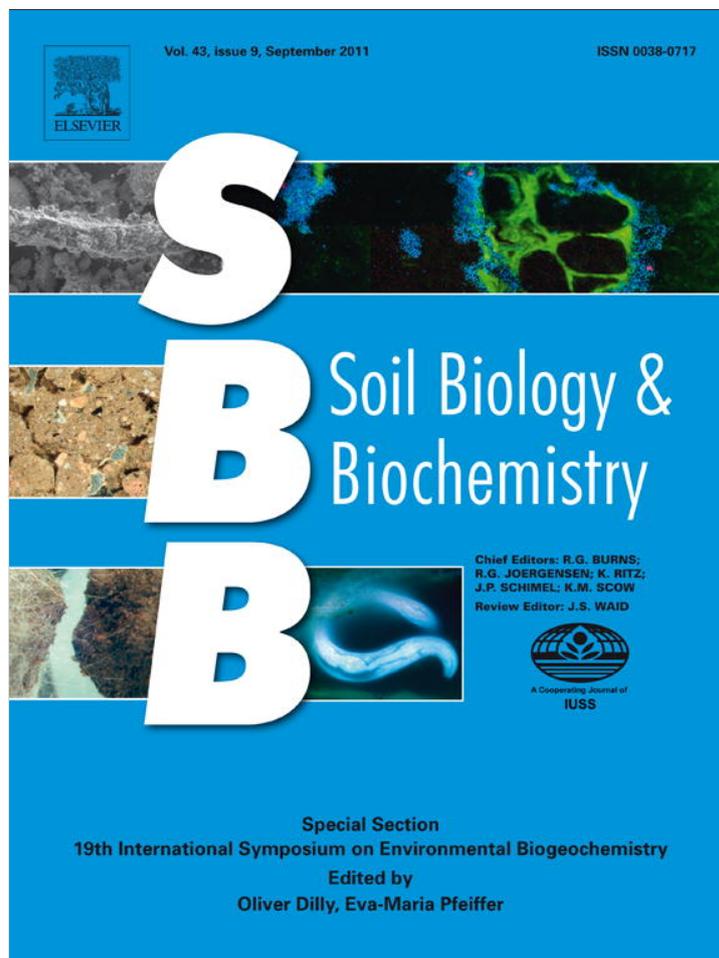


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Review

Biochar effects on soil biota – A review

Johannes Lehmann^{a,*}, Matthias C. Rillig^b, Janice Thies^a, Caroline A. Masiello^c,
William C. Hockaday^d, David Crowley^e

^a Department of Crop and Soil Science, Cornell University, Ithaca, NY 14853, USA

^b Institute for Biology, Free University of Berlin, 14195 Berlin, Germany

^c Department of Earth Science, Rice University, Houston, TX 77005, USA

^d Department of Geology, Baylor University, Waco, TX 76798, USA

^e Department of Environmental Sciences, University of California, Riverside, CA 92521, USA

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ABSTRACT

Soil amendment with biochar is evaluated globally as a means to improve soil fertility and to mitigate climate change. However, the effects of biochar on soil biota have received much less attention than its effects on soil chemical properties. A review of the literature reveals a significant number of early studies on biochar-type materials as soil amendments either for managing pathogens, as inoculant carriers or for manipulative experiments to sorb signaling compounds or toxins. However, no studies exist in the soil biology literature that recognize the observed large variations of biochar physico-chemical properties. This shortcoming has hampered insight into mechanisms by which biochar influences soil microorganisms, fauna and plant roots. Additional factors limiting meaningful interpretation of many datasets are the clearly demonstrated sorption properties that interfere with standard extraction procedures for soil microbial biomass or enzyme assays, and the confounding effects of varying amounts of minerals. In most studies, microbial biomass has been found to increase as a result of biochar additions, with significant changes in microbial community composition and enzyme activities that may explain biogeochemical effects of biochar on element cycles, plant pathogens, and crop growth. Yet, very little is known about the mechanisms through which biochar affects microbial abundance and community composition. The effects of biochar on soil fauna are even less understood than its effects on microorganisms, apart from several notable studies on earthworms. It is clear, however, that sorption phenomena, pH and physical properties of biochars such as pore structure, surface area and mineral matter play important roles in determining how different biochars affect soil biota. Observations on microbial dynamics lead to the conclusion of a possible improved resource use due to co-location of various resources in and around biochars. Sorption and thereby inactivation of growth-inhibiting substances likely plays a role for increased abundance of soil biota. No evidence exists so far for direct negative effects of biochars on plant roots. Occasionally observed decreases in abundance of mycorrhizal fungi are likely caused by concomitant increases in nutrient availability, reducing the need for symbionts. In the short term, the release of a variety of organic molecules from fresh biochar may in some cases be responsible for increases or decreases in abundance and activity of soil biota. A road map for future biochar research must include a systematic appreciation of different biochar-types and basic manipulative experiments that unambiguously identify the interactions between biochar and soil biota.

