | 57.2±8.5 | |
| | Sep14 | 58.0±4.4 | 69.8±7.5 | 71.5±7.1 | 69.9±5.7 | | 51.1±2.7 | 71.5±7.0 | 65.6±8.1 | 69.4±4.3 | |
| | Mar15 | 41.5±5.9 | 57.6±6.8 | 50.0±5.6 | 49.8±7.3 | | 32.9±2.9 | 52.79±7.2 | 40.2±2.6 | 49.8±5.9 | |
| | Aug15 | 60.7±0.9 | 69.3±8.8 | 53.3±3.6 | 72.7±7.4 | | 34.5±4.8 | 54.4±12.9 | 39.4±1.9 | 52.3±1.8 | |
| $NH_4^+$ (µg $NH_4^+$-N $g^{-1}$ dw) | Nov13 | 1.21±0.09 | 1.07±0.13 | 1.00±0.03 | 1.59±0.27 | **BC×W*** | 0.97±0.08 | 1.21±0.05 | 1.58±0.46 | 1.60±0.42 | |
| | Mar14 | 49.8±12.9 | 56.8±7.3 | 57.3±10.0 | 88.0±17.3 | | 0.71±0.12 | 0.62±0.03 | 0.97±0.19 | 0.82±0.31 | |
| | Sep14 | 1.20±0.24 | 1.27±0.20 | 1.92±0.49 | 1.70±0.43 | | 1.39±0.32 | 0.94±0.07 | 1.61±0.44 | 1.05±0.21 | |
| | Mar15 | 1.02±0.15 | 0.83±0.07 | 0.77±0.06 | 1.01±0.15 | | 1.55±0.09 | 2.00±0.43 | 1.46±0.07 | 1.74±0.17 | |
| | Aug15 | 9.05±3.83 | 20.2±8.3 | 4.38±1.85 | 7.48±2.29 | | 2.27±0.26 | 1.31±0.12 | 1.03±0.10 | 1.14±0.20 | **BC↓*** |
| $NO_3^-$ (µg $NO_3^-$-N $g^{-1}$ dw) | Nov13 | 0.07±0.04 | 0.20±0.10 | 0.00±0.00 | 0.03±0.03 | **BC↓*** | 0.68±0.02 | 0.65±0.11 | 0.30±0.08 | 0.19±0.03 | **BC↓*** |
| | Mar14 | 18.5±5.3 | 20.9±3.5 | 15.0±3.4 | 25.3±5.8 | | 3.49±0.06 | 2.87±0.19 | 1.51±0.26 | 1.94±0.54 | **BC↓*** |
| | Sep14 | 0.80±0.03 | 1.34±0.13 | 0.50±0.09 | 0.85±0.14 | **BC↓**** | 1.30±0.15 | 2.08±0.60 | 0.86±0.16 | 1.77±0.59 | |
| | Mar15 | 0.95±0.10 | 0.94±0.23 | 0.61±0.03 | 0.75±0.23 | | 1.23±0.14 | 1.15±0.08 | 1.13±0.09 | 0.94±0.07 | **BC↓*** |
| | Aug15 | 4.62±0.91 | 6.54±1.13 | 5.39±0.99 | 6.21±0.42 | | 8.77±1.01 | 13.02±1.8 | 9.65±2.14 | 7.90±1.36 | |

Arrows show the direction of single effects. $Ctrl_{Ta}$ = control soil under ambient soil temperature, $Ctrl_{Te}$ = control soil under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature. Data from 2013 and 2014 were taken from Bamminger et al. (2016).

**Crop growth**

As previously reported in Bamminger et al. (2016) for the vegetation period 2013/2014, warming increased total aboveground biomass of winter rapeseed by 45 %, but only in plots without biochar (Fig. S7.3; BC×W, $P \leq 0.05$). At the end of the second season, total aboveground biomass of spring wheat was higher by 28 % in warmed than in ambient temperature soils and 11 % higher in plots with than without biochar. However, although the effects of the factors were pronounced, they were not statistically significant due to high variation in plant growth within the field experiment.

**Greenhouse gas emissions and global warming potential**

Soil warming enhanced $CO_2$ emission rates throughout the study (Fig. 7.1a, $P \leq 0.05$) and led to an increase of 28 % of total emissions after two years (Fig. 7.1b, $P \leq 0.05$). Biochar application increased the initial $CO_2$ peak after ploughing in August 2013, especially under soil warming (Fig. 7.1a). In the following few weeks, $CO_2$ rates declined and showed no clear stimulation by biochar. This immediate $CO_2$ pulse after the experimental start in the biochar plots led to a trend of higher cumulative $CO_2$ emissions in autumn 2013 (Fig. 7.2a, $P=0.07$). No significant effect of biochar on total $CO_2$ emissions was found after two years (Fig. 7.1b). In a more detailed view, in non-vegetated periods (e.g. autumn 2014), $CO_2$ emissions were slightly decreased in biochar plots under ambient (-11 %), but not under elevated soil temperature (Fig. 7.2a, BC×W, $P=0.053$). In contrast, during vegetated periods, both soil warming (+41 %) and biochar (+6 %) significantly increased $CO_2$ emissions ($P \leq 0.05$).

Similar to $CO_2$, emission rates of $N_2O$ were initially enhanced but leveled off rapidly and this was most pronounced in biochar-amended soil under warming (Fig. 7.1c). In the first month, soil warming and biochar had an interactive effect on $N_2O$ emission rates (Fig. 7.1c), resulting in a biochar-induced decrease of 26 % of cumulative $N_2O$ emissions under ambient, but 65 % increase under elevated temperature (BC×W, $P \leq 0.05$). After two years the same pattern was observed for total $N_2O$ emissions, although the effect sizes were smaller (-8 % at Ta, +33 % at Te) (Fig. 7.1d, BC×W, $P \leq 0.05$). During the whole study, both soil warming and biochar addition showed no consistent effects on $N_2O$ rates, but seemed to interact with season (BC×W×S, $P=0.056$), which could, in addition to the $N_2O$ pulse at the beginning, be related to N-availability and precipitation events (Fig. 1c). In

spring 2014, after N-fertilization of winter rapeseed, soil warming appeared to increase cumulative emissions of $N_2O$ ($P \leq 0.07$) with highest emissions from the $BC_{Te}$ treatment, but no significant interaction between biochar and soil warming (Fig. 7.2b). In the summer of 2014, daily precipitation was quite high compared to the dry spring period before (Fig. S7.1c) and biochar increased cumulative $N_2O$ by 79 % ($P \leq 0.01$) in comparison to the control plots, showing highest $N_2O$ emissions from the $BC_{Te}$ treatment (Fig. 7.2b). In the dry summer of 2015, at the final growth stage of spring wheat and mainly after N-fertilization, soil warming led to a decrease in control plots, but enhanced cumulative $N_2O$ emissions in warmed biochar-amended soil (Fig. 7.2b, BC×W, $P \leq 0.05$). During vegetated periods in the two-year experiment, warming reduced $N_2O$ emissions by 26 % in control plots, but increased $N_2O$ by 92 % in biochar-amended soil (Fig. 7.2b, BC×W, $P \leq 0.05$).

$CH_4$ rates showed no distinct seasonal fluctuations during the experiment (Fig. 7.1e), but soil warming tended to increase total $CH_4$ uptake by 38 % (Fig. 7.1f, $P=0.07$). Soil warming had the highest impact in spring 2014, when cumulative $CH_4$ uptake was increased by 51 % and 49 % in control and biochar plots, respectively ($P=0.07$). Biochar tended to increase cumulative $CH_4$ uptake only in summer 2014 by on average 31 % ($P=0.07$), but did not affect total $CH_4$ uptake over the two years (Fig. 7.1f).

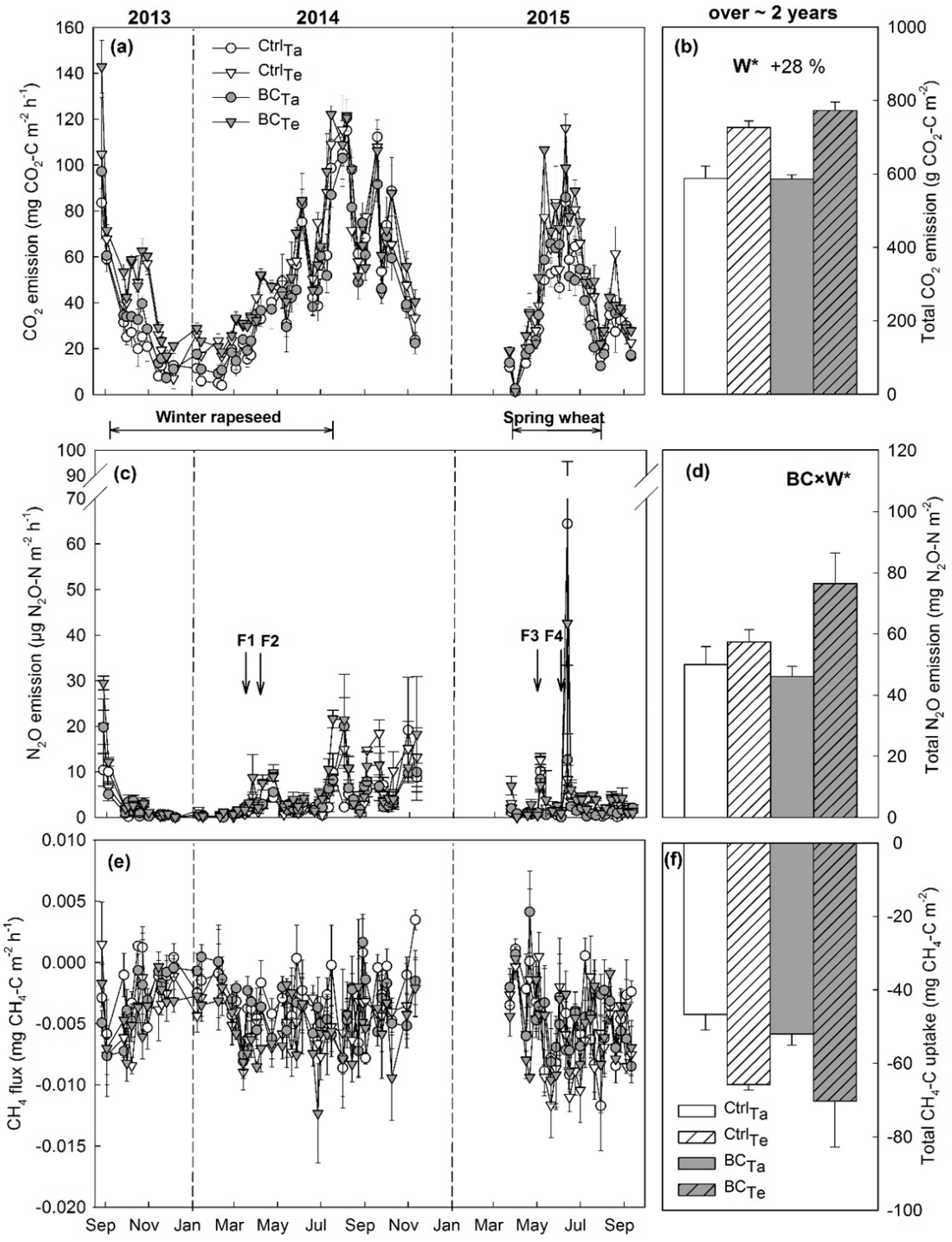


**Fig. 7.1.** Greenhouse gas flux rates (left panels) and total emissions (right panels) of $CO_2$ (a+b), $N_2O$ (c+d) and $CH_4$ (e+f) over two years (mean ± SE). The winter period 2014/2015 was excluded due to technical problems with soil heating. Significant results of linear mixed-effects model for the effects of soil warming (W) and biochar addition (BC) and their interactions are indicated by asterisks (* $P \leq 0.05$). Percent values show the size of significant single effects. F1-4 = fertilization events. $Ctrl_{Ta}$ = control soil under ambient soil temperature, $Ctrl_{Te}$ = control soil under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature.

