Soil biota interactions with hydrochar

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FOREWORD

The thesis was comprised of published papers, which is a cumulative result of the work. The following published papers were offered to in the chapters, and a reference of the introduction was put in the five chapter of this thesis.

I. Rillig, M.C., Wagner, M., **Salem, M**., Antunes, P.M., George, C., Ramke, H.G., Titirici, M.M., Antonietti, M., 2010. Material derived from hydrothermal carbonization: Effects on plant growth and arbuscular mycorrhiza. Applied Soil Ecology 45, 238–242.

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

III. **Salem, M**., Kohler, J., Wurst, S., Rillig, M.C., 2013. Impacts of hydrochar and earthworms on growth of *Plantago lanceolata* and performance of arbuscular mycorrhizal fungi (AMF). (In preparation for submission)

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DEDICATION

I dedicate this work humble to the most precious family I have in this existence, to my father and my beloved mother, who died recently, who wished to complete my education quickly and come back stay beside her.

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CHAPTER 1

General introduction

Carbonized material

Climate change due to increasing carbon dioxide is one of the biggest challenges for mankind in the 21st century (Laird, 2008; Sohi et al., 2010). Also soil degradation is also another important issue in many countries of the world, for both fundamental problems, carbonized material can be a possible solution. On the one hand, carbonized material is thought to mitigate climate change via sequestering carbon in the soil on the other hand, carbonized material can be used as a soil amendment for improving soil structure and nutrient availability. These two topics make research on carbonized materials with a major focus of ecological research (Chang et al., 2007, 2008; Lehmann and Joseph, 2009).

Some of the first and most systematic investigations about the effect of carbonized material in soil were done in the Amazonian dark earths or Terra preta de índio soils, which were formed thousands of years ago and which are characterized by a high soil fertility compared to adjacent soils (Lehmann et al., 2003).They have been associated with high levels of carbon material that has been found in large quantities in the Amazon basin (Glaser et al., 2001). Pre-Columbian indigenous people brought the carbonized material into the soil, practically a slash and char agriculture, as they burned organic matter incompletely. Additionally, they added nutrients, as the soil itself is poor in nutrients (Mann, 2002). Research on the terra preta soils showed that these soils contained high levels of carbonized material that have been stable for centuries (Glaser et al., 2003). These soils acted as an initial impulse for the use of carbonized material as biochar, which can serve as a soil additive aimed at improving soil physicochemical and ecological parameters (Glaser et al., 2002; Gundale and DeLuca, 2006; DeLuca et al., 2006).

The first experiments dealing with the carbonized material were already carried out in 1913 by Bergius, who described the transformation of cellulose to charcoal during the hydrothermal process (Sevilla and Fuertes, 2009). Berl and Schmidt in 1932 used different types of feedstock and treated them at relatively low temperatures in the presence of water (Titirici and Antonietti,

2010). But it was at the beginning of the 21st century, when research on carbonized material increased rapidly due to a possible solution to mitigate climate change (Lehmann and Joseph, 2009).

Carbonized material is usually produced via pyrolysis of treated organic feedstock at high temperatures (<700°C) under limited or without oxygen conditions in a closed system (Lehmann and Joseph, 2009). The product called biochar is carbon rich and it is used as a soil amendment (Lehmann and Joseph, 2009).

In addition, there are many studies dealing with the effect of biochar on soil biota (Lehmann et al., 2011) such as promotion of mycorrhizal fungi (Yamato et al., 2006; Rondon et al., 2007; Warnock et al., 2007; Rillig et al., 2010), or stimulation of resistance against disease agents (Prithiviraj et al., 2007; Elad et al., 2010).

Beside pyrolysis, there is another way to produce carbonized organic matter, the hydrothermal carbonization (HTC) process also known as wet pyrolysis (Titirici et al., 2007). The reaction conditions differ largely from those of dry pyrolysis. The product is called hydrochar, in analogy to biochar (Titirici et al., 2007; Hu et al., 2010; Sevilla and Fuertes, 2009). There is much less research done about hydrochar and its effect on soil than about biochar.

Hydrothermal carbonization is an aqueous pyrolysis process that converts biological biomass material (organic wastes) into a carbonaceous material similar to brown-coal. Only little gas (approx. 1–5%) is generated in comparison to other processes, and most organics remain as or are transformed into solids and liquids (Libra et al., 2011; Kammann et al 2011), known as hydrochar. Titirici (2007) found that the wet thermal carbonization process was more efficient in the production of carbonized material than by dry pyrolysis. In comparison with biochar produced by dry pyrolysis, the char produced via the wet pyrolysis is more hygroscopic.

Hydrochar is hydrophilic due to the presence of polar functional groups on the surface of the hydrochar particles. The nature of feedstock, temperature and reaction time are important factors, which can affect the elemental composition. In both (dry and wet) pyrolysis processes, char products from wet pyrolysis differs from dry pyrolysis (Libra et al., 2011). The H/C and O/C atomic ratios in the chars in both types of pyrolysis decrease with increasing temperature,

because C, H and O are lost to the liquid or gaseous phase (Amonette and Joseph, 2009 and Libra et al., 2011). Therefore, C content of the biochar product shows a steady increase with increasing temperature (Schnitzer et al., 2007; Zabaniotou et al., 2008; Lehmann and Joseph, 2009).

Also, the mineral content of the hydrochar is more concentrated than biochar owing to the higher loss of C, H and O during pyrolysis (Sevilla and Fuertes, 2009). So, hydrochar commonly, has higher H/C and O/C atomic ratios similar to bituminous coal (van Krevelen, 1993; Libra et al., 2011). In wet pyrolysis, the percentage of carbon fixed in the hydrochar is about 70–80% (Sevilla and Fuertes, 2009), while only approximately 50% of the biomass carbon is converted to biochar during dry pyrolysis (Gaunt and Lehmann, 2008).

Generally, there is a lack of available data on the conversion processes from organic material to hydrochar in comparison with biochar. As feedstock and process conditions are very variable, the product also has a wide range of variations in its characteristics.

Production of hydrochar

Hydroterminal carbonization is based on the carbonization of wet feedstock which can be waste from industrial or agricultural origin, including some nontraditional renewable biomass streams, which are produced continuously and in high quantity. This can be sewage sludges, wet animal manures and faecal sludges, aquaculture residues or algae (Libra et al., 2011); i.e. One of the most important characteristics is that the starting material can be wet. During HTC, the feedstock organic material is heated together with water at relatively mild temperatures (usually 160 to 250°C) under self generated pressure in the absence of catalysts for several hours (usually between 1 and 12 hours, but up to 72 hours) and in the presence of water (Funke and Ziegler, 2010; Rillig et al., 2010; Libra et al., 2011; George et al., 2012). The process occurs in the absence or at a low concentration of oxygen.

Physical and chemical characteristics of hydrochar

The physical and chemical properties of hydrochar change depending on the nature of the feedstocks, as they can be very heterogeneous depending on their origin (Titirici et al., 2007; Libra et al., 2011). Also, chemical properties are closely related to physicochemical characteristics of biomass feedstock and production conditions, namely temperature and time of

processing (Gajic et al., 2012). Mostly, increasing the temperature during pyrolysis reduces the O content and the O/C ratio of the hydrochar, while extension the processing time has only little effect (Gajic et al., 2012). Hydrochar is usually composed of an aromatic core containing carbonaceous polyfuran which is rich in oxygen (Titirici et al., 2012) and which is surrounded by polar function groups such as –OH, C=O and COOH (Titirici et al., 2008; Baccile et al., 2009; Rillig et al., 2010).

The aromatic structure, depleted of carbohydrates and nonpronated aromatic carbon, increases with increasing temperatures (Cao et al., 2011). Chemical composition, type and quantity of chemical bonds of hydrochar are typically similar to natural coal Libra et al. (2011). In addition, the H/C and O/C atomic ratios of the hydrochars seem to be similar to that found in natural coal (Libra et al., 2011). C content increased and the H/C and O/C ratios of hydrochar decreases compared to the original feedstocks (Gajic et al., 2012).

Effect of carbonized materials in soil

Carbonized material application or introduction the soil has proven to have different beneficial effects on the physical chemical parameters of soil: It increases soil nutrient status, although biochar does not provide many nutrients but significantly reduces leaching of nutrients. Increased nutrient retention is therefore one favorable property of Terra Preta (Glaser et al., 2002, Silber et al., 2010). It also improves pH (Glaser et al., 2003; Lehmann et al., 2003; Yamato et al., 2006; Steiner et al., 2007; Novak et al., 2009), soil water holding capacity (Glaser et al., 2002; Chan and Xu, 2009; Novak et al., 2009; Downie et al., 2009; Elad et al., 2011) and also the cation exchange capacity (CEC) of soils (Glaser et al., 2002; Yamato et al., 2006; Cheng et al., 2008; Novak et al., 2009; Brockhoff et al., 2010).

Application of hydrochar to soil

Potential of hydrochar to mitigate climate change

As mentioned at the very beginning, implementing hydrochar or biochar into soil can mitigate the rising concentration of CO_2 in the atmosphere (Lehmann, 2007b; Laird, 2008; Sohi et al., 2010), and enhance carbon sequestration through the establishment of a sustainable carbon sink

(Lehmann et al., 2006; Lehmann, 2007a; Atkinson et al., 2010; Sohi et al., 2010; Enders et al., 2012). Therefore, adding carbonized material including hydrochar to terrestrial ecosystems has been used for reducing emissions and greenhouse gases (Lehmann et al., 2006; Woolf et al., 2010; Gajic et al., 2012). Kammann et al. (2012) reported that N_2O emissions were reduced by adding hydrochars only initially but after N fertilization they significantly.

Influence of hydrochar on stability, pH and fertility of soils

Although there are only a few studies with hydrochar, it has been shown that it also can be used as a soil amendment (Fuertes et al., 2010; Kammann et al., 2012). Hydrochar application to soil can efficiently compensate the increasing crop residue removal from arable fields to restore SOC, so that soil fertility is sustained (Li et al., 2012). The improvement of SOC promotes key soil biota as Arbuscular Mycorrhizal fungi (AMF) (Rillig et al., 2010). These organisms improve themselves the aggregation of soil particles through extraradical hyphae and excretion of fungal products like glomalin. This glycoprotein may act as a binding agent to soil particles (Rillig et al., 2007). Also, hydrochar amendment indirectly influences soil aggregation (George et al., 2012).
Hydrochar significantly increases pH in soil (Rillig et al., 2010; Bargmann et al., 2012). It improves the cation exchange capacity (CEC), aeration and nutrient availability (Chandra, 2011; Libra et al., 2011; Glaser et al., 2002; Marris, 2006; Lehmann, 2007b). Soil fertility can be improved by hydrochar, because the aromatic structure of carbonized materials is protonated by functional groups (–OH, –COOH, -OOH) which contribute to an increase of the cation exchange capacity (CEC) of soils, one of the most important soil parameters related to soil fertility (Mao et al., 2012).

Hydrochar can have positive and negative effects on plant growth. Earlier studies by Rillig et al. (2010) have shown that hydrochar produced from beet root chips had positive growth effects at low dosage (1%), but deleterious effects at high dosages, over 10 % (v/v)) additions. Hydrochar derived from spent brewer's yeast also had negative effects on plant health (George et al., 2012).
Bargmann et al. (2012) observed that biomass yield of barley and beans increased by different hydrochar applications produced from sugar beet pulps and brewer's grains, but shoot biomass of leek plants decreased at the same time. Plant available nitrogen decreased with hydrochar application nearly to zero, but it was re-released after some weeks.

The decline was due to N immobilization (Gajić and Koch, 2012).Depending on the feedstock and process conditions, hydrochar usually has a low pH, meanwhile biochar has often an alkaline pH (Libra et al., 2011). This acidic nature of hydrochar facilitates the mobilization of nutrients and enhance their sorption by plant roots, although some nutrients like phosphorus also are influenced negatively in their sorption under low pH (Libra et al., 2011). This effect can be dissappear when it is integrated into the soil (Warnock et al., 2007; Rillig et al., 2010; Libra et al., 2011).

As hydrochar contains usually fewer aromatic rings and more labile carbon fractions than biochar (Steinbeiss et al., 2009; Libra et al., 2011; Cao et al., 2011), it probably decomposes faster than biochar from dry pyrolysis, but it is still much more resistant than uncarbonized material (Cao et al., 2010). In general, the decomposition and thus the stability and resistance of carbonized materials against biotic and abiotic oxidation is highly variable due to the variety of feedstock material and process conditions (Baldock and Smernik, 2002; Masiello, 2004; Kawamoto et al., 2005).

Potential effects of hydrochar on selected groups of soil biota

Hydrochar also influences soil organisms as a part of the soil environment direct and indirectly. Directly hydrochar can influence soil organisms as a food or nutrient source, indirectly through changes in soil properties as soil pH or nutrient availability.

Arbuscular mycorrhizal fungi (AMF)

Arbuscular mycorrhizae fungi (AMF) are obligate biotrophic fungi and are one of the most prevalent symbiotic fungi in terrestrial ecosystems. AM fungi colonize the host plant's roots receiving carbohydrates exclusively from the host plant (Smith and Read, 2008). In exchange plant hosts receive mineral nutrients (mainly phosphorus), and AM fungi improve nutrient availability and water uptake (Smith and Read, 2008). About 80% of all plant species are known to form this kind of symbiosis (Smith and Read, 2008).

Data available on the effects of carbonized material on AM fungi are still limited. Biochar has positive effects on mycorrhizal root colonization in soil (Warnock et al., 2007). Carbonized material can act as a provision of refuge from fungal graze and adsorb signals between AMF and

plant roots in the rhizosphere (Warnock et al., 2007). Having positive effects on AM fungi this has also indirectly positive effects on plant growth, as these kinds of organisms improves nutrient uptake of plants. Until our work, no data were available for hydrochar. We showed for the first time that freshly produced hydrochar stimulated spore germination and root colonization (Rillig et al., 2010).

Earthworms

Earthworms are important saprophagous animals and play an important role in the functioning of terrestrial ecosystems (Edwards and Bohlen, 1996). As key organisms in the soil, they are recognized as soil ecosystem engineers (Lawton, 1994; Weyers and Spokas, 2011). Their activity improves the spatial distribution of soil, and availability of nutrients (Edwards, 2004; Li et al., 2011), which can lead to increased plant uptake of inorganic nitrogen (Hawkins et al., 2000; Wurst et al., 2003) and improved plant performance (Haimi et al., 1992; Scheu, 2003). Earthworms may interact with carbonized material through feeding and burying of the material, which was clearly demonstrated in the gut contents and casts of earthworms (Weyers et al., 2008). Also, Topoliantz and Ponge (2003, 2005) observed the ingestion of biochar by earthworms in microcosm experiments. The ingested particles were transported and deposited in their casts, which lead to the incorporation into the soil profile. Until our study, there were no data available on hydrochar effects on earthworms.

Collembola

Collembola (or springtails) are among the most widespread and most abundant decomposers in soil. They often exceed individual numbers of 100.000 m^{-3} in terrestrial ecosystems (Petersen and Luxton, 1982; Bardgett et al., 1993; Chernov et al., 2010). They promote decomposition processes of organic matter in the soil (Hopkins, 1997) and soil aggregation (Siddiky et al., 2012). Also, they enhance nutrient mineralization and distribution indirectly through the stimulation of microbial biomass and activity in soil (Tiunov and Scheu, 2005; Chamberlain et al., 2006). Collembola feed on a variety of organic resources, including fungi, bacteria, decaying plant material and detritus (McMillan and Healey, 1971; Rusek, 1998; Sadaka- Laulan et al., 1998). Carbonized materials could alter the availability of food sources, by changing soil pH or

other soil characteristics (Lehmann et al., 2011).

Almost no data were available before our study on the effects of carbonized materials, either biochar nor hydrochar, on collembola. We tried to close this gap of knowledge with a series of experiments.

Effect on plant growth

There are several publications reporting the beneficial effects of biochar as a soil amendment on plant growth in terrestrial systems (Deenik et al., 2008; Chan and Xu, 2009; Graber et al., 2010; Laird et al., 2011). Addition biochar has been shown to enhance plant growth and yield under greenhouse and field conditions (Graber et al., 2010; Lehmann and Joseph, 2009). The stimulation of plant growth variates broadly depending on chemical or physical properties of the carbonized material (Elad et al., 2010) and depending on the different soil conditions such as different pH, WHC etc. (Lehmann et al., 2011). Effects of carbonized material on plant growth are based in the alteration of availability of water or nutrients, in the reduction of nutrient leaching (Lehmann et al., 2003; Laird et al., 2010) and in improved aeration in soils within the rhizosphere (Kolb, 2007).