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1. Introduction

Biochar is the product of thermal degradation of organic materials in the absence of air (pyrolysis), and is distinguished from charcoal by its use as a soil amendment (Lehmann and Joseph, 2009). Biochar has been described as a possible means to improve

soil fertility as well as other ecosystem services and sequester carbon (C) to mitigate climate change (Lehmann et al., 2006; Lehmann, 2007a; Laird, 2008; Sohi et al., 2010). The observed effects on soil fertility have been explained mainly by a pH increase in acid soils (Van Zwieten et al., 2010a) or improved nutrient retention through cation adsorption (Liang et al., 2006). However, biochar has also been shown to change soil biological community composition and abundance (Pietikäinen et al., 2000; Yin et al., 2000; Kim et al., 2007; O'Neill et al., 2009; Liang et al., 2010;

* Corresponding author. Tel.: +1 607 254 1236.

E-mail address: CL273@cornell.edu (J. Lehmann).

Grossman et al., 2010; Jin, 2010). Such changes may well have effects on nutrient cycles (Steiner et al., 2008b) or soil structure (Rillig and Mummey, 2006) and, thereby, indirectly affect plant growth (Warnock et al., 2007). Rhizosphere bacteria and fungi may also promote plant growth directly (Schwartz et al., 2006; Compant et al., 2010). The possible connections between biochar properties and the soil biota, and their implications for soil processes have not yet been systematically described.

The effectiveness of using biochar as an approach to mitigate climate change rests on its relative recalcitrance against microbial decay and thus on its slower return of terrestrial organic C as carbon dioxide (CO₂) to the atmosphere (Lehmann, 2007b). Both the composition of the decomposer community as well as metabolic processes of a variety of soil organismal groups may be important in determining to what extent biochar is stable in soils, as is known for wood decay (Fukami et al., 2010). Changes in microbial community composition or activity induced by biochar may not only affect nutrient cycles and plant growth, but also the cycling of soil organic matter (Wardle et al., 2008; Kuzyakov et al., 2009; Liang et al., 2010). In addition, biochar may change emissions of other greenhouse gases from soil such as nitrous oxide (N₂O) or methane (CH₄) (Rondon et al., 2005; Yanai et al., 2007; Spokas and Reicosky, 2009; Clough et al., 2010; Singh et al., 2010; Zhang et al., 2010; Taghizadeh-Toosi et al., 2011). Such changes may either reduce or accelerate climate forcing. The driving processes are still poorly identified (Van Zwieten et al., 2009). A more rapid mineralization of indigenous soil C or greater emission of other greenhouse gases as a result of biochar additions may counteract the benefits of reduced emissions elsewhere in the life cycle of a biochar system. A systematic examination of the ways in which different microbial and faunal populations may play a role in these biogeochemical processes is still lacking.

Biochar may pose a direct risk for soil fauna and flora, but could also enhance soil health. Biochar addition may affect the soil biological community composition as demonstrated for the biochar-rich Terra preta soils in the Amazon (Yin et al., 2000; Kim et al., 2007; O'Neill et al., 2009; Grossman et al., 2010), and has been shown to increase soil microbial biomass (Liang et al., 2010; O'Neill et al., 2009; Jin, 2010). Whether the abundance of microorganisms increases or not, as discussed for mycorrhizal fungi (Warnock et al., 2007), is likely connected to the intrinsic properties of both biochar and the soil. Biochar properties vary widely and profoundly; not only in their nutrient contents and pH (Lehmann, 2007a), but also in

their organo-chemical (Czimczik et al., 2002; Nguyen et al., 2010) and physical properties (Downie et al., 2009). The role of biochar in soil biological processes therefore represents a frontier in soil science research, with many unexplained phenomena awaiting exploration. Recent advances in our understanding of biochar warrant an evaluation of the relationship between its properties and its impact on the soil biota.

In this paper, we critically examine the state of knowledge on soil populations of archaeans, bacteria, fungi, and fauna as well as plant root behavior as a result of biochar additions to soil. We develop concepts for a process-level understanding of the connection between biochar properties and biological responses, discuss the ramifications of such changes for biogeochemical processes in soil, and develop a road map for future research.

2. Modification of the soil habitat by biochar

The material properties of biochar are very different from those of uncharred organic matter in soil (Schmidt and Noack, 2000), and are known to change over time due to weathering processes, interactions with soil mineral and organic matter and oxidation by microorganisms in soil (Lehmann et al., 2005; Cheng et al., 2008; Cheng and Lehmann, 2009; Nguyen et al., 2010). However, the relationships between biochar chemical and physical properties and their effects on soil biota and potential concomitant effects on soil processes are poorly understood. This section gives a brief overview of the unique properties of biochars compared to other compounds in soil as a background to the following sections that discuss the effects of biochar on soil biota.

2.1. Basic properties: organic and inorganic composition

Biochar composition can be crudely divided into relatively recalcitrant C, labile or leachable C and ash. The greatest chemical difference between biochar and other organic matter is the much larger proportion of aromatic C and, specifically, the occurrence of fused aromatic C structures (Table 1), in contrast to other aromatic structures of soil organic matter such as lignin (Schmidt and Noack, 2000). This fused aromatic structure of biochars in itself can have varying forms, including amorphous C, which is dominant at lower pyrolysis temperatures, and turbostratic C, which forms at higher temperatures (Keiluweit et al., 2010; Nguyen et al., 2010). It is clear that the nature of these C structures is the chief reason for the high

Table 1

Physical and chemical properties of contrasting biochars relevant to biological processes in soil (Nguyen and Lehmann, 2009; Nguyen et al., 2010; Enders, Hanley and Lehmann, unpubl. data; Hockaday, unpubl. data).