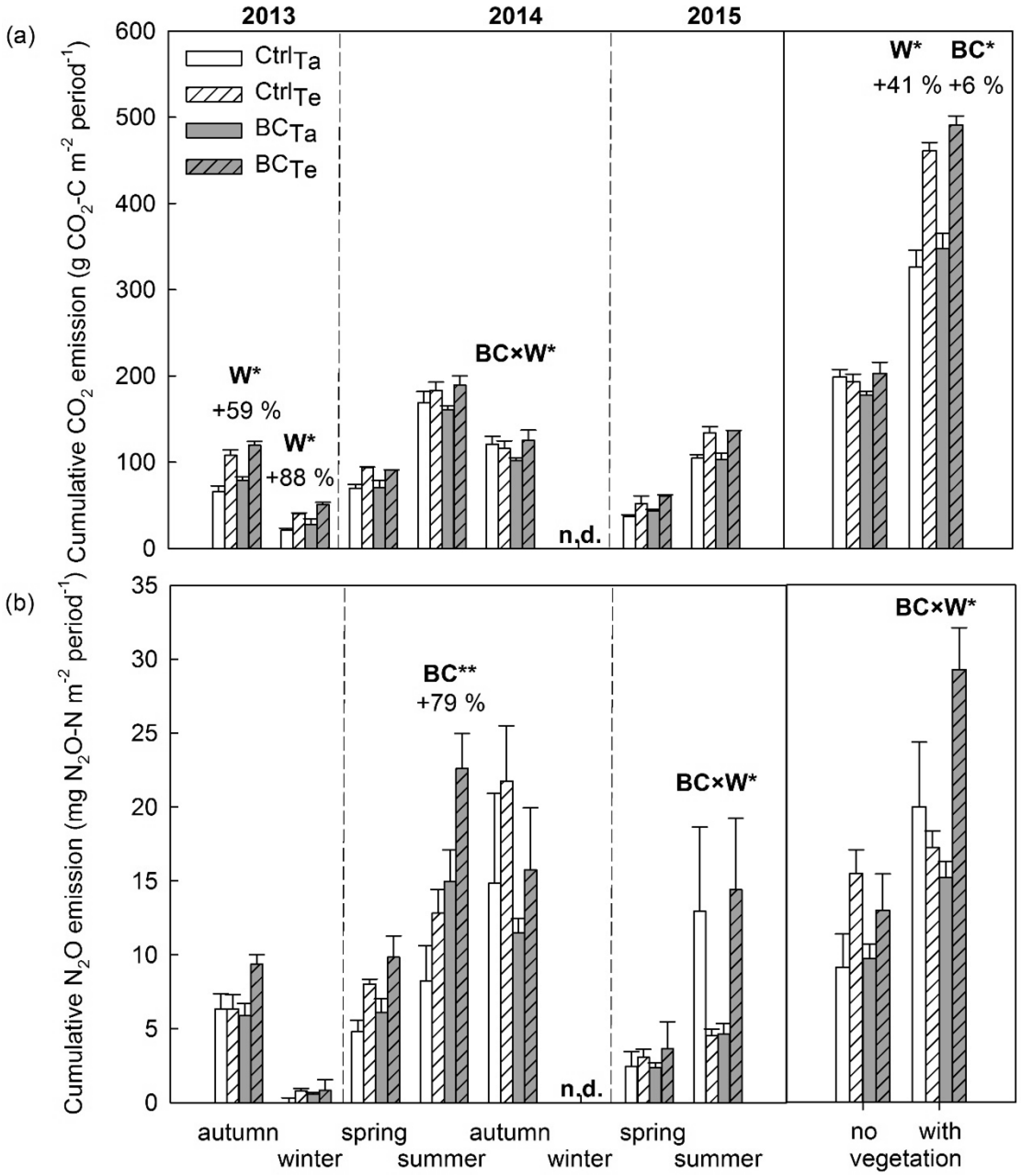


**Fig. 7.2.** Cumulative greenhouse gas emissions in single seasons as well as during crop growth periods (non-vegetated and vegetated) over two years of (a) $CO_2$-C and (b) $N_2O$-N (mean ± SE). Significant results of linear mixed-effects model for the effects of soil warming (W), biochar addition (BC) and their interactions are indicated by asterisks (* P≤0.05, ** *P*≤0.01). Percent values show the size of significant single effects. $Ctrl_{Ta}$ = control soil under ambient soil temperature, $Ctrl_{Te}$ = control soil under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature. n.d. = not determined.

$GWP_{100}$ was enhanced by 28 % in warmed soil ($P \leq 0.05$) and predominantly determined by $CO_2$ emissions, and only to a small extent by $N_2O$ and $CH_4$ emissions. Biochar had no significant effect on $GWP_{100}$ (Fig. 7.3). Annual biochar turnover in soil was estimated to be approximately 0.3 %, corresponding to a residence time of 369 years. Based on our calculations, the amount of sequestered $CO_2$ equivalents in soil by biochar application could compensate for the warming-induced enhancement of cumulative GHG emissions in a 20-year period.

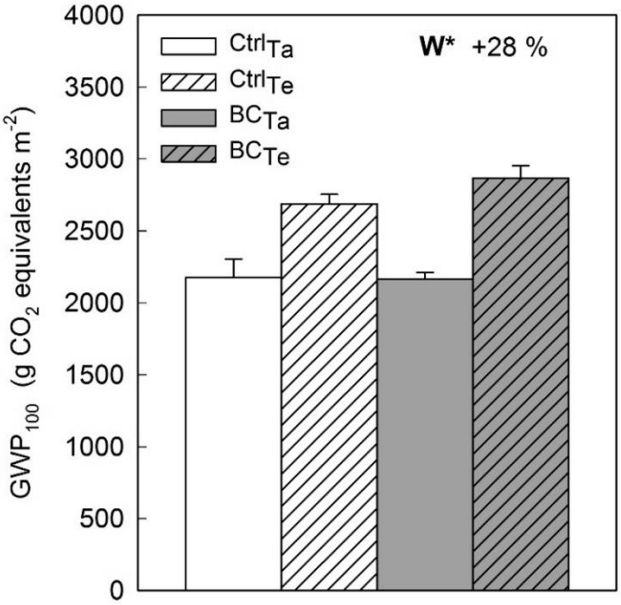


**Fig. 7.3.** Global warming potential over a period of 100 years ($GWP_{100}$) from the sum of total soil greenhouse gas emissions of $CO_2$, $N_2O$ and $CH_4$ during two years (mean ± SE). Significant results of linear mixed-effects model for the effects of soil warming (W), biochar addition (BC) and their interactions are indicated by asterisks (* $P \leq 0.05$). Percent values show the size of significant single effects. $Ctrl_{Ta}$ = control soil under ambient soil temperature, $Ctrl_{Te}$ = control soil under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature.

### Microbial biomass ($C_{mic}$) and metabolic quotient ($qCO_2$)

$C_{mic}$ was not significantly affected by soil warming or biochar at either soil depth across five sampling dates within two years after biochar application. Only in November 2013 (0-5 cm), and August 2015 (5-15 cm), biochar and soil warming showed interactive effects on $C_{mic}$, as warming led to an increase in microbial abundance in controls, but a decrease in biochar plots (Table S7.1, BC×W, $P \leq 0.05$).

Neither soil warming nor biochar consistently influenced $qCO_2$ during the experiment, and their effects depended on sampling date (Fig. 7.4, W×D, $P \leq 0.01$; BC×D, $P \leq 0.05$). In November 2013, three months after biochar application and soil ploughing, soil warming and biochar increased $qCO_2$ by 88 % ($P \leq 0.01$) and 44 % ($P \leq 0.05$), respectively (Fig. 7.4). In addition, soil warming enhanced $qCO_2$ by 31 % ($P \leq 0.05$) and 35 % ($P = 0.11$) in March and August 2015, respectively.

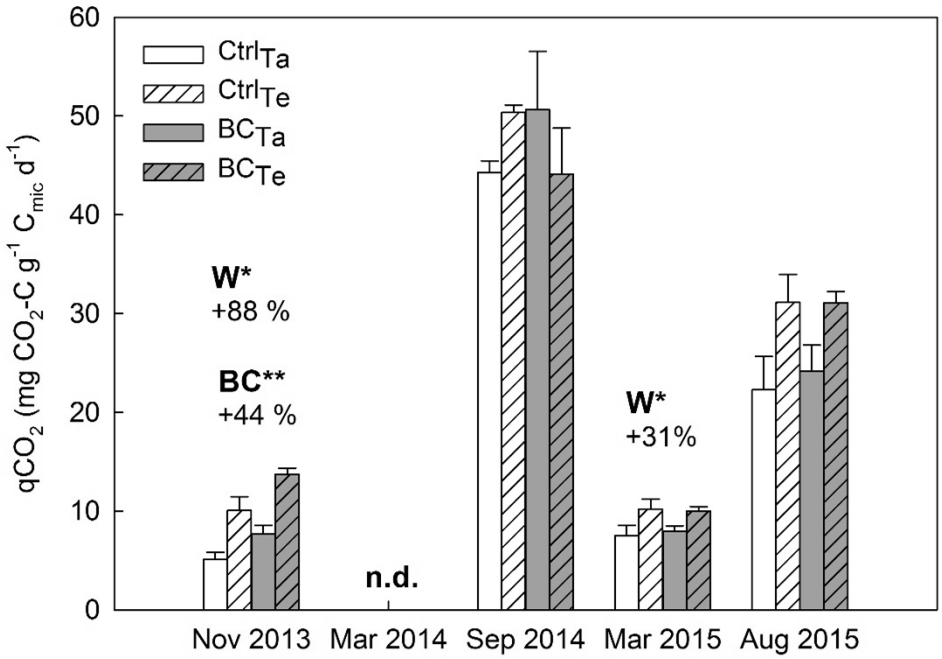


**Fig. 7.4.** Metabolic efficiency ($qCO_2$) of the soil microbial community at 0-15 cm soil depth at four soil sampling dates during the two-year experimental period (mean ± SE). Significant results of linear mixed-effects model for the effects of soil warming (W), biochar addition (BC) and their interactions are indicated by asterisks (* $P \leq 0.05$, ** $P \leq 0.01$). Percent values show the size of significant single effects. $Ctrl_{Ta}$ = control soil under ambient soil temperature, $Ctrl_{Te}$ = control soil under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature. n.d. = not determined. For calculation of $qCO_2$ in November 2013 and September 2014, $C_{mic}$ data were taken from Bamminger et al. (2016).