Plant growth responded indirectly to the amendments carbonized materials through: improvements in soil pH (Yamato et al., 2006; Steiner et al., 2007; Novak et al., 2009); increase the cation exchange capacity (CEC) of soils (Cheng et al., 2006; Yamato et al., 2006; Novak et al., 2009); resulting in an improved nutrient retention (Chan et al., 2007, 2008; Chan and Xu, 2009), and alteration in soil physical properties including water retention (Chan et al., 2008; Laird et al., 2009; Novak et al., 2009); promotion of mycorrhizal fungi (Yamato et al., 2006; Rondon et al., 2007; Warnock et al., 2007), and improved resistance against pathogens (Elad et al., 2010). In this thesis, we investigated for the first time the effect of hydrochar on plant growth. In high dosages, it is possible that hydrochar has a detrimental effect on plant growth. This can be due toxic organic compounds as hydrochar can contain phenolic and aromatic compounds (Libra et al., 2011). Another possibility is that hydrochar immobilize nutrients especially nitrogen (Gajic and Koch, 2012). Recent studies confirm these findings (George et al., 2012; Kammann et al., 2012). But until now, the interactions of hydrochar with other soil organisms as earthworms or

AMF were not taken into account. This gap of knowledge was intended to be closed with this work.

Objectives

The main objective of this thesis was therefore to test how hydrochar affect soil properties like pH, plant growth, soil organisms and their interactions.

Specific objectives were to:

i) Assess how hydrochar influences plant growth and interacts with AM fungi (chapter 2);

ii) Test if different hydrochar types can be used by collembola as a food source, and, if yes, how it will affect their life cycle, (chapter 3);

iii) Determine interactive impacts of earthworms and hydrochar on plant and AMF performance, nutrient uptake (particularly N and P) and to identify the underlying mechanisms (chapter 4).

For these objectives, we carried out a series of experiments in the laboratory and in the greenhouse, which are covered in the following chapters.

CHAPTER 2

Material derived from hydrothermal carbonization: effects on plant growth and arbuscular mycorrhiza

Abstract

Greenhouse gas mitigation options include the production of carbonized materials and their addition to soils for longer-term storage. Hydrothermal carbonization (HTC) is a novel way to produce carbonized materials. The goal here was to test if HTC material, in our case derived from beet root chips, has adverse effects on plant growth or that of root associated symbionts such as arbuscular mycorrhizal fungi. We carried out several studies, and found that increasing concentrations of HTC material could be deleterious for plant growth of *Taraxacum sect. Ruderalia*, starting at 10 vol% additions. Conversely, root colonization of the fungal symbiont was stimulated at an addition of 20 vol%. Soil pH changes occurring during the study could be traced to microbial reduction reactions, and these led to a pH increase of the medium despite the quite acidic nature of the HTC material itself. In separate assays, we showed that spore germination of the AM fungus *Glomus intraradices* was stimulated by the HTC material, suggesting that direct effects on the fungi are likely in addition to those mediated by the host plant. A third experiment with a different plant species (*Trifolium repens*) confirmed the major conclusions, and showed also neutral to stimulatory effect on nodulation. Our results suggest that HTC materials should be carefully tested and optimized to reduce negative effects on plant growth before applications in the field are undertaken, particularly at high addition rates.

Keywords: biochar, hydrothermal carbonization, soil, arbuscular mycorrhizal fungi, spore germination

Introduction

Rising concentrations of atmospheric carbon dioxide are prompting the search for potential ways of storing carbon in soils of terrestrial ecosystems. One mitigation option that has been recently discussed is the production of biochar, for example from organic waste material, and its addition to soil (Lehmann, 2007ab). This practice has been inspired by work on the *terra preta de índio* soils, the Amazonian Dark Earths (Glaser, 2007). Biochar, in addition to its potentially long resident times in the soil environment (Czimczik and Masiello, 2007), has the additional advantage of increasing soil fertility (Glaser et al., 2002) and stimulating key plant symbionts (Warnock et al., 2007), and thus enhancing plant growth and contributing to sustainably managed soils (Marris, 2006; Glaser, 2007).

Recently, the method of hydrothermal carbonization (HTC) has been proposed as a biochar production method that could be part of a new pathway for carbon sequestration (Titirici et al., 2007). Hydrothermal carbonization is a method that uses biomass and biomass-derived precursors (carbohydrates) to produce carbonaceous materials under milder conditions than any other carbonization technique (water; $160 < T < 220^{\circ}C$). The process takes place *via* a dehydration-polymerization mechanism to produce carbon particles decorated with polar functional groups (Titirici et al., 2008). During this process, unlike in the production of biochar by pyrolysis, minimal amounts of carbon dioxide are liberated; that is, this process holds particular promise as an avenue for carbon storage, albeit the residence time of the HTC material in soil (decades; Steinbeiss et al., 2009) seems to be shorter than typical for pyrolysis-derived biochar (millennia; e.g., Kuzyakov et al., 2009).

Despite the potential significance of HTC material, there is limited knowledge regarding the effects of these compounds on plants or plant-symbiotic soil biota, such as mycorrhizal associations. The HTC material may be similar to pyrolysis-derived biochar in its effects. For biochar there is a rapidly accumulating body of literature in this regard (e.g., Thies and Rillig, 2009). However, there are also potentially important differences that necessitate testing HTC material for potential negative effects. HTC material is produced in a reactor under pressure, and thus it may contain higher amounts of volatile compounds that may interfere with biological

processes; pyrolysis-derived biochar is not produced under pressure. HTC is carried out in a liquid phase, leading to a wet product; this is also a significant difference to the product from pyrolysis-derived biochar, which is dry. Finally, there are differences in the chemical structure. The structure of the HTC materials is composed of an aromatic core containing polyfurane-type units, and this aromatic core is surrounded by oxygen rich polar functional groups such as –OH, C=O and COOH. In comparison with the pyrolysis-derived biochars, the HTC materials are more hydrophilic due to the polar functional groups, more hygroscopic, and have a lower degree of graphitization (Titirici et al., 2008; Baccile et al., 2009).

Here we tested for effects of HTC-derived material derived from beet root chips on plant growth and the arbuscular mycorrhizal (AM) symbiosis. AM fungi colonize the majority of terrestrial plants; these fungi have important effects in terrestrial ecosystems (Rillig, 2004). Our working hypothesis was that the HTC material would provide beneficial effects of plant growth and AM fungal proliferation, as had been observed for various biochar amendment types (e.g., Ishii and Kadoya, 1994; Warnock et al., 2007). We used *Taraxacum sect. Ruderalia,* as a mycorrhizal test plant, and additionally carried out a study with *Trifolium repens* to also examine effects on another important root symbiont group, nodulating bacteria. To specifically test for any direct effects on the mycorrhizal fungus itself we further carried out a spore germination study using the AM fungus *Glomus intraradices*.

Materials and Methods

Production of hydrothermal carbonization material

The hydrothermal carbonization was carried out in a custom-built reactor in Höxter using beet root chips as input material. Beet root chips are an agricultural co-product (sugar processing). Production parameters were the following: temperature: 180 to 200°C, time: 11 h (of which 7 h > 180°C). No catalysts were added to the reaction (their presence is not necessary since the reaction is self-catalyzed by intermediates resulting during the process; Titirici et al., 2008). The resulting wet product was stored in closed plastic buckets; prior to the experiment it was oven-dried in

batches (80°C) to facilitate mixing with soil. Elemental analysis of the material yielded the following values: C = 53.2 %, H = 5.3 %, N = 2.3 %; the energy density of the material was 21.7 MJ kg^{-1}. The beet root chip parent material had a pH of 4.39 (1:1 in water), and the unwashed HTC product, as used in the experiment, had a pH of 4.10.

Greenhouse experiment: Taraxacum sect. Ruderalia.

We carried out a greenhouse experiment in which we examined the effects of HTC-material additions across a gradient of addition rates. HTC material was mixed thoroughly with the soil at the following addition rates: 0, 2, 4, 10, 20, 30 and 80% (v/v). Soil and plant material (*Taraxacum sect. Ruderalia* Kirschner, H. Øllg. & Štěpánek; chosen as a locally abundant species in the meadow from which the soil was taken) were collected locally from a meadow in Berlin, Germany. The soil (Albic Luvisol) we used had the following properties: 73.6% sand, 18.8% silt and 7.6% clay; 6.9 mg/100g P (calcium-acetate-lactate); 5.0 mg/100g K (calcium-acetate-lactate); 0.12% N (total); 1.87% C (total) (analyses conducted by LUFA Rostock Agricultural Analysis and Research Institute, Germany; and on a Euro EA C/N analyzer, HEKAtech GmbH, Wegberg, Germany). The soil was chosen because it had relatively high mycorrhizal inoculum potential (Rillig, unpublished). Soil was sieved (10 mm) prior to use to remove stones and root material. There were 11 replicates of each treatment. Preliminary tests showed that HTC addition rate influenced germination of *Taraxacum sect.* Ruderalia; for example in soil with the highest HTC concentration (80%), no germination was observed (data not shown). To avoid confounding effects of germination, we therefore pre-germinated achenes on moist filter paper (20ºC, light) and then transferred the seedlings to pots (initially two per pot). The experimental units were pots (Conetainers, Stuewe & Sons, Oregon, USA; filled with 165 cm^3 soil) thinned to one *Taraxacum* sect. *Ruderalia,* plant after 5d. The greenhouse (rel. hum. 50% ± 10%; min. temp. 14.5°C, max. 26.0°C) received natural light supplemented with 16 hours of artificial light. The experiment was watered to field capacity with tap water approximately every other day, and destructively harvested after five weeks. Shoots were clipped off, dried (60°C) and weighed. Roots were extracted from soil by hand, washed, dried (60°C) and weighed. Root colonization (%) by arbuscular mycorrhizal fungi was determined microscopically (200X) as described but using

ink staining (Vierheilig et al.,1998).

Soil incubations for pH effects

We carried out additional tests to elucidate the potential mechanisms underlying the pH change observed in the previous study. The goal of the experiment was to test if effects were biotically (plant or microbial) mediated. We performed incubations (n = 3 per addition rate; same addition rates as in greenhouse experiment) in 150 ml plastic cups which were either filled with mixtures of HTC material and soil (total amount 25 g) that were autoclaved (121°C, 30min) or left untreated. These were incubated at room temperature (21°C) in the dark, moistened to field capacity. Incubations were short (3 h) or longer (4d). At the end of the incubations, we measured pH (1:1 in water).

Greenhouse experiment: Trifolium repens

It is not obvious what to use as proper controls for biochar material additions (Warnock et al., 2007); we chose to carry out an additional study that uses the input material as an additional control to the 'no addition' treatment. In order to include a different plant species we used *Trifolium repens* L., which afforded us the opportunity to test for effects on nodulation. The experiment was as described above (using the same untreated soil source, containers and greenhouse), but used *T. repens* (pre-germinated on moist filter paper; one plant per pot; n = 11). We dried (80°C) and ground both beet root chip parent material and HTC-material to pass a 500 µm sieve and added it at 10% (v/v) to soil. We chose 10% since in the previous experiment that was the lowest concentration at which effects were detectable. Pots were harvested after eight weeks of growth. At harvest, we proceeded as described above, but additionally counted the number of nodules immediately at harvest using a dissecting scope at 40X magnification. The nodules were of the indeterminate type, but at time of harvest the number (rather than weight) was deemed a satisfactory representation of nodulation, since nodules were all quite small (ovoid).

Spore germination of the AM fungus Glomus intraradices

To test for effects of beet root HTC material on AM fungal spore germination, we produced

aqueous extracts/ suspensions by mixing HTC material (passed through a 500µm sieve; see above) with deionized water, adjusting the pH to 6.0 and then autoclaving for 20 min at 121°C. We held pH constant since this parameter is known to influence spore germination (Green et al., 1976). The concentrations were 0.05, 0.25, 1.25 g HTC $100ml^{-1}$ and the parent material (also ground and sieved to 500 µm), 1.25g beet root chips $100ml^{-1}$. Twelve milliliters of each HTC suspension or a control (sterile deionized water) were then added under axenic conditions to each Petri dish filled with 48g of sterilized silica sand (20 min, 121°C) for a total of 50 experimental units (n = 10). We cut small discs (6 mm diameter) of nitrous cellulose using a hole paper punch and sterilized them with 70% ethanol.

Each Petri dish, the experimental unit, received 20 discs evenly distributed across the sandy surface, and 1-3 *Glomus intraradices* Schenck & Smith spores (Premier Tech, Biotechnologies, Rivière-du-Loup, Quebec, Canada, DAOM 197198) were dispensed onto each disc. The spores were prepared as described in Antunes et al. (2008). The Petri dishes were sealed with parafilm and incubated at 25° C for 4 days in completely random positions. Following the 4 days, the spores were stained with 0.05% (w/v) trypan blue and examined under a dissecting microscope (40X) for evidence of hyphal growth, carefully distinguishing germination hyphae from remaining hyphae attached to spores. Previous trials indicated that a spore is non-viable if germination does not occur within 48 h under the conditions provided (Antunes et al., 2008).

Statistics

We tested for differences among means using analysis of variance in JMP 7.0 (SAS Institute, Inc.), verifying normal distribution of residuals using a Levene's test. When ANOVAs were significant for a response variable ($P < 0.05$), we carried out Tukey HSD tests or comparisons with control (Dunnett's test).

Results

Greenhouse experiment (Taraxacum sect. Ruderalia) and soil incubations

HTC material addition had a significant effect on biomass of *Taraxacum sect. Ruderalia*, plants at final harvest (p_{total}, p_{shoot} and p_{root} < 0.0001; Fig II.1). For both shoot and root weight, values began to diverge significantly from the control starting with 10% additions (Dunnett's test: p_{shoot} = 0.038; p_{root} < 0.0001), reflecting a plant growth inhibition at concentrations greater or equal to 10%.

Responses of root colonizing AM fungal structures, either all structures together (hyphae, vesicles, arbuscules; Fig II. 2a), or just arbuscules (Fig II. 2b), showed a different, unimodal pattern. While both response variables also differed across the treatments (p_{AMF} and $p_{arbuscules}$ < 0.0001), the 10 and 20% additions led to a significant increase compared to the control, while the 80% addition treatment exhibited a trend (albeit non-significant) towards a lower root colonization than in the control.

Soil pH at the end of the experiment exhibited a pattern of increase along the HTC additions (p < 0.0001; Fig.II.3), even though the HTC material itself was acidic (pH 4.1). To learn about this response, additional incubations were carried out in the absence of plants (data not shown): shortly (3h) after adding the HTC material to soil, the pH of the mixture in non-sterile (or sterile) soil declined linearly with the amount of HTC added (r^2 = 0.89, p < 0.0001) . After 4d, soil pH increased to over 8.3 in the HTC mixtures with non-autoclaved soil, but when the soil was autoclaved, this effect disappeared and pH decreased linearly with the HTC addition rate after 4days (r^2 = 0.96, p < 0.0001).

Greenhouse experiment with Trifolium

Comparing HTC materials with its parent material revealed that the source material itself had negative effects on plants and other parameters, compared to the no-addition control (Table II.1). For example, HTC and its parent material did not differ in their negative effect on root growth. In fact, beet root chips strongly reduced nodulation, while the HTC-transformation had less negative effects, and even stimulated nodule number on a per root weight basis. Soil pH was not significantly changed in this study. Mycorrhizal colonization followed a pattern similar to that of the first experiment and that of nodulation in this study, but the differences among the treatments were not significantly different.

Spore germination experiment

Spore germination ranged from about 50 to 80% across all treatments (Fig II. 4). The treatments significantly differed in their effect on spore germination ($p < 0.0001$). All three HTC treatments led to significantly higher spore germination than with the parent material, but only the highest addition rate produced significantly higher spore germination than the no-addition control. The parent material tended to decrease germination.

Discussion

Compared to pyrolysis-derived biochar, there is a dearth of data on the effects of HTC-material on soils. Steinbeiss et al. (2009) examined the degradation of HTC materials, and found turnover time to be in the decadal range. These authors also documented significant effects on soil microbiology, which is also known for pyrolysis-derived biochar (Thies and Rillig, 2009). However, there have been no data available on effects on plant growth or plant symbionts. Here we present evidence that HTC material can be deleterious to plant growth, at least at higher addition rates, and that symbionts, such as AM fungi, are potentially less sensitive than plants and may even be stimulated. This reduction in plant growth is an important piece of information from an applied perspective, since it may reduce the usefulness of these materials in agroecosystems, or in restoration settings. HTC materials may differ in terms of parent material and production parameters (temperature, pressure, duration, etc.), and these aspects could be quite important for the application of these materials in the field.