Feedstock	Temperature (°C)	pH (KCl)	pH (H ₂ O)	CEC ^a (mmolc kg ⁻¹)	CEC ^a (molc m ⁻²)	C (%)	C/N ratio	Total P (mg kg ⁻¹)	Ash ^b (%)	Volatiles ^b (%)	Fixed C ^b (%)	H/C ratio ^c	O/C ratio ^c	Aromatic C ^d (% of total)	Aromatic clusters	SSA ^e (m ² g ⁻¹)
Oak wood	60	3.16	3.73	182.1	ND ^f	47.1	444	5	0.3	88.6	11.1	1.48	0.72	ND	ND	ND
	350	5.18	4.80	294.2	0.65	74.9	455	12	1.1	60.8	38.1	0.55	0.20	82.8	18	450
	600	7.90	6.38	75.7	0.12	87.5	489	29	1.3	27.5	71.2	0.33	0.07	86.6	37	642
Corn stover	60	6.33	6.70	269.4	ND	42.6	83	526	8.8	85.2	6.0	1.56	0.74	2.0	6	ND
	350	9.39	9.39	419.3	1.43	60.4	51	1889	11.4	48.8	39.8	0.75	0.29	76.9	19	293
	600	9.42	9.42	252.1	0.48	70.6	66	2114	16.7	23.5	59.8	0.39	0.10	88.2	40	527
Poultry litter	60	7.53	7.53	363.0	ND	24.6	13	16,685	36.4	60.5	3.1	1.51	1.03	ND	ND	ND
	350	9.65	9.65	121.3	2.58	29.3	15	21,256	51.2	47.2	1.6	0.57	0.41	ND	ND	47
	600	10.33	10.33	58.7	0.63	23.6	25	23,596	55.8	44.1	0.1	0.18	0.62	ND	ND	94

^a Cation exchange capacity, determined at pH 7 using buffered ammonium acetate (Nguyen and Lehmann, 2009).

^b Mass % w/w analyzed using ASTM D1762-84.

^c Molar ratios.

^d In rings, determined by direct polarization ¹³C nuclear magnetic resonance spectroscopy.

^e Specific surface area, CO₂ as sorbent (courtesy A. Zimmerman).

^f Not determined.

stability of biochars (Nguyen et al., 2010) (Fig. 1). It is less clear what precise mechanisms directly or indirectly confer stability to the aromatic C structures in soil.

The chemical stability of a large fraction of a given biochar material means that microorganisms will not be able to readily utilize the C as an energy source or the N and possibly other nutrients contained in the C structure. However, depending on the type of biochar, a fraction may be readily leached and therefore mineralizable (Lehmann et al., 2009) and in some cases has been shown to stimulate microbial activity and increase abundance (Steiner et al., 2008a). At present, such fractions may be quantified by incubation studies and are frequently referred to as “volatile matter” or the labile fraction (Table 1). Volatile matter refers to an ASTM standard methodology that was developed to evaluate the quality of coals as fuels, and is only beginning to be evaluated as a material property with explanatory value for biochar stability (Deenik et al., 2010; Zimmerman, 2010). However, such quantified volatile matter (5–37% of C in the study by Zimmerman, 2010) is typically much larger than the corresponding mineralization (2–18% of C over one year). This may indicate that the mineralizable fraction is imperfectly captured by volatile matter despite often acceptable correlation results. Further improvement to capture the fraction that is potentially bioavailable may be required.

The third major component is comprised of minerals that are present as ash inclusions in biochar. These minerals include several essential macro- and micro-nutrients for biological uptake and, therefore, represent valuable resources in the soil food web. Additionally, the presence of these elements during pyrolysis plays a role in the biochar chemical structure to the extent that they are incorporated into the aromatic structure or that organo-metal reactions are thermodynamically favorable at high temperatures. For instance, N may substitute one or two C atoms in aromatic compounds (Leinweber et al., 2007) with largely unknown effects on biochar behavior in soil. Iron (Fe)-rich biochars made from peat and investigated by ^{57}Fe Mössbauer spectroscopy show the formation of Fe_3C bonds and small ferromagnetic iron clusters at

pyrolysis temperatures above 600 °C (Freitas et al., 2002). Grasses and a number of common feedstocks (rice hulls, nut shells, sewage sludge, etc.) also contain substantial quantities of amorphous silica (>2 wt %). Analyses by ^{29}Si (silicon) NMR and X-ray diffraction shows the formation of silicon carbide (Si–C) at pyrolysis temperatures above 1200 °C (e.g., Lee and Cutler, 1975; Freitas et al., 2000), temperatures that are commonly reached during biomass gasification. The Si–C bonds likely take part in cross-links between aromatic domains or crystallites (Freitas et al., 2000). At temperatures of 400–600 °C, pyrolysis alters the chemical structure of biosilicates, with a progressive increase in SiO_4 relative to SiO_{2-3} with increasing heat treatment temperature (Freitas et al., 2000). Silicates can occupy a substantial portion (>14% for corn cobs and 88% for rice hulls) of the biochar pore space (Bourke et al., 2007; Freitas et al., 2000). Nevertheless, the influence of silicates and the effects of changes in silica crystal structure on biochar structure and function have not been investigated. The bioavailability of Fe and Si in biochar is unknown, but treatments with aqueous acid solutions used in common soil tests are effective in extracting a portion of Si, Fe, S, P, K, Mg, and Ca from biochar (Freitas et al., 2002; Bourke et al., 2007; Major et al., 2010), suggesting that some fraction of these nutrients may be accessible to plants and microorganisms.