## Temperature sensitivity of soil respiration

Including all observations ('full model', n=757, $R^2=0.49$, $P \leq 0.001$), soil warming had a significant effect ($P \leq 0.05$) on the temperature response function during our two-year study, although only the γ –intercept, i.e. the magnitude of the temperature response, was affected. No interactions between warming treatment and the other model parameters ($\gamma_1$,

$\gamma_2$) were observed. In addition, biochar had no significant effect on the magnitude of the response or temperature sensitivity of $R_s$. Therefore, best fit regression curves of the single treatments were displayed with uniform shape ($\gamma_1$, $\gamma_2$ of the full model, see Fig. S7.4b), but with individual γ–intercepts, showing differences between Ta and Te plots of the control (1.374 *vs.* 1.600) and biochar (1.434 *vs.* 1.683) treatments, respectively (Fig. 7.5). In addition, the vague relationship between residuals of the full model and the VWC data (Fig. S7.4c) indicated that differences in soil moisture only weakly determined the temperature response of soil respiration.

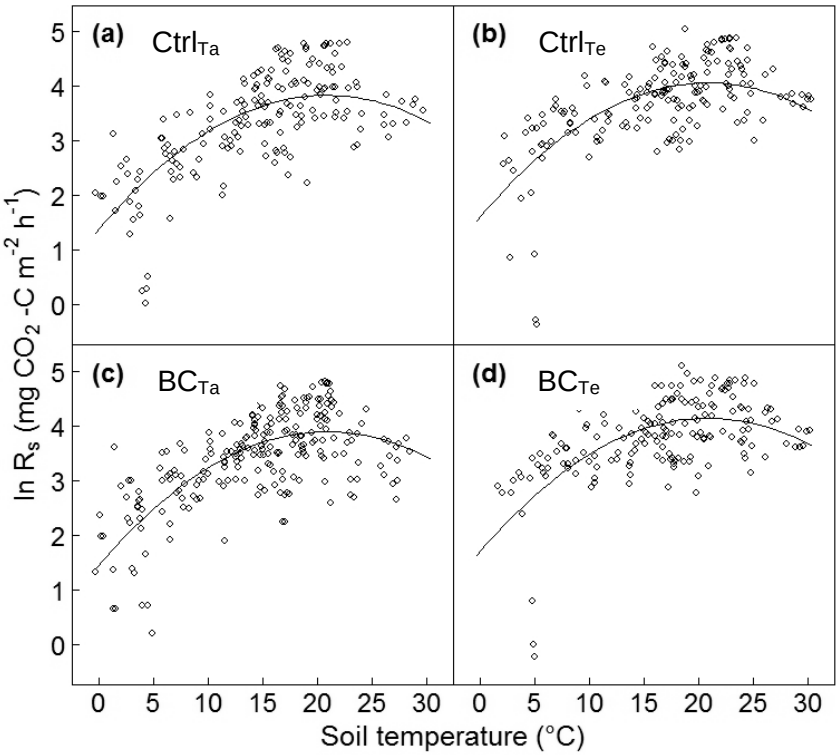


**Fig. 7.5.** Regression curve fits of ln $R_s$ (soil respiration) against soil temperature at 2 cm depth during the experiment for the single treatments. (a) $Ctrl_{Ta}$ = control soil under ambient soil temperature, (b) $Ctrl_{Te}$ = control soil under elevated soil temperature, (c) $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, and (d) $BC_{Te}$ = soil amended with biochar under elevated soil temperature.

## 7.4 Discussion

**Biochar effects on soil respiration under warming**

Climate change will affect matter cycling in soils, which may cause increases in GHG emissions and positive feedback effects to global warming (Bardgett et al., 2008). It has been suggested that biochar can sequester C in soil over the long-term (Lorenz & Lal, 2014) and thus may reduce warming-induced positive feedbacks to the global climate. This is the first study investigating the effect of biochar on soil GHG emissions in warmed soil in a temperate agricultural system. Our results during a two-year experimental period showed that soil warming enhanced total $CO_2$ emissions by 28 %. This suggests a failure of microbial adaption to elevated soil temperature even after several years, a finding also shown by Schindlbacher et al. (2015) in forest soil, but contrasts with results of several other studies in which enhanced soil respiration diminished over time (Melillo et al., 2002; Bradford et al., 2008). Biochar did not reduce total $CO_2$ emissions, but also showed no significant additional C-mineralization even under warming. This indicates high degradation resistance of the biochar, which is in line with other field experiments (e.g. Ameloot et al., 2014).

Although biochar did not influence total $CO_2$ emissions after two years, the temporal pattern of soil respiration provides insight how biochar may affect the response of C dynamics in soil to warming. Biochar initially stimulated soil respiration especially under warming. We suggest this was due primarily to readily available C substrate on the surfaces of fresh biochar, which are rapidly consumed by soil microorganisms leading to short-time enhanced $CO_2$ emissions (Smith et al., 2010). In November 2013, three months after biochar application, soil warming and biochar led to 88 % and 44 % higher metabolic quotients ($qCO_2$), respectively, showing the most pronounced increase in the $BC_{Te}$ plots. The $qCO_2$ can be used as an indicator of the metabolic efficiency of soil microorganisms affected by changes in environmental conditions (Anderson & Domsch, 2010), in this case induced by a combination of soil warming and biochar. According to Frey et al. (2013), the carbon use efficiency (CUE) of soil microorganisms was reduced with increasing temperature, including that of recalcitrant compounds, but this effect was alleviated by long-term warming. Relating this to our study, we suggest a less efficient microbial community under soil warming and prolonged microbial degradation of biochar in autumn 2013, three months after its application. The increase in microbial respiration without biomass growth in soils treated with biochar points to inefficient microbial utilization of

biochar-C. However, the simultaneous application of biochar and plant litter in warmed soil could have had an additive effect on the microbial decomposer community, increasing fungal abundances and the fungal-to-bacterial ratio in the $BC_{Te}$ treatment in November 2013 (Bamminger et al., 2016). Fungal species were found to differ in their ability to adapt to elevated soil temperature (Malcom et al., 2008), and shifts in soil microbial community composition may influence microbial C use efficiency (Bölscher et al., 2016). Nevertheless, metabolic quotients were not affected by biochar later in the first or in the second year of our experiment. Therefore, biochar may stabilize in soil with time due to the immediate mineralization of labile biochar-C and/or by the occlusion of biochar particles in soil aggregates as observed after one year (Grunwald et al., unpublished). This would explain the negligible effects on soil microorganisms in the longer term.

Nguyen et al. (2010) and Fang et al. (2014) described enhanced mineralization of recalcitrant biochar-C with increasing soil temperature. Stable SOC is generally considered to be more sensitive to increasing soil temperature than less stable SOC (Conant et al., 2011), but this theory is somewhat controversial (Fang et al., 2014). In the present study, we could not distinguish between biochar and native SOC mineralization, but we assessed the influence of biochar on the temperature sensitivity of $R_s$ in agricultural soil. We found no significant biochar effect on the temperature dependency of soil respiration during the two years. This may indicate that the temperature sensitivity of biochar-C mineralization was similar to that of SOC, as also shown by (Fang et al., 2014). Alternatively, and more likely in our case, biochar was not degraded in amounts great enough to have an effect on the temperature sensitivity of soil respiration. In addition, the $CO_2$ emission data suggest that biochar did not considerably influence SOC mineralization (priming effect) under warming in the investigated arable ecosystem. Taken together, this implies that biochar-soil warming interactions may only be important during initial degradation of biochar in the first weeks after incorporation into soil, but not in the long term. This generally confirms results from a recent two-year incubation experiment by Fang et al. (2015), which suggests that BC could be an option to store C in soils under a warming climate.

Interactions between biochar and plants likely also influence soil microbial activity and C cycling (Ventura et al., 2015). In the present study we found only minor effects of warming and biochar on $CO_2$ emissions in non-vegetated periods, whereas during vegetated periods, soil respiration was increased by 41 % and 6 % by warming and

biochar, respectively. These results corresponded well to the observed positive effects on plant growth and aboveground biomass of winter rapeseed and spring wheat. Hence, we assume that warming led also to intensified root growth and biomass as well as exudation accompanying enhanced root and microbial respiration (Trumbore, 1997). In addition, biochar application was found to be beneficial in terms of improving soil properties and increasing crop yields (Biederman & Harpole, 2013). The plant-mediated small increase in $CO_2$ emissions from the biochar plots in our study may be therefore explained by slightly improved crop growth, and related to increases in root respiration and microbial activity in the rhizosphere. In this case, mineralized C in BC plots could be derived in large part from newly assimilated $CO_2$ by plants, and only to a lesser extent from older SOC. This would underline the potential of biochar for long-term C fixation and SOC protection in cultivated soils (Weng et al., 2015) even under a predicted warming climate. However, possible interactions between SOC, biochar and plants are poorly understood to date (Whitman et al., 2014).

**Biochar influence on soil $N_2O$ and $CH_4$ fluxes under warming**

After two years, under ambient temperature, biochar decreased total $N_2O$ emissions very little. This is in contrast to several field studies which have shown that biochar reduced $N_2O$ emissions from arable soils receiving N inputs by the application of fertilizer or litter (e.g. Taghizadeh-Toosi et al., 2011; Felber et al., 2014). The lack of a reducing effect of biochar on $N_2O$ emissions in this field experiment is even more noteworthy, since in an earlier study, under controlled conditions, short-term (37d) $N_2O$ emissions from the same arable soil were found to be reduced by 42 % after combined application of 600 °C *Miscanthus* biochar and *Phacelia* litter (Bamminger et al., 2014b). The reduction of $N_2O$ emissions by biochar has been attributed mainly to suppressed denitrification rates by e.g. lower soil bulk density and related higher aeration, enhanced soil pH inaccessibility of C, sorption of inorganic N to biochar particles, microbial N-immobilization or due to more favorable conditions for complete denitrification (Clough et al., 2013). The last step of denitrification (the reduction of $N_2O$ to $N_2$) could be promoted by the creation of hotspots with optimal conditions for complete denitrification on biochar surfaces with locally higher water and N retention as well as enhanced soil pH (Harter et al., 2013; Ameloot et al., 2016). In our field experiment, biochar generally altered physico-chemical soil properties, although the slight pH increase was transient. In summary, these biochar

impacts on soil properties did not result in significant reductions in $N_2O$ emissions, which compares favorably with two other field studies using high-temperature biochars (Suddick & Six, 2013; Verhoeven & Six, 2014).