Mycorrhizal root colonization was increased with HTC material additions at an addition rate of 20%, while plant growth already declined at 10% in the *Taraxacum sect. Ruderalia*, study. A similar stimulation, albeit not significant, was observed in the *Trifolium* study. Since the HTC material diluted the fungal inoculum in the soil, root colonization is actually a slight underestimate of the promotion of the fungi. Arbuscules, as sites of nutrient delivery of the fungus to the plant cell (Smith and Read, 2008), suggest that the symbiosis was active. This result is in accordance with several studies showing a stimulation of AMF in biochar experiments (Warnock et al., 2007). The mechanisms underlying this stimulation have not yet been thoroughly addressed, but may include both the chemical properties of biochar/ HTC (in case of the spore

germination) and changes in soil physico-chemical parameters, e.g. soil nutrient status or pH, refuge effects inside biochar particles, or signaling interactions in the rhizosphere (Warnock et al., 2007). Here, higher concentrations were deleterious to the fungus either directly (e.g., inoculum dilution effect for the highest addition level); or decreased root colonization was mediated by the plants which clearly suffered from the higher addition levels and may not have been able to provide C to the mycobionts. The spore germination assay suggested that there are direct positive effects of the HTC material, and that inhibitory compounds present in the beet root parent material were rendered ineffective through the HTC transformation. An array of chemicals and nutrient conditions can cause increases in AM fungal spore germination rate (Koide and Schreiner, 1992). Nodulation (only nodule number per plant weight) was also slightly stimulated by HTC additions. The reasons may be equivalent to those proposed for AMF because AMF and rhizobia share common signaling pathways (Antunes and Goss, 2005). In addition, it is possible that the effect in nodulation was indirect, resulting from the stimulation of AMF (Antunes et al., 2006).

Pyrolysis-derived biochar material often has a basic pH (Chan and Xu, 2009); the material produced here was acidic, which is probably due to the residues on the surface of HTC material (Baccile et al., 2009). It was therefore initially surprising that the pH at the end of our experiment was higher than that of the soil at the beginning or without additions (Fig II. 3). We carried out incubations in the absence of the plant, showing that this response was likely not plant-mediated. Sterilizing the HTC/ soil mixture eliminated this response (i.e. led to increasingly acidic soils); therefore, this occurrence in our experiment must have been the result of a microbial activity. Proton-consuming reactions are reductions (Van Breemen et al., 1983), and the addition of the HTC material must therefore have caused a microbial reduction reaction. This illustrates the importance of further examining soil microbial reactions to the addition of this material, which was beyond the scope of this study; our result also suggest that it is important to consider pH when carrying out experiment with or planning field application of HTC materials.

The HTC-derived material used in these experiments was not pre-washed to remove constituents sorbed to the more resistant core molecular structure. Since the material was, for practical reasons, dried prior to its use in the study, it would also have contained dissolved chemical

components that are part of the liquid production phase. However, in an actual field use of this material the material would not be pre-washed either, and therefore our experimental setup was realistic in terms of the chemical components of HTC materials included. Nevertheless, future studies should also attempt to pre-wash the materials in order to gain insights into the effects of the actual macromolecular core.

Our experiments were designed to detect short-term effects of this material on plant growth and symbionts; these deliver valuable information about the performance of seedlings to young plants, often a particularly sensitive life history stage in many plants. It will also be necessary to carry out studies with a more long-term perspective that will then include effects of the HTC material as it is aged in the soil (for biochar: Cheng et al., 2008). Nevertheless, our study clearly showed that symbionts and plant growth may respond in opposite ways to such soil additives, and if a goal of such additions is to stimulate plant growth we recommend a cautious approach in the use of HTC-derived materials.

Acknowledgements

We thank Freie Universität Berlin and Deutsche Bundesstiftung Umwelt for providing partial funding for this study.

Table II.1 Effects of HTC-beet root chips and beet root chip parent material on plant (*Trifolium*), soil and microbial parameters.

	No addition (control)	Beet root chips	HTC beet root	*p* value
Shoot weight (mg)	402.9 (30.2)a	371.7 (30.0)a	265.8 (17.4)b	
Number of leaves plant^{-1}	28.3 (1.83)	34.0 (3.84)	25.5 (1.54)	0.11
Root weight (mg)	217.7 (13.1)a	111.5 (13.8)b	119.8 (13.1)b	
Total plant weight (mg)	620.7 (43.9)a	483.3(42.3)b	385.7 (23.3)b	
Soil pH (1:1 in H_2O)	7.55 (0.03)	7.53 (0.03)	7.61 (0.03)	0.09
Nodules (number root system^{-1})	52.8 (3.6)a	9.6 (3.8)b	44.6 (3.6)a	
Nodule number per root weight (mg^{-1})	0.26 (0.03) b	0.08 (0.02)c	0.38 (0.03)a	
AM fungal root colonization (%)	76.3 (4.7)	69.3 (6.6)	80.4 (3.6)	0.31

Figure II.1 Effects of different HTC-material addition rates on *Taraxacum sect. Ruderalia* biomass (means, standard errors). Bars followed by the same letter are not significantly different ($p < 0.05$).

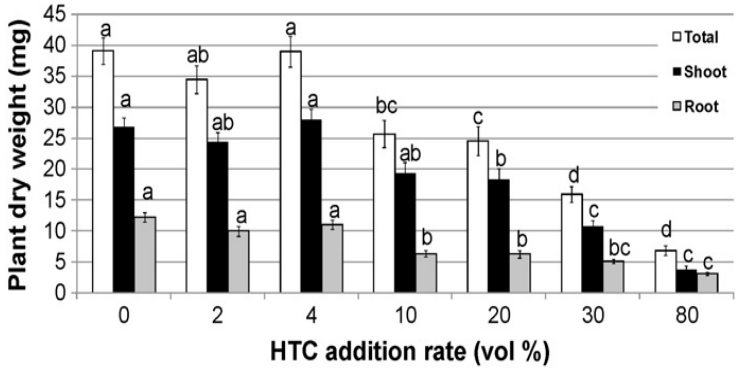


Figure II.2 Effects of different HTC-material addition rates on root colonization with arbuscular mycorrhizal fungi ((A) all structures; (B) arbuscules) in *Taraxacum sect. Ruderalia* (means, standard errors). Bars followed by the same letter are not significantly different ($p < 0.05$).

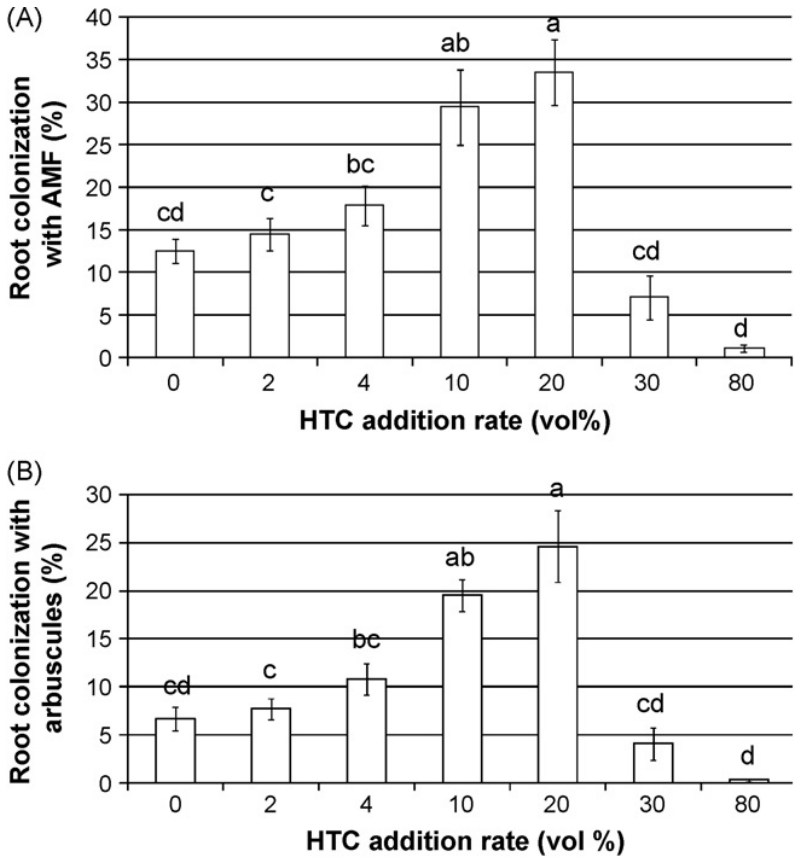


Figure II.3 Soil pH measured at the end of the experiment with *Taraxacum sect. Ruderalia*. Bars followed by the same letter are not significantly different ($p < 0.05$).

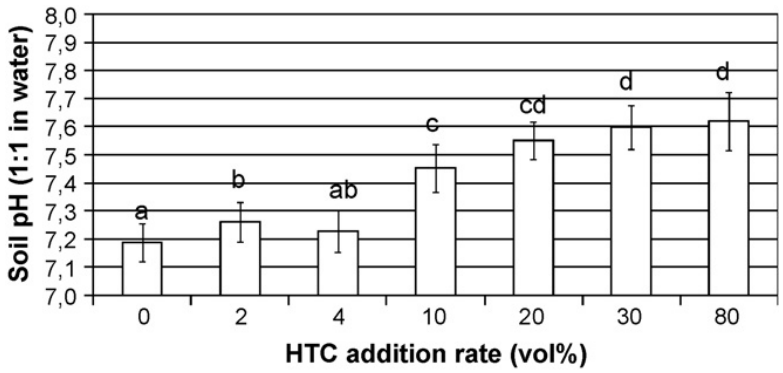
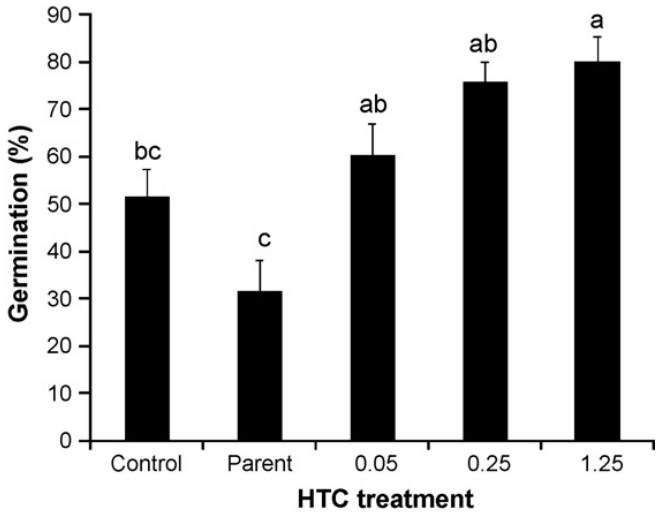


Figure II.4 Effect of HTC material and parent material (beet root chips) on arbuscular mycorrhizal fungal spore germination (*Glomus intraradices*) after 4 d. HTC addition rates were at concentrations of 0.05, 0.25, 1.25 g HTC 100mL^{-1}. Bars represent the mean (n = 10, except n = 9 for the parent treatment) ±1SE; bars followed by the same letter are not significantly different ($p < 0.05$).



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Chapter 3

Earthworms can modify effects of hydrochar on growth of *Plantago lanceolata* and performance of arbuscular mycorrhizal fungi

Abstract

Hydrothermal carbonization (HTC) is a method to produce carbonized material at relatively low temperatures (180 to 250 °C) under pressure and aqueous conditions. The product is called hydrochar and can be used as a soil amendment. However, applied in high dosages it may have detrimental effects on plants or soil biota. The potential impact of hydrochar amendment on beneficial soil organisms such as arbuscular mycorrhizal fungi (AMF) and earthworms and their interactions are not well understood. The goal of the present study was to determine effects of hydrochar on plant growth and soil biota and to evaluate interactions of earthworms and hydrochar on plant and AMF performance and to identify underlying mechanisms. In a greenhouse experiment, we investigated the effect of hydrochar at different addition rates (control, 1%, and 10% v/v) with or without the earthworm *Aporrectodea caliginosa* on the growth of *Plantago lanceolata* L. and the performance of its AMF. We observed a positive interaction between earthworms and 10% hydrochar on shoot and root biomass: added as a single treatment hydrochar had a negative effect on plant growth at this dosage, but plant biomass increased significantly when hydrochar was added together with earthworms. Root colonization by AMF increased significantly with increasing concentration of hydrochar, but was not affected by earthworms. Contrastingly, extraradical hyphal length of AMF was reduced by earthworms, but not affected by hydrochar. Thus, hydrochar and earthworms affected the performance of AMF, albeit of different AMF structures and in different directions. Our results indicate that earthworms may play an important role in alleviating the negative impacts of high dosages of hydrochar on plant growth; such interactions should move into focus of future research on potential effects of HTC materials.

Keywords: hydrochar, hydrothermal carbonization, earthworms, AMF, *Plantago lanceolata*, biochar, bioturbation

Introduction

When searching for solutions to mitigate the greenhouse effect, carbon storage in soil can be a potential way to curtail the increasing concentration of CO_2 in the atmosphere. One possible solution is soil amendment with carbonized material. Carbonized materials are produced by thermal decomposition of organic material under limited oxygen supply (Lehmann and Joseph, 2009) and are characterized by a high C content with aromatic compounds which are relatively stable in soil (Noguera et al., 2010). The product of this pyrolysis is called biochar, which is obtained by heating organic materials (up to 700° C) in a closed system (Lehmann and Joseph, 2009). Hydrothermal carbonization (HTC) is a recently rediscovered method to produce carbonized material at relatively low temperatures (180 to 250° C) in the presence of water and under pressure (Titirici et al., 2007; Libra et al., 2011). The product is called hydrochar (or HTC-biochar), and likely decomposes more rapidly in soil than biochar (Steinbeiss et al., 2009; Libra et al., 2011). Nevertheless, hydrochar is thought to have broadly similar characteristics as the widely used biochar improving soil biological activity, infiltration, water holding capacity, and cation exchange capacity, which leads to a higher soil fertility and nutrient use efficiency (Lehmann, 2007; Libra et al., 2011). Biochar including hydrochar is thought to act primarily as a soil conditioner and driver of nutrient transformation (DeLuca et al., 2009). However, in high dosages there are some reports of negative effects of hydrochar on plant growth (Gajic and Koch, 2012, George et al., 2012). But there is still little information on how the application of carbonized materials affects soil properties, plant productivity and soil biota (Lehmann et al., 2011). In particular, the potential impact of hydrochar amendment on beneficial soil organisms such as earthworms and arbuscular mycorrhizal fungi (AMF) and their effect on plant growth are not yet sufficiently studied (Rillig et al., 2010; George et al., 2012).

Earthworms are important saprophagous animals in terrestrial ecosystems (Edwards and Bohlen, 1996) and recognized as ecosystem engineers (Lawton, 1994; Lavelle et al., 1997; Weyers and Spokas, 2011). Their activity improves the spatial distribution and availability of nutrients (Edwards, 2004), which can enhance nitrogen uptake of plants and improve plant

performance (Haimi et al., 1992; Scheu, 2003). Several studies on the effect of biochar on earthworms indicate that the presence of charcoal affects earthworms and their activity, depending on the kind of charcoal, the earthworm species, and soil characteristics such as pH (reviewed by Weyers and Spokas, 2011). Some studies showed that earthworms can ingest charcoal (Eckmeier et al., 2007; Weyers and Spokas, 2011). By ingesting carbonized material, transferring and burying it into the soil, earthworms can improve soil structure and the function of carbonized material in the soil (Topoliantz and Ponge, 2003). As far as we are aware, there are no studies on combined effects of hydrochar and earthworms on plant performance.

Earthworm activity may also influence other soil biota. For example, earthworms may impact the extraradical hyphal network of arbuscular mycorrhizal fungi (AMF) as they disrupt the fungal network through their feeding and burrowing activity (Bonkowski et al., 2000; Lawrence et al., 2003; Wurst et al., 2011). However, the absence of significant effects of earthworms on AMF root colonization has also been documented (Wurst et al., 2004a; Eisenhauer et al., 2009; Wurst et al., 2011; Wurst and Rillig, 2011). AMF are one of the most important plant symbionts in terrestrial ecosystems. AMF colonize a large number of plant species, receiving carbohydrates from their host plant (Smith and Read, 2008) and providing their hosts in exchange with nutrients. Thereby they enhance nutrient availability, especially of phosphorus, and improve water uptake (Newsham et al., 1995; Smith and Read, 2008). There are few data available on effects of hydrochar on AMF, not yet permitting broad generalizations. Rillig et al. (2010) found positive effects of hydrochar on AMF root colonization. However, George et al. (2012) documented clear negative effects on AMF root colonization.