2.2. Biochar surface properties and sorption

Fresh biochars can have net positive or net negative surface charge, but typically have initially low cation exchange capacities (CEC) compared to soil organic matter on a mass basis (Table 1; Lehmann, 2007a; Chan and Xu, 2009). Notable is the initially measurable anion exchange capacity which disappears over time in soil (Cheng et al., 2008), and in some cases strong interaction with phosphates (Beaton et al., 1960). High-ash biomass generates biochars with slightly greater CEC and charge density upon normalization of CEC to surface area (Table 1). On the other hand, greater pyrolysis temperatures cause a decrease in CEC, especially in charge density as a result of the greater surface area produced at high temperatures of up to 600 °C and loss of volatile matter (Table 1), which may contain a substantial portion of the negative charge and CEC as organic acids. Sorption of comparatively polar organic matter such as catechol or humic acid extracts to wood biochars increased in the range from 400 to 650 °C, likely due to greater nanopore surface area (Kasozzi et al., 2010). At even higher temperatures of up to 1000 °C (typically to produce so-called “activated carbon”, but also gasification biochars), carbons are mainly hydrophobic and do not sorb appreciable amounts of nutrients or polar organic substances, such as sugars (Yam et al., 1990). The same has also been observed for naturally occurring black C (Cornelissen et al., 2005). Rather, such high-temperature carbons sorb mainly non-polar or weakly polar organic solutes, notably those bearing aromatic structures, as have been described in a large number of studies in the scientific literature (Moreno-Castilla, 2004). These also include enzymes (Cho and Bailey, 1978) and other substances important for microbial processes in soil. This body of information is not fully transferable to biochars that are produced at much lower temperatures, and without the addition of chemicals that are commonly used to increase the surface area of activated carbons. Nonetheless, prior research on activated carbon may be informative for describing the properties of biochars produced at comparatively high temperatures such as during some gasification procedures and may even apply for understanding some surface properties of low-temperature biochars. In soil, biochars (those produced at or below 600–700 °C) seem to oxidize rapidly and attain greater amounts of CEC (Cheng et al., 2008; Nguyen et al., 2010), but initially still retain a significant proportion of non-polar surfaces (Smernik, 2009).

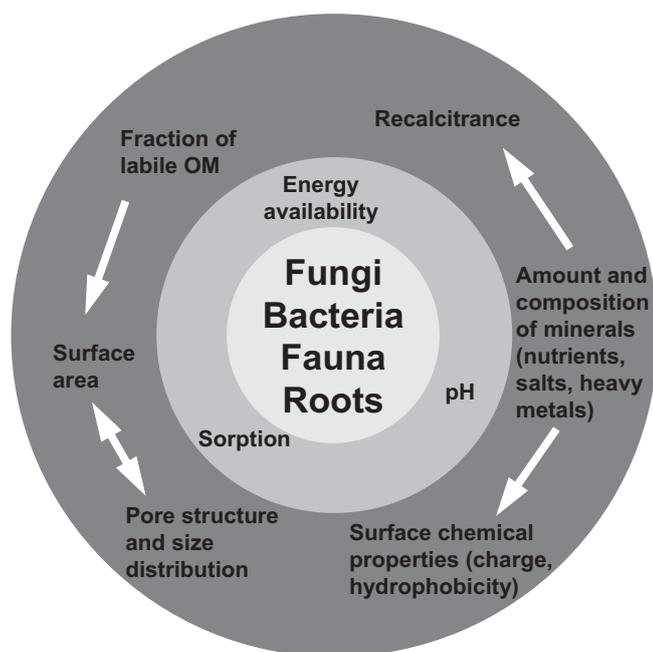


Fig. 1. Schematic overview of the connection between primary biochar properties (outer circle), the soil processes they may influence (intermediate circle) and the soil biota (inner circle) (shorter distance give a qualitative estimate of the strength of the connection). White arrows indicate influence between biochar properties.

Given that different feedstock properties range from mineral-poor woody materials to mineral-rich manures or crop residues, such as rice hulls, the resulting pH is highly variable from below pH 4 to above pH 12, even for the same biomass type (Lehmann, 2007a). Typically, biochars with high mineral ash content have greater pH values than those with lower ash contents (Table 1). For all feedstocks, pH increases with greater pyrolysis temperature. Over time, the pH of biochars may change and either decrease or increase depending on type of feedstock. Nguyen and Lehmann (2009) observed a pH decrease with mineral-poor oak wood biochar from pH 4.9 to 4.7, but an increase with mineral-rich corn stover biochar from pH 6.7 to 8.1 over the course of a one year incubation. The driving force behind a pH decrease is oxidation of C to form acidic carboxyl groups (Cheng et al., 2006), whereas the increase in pH is likely related to the dissolution of alkaline minerals.