In contrast, under soil warming, biochar increased total $N_2O$ emissions by 33 % over the two year period. Interactive effects of biochar and warming on field-scale $N_2O$ emissions have not yet been evaluated by others, but may be highly relevant to agricultural soils. Soil $N_2O$ is produced primarily by denitrification and nitrification processes, which can occur simultaneously at different locations in soil, depending on water content and the availability of oxygen, mineral N and C sources (Bateman & Baggs, 2005). Especially in the $BC_{Te}$ treatment we observed a short-term increase in $N_2O$ rates in the first weeks after biochar application and soil ploughing, which was analogous to increased $CO_2$ emissions. After this initial stimulation, the main or interactive effects of biochar and warming on $N_2O$ emissions were mainly associated with the combination of N-fertilization and precipitation events in dry periods (spring 2014 and summer 2015) and extensive precipitation, leading to marked increases in soil moisture and microbial activity (summer 2014). Our results, increased $N_2O$ emissions in warmed biochar plots, may be explained by the combination of several mechanisms potentially affecting denitrification/ nitrification in soil: (i) sorption of inorganic N ($NO_3^-$, $NH_4^+$) onto biochar surfaces, thus preventing N-leaching while maintaining bioavailability in the upper soil layers (Clough et al., 2013; Zheng et al., 2013), which could further enhance N-cycling due to soil warming (Bai et al., 2013); (ii) stimulated SOM mineralization under warming, providing more available C in soil and, at the same time, oxygen depletion creating anaerobic zones in soil for denitrification (Butterbach-Bahl et al., 2013); (iii) increased soil moisture with biochar, especially during dry periods (Saarnio et al., 2013) and enhanced aeration at the same time (Karhu et al., 2011) potentially promoting nitrification (Verhoeven & Six, 2014); or (iv) shifts in the N-cycling microbial community due to soil warming (Cantarel, et al., 2012) and biochar application (Prommer et al., 2014). Another mechanism resulting in increased $N_2O$ emissions could be improved transport of $N_2O$, produced in deeper soil layers, to the atmosphere due to lower bulk density of the silty-loam soil after biochar addition. This could have prevented the reduction of $N_2O$ to $N_2$ in the topsoil, the assumed reason for decreased $N_2O$ emissions observed in other studies (e.g. Harter et al., 2013).

Intensively managed agricultural soils typically have only minimal sink potential for methane (Jeffery et al., 2016). Likewise in our agroecosystem, methane fluxes were

generally low and showed only small seasonal fluctuations but high variation between plots, which likely explains the lack of significant effects of biochar (Castaldi et al., 2011) and warming or their interaction on $CH_4$ fluxes. However, soil warming tended to increase total $CH_4$ uptake by, on average, 38 % over the two years and this was most pronounced during the dry spring period in 2014. Soil drying, enhanced oxygen supply, and increased diffusion of $CH_4$ into the soil under warming may explain this effect (Dijkstra et al., 2012). Biochar tended to increase $CH_4$ uptake only in summer 2014 when extensive precipitation increased soil moisture. Karhu et al. (2011) proposed that biochar could increase water holding capacity and air-filled pore space at the same time, which may either reduce $CH_4$ production and/or increase $CH_4$ oxidation, thus increasing $CH_4$ uptake. Overall, the low $CH_4$ fluxes during the two-year experimental period indicate that methane is of minor importance for the GHG budget of the investigated agroecosystem.

**Biochar C-sequestration vs. enhanced GHG emissions under global warming**

Soil warming increased $GWP_{100}$ by 28 % over two years (during the 6th and 7th year of the warming experiment), supporting predictions of further increased GHG emissions under future elevated soil temperatures (Ciais et al., 2013). Although biochar was not effective in reducing warming-induced enhanced soil GHG emissions, its application could mitigate climate change by long-term C sequestration and increasing SOC stocks in arable soils (Hernandez-Soriano et al., 2016). We estimated that the high-temperature biochar used in our study is mineralized slowly (about 0.3 % per year) and therefore should persist for about 369 years in warmed soil. Based on our calculations, the amount of sequestered $CO_2$ equivalents by biochar incorporation into soil may counterbalance the increased soil GHG emissions due to warming for two decades. This underscores the value of biochar as a tool to offset soil GHG emissions at predicted elevated temperatures in temperate arable soils, even though biochar and warming interactively increased $N_2O$ emissions. However, the climate change mitigation potential of biochar-C sequestration is constrained by the degree of its resistance to decomposition, potential positive or negative feedbacks on native SOC, and the sustainability of biochar production systems (Wang et al., 2016b; Woolf et al., 2016). Clearly, the linkages between biochar stability, soil microbial communities, GHG emissions and crop growth in agroecosystems need to be further explored in long-term experiments to evaluate biochar as a C sequestration and GHG mitigation tool, especially under predicted global warming.

## 7.5 Acknowledgements

Special thanks to M. Kempe and R. Kahle for assistance during GHG samplings. In addition, we thank the members of the soil biology group at our institute for their assistance in maintaining the field experiment and Kathleen Regan for English corrections. C. Bamminger was funded by a PhD scholarship (Landesgraduiertenförderung Baden-Württemberg) awarded by the faculty of Agricultural Sciences at the University of Hohenheim. The authors declare that there is no conflict of interest.

## 7.6 Supplementary material

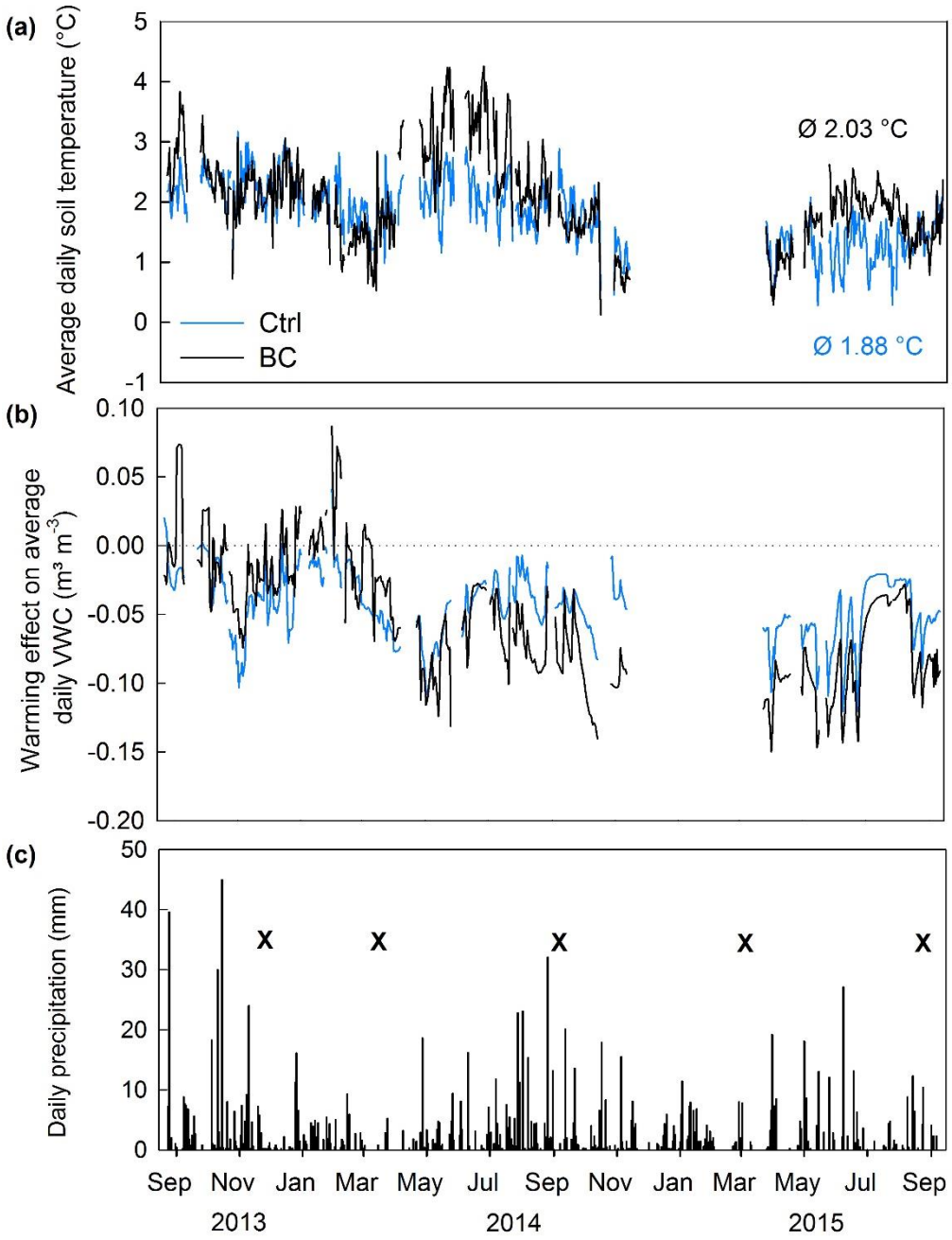


**Fig. S7.1.** Effect of soil warming on (a) average daily soil temperature and (b) average daily volumetric soil water content in control (Ctrl) and biochar (BC) plots as well as (c) daily precipitation amount for the weather station "Hohenheim" during the two-year experimental period. Data on soil temperature and VWC during the winter period 2014/2015 were excluded due to technical problems with soil heating. Precipitation data was provided by the agricultural technology center in Baden-Württemberg, Germany (LTZ Augustenberg). X = soil sampling. Data on soil temperature and VWC between August 2013 and September 2014 were taken from Bamminger et al. (2016).