In view of the scarce information on the impact of hydrochar on beneficial soil organisms and their interactions, we studied the individual and combined impacts of hydrochar amendment and earthworms on plant growth, nutrient uptake, and the performance of AMF. Thus, a central hypothesis was that hydrochar effects would be changed by earthworms, with earthworms potentially alleviating negative effects of high doses of hydrochar.

Material and Methods

Earthworms

We used the endogeic earthworm *Aporrectodea caliginosa* Savigny (Lumbricidae) which was collected from an experimental field of Freie Universität Berlin, Germany. We chose this earthworm species because it is relatively abundant in agriculture systems and gardens (Edwards and Bohlen, 1996) and it has frequently been used as a study organism (e.g. Wurst et al., 2004b; Partsch et al., 2006). Only adult earthworms with a mean weight of 0.6 ± 0.02 g were selected for the experiment. The animals were collected in the field by hand sorting, and were kept in plastic containers with moist soil at 4°C for one week until addition to the experimental containers.

Hydrochar preparation

Hydrochar was produced by hydrothermal carbonization (Titirici et al., 2007). Beet root chips (BRC), a by product of sugar processing, with a pH of 4.39 were used as parent material. The carbonization was carried out in the department "Abfallwirtschaft und Deponietechnik" at the Hochschule Ostwestfalen/Lippe in Höxter, Germany, in a custom-built reactor heated to 180-200°C during 11h, of which at least 7h were higher than 180°C. The hydrothermal carbonization was carried out without added catalysts in the presence of water, yielding a wet product with high H/C and O/C ratios (Ramke et al., 2009). Elemental analysis of the material showed the following properties: C = 53.2%, H = 5.3%, N = 2.3%; C/N ratio 23.1. The energy density of the material was 21.7 MJ kg^{-1}. The unwashed HTC product, as used in the experiment, had a pH of 4.1 (1:5 dilution in water, v/v). Hydrochar was kept in closed plastic buckets at room temperature until use.

Soil

A fresh loamy sandy mineral soil (Albic Luvisol; N = 0.12%, C = 1.87%, C/N ratio 15.58) was collected from a meadow in Dahlem (Berlin, Germany). Soil was collected from 10-40 cm below the surface, air dried, and then passed through a 1 cm-sieve for homogenization and to exclude earthworms, stones and root material. We chose this soil because it contains relatively

high abundances of AMF (Rillig et al., 2010).

Experimental set-up and measurements

The experiment was conducted in a greenhouse at Freie Universität Berlin and was set up in a randomized fully factorial design with two categorical treatment factors and eight replicates. The first factor was the application of hydrochar with three levels (control (none), 1%, and 10% v/v), the second factor was the addition of earthworms with two levels (without and with earthworms). A total of 48 plastic pots (diameter 10 cm and 15 cm height) were set up in the greenhouse (16 h light, a night/day temperature of 18-20°C and a relative humidity of 60-70%). Seeds of *Plantago lanceolata* L., purchased from Appels Wilde Samen GmbH (Darmstadt, Germany), were sown on wet paper in plastic containers in a climate chamber at 20°C and 16h light. Seedlings were transplanted six days after ermination into the experimental pots filled with soil mixed with hydrochar in different concentrations. One seedling was planted into each pot closed at the bottom by a 1 mm mesh preventing the possible escape of earthworms but allowing water drainage. Hydrochar was thoroughly mixed with the soil at the rates corresponding to the treatment levels. The addition levels were chosen due to the results of a previous study (Rillig et al., 2010) where 10% addition of hydrochar had beneficial effects on plant growth and colonization of roots of *Taraxacum*. We filled 900 g of the respective soil: hydrochar mixture into each experimental pot.

Two weeks after planting, three adult specimens of the endogeic earthworm *Aporrectodea caliginosa* Savigny (Lumbricidae) were weighed and subsequently added to half of the pots (earthworm treatment), while the other half received no earthworms. Sixteen weeks after planting, the plants were harvested at ground level and separated into shoots and roots. Thereafter, shoots and roots were thoroughly washed, dried at 60 °C for 48 hours and weighed.

Earthworms were collected during the root washing procedure; they were washed to remove attached soil particles, dried for 1 min on filter paper, counted and weighed. To determine the root colonization by AMF, fresh root samples were collected, stained with ink as described by Vierheilig et al. (1998) and the percentage of AMF root colonization was

determined using the gridline intersect method (McGonigle et al.,1990; Rillig et al., 1999).
Hyphal length was determined from 4.0 g of fresh soil per mesocosm by an aqueous extraction and membrane filter technique modified after Jakobsen et al. (1992). Hyphae of AMF were distinguished microscopically at (200X) as described by Rillig et al. (1999). Dried shoot samples were crushed with a mill (Retsch GmbH, Haan, Germany) and approximately 2-3 mg was weighed into tin capsules to analyze nitrogen and carbon concentration by an Elemental Analyser (EuroEA, HekaTech, Germany) with acetanilide (Merck, Darmstadt, Germany) as internal standard. Shoot phosphorus was determined spectrophotometrically (Chapman and Pratt, 1961). Soil pH was assessed at the end of the experiment with a pH-meter (Knick 761 Calimatic) in a 1:5 (w/v) aqueous dilution.

Statistical analysis

Treatment effects were tested in R (Version 2.14.1) using a general linear model (Zuur et al., 2009). We tested the normal distribution of residuals and the homogeneity of variance by using the Shapiro-test and Levene's-test, respectively. For the analysis of pH and shoot biomass, residuals deviated slightly from normality but given the balanced experimental design and the conformation of data to homoscedasticity this was deemed acceptable. The pH and shoot biomass data were log-transformed as necessary to meet the assumptions of ANOVA.
We used Tukey-Kramer HSD to conduct multiple comparison tests. The relationship among hyphal length, AMF root colonization, plant performance, shoot nutrient concentrations (N, P, C) and soil pH were tested by Pearson correlation coefficients.

Results

Earthworms

In total 88% of the 72 added individuals of *A. caliginosa* survived until the end of the experiment. The total biomass of *A. caliginosa* decreased on average by 30.32% ±0.29 during

the course of the experiment. The decrease in biomass was not affected by hydrochar addition (Table 1).

Plant performance and nutrients

There was a negative effect of hydrochar at high dosage on plant growth without earthworms. We found significant positive interactions between earthworms and hydrochar on plant shoot and root biomass (Figures 1a and 1b, Table 1). Earthworms significantly increased shoot and root biomass of *P. lanceolata* only in the high hydrochar addition treatment. In the control (without hydrochar) and the low hydrochar addition treatment, earthworms had no effect. Furthermore, root biomass was lowest in the absence of earthworms at high hydrochar addition.

Addition of hydrochar had a significantly positive effect on shoot nitrogen concentration with significant interactions between the factors earthworm and hydrochar (Figure 2a, Table 1). The presence of earthworms at low hydrochar level had a positive significant effect on shoot nitrogen content. Leaf N concentration was positively correlated with leaf P concentration, but was negatively correlated with root biomass and soil pH (Table 2).

There was a significant difference in leaf phosphorus (P) concentration due to hydrochar addition. High dosage of hydrochar significantly increased shoot P concentration of *P. lanceolata* compared to the control and the low hydochar dosage, while earthworms did not affect the leaf P concentration, and no interactions with hydrochar was detected (Figure 2c, Table 1). Leaf P concentration was positively correlated with hyphal length and leaf N concentration, but negatively correlated with soil pH (Table 2).

Arbuscular mycorrhizal fungi

We assessed the impact of hydrochar and earthworms on root colonization and hyphal length of AMF in soil. We found that hydrochar addition significantly increased AMF root colonization (Figure 3a, Table 1). Earthworms had a negative effect on hyphal length in soil, whereas hydrochar had a positive effect, while no interaction was detected (Figure 3b, Table 1). Root colonization, hyphal length and carbon correlated negatively with soil pH (Table 2). We also detected a positive correlation between AMF root colonization and hyphal length

(Table 2).

Soil pH

We observed a significant interaction between hydrochar and earthworms on soil pH (Table 1). There was an increase with hydrochar addition in the presence of earthworms, while without earthworms the pH was significantly higher at hydrochar addition (1% and 10%) compared to the control (Figure 4, Table 1).

Discussion

Although there are studies exploring the effects of biochar on plant growth and interactions with soil biota, there are only very few studies with hydrochar as carbonized material (Rillig et al., 2010, George et al., 2012). We showed here for the first time that earthworms could alleviate negative effects of fresh hydrochar on plant growth.

Effects of hydrochar and earthworms on plant biomass and available nutrients

Hydrochar at high dosage had a detrimental effect on plant growth, especially on shoot biomass, when no earthworms were applied. This can be through immobilization of nutrients especially nitrogen cations adsorbed on hydrochar particles (Gajic and Koch, 2012), but we did not observe a lower N shoot content at the highest dosage of hydrochar. Potentially nutrients other than N were affected, but we found no evidence in support of this. Another possibility of reduced plant growth could be toxic organic compounds in the hydrochar, as hydrochar can contain phenolic and aromatic compounds (Libra et al., 2011). Especially in fresh hydrochar negative effects on plant growth are associated with the emission of phytotoxic volatile substances (Busch et al., 2012).

Earthworms can have positive effects on plant growth by improving soil structure through their burying activity and enhancing the mineralization of organic matter (Scheu, 2003). However, in our study we did not observe positive effects of earthworms in the absence of hydrochar. This may be related to the sandy texture of the soil and its relatively low organic matter content.

Interestingly we observed an increase in plant biomass with earthworms and high dosages of hydrochar. Earthworms and hydrochar may interact directly: earthworms ingest carbonized particles and excrete them in their casts, which leads to spatial distribution of nutrients adsorbed on carbonized particles (Edwards, 2004; Milleret et al., 2009). Earthworms also enhance bioturbation and transport of carbonized particles (Eckmeier et al., 2007; Hammes and Schmidt, 2009; Weyers and Spokas, 2011). Although there are no empirical data available, this is likely also true for hydrochar. Probably earthworms led to an increased decomposition of hydrochar due to their feeding activity (Edwards and Bohlen, 1996) and an increased aeration of the soil by their burrowing activities. Ingesting hydrochar particles may lead to the above mentioned aging process through decomposition, as microbial activity is likely increased in earthworm casts (Devliegher and Verstraete, 1997). This aging process could neutralize possible phytotoxic volatile substances and leads to a more hydrophilic behavior (Busch et al., 2012) and to a higher availability of nutrients (Noguera et al., 2010).

We found highly significant effects of hydrochar on N shoot content with also significant interactions with the factor earthworm. A negative effect of low dosage of hydrochar on N shoot content of *P. lanceolata* could be observed without but not with earthworms. Hydrochar is known to immobilize N in soil, but this effect may depend on the added concentration (Libra et al., 2011). Probably due to earthworm activity the mineralization of organic matter increased in the soil (Wurst et al., 2004b), thus outweighing this N immobilization effect (Noguera et al., 2010). The relatively high N content at high dosage of hydrochar without earthworms can be explained by the fact that the plants were smaller and therefore the N content was more concentrated.

Addition of carbonized materials into agricultural soils can have positive (Lehmann et al., 2003) or negative effects (Steiner et al., 2007; Rillig et al., 2010) on plant available N and yield. Probably, N is immobilized through the adsorption of NH_4^+ on hydrochar particles. This leads on the one hand to a reduction of N leaching and an increase of N fertility over time in surface soils (Lehmann et al., 2003). On the other hand, an immobilization of N can also have a negative effect on plant growth. This was especially observed in soils amended with

hydrochars with a high C/N ratio (Gajic and Koch, 2012). As in our study the hydrochar used had a relatively low C/N ratio of 23, the positive effect on soil fertility prevailed, especially when earthworms were present.

Effects of treatments on performance of arbuscular mycorrhizal fungi and soil pH

Hydrochar had a positive effect on AMF root colonization, also at a high dosage, a finding consistent with a previous study (Rillig et al., 2010). Though underlying mechanisms behind these effects remain unclear, it is probably due to changes in the soil pH and changes in the nutrient availability like P, K, Ca or Mg (DeLuca et al., 2006; Warnock et al., 2007, Gaskin et al., 2010). Interestingly the addition of hydrochar increased the soil pH despite of itself being acidic. This was likely due to microbial reduction reactions which lead to an increased microbial activity (Rillig et al., 2010; Libra et al., 2011). Nevertheless, pH was still in the neutral range, so the effects of a pH increase on microbial activity will be minimal. Another possible explanation for the increase in pH is the addition of base cations to soil with the hydrochar which leads to better plant growth (Warnock et al., 2007, Gaskin et al., 2010). Indeed, we also found higher P and N concentrations in the plant tissue which was amended with high levels of hydrochar. This is probably also due to the better performance of AMF, which have a main role in providing P to their host (Smith and Read, 2008).

Hydrochar with its porous structure also can act as a refuge for hyphae protecting them from grazers (Warnock et al., 2007) and thus indirectly leading to the observed higher AMF root colonization. Earthworms reduced hyphal length in soil, probably by grazing on them and disrupting the hyphal network, nevertheless they did not affect the mycorrhizal colonization in roots. Finding no effects of earthworms on AMF root colonization is not unusual (Wurst et al., 2004a; Eisenhauer et al., 2009; Wurst et al., 2011; Wurst and Rillig, 2011). Earthworms can also influence AM fungi positively by contributing to the dispersal of spores (Lawrence et al., 2003; Ortiz-Ceballos et al., 2007).

Conclusions

We showed effects of earthworms and hydrochar on plant performance and root symbionts

with positive interactive effects on plant biomass. Earthworms and hydrochar significantly affected AMF, but these effects were mainly independent of each other. High dosage of hydrochar addition enhanced AMF root colonization and P uptake, and earthworms significantly decreased AMF hyphal length in soil.

Our findings also suggest that results from highly controlled pot experiments (which tend to exclude earthworms) may overestimate the initial negative effects of carbonized materials; this exemplifies the complex interactions of hydrochar with other soil biota, which may result in positive effects on plant growth. Therefore, our results suggest that interactions between soil fauna and carbonized materials could play an important role in the management of carbonized materials.

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Table 1 ANOVA F values for the effects of hydrochar (H) and earthworms (EW) and their interaction on earthworm biomass and numbers, shoot biomass, root biomass (dw, dry weight), soil pH, AMF hyphal length, AMF root colonization, and N, C, P concentration in leaves of *P. lanceolata* (* = p<0.05; **= p <0.01; *** = p <0.001).

Variable	Earthworms	Hydrochar (H)	Interaction
Biomass EW	432.21***	0.35	0.35
Number EW	0.88	0.91	0.91
Shoot biomass (g dw)	0.07	2.79	5.16**
Root biomass (g dw)	0.69	0.39	4.04**
Soil pH	8.75 ***	192.19 ***	5.45***
Hyphal length (m g^{-1}	4.68*	4.84*	0.02
Root colonization	2.26	15.37***	1.3
N (mg g^{-1} dw)	2.61	5.48**	3.25*
C (mg g^{-1} dw)	0.08	1.19	2.86
P (µg g^{-1} dw)	0.24	6.25***	0.08

Table 2 Pearson's correlation coefficients (r) for the effects of hydrochar (H) and earthworms(EW) and their interaction on all variables (* = p <0.05; ** p <0.01) (n=8).

	pH	**Dw shoot**	**Dw root**	**Hyphal length**	**Root colonization**	**Nitrogen**	**Carbon**	**Phosphorus**
Dw (shoot)	*NS*	-	***0.878(**)***	*NS*	*NS*	***-0.316(*)***	*NS*	*NS*
Dw (root)	*NS*	***0.878(**)***	-	*NS*	*NS*	***-0.564(**)***	***0.388(**)***	*NS*
Hyphal length	***-0.310(*)***	*NS*	*NS*	-	***0.302(*)***	*NS*	*NS*	***0.301(*)***
Root colonization	***-0.522(**)***	*NS*	*NS*	***0.302(*)***	-	*NS*	***-0.351(*)***	*NS*
Nitrogen	***-0.316(*)***	***-0.316(*)***	***-0.564(**)***	*NS*	*NS*	-	*NS*	***0.470(**)***
Carbon	*NS*	*NS*	***0.388(**)***	*NS*	***-0.351(*)***	*NS*	-	*NS*
Phosphorus	***-0.453(**)***	*NS*	*NS*	***0.301(*)***	*NS*	***0.470(**)***	*NS*	-

Figure. 1 Effects of different HTC material addition rates (control, 1 or 10%) and earthworms on (a) shoot and (b) root biomass (dw, dry weight) of *Plantago lanceolata*. Shown are means and standard errors (n = 8). Different letters indicate significant difference between the treatments at $p < 0.05$ according to the Tukey-Kramer HSD test.