2.3. Biochar physical properties

The effects of biochar on soil biota may be driven as much by its physical properties as by its chemical properties. The differences in physical structure between biochar and soils lead to altered soil tensile strength, hydrodynamics, and gas transport in a soil–biochar mixture; all of which can be expected to have major impacts on soil biota. The extent of these effects will depend on the biochar production conditions and feedstock, which together control the macro- and micro-structure of biochar particles (Downie et al., 2009). Whether these effects are merely a result of a mixture of two very different materials (soil and biochar) or whether biochar has a distinct effect on soil properties on a fine spatial scale has not been focus of experimentation.

When the tensile strength of biochar is less than that of soils (e.g., for clay-rich soils), biochar addition can reduce the overall tensile strength of the soil. In a pot trial with a hard-setting Chromisol (an Alfisol in USDA nomenclature), Chan et al. (2007) found a decrease in soil tensile strength from an initial, biochar-free value of 64.4 kPa–31 kPa at an amendment rate of 50 t biochar ha⁻¹; the tensile strength was again reduced to 18 kPa at 100 t biochar ha⁻¹ (Chan et al., 2007). Mechanical impedance is one of the main factors determining root elongation and proliferation in soil (Bengough and Mullins, 1990). Reductions in soil tensile strength may therefore make root and mycorrhizal nutrient mining more effective, as well as allow seeds to germinate more easily. Reduced soil tensile strength may also make it physically easier for invertebrates to move through the soil, altering predator/prey dynamics. Because a reduction in tensile strength could facilitate both increased root growth and increased root predation, it is not clear what the net effect of a reduction in tensile strength would be on root systems.

Biochar application can also change soil bulk density (e.g., Major et al., 2010); with possible effects on soil water relations, rooting patterns and soil fauna. This occurs both because the density of biochar is lower than that of some minerals, and because biochar contains macro- and micropores (Downie et al., 2009), which can hold air or water, greatly reducing the bulk density of the entire biochar particle. Surprisingly little bulk density data have been published for biochar or natural char samples. Density measurements for biochar should distinguish between the true, solid particle density and the bulk density of the biochar particles plus their pore space. Published true biochar densities are high, between 1.5 and 2.1 g cm⁻³ for a range of feedstocks (Brewer et al., 2009), whereas, bulk densities typically lie between 0.09 and 0.5 g cm⁻³ (Karaosmanoglu et al., 2000; Özçimen and Karaosmanolu, 2004; Bird et al., 2008; Spokas et al., 2009), values much lower than those of soils.

2.4. The biochar-aggregate analogy

In contrast to other organic matter in soil, biochars remain particulate over long periods of time (Skjemstad et al., 1996; Lehmann et al., 2005, 2008b), even though particle sizes may decrease on a decadal time scale (Nguyen et al., 2008). Although biochar apparently has a monolithic structure on the millimeter scale, it can be viewed, on the micro- and nanometer scale, as a disordered mixture of C clusters and mineral elements (i.e., ash inclusions). In addition, biochar particles have large internal surface areas and pores that may be important for biological processes. In this sense, the biochar particle can be compared to a soil aggregate. Biochar “aggregates” may provide similar functions such as protection of organic matter, habitat for soil biota, or retention of soil moisture and nutrients as described for aggregates made from minerals and organic matter (Tisdall and Oades, 1982).

Biochar properties such as total surface area and pore size distribution are known to vary with feedstock properties and pyrolysis temperatures (Downie et al., 2009; Table 1). In addition, surface area and pore volume may change upon contact with soil by pore clogging from sorbed organic (Pignatello et al., 2006) and mineral material (Joseph et al., 2010) or, conversely, possibly by mineralization of volatile matter that may be blocking pores. These properties have shown to change sorption behavior of mineral (Liang et al., 2006) and organic matter (Kasozi et al., 2010) which in turn may influence energy and pore space available to soil biota (Fig. 1).

Many soil microorganisms are specialists living in microhabitats that provide resources for their specific metabolic needs. For instance, aerobic microbes live at the surface of soil aggregates, while denitrifiers and semi-aquatic species dwell within the moist interior of soil peds (Sexstone et al., 1985). Organic matter decomposition rates are higher at the surface of soil aggregates than in the core of aggregates due to higher influx of resources at the surface (organic matter, moisture, and O₂). This is evident from depleted C concentrations and C-to-N ratios, as well as the oxidation of lignin phenols and the accumulation of microbial polysaccharides at the aggregate surface relative to the aggregate core (Amelung and Zech, 1996). Similarly, the exterior surfaces of biochar particles in the soil are significantly more oxidized than the particle interior or core (Lehmann et al., 2005; Liang et al., 2006; Cheng et al., 2008). This is due to sorption of organic matter on the biochar surface and the oxidation of the biochar C itself (Liang et al., 2006), both biotically and abiotically mediated via reactions with O₂ (Cheng et al., 2006, 2008). Similar to soil aggregates, the preferential oxidation of the biochar particle surface relative to the particle interior implies a limited diffusion of O₂ to the interior of biochar particles. Such differential redox conditions not only influence organic matter oxidation but also metal transformation.