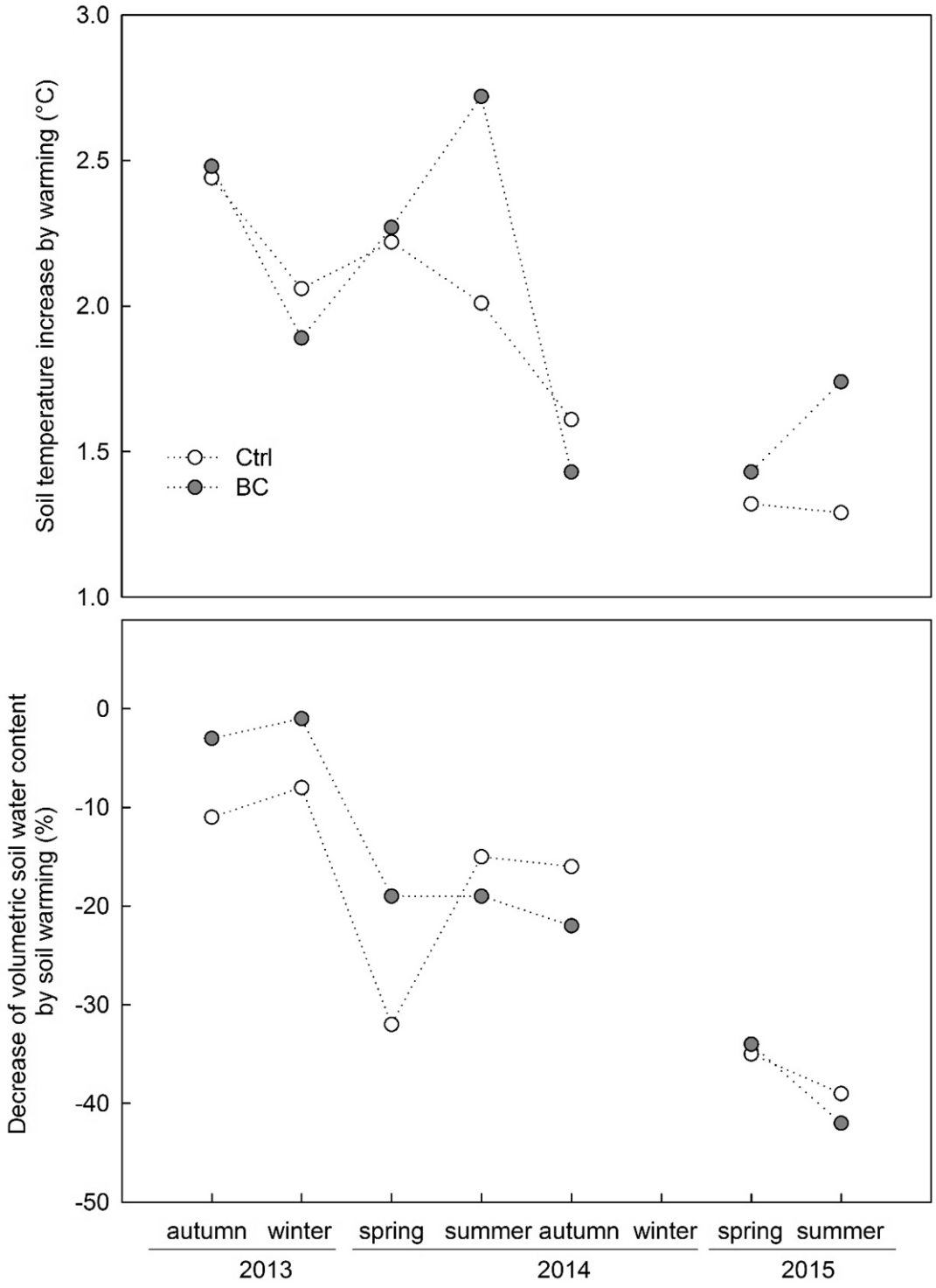


**Fig. S7.2.** (a) Average seasonal soil temperature increase by warming and (b) average seasonal decrease of volumetric soil water content by warming in control (Ctrl) and biochar (BC) plots. Please note that data for the winter period 2014/2015 were removed due to technical problems with soil heating.

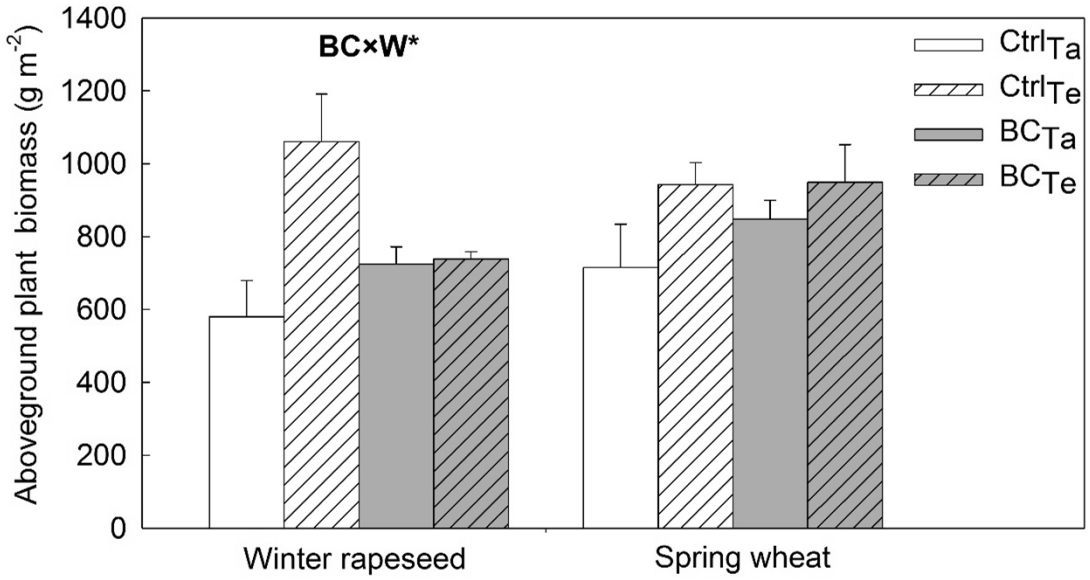


**Fig. S7.3.** Final aboveground biomass at crop maturity of winter rapeseed and spring wheat (mean ± SE). Results of linear mixed-effects model for the effects of soil warming (W), biochar addition (BC) and their interactions separately for each crop are shown when significant (* $P \leq 0.05$). $Ctrl_{Ta}$ = control soil under ambient soil temperature, $Ctrl_{Te}$ = control soil under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature. Data on aboveground biomass of winter rapeseed were taken from Bamminger et al. (2016).

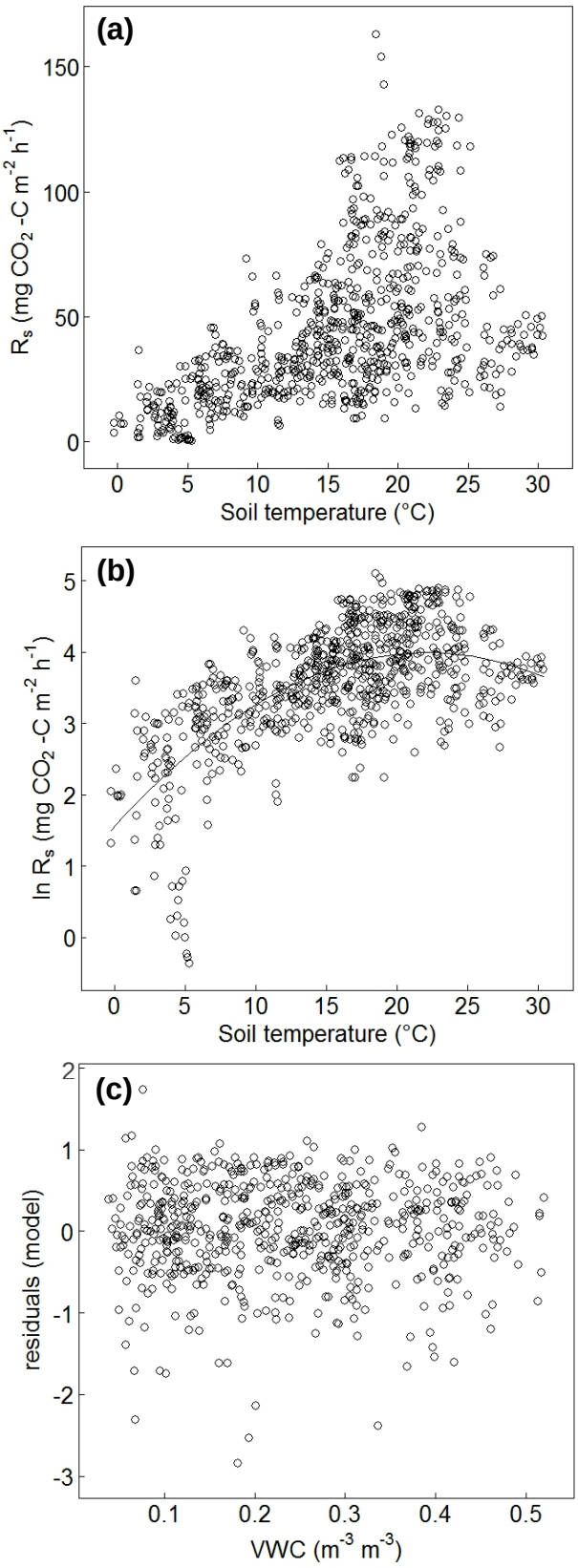


**Fig. S7.4.** (a) Soil respiration ($R_s$) as a function of soil temperature across all treatments, (b) ln $R_s$ as a function of soil temperature fitted with a log-quadratic model and (c) residuals of the regression model used in (b) against volumetric soil water content (VWC).

**Table S7.1.** Microbial biomass C (µg-1 dw soil) at 0-5 and 5-15 cm in 2013 (November), 2014 (March and September) and 2015 (March and August) (mean ± SE). Significant results of linear mixed-effects models for the effects of soil warming (W) and biochar addition (BC) and their interactions separately for each sampling date and soil depth are indicated by asterisks (* P≤0.05).

| month / year | 0-5 cm | | | | | 5-15 cm | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | $Ctrl_{Ta}$ | $Ctrl_{Te}$ | $BC_{Ta}$ | $BC_{Te}$ | **Effects** | $Ctrl_{Ta}$ | $Ctrl_{Te}$ | $BC_{Ta}$ | $BC_{Te}$ | **Effects** |
| Nov13 | 270.2±12.4 | 316.4±21.2 | 292.8±8.6 | 285.7±22.4 | **BC×W*** | 248.2±11.3 | 297.7±18.5 | 263.9±28.8 | 274.2±2.4 | |
| Mar14 | 247.0±18.9 | 218.1±21.0 | 229.2±16.6 | 214.2±13.0 | | 157.5±24.0 | 186.3±17.1 | 172.6±10.4 | 173.1±17.1 | |
| Sep14 | 239.1±30.1 | 262.1±17.3 | 223.6±11.1 | 253.0±9.6 | | 180.3±15.1 | 180.0±21.1 | 172.2±25.5 | 172.9±20.9 | |
| Mar15 | 201.6±16.7 | 181.7±19.5 | 168.3±14.7 | 189.9±9.6 | | 163.3±10.9 | 207.4±29.5 | 181.2±5.6 | 186.0±10.2 | |
| Aug15 | 183.5±12.0 | 225.8±29.0 | 219.2±14.7 | 199.3±12.1 | | 175.8±10.7 | 216.9±11.9 | 210.0±8.9 | 182.4±13.3 | **BC×W*** |

Arrows show the direction of single effects. $Ctrl_{Ta}$ = control soil under ambient soil temperature, $Ctrl_{Te}$ = control soil under elevated soil temperature, $BC_{Ta}$ = soil amended with biochar under ambient soil temperature, $BC_{Te}$ = soil amended with biochar under elevated soil temperature. Data from 2013 and 2014 were taken from Bamminger et al. (2016).