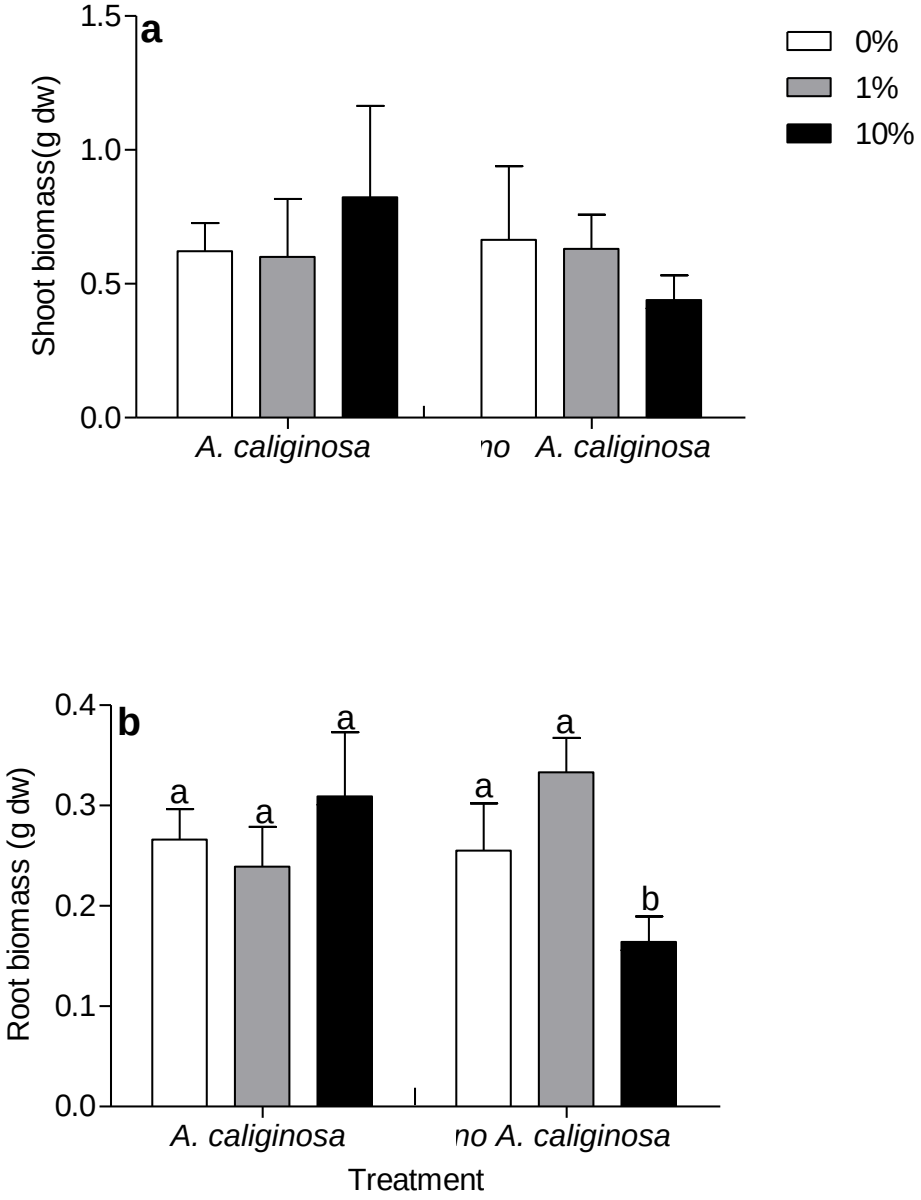


Figure. 2 Effects of different HTC-material addition rates ((control, 1 or 10%) and earthworms on (a) nitrogen (b) carbon and (c) phosphorus concentration of leave tissue of *Plantago lanceolata*. Shown are means and standard errors (n = 8). Different letters indicate significant difference between the treatments at $p < 0.05$ according to the Tukey-Kramer HSD test.

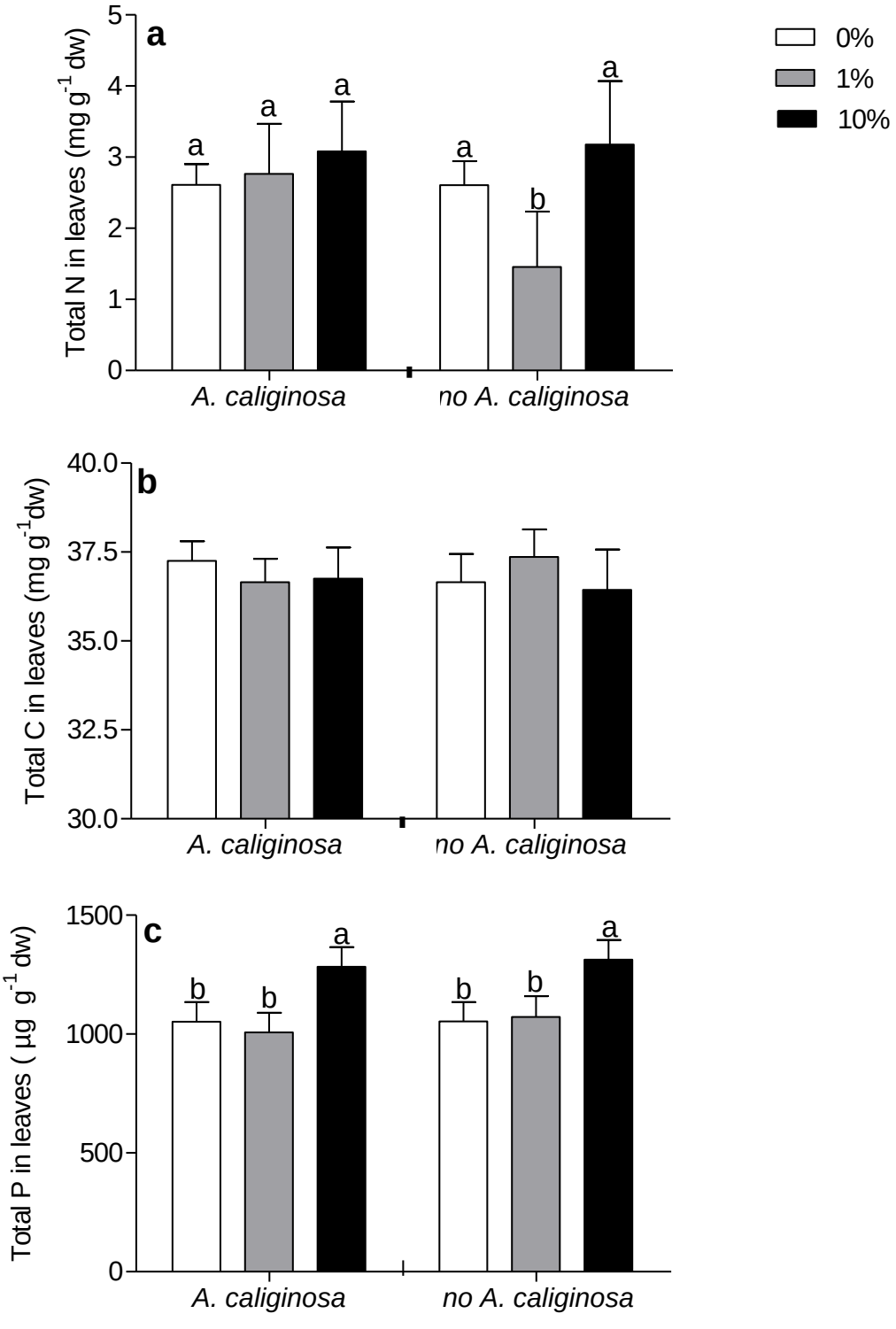


Figure. 3 Effects of different HTC-material addition rates (control, 1 or 10%) and earthworms on (a) root colonization and (b) hyphal length of AM fungi in *Plantago lanceolata*. Shown are means and standard errors (n = 8). Different letters indicate significant difference between the treatments at $p < 0.05$ according to the Tukey-Kramer HSD test.

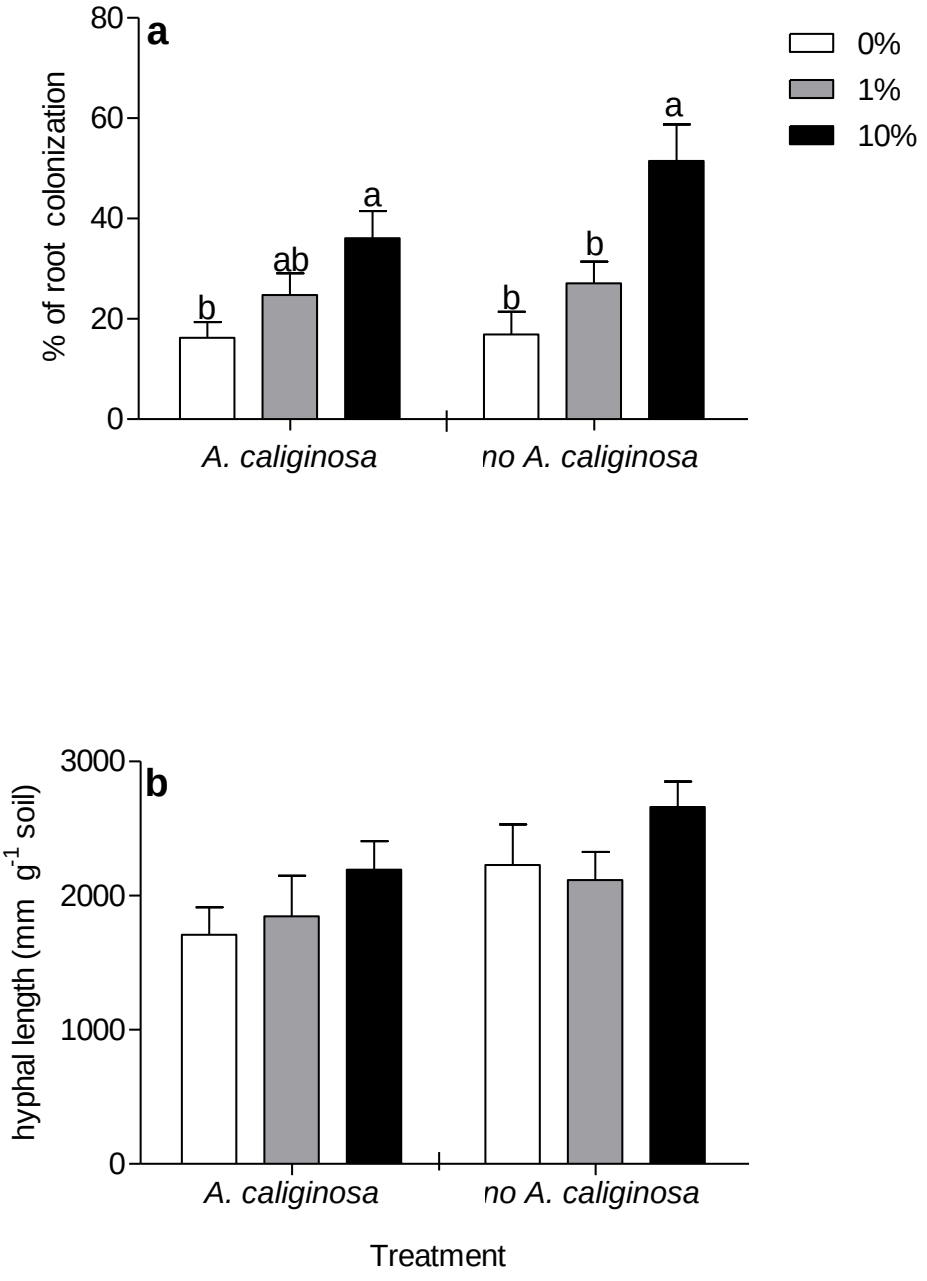
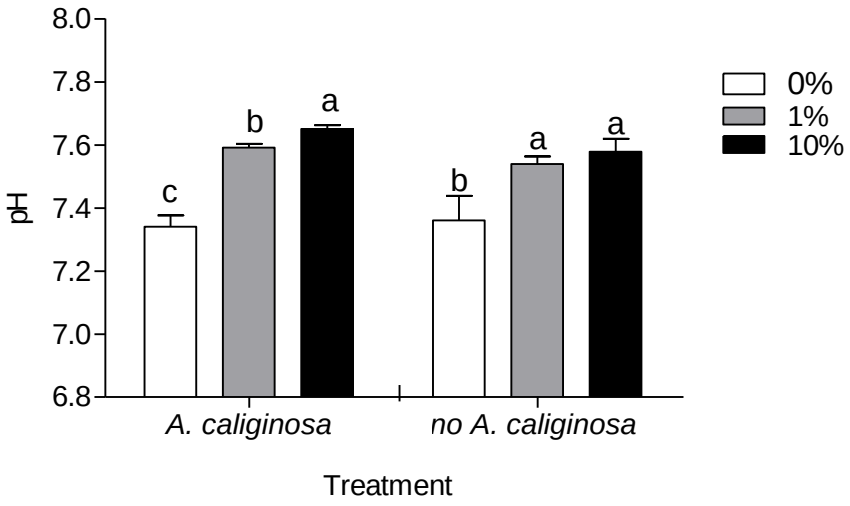


Figure. 4 Effects of different HTC-material addition rates (control, 1 or 10%) on soil pH, as measured at the end of the experiment with *Plantago lanceolata*. Shown are means and standard errors (n = 8); note y-axis break. Different letters indicate significant difference between the treatments at $p < 0.05$ according to the Tukey-Kramer HSD test.



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CHAPTER 4

Palatability of carbonized materials to collembola

Abstract

Amendments with carbonized materials can improve soil carbon storage and fertility. While data on effects of such additions on soil properties or plant growth are becoming increasingly available, we do not yet know how carbonized materials affect key soil biota groups, such as collembola. Hydrochar is one type of carbonized product, produced by hydrothermal carbonization, an alternative method to pyrolysis. The goal of this study was to examine the palatability of hydrochars to collembola. As part of this study, we evaluated effects on palatability of different particle size, different types of hydrochar, and the effects of pre-washing the product. Finally, we analyzed the effect of hydrochar on fitness parameters such as molting, number of eggs, survivors and hatchlings of collembola. We conducted all these laboratory tests using two different species *Coecobrya tenebricosa* (Folsom) Gruia, and *Folsomia fimetaria* L. Both species were able to consume hydrochar, even though palatability was relatively low. Both species were able to complete their life cycle with hydrochar as the sole food source. Neither pre-washing, nor type and particle size of hydrochar significantly influenced palatability in the ranges of properties we examined. Our results suggest that these animals, since they can ingest these materials, can play an important role in the degradation (by comminution) and distribution of carbonized materials in soil.

Keywords: hydrochar, hydrothermal carbonization, palatability, springtails, *Coecobrya tenebricosa, Folsomia fimetaria*

Introduction

In searching for solutions to mitigate the rising atmospheric CO_2 concentration there is a relatively new research focus on carbonized materials produced from biomass (biochar). The interest arises because of the relative recalcitrance of this material in the soil and its potentially beneficial soil fertility effects (Lehmann, 2007a; Lehmann, 2007b). For thousands of years humans have used the process of pyrolysis turning wood into charcoal (Antal and Gronli, 2003). Investigating the terra preta do Indio soils, the highly fertile Amazonian Dark Earths (Lehmann et al., 2003; Glaser, 2007), has shown that biochar can be used as a promising soil additive which can promote plant growth and carbon storage (Glaser et al., 2002; Glaser and Woods, 2004; Lehmann et al., 2006; Glaser, 2007). Biochar is obtained by pyrolysis, where organic matter is heated (<700° C) under controlled conditions in a closed system (Lehmann and Joseph, 2009). Beside pyrolysis, there is another way to carbonize organic matter, the hydrothermal carbonization (HTC) process (Titirici et al., 2007). Here, organic feedstock is heated for several hours in the presence of water at much lower temperatures (180 to 250 °C) under pressure (Funke and Ziegler, 2010; Libra et al., 2011). Hydrochar, the product of HTC processes, has a less aromatic structure than biochar, and possesses also a higher percentage of labile C fractions, therefore it is likely less stable and decomposed in soil more rapidly than biochar from dry pyrolysis (Steinbeiss et al., 2009; Cao et al., 2011; Libra et al, 2011). Nevertheless, hydrochar may have broadly similar effects on soil properties as biochar, enhancing water-holding capacity and increasing the cation exchange capacity (Libra et al., 2011).

Although there are many reports of effects of biochar on plant growth, soil properties and soil biota, there is a dearth of information of the response of soil microarthropods, such as collembola, to biochar (Lehmann et al., 2011). This is unfortunate, because collembola are important members of the belowground food web. As a group they are considered to be unspecialized feeders as they feed on a great variety of resources, including fungi, bacteria, decomposing vegetation and detritus, among others, even though species-level feeding preferences exist (McMillan and Healey, 1971; Rusek, 1998; Sadaka-Laulan et al., 1998).