3. Responses of the soil biota to biochar

The application of biochar as a targeted strategy for managing soil biota is a topic of growing interest, and inadvertent changes of soil biota as a result of biochar application are of equally strong concern. This line of research is an important one, as the health and diversity of soil microbial populations are critical to soil function and ecosystem services, which, in turn have implications for soil structure and stability, nutrient cycling, aeration, water use efficiency, disease resistance, and C storage capacity (e.g., Brussaard, 1997). Brussaard et al. (2007) suggest that organic amendments are perhaps the most important means of managing biodiversity in soils. It is well-known that the quantity, quality, and distribution of organic amendments each affect the trophic structure of the soil food web (Moore et al., 2004). Therefore, all three of these aspects

should be considered in the use of biochar as a soil management tool. Early research has focused on the effects of biochar properties at the level of primary decomposers (bacteria and fungi). Other functional groups, including secondary decomposers, predators, and soil animals, also play important roles in nutrient and energy cycling. In the following sections we consider how biochar affects soil biota on several trophic levels, including root dynamics, and discuss the reasons behind observed changes with respect to different biochar properties.

3.1. Abundance of microorganisms

Microbial abundance has been determined in biochar-amended soil by various methods including, total genomic DNA extracted (O'Neill, 2007; Grossman et al., 2010; Jin, 2010), culturing and plate counting (Jackson, 1958; O'Neill et al., 2009), substrate-induced respiration (Zackrisson et al., 1996; Steiner et al., 2004, 2009; Wardle et al., 2008; Kolb et al., 2009), fumigation–extraction (Jin, 2010; Liang et al., 2010), phospholipid fatty acid (PLFA) extraction (Birk et al., 2009), staining and direct observation of individual biochar particles (Jackson, 1958; Pietikäinen et al., 2000; Warnock et al., 2007; Jin, 2010; Fig. 2). The microbial reproduction rate has also been shown to increase in some biochar-amended soils (Pietikäinen et al., 2000; Steiner et al., 2004), and in waste water (Koch et al., 1991). Similarly, in biodigesters used to generate methane (CH₄) as an energy source, additions of biochar (commercial wood charcoal) led to an increase in anaerobic and cellulose-hydrolyzing bacterial abundance (Kumar et al., 1987).

The reasons for changes in microbial abundance may differ for different groups of microorganisms. The two most commonly occurring types of mycorrhizal fungi (arbuscular [AM] and ectomycorrhizal [EM]), are often positively affected by biochar presence, as reviewed in Warnock et al. (2007). Mycorrhizal response in the host plant is most commonly assessed by measuring root colonization; that is, the abundance of fungal tissue in the host. Both formation rate and tip number of EM infection of larch seedling roots was increased by 19–157% with biochar additions

(Makoto et al., 2010). Likewise, AM colonization of wheat roots was found to increase to 20–40% two years after *Eucalyptus* wood biochar additions of 0.6–6 t ha⁻¹, in comparison to a colonization rate of 5–20% in unamended controls (Solaiman et al., 2010). It is far less clear how the soil-borne phase of the fungus, the extraradical mycelium, is affected by biochar. Direct interactions with biochar particles could be important. For example, the internal pore systems of biochar particles may protect the extraradical mycelium from grazers (Warnock et al., 2007; Table 2 for a summary of mechanisms; Fig. 2). Sorption of signaling compounds, detoxification of allelochemicals, soil physico-chemical properties or indirect effects through alterations of other soil microbial populations have also been discussed (Warnock et al., 2007; Elmer and Pignatello, 2011). Recently, Rillig et al. (2010) found that biochar produced via hydrothermal carbonization (here called “hydrochar” due to its properties that differ greatly from pyrolysis chars) could stimulate spore germination of AM fungi, which is another mechanism potentially leading to increased populations of these symbionts in soil.

Decreases in AM abundance or relative proportion have also been observed after additions of biochar (Gaur and Adholeya, 2000; Birk et al., 2009; Warnock et al., 2010). The reasons for such decreases are not entirely clear but could stem from: (i) a reduced requirement for mycorrhizal symbiosis due to increased nutrient and water availability to plants; decreases in mycorrhizal abundance have been observed, for example, with greater P availability in soil (Corbin et al., 2003; Covacevich et al., 2006; Gryndler et al., 2006); (ii) changes in soil conditions, e.g., due to modifications of pH or water relations (discussed below); or (iii) direct negative effects from high contents of mineral elements or organic compounds detrimental to the fungi, such as high salt or heavy metal contents (Killham and Firestone, 1984; Killham, 1985). (iv) Sorption of organic C and organically-bound nutrients may influence their availability (Pietikäinen et al., 2000; Chan and Xu, 2009). The first two scenarios are specific to a certain soil environment, whereas the third is primarily a result of biochar properties which can significantly vary.