# 8 General discussion

The objectives of this thesis were to evaluate the biological stability of *Miscanthus* biochar from slow-pyrolysis and its potential for long-term C sequestration and GHG mitigation in a temperate agricultural ecosystem under predicted soil warming, which is closely related to its influence on soil properties, plant growth and soil microorganisms.

## 8.1 Soil microbial community influenced by biochar application and warming

In a microcosm study (Chapter 5), biochar from *Miscanthus* (600 °C, 30 Min) was added at a rate of 30 t $ha^{-1}$ to a temperate agricultural soil and incubated for short time (37d) at constant soil temperature (20 °C) in the laboratory, including earthworm and *Phacelia* litter treatments, but excluding living plants. Soil from the ensuing BC-HoCC field experiment (Chapter 6 and 7) was used to evaluate biological effects and the compatibility of such biochars for field application. In this pre-experiment, it was aimed to assess the interactive effects between slow-pyrolysis *Miscanthus* biochar, soil microorganisms and earthworms. Most striking results were that biochar led to short-term increased microbial abundances and shifts in the fungal-to-bacterial PLFA ratio as well as Gram-positive-to-Gram-negative bacterial ratio in treatments with litter. Without litter, biochar increased the Gram-positive-to-Gram-negative bacterial ratio, but did not affect single microbial groups (Table 5.2, 5.3; Fig. 5.3). Similarly, in the study of Prayogo et al. (2014) biochar affected fungal biomass only when applied to soil together with litter. This suggests that fungi as primary decomposers of plant litter may profited most from better living conditions created by biochar. The observed shift in the bacterial community toward Gram-positive bacteria in both litter treatments were attributed to the recalcitrance of the biochar, which generally favors Gram-positive over Gram-negative bacteria (Ameloot et al., 2013a; Farrell et al., 2013). Increased microbial biomass in soil amended with biochar could also be connected to improvements of physical and chemical soil properties due to biochar (Gul et al., 2015). In our microcosm study, changes in microbial abundances and community composition with biochar could not be entirely explained by improved soil properties. Biochar did not affect soil water content at 60 % WHC compared to the control soils as measured by water retention (pF) curves (Fig. S5.3; Table S5.2). In addition, biochar had no effect on EOC contents in both litter treatments, thus may not enhance C

availability to microorganisms. In the presence of N-rich litter, where highest effects of biochar on microbial abundances were detected, biochar did not significantly influence pH, but reduced the extractability of $NH_4^+$-N and $NO_3^-$-N (Table 5.2, 5.3; Fig. 5.2). Therefore, it was proposed that enhanced N immobilization by soil microorganisms could be the most likely responsible mechanism for the extended microbial growth in the 'with litter' treatments. Moreover, the often suggested co-location of substrates and microorganisms on biochar surfaces, which leads to more efficient microbial C use (Lehmann et al., 2011; Jin, 2010), may also explain our results and further changes in the functionality of soil microorganisms (see Chapter 8.2). Without litter, microbial groups were positively influenced by biochar × earthworm interactions. Although, biochar particles were found in earthworm casts, the earthworms did not incorporate biochar-C in relevant amounts and thus had no direct benefit from the presence of biochar in soil (Fig. S5.1). This is in concert with several other studies (e.g. Topoliantz & Ponge, 2003). However, it was assumed that biochar associated to OM in earthworm-worked soil may provide a more advantageous microbial growth habitat as supported by the study of Augustenborg et al. (2012). Such interactions between biochar, mesofauna and microorganisms were scarcely considered by others when evaluating the biological effects of biochar in soil (Ameloot et al., 2013b).

Information obtained from this microcosm experiment was compared with short-term microbial responses at the same biochar addition rate (30 t $ha^{-1}$) under field conditions. In August 2013, a field experiment with another slow-pyrolysis *Miscanthus* biochar (850 °C, 30 Min) was established on an already existing climate change experiment (Chapter 6), where soil temperature is enhanced by 2.5 °C since 2008 (Poll et al., 2013). The effect of biochar and soil warming on microbial abundances and community composition was monitored throughout the first year after biochar application under winter rapeseed crop. It was hypothesized that the used biochar would be stable against microbial degradation and incorporation into microbial biomass even under soil warming.

Incorporation of biochar-derived C into microbial biomass could not be determined by stable isotopes ($^{13}C$) due to the high variability of the data (Fig. 6.4), which was particularly explained by the heterogeneous spatial distribution of biochar particles in field soil. However, fungal PLFA abundances and the fungal-to-bacterial ratio showed that fungal growth was promoted in biochar-amended soil under warming three months after biochar application and that fungi were advantaged over bacteria under these conditions

(Fig. 6.3b; Table 6.3). Based on these results, it was proposed that fungi were responsible for initial degradation of stable biochar compounds, because some of them are known to metabolize stable biochar-C (Ascough et al., 2010; Ameloot et al., 2013b). Such fungal-driven biochar mineralization at elevated soil temperature may be only short-lived and related to the presence and degradation of spring barley litter, as enhanced fungal biomass was not observed after seven and twelve months. It is still debated whether the mineralization of recalcitrant SOM such as biochar is more sensitive to temperature elevation than labile C pools Fang et al. (2014). However, other potential reasons for enhanced fungal biomass in soils amended with biochar such as changes of pH or alterations of other physico-chemical soil properties (Lehmann et al., 2011) were not pronounced in the warmed BC treatment ($BC_{Te}$) and therefore expected to marginally explain the described results.

Interestingly, after immediate microbial utilization of labile C following biochar incorporation, fungal biomass was increased on the short term under laboratory (after 37 days) and field conditions (after 3 months) in the same soil applied with comparable slow-pyrolysis *Miscanthus* biochars and plant litter. Indeed, the positive effect of biochar on fungi in the field was restricted to the warmed plots. Nevertheless, these results may be explained by some general mechanisms, which initially favor fungi over bacteria in this temperate arable soil applied with biochar and litter:

(I) Differences between bacteria and fungi in their mobility and colonization of biochar cracks and pores (Lehmann et al., 2011; Ascough et al., 2010; Jaafar et al., 2014) and the fact that some fungal species can decompose stable biochar-C (Thies et al., 2015).

(II) Higher carbon-use-efficiency of fungi compared to bacteria, thus favoring fungi after the addition of biochar with high C:N ratio to soil (Ng et al., 2014).

(III) Fungal dominance in plant litter decomposition, thereby potentially utilizing recalcitrant biochar-C by co-metabolism (Hamer et al., 2004).

(IV) Faster response of bacteria compared to fungi to easily available substrate (Six et al., 2006) such as labile biochar-C, with a maximum bacterial growth that was not captured by sampling scheme in this thesis.

In the present thesis, only short-term increases of fungal abundances were observed, which may indicate an overall high degradation stability of the investigated *Miscanthus* biochars. If the growth of fungi (especially of mycorrhizae) would be enhanced on longer

terms and stimulate crop growth without increasing the mineralization of SOC and biochar-C, this would benefit agricultural soils to serve for long-term C sequestration (Warnock et al., 2007). The observed microbial community shifts toward fungi in this thesis stand in contrast to other studies which revealed relative higher bacterial abundances after biochar addition (Jones et al., 2012; Chen et al., 2013). Such bacterial-dominated microbial communities may indicate faster C cycling and reduced C sequestration potential of biochar (Six et al., 2006; Jones et al., 2012). These results point out that distinct biochars in soils with varying physical and chemical properties differ in their impact on microbial groups. This might have influence on the effectiveness of biochar to increase C stocks in soils.

Another benefit of biochar field application could be the mitigation of negative effects of warming on soil moisture by enhancing water retention in soil, especially in dry periods, thereby positively influencing microbial abundances and plant growth. In the short-term field study (Chapter 6), it was shown that biochar attenuated soil water loss in the dry spring period in 2014 (Figs. 6.1, S6.2b) and the reduction of microbial abundances under drought and warming in March 2014 (Fig. 6.3). For example, the decrease in fungal biomass was by up to 80 % less pronounced in biochar-amended soil at elevated temperature compared to the respective control soil. In addition, biochar field application slightly shifted the bacterial community toward Gram-positive bacteria (Table 6.3) which was likely induced by the higher moisture sensitivity of Gram-positive compared to Gram-negative bacteria (Schimel et al., 2007). In another study, the drought tolerance of soil microorganisms was similarly enhanced possibly due to better habitat conditions in biochar-amended tropical soil (Liang et al., 2014). In addition, some studies also showed positive effects of biochar on plant growth under drought conditions (Kammann et al., 2011; Paneque et al., 2016). In the present thesis, the positive effect of biochar on soil moisture regime also improved the early growth of winter rapeseed, but only at ambient temperature in spring 2014 (Fig. 6.2a). This could be connected to the greater influence of biochar on smaller plants in the ambient plots, whereas plants under warming were bigger and may exhibited a more widespread rooting system, which exceeded the soil depth (0-20 cm) to which biochar was applied. Hence, only limited additional indirect biochar effects on the soil microbial community by enhanced plant growth were assumed under warming. Like observed for microbial abundances, the observed beneficial effects of biochar on crop growth were not long lasting and disappeared during the first year after biochar application and were scarce in the second year under spring wheat (Table S7.2;

Fig. S7.3). This reflects that positive biochar effects on soil properties, microbial abundances and crop growth were limited in the investigated ecosystem as determined in other temperate, fertile arable soils (Crane-Droesch et al., 2013; Ameloot et al., 2014), but partly highlighted under extreme conditions (e.g. drought).

## 8.2 Potential of biochar to reduce soil GHG emissions under warming

Biochar application to soil is thought to mitigate soil GHG emissions (e.g. Case et al., 2014) and in this thesis, it was aimed to investigate whether biochar could reduce GHG emissions from temperate agricultural soil under predicted global warming. In the above mentioned laboratory pre-experiment (Chapter 5), the emissions of $CO_2$ and $N_2O$ were measured during a 34-d period. Under these short-term lab conditions without plants, cumulative fluxes of $CO_2$ were significantly reduced by 43 % and 27 % in treatments without and with litter, respectively (Fig. 5.1a). Further, biochar led to negative priming effects and decreased the mineralization of native SOM by 56 % on average in treatments without litter during the second half of the incubation. Such high suppression of soil $CO_2$ emissions after biochar addition is comparable to other incubation studies (e.g. Ameloot et al., 2013a; Bamminger et al., 2014a). The increase of microbial abundances and decrease in soil respiration resulted in lower metabolic quotients ($qCO_2$) with biochar (Table 5.2), indicating enhanced respiratory efficiency of the soil microbial community. This phenomenon was often observed in different soils amended with biochar (Steiner et al., 2008a; Jin, 2010; Domene et al., 2014) and is attributed to the close co-location of substrates and microorganisms on biochar surfaces (Lehmann et al., 2011). Unfortunately, there is no study providing clear evidence for this hypothesis. Moreover, the effect of biochar on the metabolic efficiency was only determined in incubations under nearly optimal conditions with soil samples derived from laboratory or field experiments, but not investigated under soil warming.