Through their feeding activity they promote decomposition processes in the soil (Hopkin, 1997).

Collembola are also important for other important soil-borne processes, including soil aggregation (Siddiky et al., 2012) or distribution of materials in the soil, including propagules of other biota (Filser, 2002; Kreuzer et al., 2004). To our knowledge, there are no experimental data available on the effects of carbonized materials, neither biochar nor hydrochar, on collembola.

There are some incidental observations that suggest that carbonized materials can be ingested and perhaps used by soil arthropods (Lehmann et al., 2011). For example, Phillips et al. (2000) found large amounts of fecal pellets of arthropods and earthworms in the charcoal layer of burned soils. Also, Uvarov (2000) reported that the density and diversity of collembola and other soil biota was increased in soils next to charcoal kilns. However, we do not know how palatable these materials are, if ingestion of carbonized material has any effect on fitness components of collembola, or if ingestion is rather more incidental (Lehmann et al., 2011). In a preliminary experiment, we tested feeding preference of collembola in the presence of hydrochar and an alternate food source and showed that, indeed, they consumed hydrochar, but in a relatively small amount (data not shown). Hydrochar also can contain adsorbed soluble organic compounds (Rillig et al., 2010; Cao et al., 2011) which could have a negative influence on soil biota, so we also included a washing pretreatment in one of our experiments. Furthermore, parent material and production parameters influence the physical and chemical characteristics of hydrochar (Joseph et al., 2009). Therefore, we tested the palatability of different types of hydrochar to collembola.

Thus, the aim of this study was to investigate in lab tests (i) the effect of pre-washing the hydrochar on palatability; (ii) the role of particle size of hydrochar on palatability; (iii) the effect of different hydrothermal carbonization production conditions on palatability; and (iv) if these preferences are reflected in survival rate or fecundity of the animals when these are fed exclusively carbonized materials.

Material and Methods

Collembola

In all experiments we used two species, Coecobrya tenebricosa (Folsom) Gruia, and *Folsomia fimetaria* L., which we reared as laboratory cultures. The cultures both originated from soils of Northern Germany. We selected these species as they have a translucent body, which facilitiates observation of the ingested food under the microscope. Another reason for selecting these species was that they are both relatively unspecific feeders of organic material in soil (Fjellberg, 2007). Both species have similarly shaped mouthparts (Chen et al., 1996). F. *fimetaria* belongs to the Isotomidae, whose members inhabit peat and pasture, *C. tenebricosa* is a member of the Entomobryidae, a family whose members are often found in pasture and sandy soils (Gudleifsson and Bjarnadottir, 2008). This species also thrives in flowerpots and greenhouses (Fjellberg, 2007).

The animals were kept in plastic containers with a plaster of Paris / charcoal mixture (9:1, v:v), incubated at 20 ± 1 °C in the dark and fed with dried baker's yeast. Before starting each experiment, the animals were starved for six days in order to empty the digestive tract. All individuals used in assays were sexually mature, since they are likely to be more efficient feeders and display higher growth rates than immature or senescent individuals (Johnson and Wellington, 1983).

Production of hydrochar

The hydrothermal carbonization process was carried out in a custom-built reactor in the department of Abfallwirtschaft and Deponietechnik of the Hochschule Ostwestfalen/Lippe (Höxter, Germany) using beet root chips (BRC) as input material in all experiments except in experiment 3, see below). BRC are a waste product from sugar processing. The material was hydrothermally carbonized at relatively mild temperatures (180-200 °C, 11h of which 7h>180 °C) in the absence of catalysts. The resulting hydrochar was wet and had high H/C and O/C ratios. Elemental analysis of material yielded the following values: C = 53.2%, H = 5.3%, N = 2.3%; C/N ratio = 23.1. The energy density of the material was 21.7 MJ kg^{-1}. The beet root chip parent material had a pH of 4.39 (1:1 in water), and the unwashed HTC product, as used

in the experiment, had a pH of 4.10. Hydrochar was kept in closed plastic buckets until use.

For experiment 3 (see below) we produced ten different types of hydrochar in the same reactor. They differed in input material (BRC or spent brewers grains (SBG)) and production temperatures (180 °, 200 ° or 220 °C) and carbonization times (2, 4, 8 or 12 h). In the hydrochar type 10 the processing temperature was gradually increased up to the end processing temperature (VA+). This yielded a series of carbonized materials with different properties, which are summarized in Table IV. S1 (supplementary online material).

Experiment description

We designed a series of four laboratory experiments, described below, aimed at comprehensively exploring the effects of carbonized materials on collembola. All experiments had a two factorial design with the first factor the collembola species and the second factor an aspect of the food source depending on the question, as described below. Experiments 1 to 3 were set up with ten individuals of one of the two collembola species placed in one Petri dish containing the different food sources. Each experiment was replicated ten times, except experiment 2, where we had eleven replicates. Experiment 4 was carried out in plastic vessels as described below.

At the start of each experiment the animals were transferred to a bottomless plastic cylinder (1 cm diameter) placed in the center of each Petri dish (9 cm diameter) which had been filled (3-5 mm thick layer) with plaster of Paris as substrate. Round, equidistant holes (internal hole diameter: 0.4 mm, distance of holes from side of Petri dish: 1.5 cm) were punched in the plaster of Paris, and were filled randomly with discs of the different food sources. In these feeding stations we always placed the same amount of yeast either on its own or mixed with hydrochar. We added 1-3 drops of food coloring "Green (E131/102)" to the yeast before placing the material into the hole (Thiele and Larink, 1990). The food coloring does not affect feeding preferences (Schreiner and Bethlenfalvay, 2003).

All Petri dishes (for experiments 1-3) and small plastic vessels (experiment 4), respectively, were checked right after transferring the animals to make sure that they were still alive. Then the dishes were sealed with a strip of parafilm and incubated in darkness at 20 ± 1 °C. As an index of palatability we counted the number of fecal pellets after 24h and 72h for experiments

2 and 3 and only after 72h for experiment 1. Fecal pellets were recorded within a 5mm radius around each hole for attributing them to the respective food station. For all measurements, we used a stereo microscope (GSZ Jenoptik, 10 X magnification) with a cold light source (KL 1500 electronic, Schott).

Experiment 1: Effect of particles size distribution of hydrochar on feeding preference

In the second two-factorial experiment, we used species and the particle size of hydrochar as factors. For this purpose, hydrochar was sieved to three size classes: < 20µm, 20- 212µm and 212-500µm. The preparation of animals and dishes was as described above. Each Petri dish contained four holes, either with one of the three particle sizes and the fourth remained empty as a control. The animals were placed inside the dish and fecal pellets were counted after 72 h.

Experiment 2: Effect of different hydrochars

In this experiment, the second factor beside the species was the type of hydrochar. Ten different types of hydrochar were used as explained above (Table IV. S1). Thus, each Petri dish had 12 holes, filled with each of the different food sources plus two control disks (yeast and empty) respectively. To avoid positional effects, a randomized code was used for the order of the feeding stations inside the Petri dish. The animals were placed inside the dish and fecal pellets were counted as described above.

Experiment 3: Effect of pre-washing on hydrochar palatability

For testing the effect of washing of hydrochar on feeding preference, we carried out again a two-factorial experiment with species and pre-washing (with the levels washed and not washed) as factors, using yeast as control. Forty gram wet weight of BRC hydrochar was washed with 0.1M HCl by stirring for 1.5 hours, then rinsed three times with distilled water, dried for 24h at 80 °C and then ground and finally passed through a 0.44µm sieve, as described by Minori et al. (2010). The animals were placed inside the Petri dish with the punched holes filled with either the washed, unwashed hydrochar or yeast. We counted fecal pellets as described above.

Experiment 4: Influence of hydrochar diet on fitness parameters

This two-factorial experiment to test the influence of hydrochar on survival rate, molting rate and fecundity of collembola differed from the previous experiments in using small plastic vessels with a press-on lid (4.5 cm diameter, height 3.0 cm) with a plaster of Paris substrate at the bottom. The surface was carefully smoothed to prevent the appearance of fissures and holes where the insects could hide their eggs. Few drops of distilled water were added to the culture twice a week to maintain high relative humidity. The experimental factors were the two species and the food source. Each container was filled with ten individuals of each species and one disc of a single food source, either hydrochar, yeast or a mixture of both. Each treatment was replicated ten times for a total of 60 containers. We incubated them for 31 days at 20 ± 1 °C in the dark (Larsen et al., 2007). Survivorship, molting rate and egg production as well as number of hatchlings were measured as indicators of performance. We counted surviving animals every 72 hours. Numbers of exuviae (molting rate), eggs and hatchlings were recorded at the end of the experiment. We removed dead animals and exuviae; missing animals were counted as dead (Van Amelsvoort and Usher, 1989).

Statistical analysis

We tested for differences among means using linear mixed effect models (Zuur et al. 2009) analysis of variance in R version 2.14.1 verifying normal distribution of residuals using Shapiro-test and Levene‘s-test to test for homogeneity of variances. The factor “arena” was the random effect accounting for data correlation within each arena. In the cases where the data were not normal, we used log-transformations. For the experiment “pre-washing” we used a Poisson distribution to properly model data. All parameters were analyzed using two-way ANOVA. For experiment 2 and 3, we used a Tukey's HSD-test to test for differences among the food sources. Differences between the food sources were analyzed by single factor ANOVA including all the data.

Results

Effect of different size of HTC particles (experiment 1)

Both species consumed hydrochar, which we could also directly observe due to the animals' translucent digestive tract (Figure IV. S1, supplementary online material). We observed that *F. fimetaria* overall consumed significantly less hydrochar compared to *C. tenebricosa* ($p < 0.1$; Table IV.1, Figure IV. 1). However, both species produced fecal pellets at all feeding stations with hydrochar without discriminating among the different particle sizes. There were no interactions between the factor species and the particle size of hydrochar (Table IV.1).

Effect of different hydrochars (experiment 2)

Assessing the effect of different hydrochar types on collembola, we found that the food source had a significant effect on fecal pellets at both sampling times including the yeast and the empty feeding stations, while we did note a species effect only after 72 h (Table IV.1). The effect of the food source was mainly due to the yeast and the empty control, because we found no difference among hydrochar types in both species, neither after 24 h nor 72 h. *F. fimetaria* consumed significantly more hydrochar compared to *C. tenebricosa* (Table IV. 1, Figure IV. 2).

Effect of pre-washing on hydrochar palatability (experiment 3)

After 24 h there were no differences in the feeding preference of *C. tenebricosa*, but after 72h there was a clear increase of fecal pellets for animals feeding on yeast but not on either of the hydrochar sources. *F. fimetaria* had a preference on yeast from the beginning of the experiment, but we also could observe an increase of the fecal pellets with hydrochar. Although there was a significant effect of the food source, this was due to the high palatability of the yeast. Testing the effect without yeast, there was no difference in the consumption of washed and unwashed hydrochar for any species or sampling time (Figure IV. 3, Table IV. 1).

Influence of hydrochar diet on fitness parameters (experiment 4)

In the fecundity experiment with separate vessels for each food source, we recorded the data for survivorship, exuviae (molting rate), eggs and hatchlings every 72 h, up to four weeks. For all these parameters, the food source had a significant effect; the factor species furthermore had a significant effect on the eggs and the hatchlings. There were no significant interactions between these two factors for any parameter (Table IV.2).

Both collembola species had the highest survivorship when fed with yeast and the lowest in the container with pure hydrochar. With hydrochar as the sole food source, about 25 % of *C. tenebricosa* individuals survived after four weeks, whereas *F. fimetaria* had a higher survivorship of 38 % (Figure IV. 5). The molting rate was three times higher in the containers with yeast compared to the hydrochar diet for both species, but the differences between the treatments in the number of eggs and hatchlings were much less pronounced. The number of eggs did not depend on the food source for *F. fimetaria* but was negatively affected by hydrochar in *C. tenebricosa* (Figure IV. 4).

Discussion

Feeding preferences

Carbonized materials can affect soil fauna such as springtails indirectly or directly. Indirectly, carbonized materials could change pH or other soil parameters, which could alter the availability of fungi, an important food source of collembola (Lehmann et al., 2011). In terms of direct effects, the focus of the present study, we showed in our controlled laboratory experiments that springtails consumed and ingested char particles.

In experiment 1 with hydrochar as the only food choice, both species were able to consume hydrochar to a significant degree irrespective of difference in particle size; an observation which we confirmed also visually (Figure IV. S1). Perhaps they use it like an “emergency food source”, when other options are unavailable. We showed that the particle size of hydrochar did not influence palatability. This is probably due to their manner of rasping food sources with the tips of their mandibles, which are strongly toothed (Hopkin, 1997); this is the case for members of the families of both species (Chen el al., 1997).

The C:N ratio of a food source for soil animals generally reflects the resource quality, with low C:N ratios being associated with high, and high C:N ratios with lower quality (Haubert, 2004; Larsen et al., 2011). As hydrochar has a huge variety in quality and chemical characteristics due to their feedstock and production conditions (Libra et al., 2011), we tested if different types had an influence in their consumption behavior.

Although different production temperature and feedstock influenced the C content of hydrochar and it's C: N ratio (Table IV. 1 of supplemental material), this did not influence palatability of these different types of hydrochar. Perhaps the species were not sensitive to the C:N ratio across the range examined here.

Hydrochar can contain phenolic and aromatic compounds (Libra et al., 2011), which may be toxic for collembola (Sverdrup et al., 2001). Some species are known to avoid certain food sources because they contain toxic substances and volatile compounds which they detect by their olfactory sense (Bengtsson et al., 1988; Scheu and Simmerling, 2004; Böllmann et al., 2010; Staaden et al., 2011). Therefore, they probably also detect hydrochar as it contains relatively high amounts of volatile compounds, because of its production under pressure (Rillig et al., 2010). Consequently, our hypothesis was that a pre-washing step of hydrochar will eliminate some of these compounds, enhancing palatability. Nevertheless, we did not find any differences, although *F. fimetaria* tended to prefer the feeding stations with washed hydrochar, but this trend was not significant. As they can detect volatile compounds to very low concentrations (Bengtsson et al., 1991), our prewashing probably was not sufficient to remove all volatiles from hydrochar.

There is a possibility that collembola just fed on the hydrochar for accessing fungi or other microbes which could grow inside the particles, as these could not be kept sterile. However, we never observed fungal growth on the hydrochar particles during any of the experiments. Also, pre-washing did not have an effect, and this step could be expected to removed easily-soluble organic material on which microbes may have been able to better proliferate.

Fitness consequences of consumption of carbonized materials

From previous observations, it was not clear if collembola incidentally take up carbonized

materials, which then just passes their gut unutilized, or if they can derive a nutritional value from these materials. To address this question, we also measured the influence of a hydrochar diet on a set of fitness parameters. Yeast, as part of the normal diet of many collembola species, resulted in higher fitness parameters and survivorship in both species, as consumption of better quality food leads to better fitness (Hopkin, 1997). Feeding on hydrochar, *F. fimetaria* had higher fitness parameters compared to *C. tenebricosa,* but both species could complete their life cycle. The results suggest that the material had some nutritional quality to the animals; the low nitrogen content did not influence palatability as also shown in experiment 3, but it negatively influenced aspects of fitness. Importantly, this shows for the first time that collembola not only ingest carbonized materials, but also can derive some nutrition from it.

Potential consequences of consumption of carbonized materials

Hydrochar is decomposed in soil more rapidly than biochar, because of its less aromatic structure and higher percentage of labile C fractions like carbohydrates and carboxylates (Cao et al., 2011; Libra et al, 2011). So far, fungi are considered the main agents in the decomposition of carbonized materials (Libra et al., 2011). While this is likely correct, our findings suggest that the role of microarthropods in the decomposition process via consumption and ingestion should be kept in mind. Food material passing the gut of collembola increases its surface area and can condition it for subsequent fungal and bacterial attack (Hopkins, 1997), which can in turn lead to the release of nutrients from the carbonized material. As we discussed above, they consume and ingest food material by rasping food materials surfaces, and chewing and grinding it with their mandibles and maxillae (Chen et al., 1997), which also leads to an increased surface area.

Even though we did not directly test for this here, collembola could play a role in the transport of carbonized materials through their feeding activity. As mobile soil biota they lead to a translocation of organic carbon from litter to soil (Chamberlain et al., 2006), which is also plausible for hydrochar.

Recently it was shown that collembola can play an important role in improving soil structure and functioning through soil aggregation due to their fecal pellets (Siddiky et al., 2012).