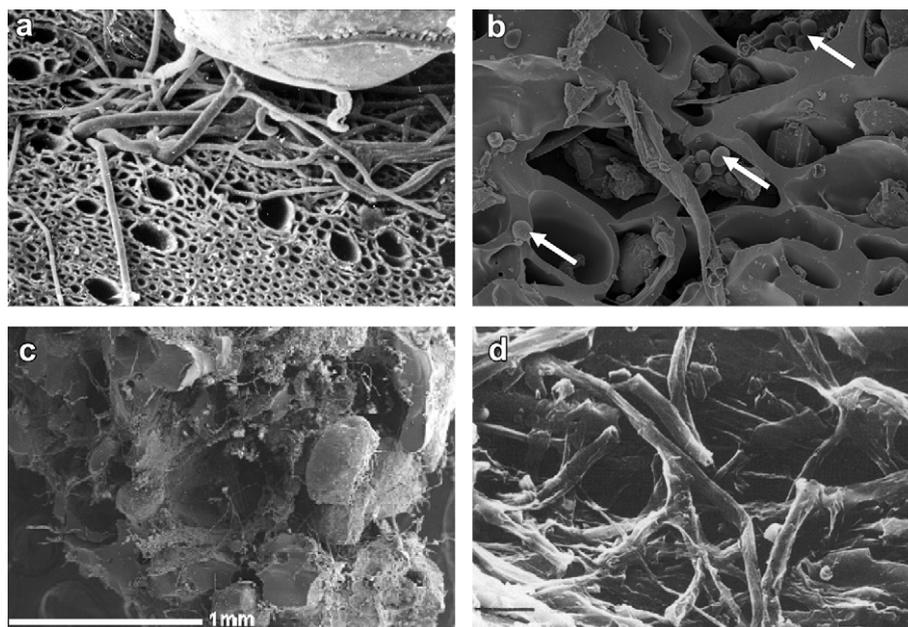


Fig. 2. Visual observation of spatial association and colonization of biochar by microorganisms. (a) fresh biochar showing fungal hyphae (Lehmann and Joseph, 2009; with permission); (b) fresh corn biochar showing microorganisms in pores (arrows) (Jin, 2010; with permission). (c) 100-year-old char from a forest fire isolated from a frigid entic Haplorthod (Hockaday et al., 2007; with permission); (d) 350-year-old char from a forest fire in a Boreal forest soil (Zackrisson et al., 1996; with permission).

Table 2

Summary of possible mechanisms by which microbial abundance is affected by biochar additions to soil; (+) indicates that relative abundance may increase (not necessarily better growth conditions), (–) indicates that relative abundance decreases.

Mechanism	Rhizobia	Other bacteria	Mycorrhizal fungi	Other fungi
Protection from grazers	nc	(+)	(+)	(+)
Improved hydration	+	+	?	? or ±
Greater P, Ca, Mg, K availability	+	+	–	–
Greater micronutrient availability	+	+	–	?
Higher pH	+	+	nc	nc
Lower pH	–	–	nc	nc or –
Sorption of signaling compounds	? or –	?	?	?
Greater N availability (also through sorption of phenolics and increased nitrification)	–	+ or –	nc	nc
Sorption of microorganisms	nc	+	nc	nc
Biofilm formation	+	+	?	?
Sorption of inhibitory compounds	?	+	?	?
Sorption of dissolved OM as an energy source for microorganisms	?	?	nc	?

nc, no change.

?, reaction not known.

Parentheses, weak circumstantial evidence.

3.1.1. Influence of nutrient and carbon availability on microbial abundance

Nutrient additions by fertilizers reduced the enhancing effect of biochars on microbial reproduction rates (Steiner et al., 2009). Similarly, Blackwell et al. (2010) found significant increases in the proportion of root colonization of wheat with AM in biochar-amended soils at no or low fertilizer additions, but no significant increases when large amounts of nutrients were applied. This effect depends on the type of fertilizer applied and the particular microorganism group. Mycorrhizal infection was reduced by P-containing fertilizers despite the presence of biochar, whereas this was not observed with fertilizers that only contained N. The reverse was observed for nodule formation by rhizobia (Ogawa and Okimori, 2010). This can be explained by the different need of the plant to form symbiotic relationships with microorganisms under changing nutrient limitations. With N fertilizer additions, the plant may not need to rely on biological N₂ fixation as much as under N limitation. Similar explanations may hold for the effect of what is likely increased C supply by exudation or root turnover in the rhizosphere and C as energy sources for heterotrophic microorganisms. Consequently, Jin (2010) found greater enhancement of microbial abundance by biochar additions in the rhizosphere than in bulk soil, whereas Graber et al. (2010) reported the opposite.

On the other hand, microbial abundance, especially that of non-symbiotic microorganisms under nutrient limiting conditions, may be increased by slightly greater nutrient availability (Lochhead and Chase, 1943; Taylor, 1951), either due to biochar-driven improvements in nutrient retention or due to nutrients that are released by the biochar. However, the abundance of symbionts such as mycorrhizal fungi has been shown to improve by alleviating N and P limitations of the fungi themselves (Treseder and Allen, 2002). In most cases, though, reductions in abundance have been found to occur for rhizobia and mycorrhizal fungi as a result of significantly greater N and P availability, respectively. Little direct evidence is available for nutrient-related effects of biochar on microorganisms (Warnock et al., 2010).

Increased micronutrient concentrations, namely of molybdenum (Mo) and boron (B), were thought to be responsible for enhanced biological N₂ fixation (BNF) by rhizobia in legumes grown in biochar (Rondon et al., 2007), but the same may not hold for abundance of rhizobia. For example, Vantsis and Bond (1950) did not find that the Mo in biochar improved nodule dry weight and activity; instead, they favored an explanation whereby biochar enhanced BNF by sorbing toxic substances, which was confirmed by Nutman (1952). Similarly, Turner (1955) identified sorption of inhibitory compounds to wood biochar to increase nodule

production and to decrease the time between inoculation and first appearance of nodules. Gibson and Nutman (1960) attributed similar findings to nitrate adsorption, which is unlikely to be the case given the low amount of anion exchange capacity typically found in biochars (Cheng et al., 2008).