In the field experiment of this thesis, biochar enhanced initial $CO_2$ rates especially under warming (Fig. 7.1a). Labile biochar-C pools were likely already consumed before November in 2013 as indicated by declining $CO_2$ rates in the first weeks (Fig. 7.1a). Metabolic efficiency of the soil microbial community was investigated by relating *in-situ* soil respiration to microbial biomass at 0-15 cm depth. The increase of $qCO_2$ in the $BC_{Te}$ treatment after three months in November (Fig. 7.4) suggests a less efficient microbial C use and may support the outcome of the second study of this thesis, the potential

mineralization of recalcitrant biochar-C under warming mainly by fungi (see Chapters 6 and 8.1). However, further remarkable changes of microbial abundances, respiration rates and $qCO_2$ induced by biochar could not be observed during later stages of the experiment (Table S7.1; Figs. 7.1a, 7.4). Additionally, biochar neither did significantly influence total $CO_2$ emissions or the temperature sensitivity of soil respiration (Figs. 7.1b, 7.5) after two years. Hence, it was argued that a temporal biochar mineralization under soil warming is negligible under the aspect of long-term C sequestration. Concluding from this two-year study, this means that biochar could be persistent and contribute to C sequestration in soil even under predicted elevated soil temperature, thus confirming results from recent incubation studies (Fang et al., 2014, 2015).

Even though the used biochar was considered to be relatively stable against microbial mineralization on the short term (Chapter 5 and 6) to medium term (Chapter 7), it has been shown that the large reduction of $CO_2$ emissions and the increased microbial efficiency observed in the incubation experiment could not be observed in field soil as well. No, or only slight effects of biochar on $CO_2$ emissions were also shown for other field experiments in agroecosystems (e.g. Castaldi et al., 2011). Potential reasons for the differences between laboratory and field experiments may be constant optimal incubation conditions vs. fluctuating weather and soil conditions (temperature, moisture), homogenized soil vs. soil heterogeneity in field soil, or plant-free microcosms vs. soil-plant interactions in the field. In non-vegetated periods in the field experiment, biochar reduced $CO_2$ emissions by 11 % in ambient plots, but had no effect under soil warming. Under vegetation, biochar overall increased $CO_2$ emissions by 6 % (Fig. 7.2a). If enhanced $CO_2$ emissions with biochar in cultivated soil originated from higher plant root activity and exudation, this would mean that native SOC remained mainly unaffected. Despite the fact that biochar just slightly stimulated crop growth and soil respiration in the vegetated plots of our field experiment, this highlights that interactions between soil, biochar and plants could have impact on microbial activity, C-cycling and C sequestration in agricultural soils (Biederman & Harpole, 2013; Whitman et al., 2014). Incubation studies without plants are therefore limited in predicting potential effects of biochar on C and N cycling in cultivated field soils (Weng et al., 2015).

$N_2O$ emissions were only significantly reduced by biochar in the treatments with litter (by 42 %) in the incubation experiment, when decreased mineral N and enhanced microbial abundances were observed (Chapters 5 and 8.1; Figs. 5.1b, 5.2 and 5.3). Several

laboratory studies confirm the high suppression of $N_2O$ emission from biochar-amended soil (Ameloot et al., 2013a; Harter et al., 2013). Most likely explanations for the reduced $N_2O$ emissions in the microcosm study (Chapter 5) could be the adsorption of nitrate and ammonium on biochar sites as well as enhanced microbial N immobilization which could have decreased the availability of N for denitrification and nitrification. Additional factors such as C availability, soil pH or moisture (Clough et al., 2013) were rather marginally affected (Tables 5.2, 5.3; Fig. S5.3) and not considered to be major responsible for the observed results. Alternatively, biochar has been shown to foster the growth and activity of $N_2O$-reducing bacteria containing the nosZ gene, promoting complete denitrification and the production of $N_2$ (Harter et al., 2013, 2016). It cannot be excluded that $N_2O$ was also further reduced and emitted from soil via $N_2$ during the incubation of the first study of this thesis. However, evidence for such mechanistic explanation for reductions in $N_2O$ emissions is still lacking from field experiments (Ameloot et al., 2016).

In the BC-HoCC experiment, we found that biochar decreased cumulative $N_2O$ emissions by 26 % at ambient soil temperature in the first month in non-vegetated plots when no N-fertilizer was added. This short-term reduction of $N_2O$ is smaller than observed in the laboratory study (Chapter 5). This can likely be explained by the constant and favorable soil water conditions for $N_2O$ production as well as the addition of N-rich litter in the microcosm experiment, rather enabling the reduction of $N_2O$ emissions by biochar. After two years, the mitigating effect of field-applied biochar on total $N_2O$ emissions at ambient temperature was rather low (8 %). No relevant effects were shown in other field studies using high-temperature biochars (e.g. Verhoeven & Six, 2014) as in our field experiment. The lack of prolonged reduction of field $N_2O$ emissions due to biochar may be related to biochar ageing and changes of its impact on soil pH, soil aggregation, water holding capacity and nutrient retention (Spokas, 2013; Heitkötter & Marschner, 2015). In fact, in the present field experiment the biochar-induced slight increase of soil pH disappeared during two years (Table 7.1), but increased soil moisture and nutrient retention (derived from extractable mineral N) as well as reduced bulk density in soil applied with biochar were still detectable. Other factors may also weaken the mitigating effect by biochar such as soil-biochar-plant interactions and soil disturbances through changes of weather and soil conditions (heavy rainfall, soil temperature and moisture, drying-rewetting cycles) as well as land management (ploughing and fertilization). However, it is questionable and only scarce evidence exists that biochar has the potential to effectively mitigate $N_2O$ emissions from agricultural soils on longer terms (Hagemann et al., 2016).

So far, it is also unknown how this will be at elevated soil temperature in the future. Biochar raised cumulative $N_2O$ fluxes under warming in the first months and total $N_2O$ emissions after two years by 33 % (Figs. 7.1d, 7.2b). These results imply that biochar addition to fertilized agricultural soils could be a serious problem with respect to $N_2O$ emissions under predicted global warming scenarios. The interactive effect of biochar and warming on $N_2O$ was mainly driven by precipitation events, soil moisture fluctuations and fertilizer additions. Biochar × warming interactions seem to stimulate soil microorganisms involved in N-cycling by the aforementioned alterations of several soil properties with biochar (e.g. Atkinson et al., 2010) and accelerated N cycling in warmed soil (Butterbach-Bahl et al., 2013). The combination of these effects might also have impact on the composition of N-cycling microbial communities. Moreover, competition between microorganisms and plants for available N in biochar-applied soil could have also influenced $N_2O$ emissions (Saarnio et al., 2013), but this was not investigated in detail. More research is definitely needed to identify the mechanisms involved in the impact of biochar on N-cycling microbial communities, plants and $N_2O$ emissions under current and predicted climate conditions.

The effect of biochar on methane emissions from soil was only evaluated on the field scale. Agricultural soils are mostly small sinks or sources for $CH_4$ and could be also influenced by biochar amendment (Jeffery et al., 2016). Effects of biochar on methane fluxes are expected to be derived from increased water and nutrient retention, enhanced aeration, changes of soil pH or adsorption of C and N (Feng et al., 2012). Our agricultural soil served as a small sink for $CH_4$ during the two-year experimental period, but was only scarcely influenced by biochar addition, while warming tended to increase total $CH_4$ uptake by 38 % (Figs. 7.1e,f). The warming-induced increase of $CH_4$ uptake may be linked to enhanced activity of methane oxidizers in soil and soil drying respective higher aeration as well leading to increased diffusion of methane from the atmosphere into the soil (Smith et al., 2003). It was concluded that methane fluxes were mostly too low to be affected by biochar amendment in the present agricultural ecosystem. At least, biochar did not increase $CH_4$ emissions, which is another positive issue with respect to the C sequestration potential of biochar in the investigated arable soil.

The global warming potential of soil GHG emissions over a period 100 years ($GWP_{100}$) was enhanced by 28 % by soil warming, but was not affected by biochar (Fig. 7.3). Hence, even after seven years of warming, especially microbial C cycling was still enhanced under

elevated soil temperature suggesting a lack of thermal adaption and/or no limiting effect of substrate depletion (Schindlbacher et al., 2015). In contrast to Case et al. (2014), biochar could not reduce the global warming potential of soil GHG in the present field experiment. However, atmospheric $CO_2$ fixed in plant biomass can be converted to less biodegradable biochar via pyrolysis and subsequently stored in soil for long time (Lorenz & Lal, 2014). For the present experiment, it was estimated that the sequestration of highly stable biochar-C into arable soil could offset the amount of warming-induced GHG emissions of two decades.

# 9 Final conclusions and perspectives

Biochar has gained increasing attention under the aspect of climate change due to its potential long-term stability in soil and suggested positive effects on soil properties, plant growth, microbial abundances, metabolic efficiency and soil GHG mitigation. To date, there is a scarcity of knowledge about such biochar effects in soil under predicted global warming. This thesis helps to better understand how biochar acts in soil and provides novel knowledge about combined effects of biochar application and soil warming on abiotic and biotic soil properties, crop growth and GHG emissions on the field scale.

On the short term, it could be shown that biochar increased fungal abundances and induced soil microbial community shifts toward fungi in the presence of plant litter both in the laboratory and in warmed plots in the field experiment. As bacteria are rather known to quickly respond to labile C supply, this may be a hint for the initial degradation of recalcitrant biochar-C by fungal species. Under controlled conditions, however, biochar simultaneously reduced the cumulative emissions of $CO_2$ and $N_2O$, the mineralization of SOM as well as $qCO_2$ rather suggesting the potential for GHG mitigation and enhanced microbial efficiency. In addition, the observed interactions between biochar and earthworms on microbial abundances indicate that biochar could increase overall soil fertility of temperate arable soils. In the soil warming field experiment, biochar was shown to mitigate seasonal effects of climate change on soil moisture, microbial abundances and plant growth in the first year and thus could sustain the fertility of agricultural soils in the future. Nevertheless, in the investigated agroecosystem, biochar had limited effects on final aboveground biomasses of winter rapeseed and spring wheat during two vegetation periods. After two years, total $CO_2$ emissions, the temperature sensitivity of soil respiration and $qCO_2$ remained mainly unaffected by biochar. These results indicate high stability of the biochar and support the concept of long-term C sequestration in temperate agricultural soils by biochar amendment even under future soil warming. However, this thesis showed, for the first time, that biochar amendment to warmed soil could be a serious problem with respect to $N_2O$ emissions from soil. $CH_4$ uptake was not significantly influenced by biochar or soil warming and less important in the investigated agroecosystem. Finally, the incorporation of stable *Miscanthus* biochar to soil was estimated to counterbalance the expected warming-induced higher GHG emissions of two decades. Such climate change mitigation could be the minimum benefit of biochar application to fertile temperate

agricultural soils, even when further proposed positive effects on soil properties, microorganisms and crop growth are non-existent or short-lived.