Application of hydrochar to soil had a positive effect on soil aggregation (George et al., 2012), even though this is not always the case for carbonized materials (Peng et al., 2011). It is possible that hydrochar particles inside the fecal pellets can form part of a microaggregate, and that therefore collembola are involved in stabilization of this material in the soil.

Conclusions

The palatability of carbonized materials to soil microarthropods such as collembola has not been previously studied. We showed for the first time that different collembola species in principle can use it as a food source, although they have a reduced survivorship compared with yeast. Our results suggest that microarthropods could play an important role in the fate of carbonized materials in the soil system and their effects on soil processes; their interactions with these materials should become a future focus of biochar research.

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Table IV.1 ANOVA table (F values) based on linear mixed effect models (Zuur et al., 2009) for the effects of food source and Collembola species on number of fecal pellets (**P*<0.05; ***P*<0.01; ****P*<0.001) (n=10). In the type and pre-washing experiment, we used time as a cofactor.

Experiment	Duration	Species (S)	Food source (F)	Interaction (S×F)
Particle size	24h	3.61	31.4***	0.19
Hydrochar type	72h	8.71**	35.9***	1.84*
Pre-washing	72h	4.66*	18.35***	2.2

Table IV. 2 ANOVA table (F values) for the effects of food source, species and their interaction on fecundity after 4 weeks. Generalized linear model (*p*-values) using a binomial distribution for the effects of food source, species and their interaction on survivorship after 4 weeks. (**P*<0.05; ***P*<0.01; ****P*<0.001) (n=11)

	Species (S)	Food source (F)	Interaction (S×F)
Exuviae	**0.60**	**10.1*****	**0.19**
Eggs	**6.00***	**3.49***	**1.28**
Hatchlings	**4.22***	**7.52****	**1.93**
Survivors	**0.821**	**<0.001*****	**<0.001*****

Figure IV. 1 Effect of different size of particles of HTC from beet root chips on the number of fecal pellets produced by ten individuals of *Coecobrya tenebricosa* and *Folsomia fimetaria* after 24 hours (Experiment 2). Sizes of hydrochar were < 20µm, 20- 212-µm and 212-500µm, plus an empty feeding station. Shown are means and standard errors (n=10).

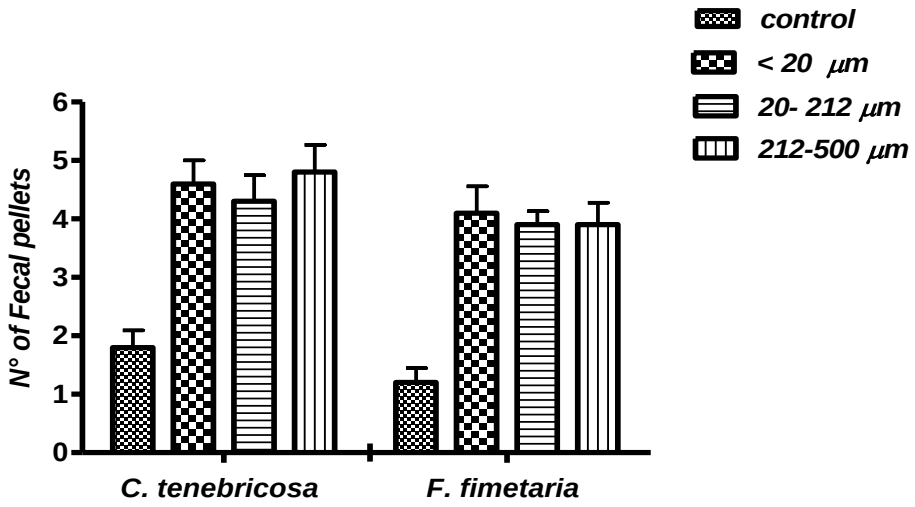


Figure IV.2 Effect of yeast and ten different types of carbonized materials and control (empty feeding station) on the number of fecal pellets produced by ten individuals of *Coecobrya tenebricosa* and *Folsomia fimetaria* after 24 h (a) and after 72 hours (b) (Experiment 3). Shown are means and standard errors (n=11). SBG, spent brewers grains; BRC, beet root chips. Bars with the same letter are not significantly different (Tukey's HSD-test, $p<0.05$).

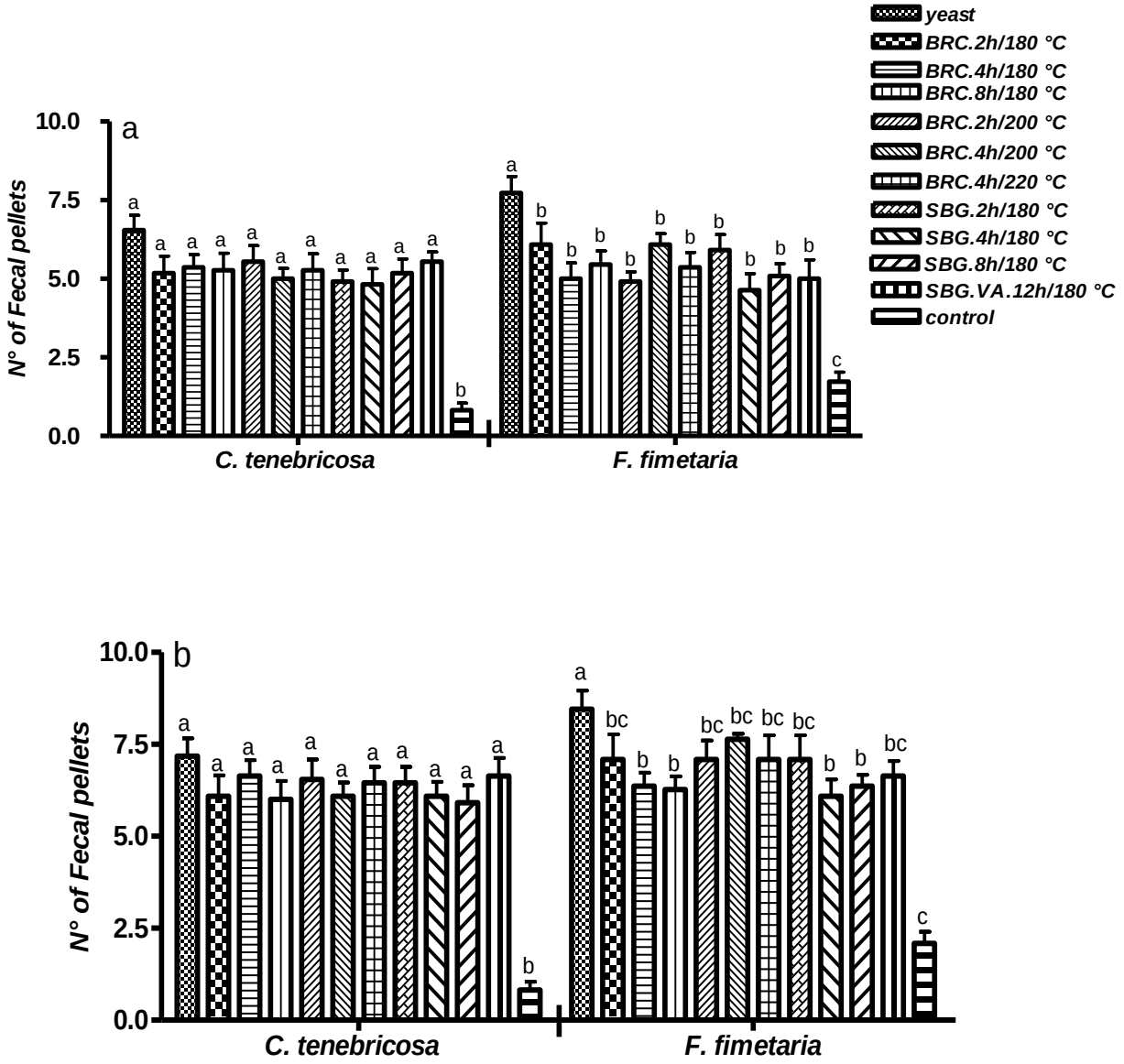


Figure IV.3 Effect of three different materials (yeast, pre-washed HTC from beet root chips and unwashed HTC) on number of fecal pellets produced by ten individuals of *Coecobrya tenebricosa* and *Folsomia fimetaria* after 24 and 72h (Experiment 4). Shown are means and standard errors (n=10). Bars with same letter within the species and sampling time are not significantly different (Tukey's HSD- test, $p<0.05$).

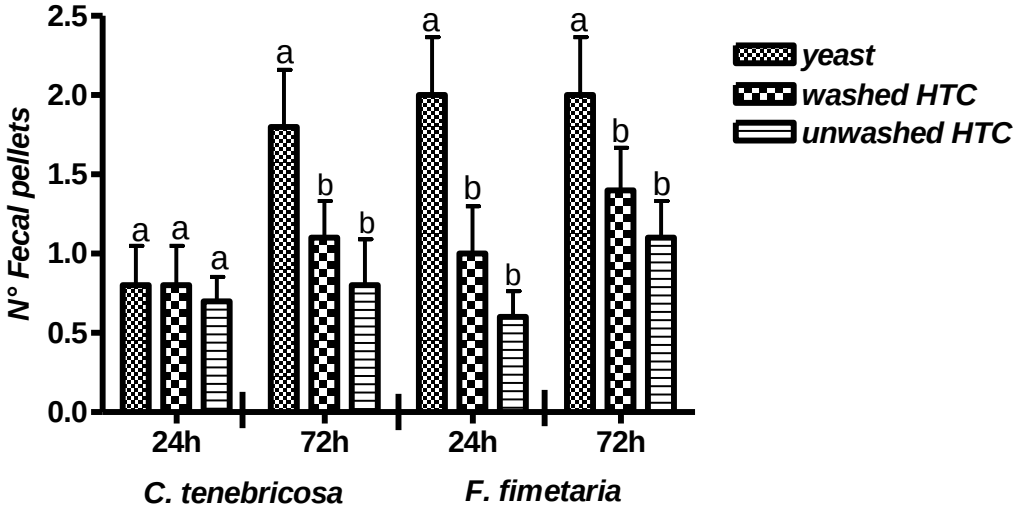
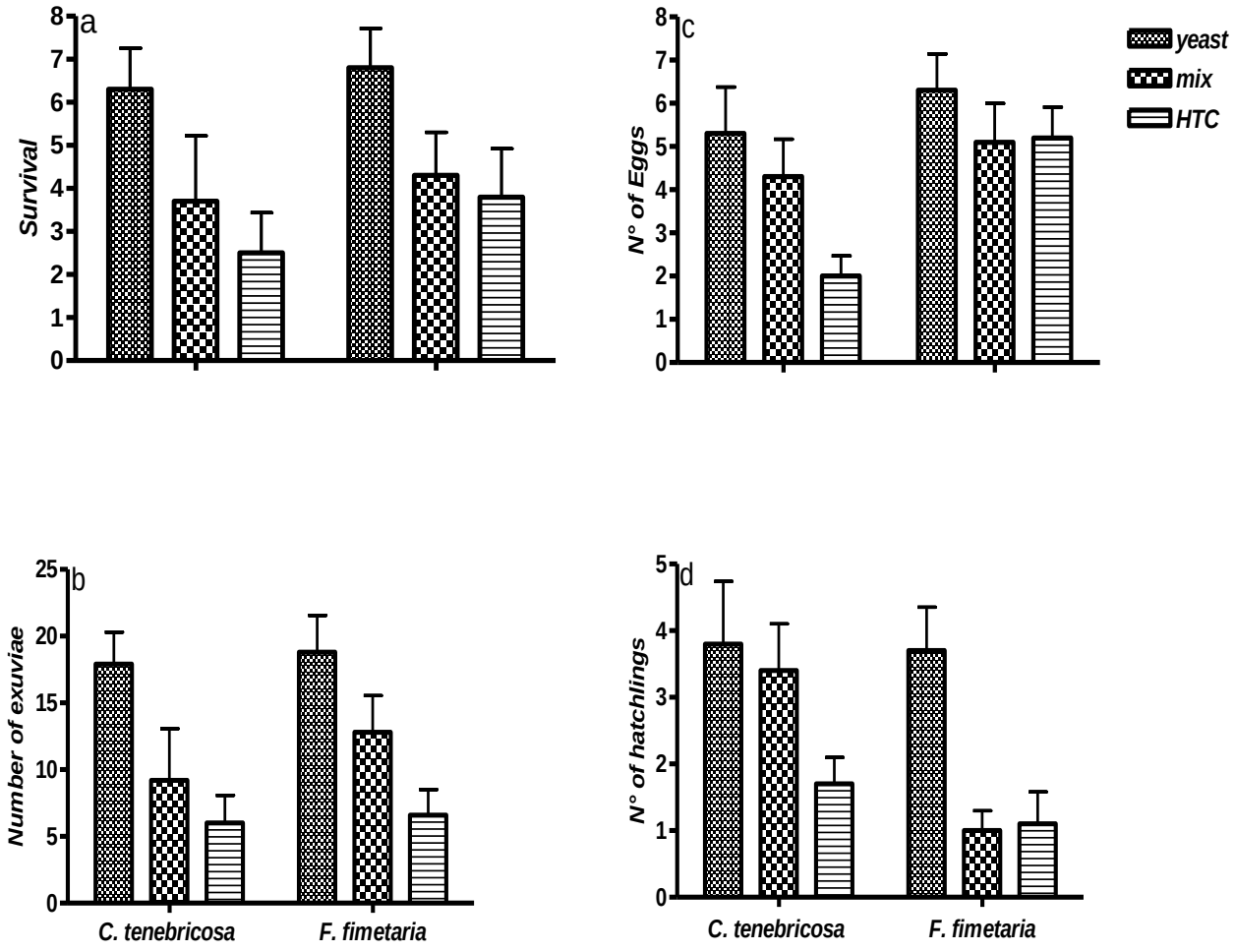


Figure IV. 4 Effect of three different materials (yeast, HTC from beet root chips and mix of HTC and yeast, 1:1 w: w) on number of survivors over a period of four weeks (a), exuviae (b), eggs (c) and hatchlings (d), starting with ten individuals of *Coecobrya tenebricosa* and *Folsomia fimetaria* (n=10) 4 weeks after the beginning of the experiment (Experiment 5). Shown are means and standard errors (n =10).



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CHAPTER 5

Summary

One of the possible solutions to mitigate climate change is carbon sequestration via the net removal of CO_2 from the atmosphere in terrestrial or marine ecosystems. Among other possibilities as reforestation, carbon can be sequestered in soil via carbonization of organic material for longer term carbon storage. This can be done via pyrolysis at high temperature and absence of oxygen. The product biochar can be used as soil amendment.

A rediscovered method to carbonize organic material is the hydrothermal carbonization (or wet pyrolysis) at relatively low temperatures ranging from (180 to 250°C) in a closed system in absence of oxygen and aqueous conditions. The product is known as hydrochar and can be used a soil amendment to improve soil properties, fertility and as long-term carbon storage in soil. However, applied hydrochar in high concentration may have negative effects on plant growth or soil biota. Little is known about the potential impact of hydrochar improvement on plant growth and soil organisms such as arbuscular mycorrhizal fungi (AMF) and earthworms or collembola and their interactions. On some soil biota as collembola, there is even no data available about the effects of hydrochar on these groups until this work.

The main objective of this thesis was therefore to test how carbonized materials like hydrochar affect soil properties, plant growth, soil organisms and their interactions. So, the specific objectives were i) assess how hydrochar influences plant growth and interacts with soil micro-organisms, such as AM fungi and nodulating bacteria ii) test if different hydrochar types can be used by collembola as a food source, and, if yes, how it will affect their life cycle, and iii) determine interactive impacts of earthworms and hydrochar on plant, nutrient uptake (particularly N and P) and AMF performance and to identify the underlying mechanisms.

For these objectives, we carried out a series of experiments:

For objective i) a laboratory test was conducted on spore germination of the AM fungus *Glomus intraradices* at different concentrations of HTC (0.0, 0.05, 0.25, 1.25 g $100mL^{-1}$ and

parent material respectively) in Petri dishes. We showed that spore germination of *Glomus intraradices* was stimulated by the HTC material, suggesting that direct effects of the fungi are likely in addition to those mediated by the host plant. In an additional pot experiment, I tested the effect of hydrochar at different concentrations (0, 2, 4, 10, 20, 30 and 80% (v/v)) on the root colonization of *Taraxacum sect. Ruderalia*, and I found that root colonization of the fungal symbiont was stimulated even at an addition of 20 % (v/v) despite the quite acidic nature of the HTC material itself. Therefore, the results suggest that HTC materials should be carefully tested and optimized to reduce negative effects on plant growth before applications in the field are undertaken, particularly at high addition rates.

For objective ii), I evaluated the effects on palatability of different particle sizes and different types of hydrochar (depending on feedstock and production conditions), and the effects of pre-washing the product on the important soil biota of collembola (springtails). In addition, I analyzed the effect of hydrochar on fitness parameters such as molting, number of eggs, survivors and hatchlings of collembola. I conducted all these laboratory tests using the two different collembola species *Coecobrya tenebricosa* (Folsom) Gruia and *Folsomia fimetaria* L. Both species were able to consume hydrochar, even though palatability was relatively low. Both species were also able to complete their life cycle with hydrochar as the sole food source. Neither the pre-washing treatment, nor type and particle size of hydrochar significantly influenced palatability in the ranges of properties we examined.

For objective iii) I carried out another greenhouse experiment (mesocosms), where we investigated the effect of hydrochar at two different addition rates (1%, and 10% v/v) and with or without the earthworm *Aporrectodea caliginosa* on the growth of *Plantago lanceolata* L. and the performance of its AMF. We observed a positive interaction between earthworms and 10% hydrochar addition on shoot and root biomass: added as a single treatment hydrochar had a negative effect on plant growth at this dosage, but plant biomass increased significantly when hydrochar was added together with the earthworms.

Root colonization by AMF increased significantly with increasing concentration of hydrochar, but was not affected by earthworms. Contrastingly, extraradical hyphal length of AMF was

reduced by earthworms, but not affected by hydrochar. Thus, hydrochar and earthworms affected the performance of AMF, albeit of different AMF structures and in different directions. Our results indicate that earthworms may play an important role in the bioturbation or conditioning of carbonized materials, alleviating the negative impacts of high dosages of hydrochar on plant growth.

The major findings of this dissertation show that

i) hydrochar could stimulate even at the highest concentration 20 % (v/v) of root colonization of AM fungi although plant growth was decreased at the highest level. Spore germination of the AM fungus *Glomus intraradices* was also stimulated by the HTC material. This shows that hydrochar can have very different effects on different soil organisms, which is why these types of materials always should be tested on the whole ecosystem level.

ii), different species of collembola were able on ingest the hydrochar in relatively small amounts, despite the differences in the size and types of hydrochar. Also, pre-washing of hydrochar did not significantly influence palatability hydrochar. Collembola were able to complete their life cycles with hydrochar. Therefore, soil biota as collembola, since they can ingest these materials, can play an important role in the degradation and distribution of carbonized materials in soil.

iii) We found significantly increased plant biomass of *Plantago lanceolata* when 10% hydrochar was added together with the earthworms. Increased concentration of hydrochar improved root colonization, but this was not affected by earthworms. Contrastingly, extraradical hyphal length of AMF was reduced by earthworms, but not affected by hydrochar. Again, this shows the important interactions of different soil groups in the application of hydrochar to soil.

Future perspectives:

Future research is necessary to examine whether results from this mesocosm systems are applicable under field conditions and /or under different environmental stresses.

Moreover, as adverse impacts of hydrochar on plant growth at high doses were found in this thesis, further research is needed to test how these negative effects can be mitigated, for example

through a washing step of hydrochar or pre-incubation (ageing). In addition, as collembola can consume carbonized materials like shown within this dissertation (chapter 4), interactions of hydrochar with soil biota and plants should be examined in pots and also field experiments.
Short-term laboratory and pot experiments, as performed in the studies above, are useful to develop first ideas for guidelines on the use of hydrochar or carbonized material in general.
These can only give insights into initial responses to carbonized materials. Therefore, it is essential that these be complemented with field experiments on the potential long-term effects of hydrochar application in different ecosystems, and plant and soil biota.

Zusammenfassung

Einer der möglichen Lösung zur Eindämmung des Klimawandels ist Kohlenstoffbindung über das Netz Entfernung von CO_2 aus der Atmosphäre in terrestrischen oder marinen Ökosysteme. Neben anderen Möglichkeiten, wie Wiederaufforstung, kann Kohlenstoff in Böden über Karbonisierung von organischem Material für die längerfristige Speicherung von Kohlenstoff sequestriert werden. Dies kann über Pyrolyse bei hohen Temperaturen und in Abwesenheit von Sauerstoff durchgeführt werden. Das Produkt Biokohle kann zur Bodenverbesserung eingesetzt werden. Eine Methode, um wiederentdeckt organisches Material carbonisieren ist die hydrothermale Carbonisierung (bzw. nassen Pyrolyse) an bei relativ niedrigen Temperaturen im Bereich von (180 bis 250°C) in einem geschlossenen System in Abwesenheit von Sauerstoff und wässrigen Bedingungen. Das Produkt wird als hydrochar bekannt und können verwendet eine Bodenverbesserung Bodeneigenschaften, Fruchtbarkeit und als langfristige Speicherung von Kohlenstoff im Boden zu verbessern. Allerdings galt hydrochar in hohen Konzentrationen kann negative Auswirkungen auf das Pflanzenwachstum und Bodenleben haben. Wenig ist über die möglichen Auswirkungen der hydrochar Verbesserung des Pflanzenwachstums und Bodenorganismen wie arbuskulärer Mykorrhizapilze (AMF) und Regenwürmer oder Collembolen und deren Wechselwirkungen bekannt. Auf einigen

Bodenorganismen wie Collembolen, gibt es sogar keine Angaben über die Auswirkungen der hydrochar auf diese Gruppen bis zu diesem Werk.

Das Hauptziel der vorliegenden Arbeit war es daher, zu testen, wie verkohlte Materialien wie hydrochar Bodeneigenschaften, Pflanzenwachstum, Bodenorganismen und ihre Wechselwirkungen beeinflussen. So waren die spezifischen Ziele

i) abschätzen, wie hydrochar Pflanzenwachstum beeinflusst und interagiert mit Mikroorganismen im Boden, wie AM-Pilzen und Bakterien nodulating ii) Test, wenn verschiedene hydrochar Typen können durch Collembolen als Nahrungsquelle verwendet werden, und, wenn ja, , wie es auf ihre Lebensdauer und iii) die interaktive Auswirkungen von Regenwürmern und hydrochar am Werk, die Nährstoffaufnahme (besonders N und P) und AMF Leistung und die zugrunde liegenden Mechanismen zu identifizieren.
Für diese Ziele, die wir führten eine Reihe von Experimenten:

Für Ziel i) ein Labortest wurde auf Sporenkeimung der AM *Glomus intraradices* bei verschiedenen Konzentrationen von HTC (0,0, 0,05, 0,25, 1,25 g^{-1} und 100 ml Ausgangsmaterial bzw.) in Petrischalen durchgeführt. Wir zeigten, dass Sporenkeimung von *Glomus intraradices* vom HTC-Material stimuliert wurde, was darauf hindeutet, dass die direkte Wirkung der Pilze wahrscheinlich sind zusätzlich zu denen, vermittelt durch die Wirtspflanze. In einer zusätzlichen Gefäßversuch I getestet den Effekt hydrochar bei verschiedenen Konzentrationen (0, 2, 4, 10, 20, 30 und 80% (v / v)) auf der Wurzel Kolonisierung Taraxacum sect. Ruderalia, und ich fand, dass Wurzelbesiedlung des pilzlichen Symbionten auch bei einem Zusatz von 20% (v / v) wurde trotz der sehr sauren Natur des HTC Material selbst angeregt. Daher legen die Ergebnisse nahe, dass HTC Materialien sorgfältig getestet und optimiert werden, um negative Auswirkungen auf das Pflanzenwachstum, bevor Anwendungen im Bereich unternommen zu reduzieren, insbesondere bei hohen Zugabemengen.

Für objektive ii), I die Auswirkungen auf die Schmackhaftigkeit unterschiedlicher Partikelgrößen und verschiedene Arten von hydrochar (je nach Rohstoff-und Produktionskosten Bedingungen) ausgewertet, und die Auswirkungen der Vorwäsche das Produkt auf dem wichtigen Bodenorganismen von Collembolen (Springschwänze). Darüber hinaus analysierte ich die Wirkung von hydrochar auf Fitness Parameter wie Häutung, Anzahl der Eier, Hinterlassenen-und Jungtiere von Collembolen. Ich führte alle diese Labortests mit den beiden anderen Collembolen Arten *Coecobrya tenebricosa* (Folsom) Gruia und *Folsomia fimetaria* L. Beide Arten konnten hydrochar verbrauchen, obwohl die Schmackhaftigkeit war relativ gering. Beide Arten konnten auch ihren Lebenszyklus mit hydrochar abzuschließen als einzige Nahrungsquelle. Weder die Vorwäsche Behandlung, noch Art und Teilchengröße hydrochar signifikant Schmackhaftigkeit in den Bereichen von uns untersuchten Eigenschaften beeinflusst.

Für objektive iii) führte ich ein weiteres Gewächshaus Experiment (Mesokosmen), wo wir untersuchten die Wirkung von hydrochar an zwei verschiedenen Zugabemengen (1%, und 10% v / v) und mit oder ohne dem Regenwurm *Aporrectodea caliginosa* auf das Wachstum von Plantago lanceolata L. und die Leistung seiner AMF. Wir beobachteten eine positive interaktion zwischen Regenwürmern und 10% hydrochar zusätzlich auf Spross und Wurzel Biomasse: hinzugefügt eine einzige Behandlung hydrochar hatten einen negativen Einfluss auf das Pflanzenwachstum bei dieser Dosierung, sondern pflanzliche Biomasse deutlich erhöht, wenn hydrochar wurde gemeinsam mit den Regenwürmern aufgenommen. Wurzelbesiedlung von AMF stieg signifikant mit zunehmender Konzentration von hydrochar, wurde aber nicht durch Regenwürmer betroffen. Im Gegensatz dazu wurde extraradical Hyphenlänge von AMF von Regenwürmern reduziert, aber nicht durch hydrochar betroffen. Somit beeinflusst hydrochar und Regenwürmern die Leistung AMF, allerdings unterschiedlicher AMF Strukturen und in verschiedene Richtungen. Unsere Ergebnisse zeigen, dass Regenwürmer können eine wichtige Rolle in der Bioturbation und Konditionierung von verkohlten Materialien spielen, Linderung der negativen Auswirkungen von hohen Dosierungen von hydrochar auf das Pflanzenwachstum.Die wichtigsten Ergebnisse dieser Arbeit zeigen, dass

i) hydrochar könnte sogar zu stimulieren bei der höchsten Konzentration von 20% (v / v) Wurzelbesiedlung der AM-Pilze, obwohl das Pflanzenwachstum auf höchstem Niveau verringert wurde. Sporenkeimung der AM Pilzes *Glomus intraradices* wurde auch von der HTC-Material stimuliert. Dies zeigt, dass hydrochar können sehr unterschiedliche Auswirkungen auf die verschiedenen Bodenorganismen, weshalb diese Arten von Materialien immer auf das gesamte Ökosystem-Ebene geprüft werden sollte, ist zu haben.

ii) wurden verschiedene Arten von Collembolen Lage auf den In gest hydrochar in relativ geringen Mengen, trotz der Unterschiede in der Größe und Art der hydrochar. Außerdem hat Vorwaschen von hydrochar nicht signifikant beeinflussen Schmackhaftigkeit hydrochar. Collembolen konnten ihre Lebenszyklen mit hydrochar abzuschließen. Daher Bodenbiota als Collembolen, da sie diese Materialien aufnehmen, kann eine wichtige Rolle beim Abbau und Verteilung der carbonisierten Materialien im Boden.

iii) Wir fanden signifikant erhöhte pflanzlicher Biomasse von Plantago lanceolata, wenn10% hydrochar wurde gemeinsam mit den Regenwürmern aufgenommen. Erhöhte Konzentration von hydrochar verbesserte Wurzelbesiedelung, aber dies war nicht von Regenwürmern betroffen. Im Gegensatz dazu wurde extraradical Hyphenlänge von AMF von Regenwürmern reduziert, aber nicht durch hydrochar betroffen. Wiederum zeigt dieser wichtigen Wechselwirkungen verschiedener Bodenarten in der Anwendung von hydrochar zu Boden.

Zukunftsperspektiven:

Zukünftige Forschung ist notwendig, um zu prüfen, ob die Ergebnisse von diesem Mesokosmen-Systeme anwendbar unter Feldbedingungen und / oder unter verschiedenen Umwelteinflüssen sind.
Außerdem ist, wie nachteilige Auswirkungen auf das Pflanzenwachstum hydrochar bei hohen Dosen in dieser Arbeit gefunden, wird die weitere Forschung benötigt, um zu testen, wie sich diese negativen Effekte abgemildert werden, beispielsweise durch einen Waschschritt oder hydrochar Vorinkubation (Alterung). Darüber hinaus, wie Collembolen verbrauchen kann

verkohlten Materialien in dieser Dissertationwiegezeigt (Kapitel 4), sollten Wechselwirkungen hydrochar mit Bodenorganismen und Pflanzen in Töpfen und Feldversuchen untersucht werden Kurzfristige Labor und Gefäßversuchen, wie in den obigen Studien durchgeführt, sind nützlich, um erste Ideen für Richtlinien für die Verwendung von hydrochar oder carbonisierten Materials allgemein entwickeln.

Diese können nur Einblicke in ersten Reaktionen auf verkohlten Materialien. Daher ist es wichtig, dass diese mit Feldversuchen auf den möglichenlangfristigen Auswirkungen von hydrochar Anwendung in verschiedenen Ökosystemen sowie Pflanzen-und Bodenorganismen ergänzt werden

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CONTRIBUTION TO CHAPTERS

Chapter 2: Rillig, M.C., Wagner, M., **Salem, M**., Antunes, P.M., George, C., Ramke, H.G., Titirici, M.M., Antonietti, M., 2010. Material derived from hydrothermal carbonization: Effects on plant growth and arbuscular mycorrhiza. Applied Soil Ecology 45, 238–242. http://dx.doi.org/10.1016/j.apsoil.2010.04.011

Own contributions: Design of the laboratory experiment to test the effect of hydrochar on the germination of spores, statistical analyses for data and writing of "Material and methods" of the paper published (together with Dr. Antunes, P.M.,). Collection of the material, performation of the experiment in the incubator. Estimation and calculation of root colonization (%) by arbuscular mycorrhizal fungi by using ink staining and counting.

Chapter 3: Salem, M., Kohler, J., Rillig, M.C., 2013. Palatability of carbonized materials to collembola. Applied Soil Ecology 64, 63-69. http://dx.doi.org/10.1016/j.apsoil.2012.10.009

Own contributions: Design of work (together with Prof. MC Rillig), collection of materials, performation of the experiments in the laboratory, and statistical analyses. Writing the manuscript (together with Dr. Kohler, J).

Chapter 4: Salem, M., Kohler, J., Wurst, S., Rillig, M.C., 2013. Impacts of hydrochar and earthworms on growth of *Plantago lanceolata* and performance of arbuscular mycorrhizal fungi (AMF). (In preparation for submission).

Own contributions: Design of work (together with Prof. MC Rillig), collection of materials, performation of greenhouse experiment, and statistical analyses. Writing the manuscript (together with Dr. Kohler, J).

CONGRESS CONTRIBUTION

Salem M, Kohler J, Ramke, H.G., Wurst, S., Rillig, MC.: Impacts of hydrochar and earthworms on growth of *Plantago lanceolata* and performance of arbuscular mycorrhizal fungi (AMF). Panel.7th International Symbiosis Society Congress. Kraków, July 22-28 2012.

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APPENDIX

Supplemental Table S1 and Figure S1 to Chapter 4

Supplemental Table IV.S1 Feedstock, production conditions and chemical characteristics of different types of hydrochar (SBG spent brewers grains; BRC beet root chips) used in experiment 3. VA+ means a gradual increase of the temperature.

Feedstock	Carbonization time	Processing temperature	C %	H %	N %	O%	C/N.	pH
BRC	2h	180°C	45.53	4.73	1.48	24.20	30.80	4.7
BRC	4h	180°C	48.29	4.71	1.47	25.31	32.75	4.8
BRC	8h	180°C	46.92	4.74	1.42	25.69	33.02	4.8
BRC	2h	200°C	47.72	4.50	1.49	23.64	31.94	4.7
BRC	4h	200°C	47.92	4.62	1.43	24.19	33.42	4.7
BRC	4h	220°C	49.75	4.36	1.59	19.79	31.27	4.7
SBG	2h	180°C	55.50	6.38	3.81	25.89	14.57	4.7
SBG	4h	180°C	56.80	6.14	3.61	24.05	15.76	5.1
SBG	8h	180°C	56.92	6.20	3.65	23.78	15.58	5.0
SBG	12h	180°C VA+	59.68	6.04	3.86	20.08	15.45	5.3

Supplemental Figure IV. S1. Observation (50x magnification) of the passage of HTC material from beet root chips through the gut in *F. fimetaria* 24 h after the beginning of the first experiment.