The present thesis provides a basis for further research on biochar as C sequestration tool in arable soils to face climate warming and related implications on plant growth, soil microorganisms and GHG emissions. However, impacts on different soil properties after biochar application need to be further studied on longer terms in field experiments to disentangle the roles of biochar, warming, soil microorganisms and plants and their interactions in affecting soil C sequestration and GHG emissions under global change. Biochar research was yet not systematic and too widespread, spanning over dozen of feedstocks, production techniques and application rates making it difficult to compare results. In addition, many studies are still lacking to adequately describe the physical and chemical properties of biochars that were used in experiments on biological effects and mitigation of GHG emissions in soil. It is essential to characterize biochar for field application to get a more comprehensive understanding of possible interactions with soil and its organisms. As biochar is once added to soil, it cannot be easily removed in case of adverse effects. This has to be considered when discussing about biochar as soil amendment and C sequestration tool.

There are some methodological issues associated to extraction-based analyses of microbial abundances such as phospholipid fatty acid analysis (PLFA), chloroform-fumigation-extraction (CFE) or DNA extraction from soils amended with biochar. Due to its large surface area, functional groups and high adsorption capacity, biochar may adsorb organic C which could lead to underestimation of microbial abundances (Lehmann et al., 2011), but also to overestimation by stabilization of dead microbial biomass on biochar. Therefore, some authors suggested to determine the extraction efficiency of microbial C, PLFAs or DNA to overcome these methodological problems (Liang et al., 2010; Durenkamp et al., 2010; Gomez et al., 2014; Hale & Crowley, 2015). For example, Liang et al. (2010) added $^{13}C$ enriched microbial culture to soil and assessed the recovery rate of microbial biomass by CFE. This approach would be hardly possible in our experiments where $^{13}C$-labeled biochars were applied to soil, thus making source partitioning difficult. In this thesis, it was assumed that the interference of biochar with the used extraction methods is rather negligible as texture of the investigated soil (silty-loam) suggests that clay minerals may act as sorption sites as well (Gomez et al., 2014). Surely, adsorption of microbial-derived C onto biochar surfaces cannot be completely excluded.

This thesis followed the approach to trace the fate of biochar-C into different C pools such as $CO_2$, earthworm and microbial biomasses by the use of naturally labeled *Miscanthus* biochar and the application of the stable isotope technique ($^{13}C$). Unfortunately, quantification of biochar-derived C in the different compartments ($CO_2$ and $C_{mic}$) was accompanied by high variability and uncertainty in the field experiment. This was mainly explained by the heterogeneity of spatial distribution of biochar in the soil profile. In general, biochar can be applied to soil in different forms such as large-sized particles, milled, co-composted or together with liquid manures or fertilizers and can be incorporated, top-dressed or added by banding (Blackwell, 2010). In the field experiment, we followed a more practical approach and added unsieved, heterogeneous biochar to our arable soil and incorporated it into 0-20 cm depth. A compromise has to be made in future studies to allow investigation into biochar mechanisms in soil while maintaining realistic environmental conditions and practicability for farmers.

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# Curriculum Vitae

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## Studies

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| 2013 – 2016 | PhD student, Institute of Soil Science and Land Evaluation, Soil Biology Section, University of Hohenheim, Stuttgart; partly funded by a two-year PhD scholarship of the Landesgraduiertenförderung Baden-Wurttemberg, awarded by the faculty of Agricultural Sciences at the University of Hohenheim |
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| 2009 – 2012 | Studies of Geography (M.Sc.) with specialization on "Urban and Landscape Ecology", Ruhr-University Bochum |
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## Professional experience

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| 04/2017 – 03/2018 | Consultant in the division of Preventive Soil Protection and Soil Management, Ahu AG, Aachen |
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| 11/2012 – 12/2012 | Scientific assistant, Institute of Soil Science and Land Evaluation, Soil Biology Section, University of Hohenheim, Stuttgart |
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| 2006 | General qualification for university entrance (Abitur) |
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Düsseldorf, 16th March 2018 Chris Bamminger

## Publications, Presentations and Supervisions

Parts of the PhD thesis and other related projects were published and presented at national and international scientific conferences. In addition, I supervised and co-supervised several student projects in the frame of my PhD thesis:

### Peer-reviewed publications

**Bamminger, C**., Poll, C., Marhan, S. 2018. Offsetting global warming-induced elevated greenhouse gas emissions from an arable soil by biochar application. Global Change Biology 24, e318-e334. DOI: 10.1111/gcb.13871.

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Schimmelpfennig, S., Kammann, C.I., Mumme, J., Marhan, S., **Bamminger, C**., Moser, G., Mueller, C. 2017. Degradation of *Miscanthus* × *giganteus* biochar, hydrochar and feedstock under the influence of simulated weather events and priming. Applied Soil Ecology 113, 135-150.

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**Bamminger, C**., Poll, C., Marhan, S. Can biochar reduce $CO_2$, $N_2O$ and $CH_4$ fluxes in a temperate agricultural ecosystem under soil warming? Submitted to Global Change Biology.

Carey, J.C., Tanga, J., Templer, P.H., Kroeger, K.D., Crowther, T. W., Burton, A., Dukes, J.S., Emmett, B., Frey, S., Heskel, M., Jiang, L., Machmuller, M., Mohan, J.E., Panetta, A.M., Reich, P.B., Reinsch, S., Wang, X., Allison, S.D., **Bamminger, C**., Bridgham, S.D., Collins, S.L., de Dato, G., Eddy, W.C., Enquist, B.J., Estiarte, M., Harte, J., Henderson, A., Johnson, B.R., Larsen, K.S., Luo, Y., Marhan, S., Melillo, J., Peñuelas, J., Pfeifer-Meister, L., Poll, C., Rastetter, E.B., Reinmann, A., Reynolds, L. L., Schmidt, I.K., Shaver, G.R., Strong, A.L., Suseela, V., Tietema, A. 2016. Temperature response of soil respiration largely unaltered with experimental warming. Proceedings of the National Academy of Sciences 113, 13797-13802. . DOI: 10.1073/pnas.1605365113.

## Presentations

### 2016

Kaiser, M., Grunwald, D., Marhan, S., Poll, C., **Bamminger, C.**, Ludwig, B. Effect of biochar application and soil temperature on characteristics of organic matter associated with aggregate-size and density fractions. Presentation, EGU 2016, Wien, 17.-22.04.2016.

### 2015

**Bamminger, C.**, Grün, F., Wüst, D., Marhan, S. Divergent effects of pyrochar and hydrochar on greenhouse gas emissions and microbial abundances in an arable soil. Poster presentation, Ecology of Soil Microorganisms 2015: "Microbes as important drivers of soil processes", Prag, 29.11-03.12.2015.

Marhan, S., Grün, F., Wüst, D., **Bamminger, C.** Effects of pyrochar and hydrochar on greenhouse gas emissions and microbial abundances in an arable soil. Presentation, SOM 2015, 5th International Symposium on Soil Organic Matter, Göttingen, 20.-24.09.2015.

Poll, C., **Bamminger, C.**, Marhan, S., Kandeler, E. Impact of climate change on carbon cycling and soil microorganisms in an arable ecosystem. Presentation, SOM 2015, 5th International Symposium on Soil Organic Matter, Göttingen, 20.-24.09.2015.

**Bamminger, C.**, Poll, C., Marhan, S. Unterschiedliche Effekte von Biochar und erhöhter Bodentemperatur auf die $CO_2$-, $N_2O$- und $CH_4$-Emissionen eines Ackerbodens. Presentation, Annual meeting of the German Soil Science Society, München, 05.-10.09.2015.

Grunwald, D., Kaiser, M., **Bamminger, C.**, Poll, C., Marhan, S., Ludwig, B. Einfluss von Pflanzenkohle und Bodentemperatur auf die Aggregatdynamik, C-Fraktionen und Basalatmung eines Lössbodens. Poster presentation, Annual meeting of the German Soil Science Society, München, 05.-10.09.2015.

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**Bamminger, C.**, Poll, C., Högy, P., Kandeler, E., Marhan, S. The role of biochar and elevated soil temperature in affecting microbial abundance and growth of *Brassica napus* in an agroecosystem. Presentation, "Soil biota: diversity and habitats" conference of the Commission III of the German Soil Science Society, Bremen, 17.-18.03.2015.

**2014**

**Bamminger, C.**, Poll, C., Marhan, S. Effects of biochar and elevated soil temperature on soil microbial activity and abundance in an arable field. Poster presentation, EGU 2014 in Wien, 27.04.-02.05.2014.

**2013**

**Bamminger, C.** Influence of biochar and elevated soil temperature on soil microbial activity and abundance in an arable field. Presentation, Colloquium of the Soil science/Soil ecology group, Geographical institute, Ruhr-Universität Bochum, November 2013.

**Bamminger, C.**, Zaiser, N., Zinßer, P., Lamers, M., Kammann, C., Marhan, S. Potential von Pflanzenkohle zur Verminderung von $CO_2$- und $N_2O$ Emissionen aus einem Ackerboden und Interaktion mit Regenwürmern. Presentation, Annual meeting of the German Soil Science Society, Rostock, 07.-12.09.2013.

**Supervised and co-supervised Bachelor and Master theses**

„Veränderung des Einflusses von Pflanzenkohle auf das Wachstum von Sommergerste durch die Anwesenheit von Regenwürmern“ (B.Sc. thesis, Anne-Ruth Hanemann)

“Influence and comparison of biochar, biological activated biochar and compost amendment to soils on plant growth of spring barley and soil properties” (B.Sc. thesis, Robert Fischle)

„Einfluss von Pyrolyse- und HTC-Kohle auf mikrobielle Abundanz und Treibhausgasemissionen eines sandigen Ackerbodens“ (M.Sc. thesis, Franziska Grün)

„Biochar und erhöhte Bodentemperatur - Wechselwirkungen und Einfluss auf Winterraps und Bodeneigenschaften“ (M.Sc. thesis, Christina Sixt)

**Other student projects**

**Project in soil science:**

“Interaction of biochar with phacelia litter and its effect on germination and early plant growth of barley” (Imke Harms)

“The effect of increased soil temperature and biochar amendment on soil microbial community composition and potential enzyme activity” (Vera Baumert)

**“Humboldt reloaded: Wissenschaftspraxis von Anfang an” (funded by BMBF):**

„Einfluss verschiedener Pflanzenkohlen aus *Miscanthus* auf Mikroorganismen in einem sandigen Ackerboden“ (Samuel Schlichenmaier)

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## Eidestattliche Erklärung

Ich erkläre hiermit an Eides statt, dass ich die vorliegende Dissertation selbstständig angefertigt, nur die angegebenen Quellen und Hilfsmittel benutzt und inhaltlich oder wörtlich übernommene Stellen als solche gekennzeichnet habe. Ich habe noch keinen weiteren Promotionsversuch unternommen.

Düsseldorf, 16th March 2018 Chris Bamminger