The available research appears to indicate that nutrient and C availability changes may both increase or decrease microbial biomass, depending on (i) the existing nutrient and C availability in soil; (ii) the magnitude of change; and (iii) the microorganism group. This response may prove to be predictable with further experimental evidence.

3.1.2. Influence of pH on microbial abundance

Microbial biomass increases with rising pH values have been shown for a gradient from pH 3.7 to 8.3 under otherwise identical environmental conditions by Aciego Pietry and Brookes (2008). However, fungal and bacterial populations react differently to changes in pH. Bacteria are likely to increase in abundance with rising pH up to values around 7, whereas, fungi may show no change in total biomass (Rousk et al., 2010), or potentially dramatically reduce their growth at higher pH (Rousk et al., 2009). This may also apply to rhizobial infection of legumes (Angelini et al., 2003).

After biochar additions, the pH of soils may increase or decrease, depending on the pH and liming value of the biochar. Biochars can have pH values of below 4 or above 12, depending on feedstock type, pyrolysis temperature (Lehmann, 2007a; Chan and Xu, 2009) and degree of oxidation (Cheng et al., 2006), which generates very different living conditions for microorganisms in biochar pore spaces. Given the often observed spatial proximity of microorganisms and biochar surfaces (Fig. 2), the pH of biochars may therefore have a very important influence on total microbial abundance.

Rillig et al. (2010), working with hydrochars (which had an acidic pH of 4.10) observed a microbial activity-dependent increase in soil pH, due to some unidentified microbially-mediated reductions of substrates or electron acceptors. This illustrates that secondary processes may also be responsible for soil pH changes following addition of biochar. Microbial biomass assessed by substrate-induced respiration (SIR) correlated positively with pH in an acid Xanthic Ferralsol of Brazil after biochar applications (Steiner et al., 2004). This likely indicates a stimulation of microbial reproduction when the pH of these acid soils was increased by adding biochar. Similar to nutrient and C changes, the effects of pH changes induced by biochar will largely depend on the pre-existing soil pH, the direction and magnitude of change. This may be predicted from available literature but must recognize that pH measurements of

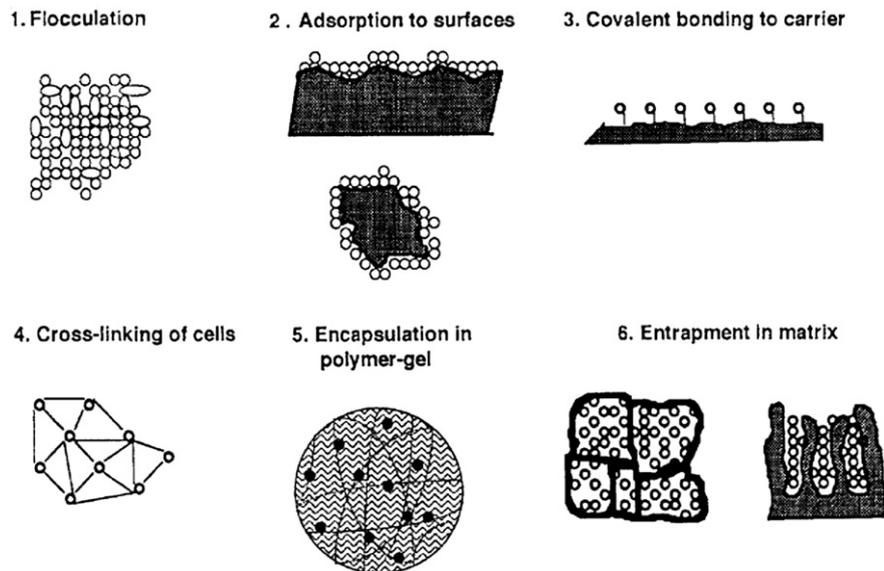


Fig. 3. Processes for possible attachment of viable microbial cells to surfaces (Cassidy et al., 1996; with permission).

bulk soils does not reflect pH values experienced by microorganisms located around biochar particles.

3.1.3. Influence of bacterial adhesion to biochar on microbial abundance

Bacteria may sorb to biochar surfaces, rendering them less susceptible to leaching in soil (Pietikäinen et al., 2000). This would increase bacterial abundance but likely has no effect on fungal abundance, as fungi will be less mobile owing to their hyphal network. Microbial sorption (called “immobilization” in the relevant literature) has been studied and used in industrial and scientific applications (Cassidy et al., 1996). The main processes leading to attachment are (1) flocculation, (2) adsorption on surfaces, (3) covalent bonding to carriers, (4) cross-linking of cells, (5) encapsulation in a polymer-gel, and (6) entrapment in a matrix (Fig. 3).

Adsorption to biochar may occur via different processes including hydrophobic attraction or electrostatic forces. At the iso-electric point, adsorption of *Escherichia coli* to demineralized activated carbon was negligible and increased with increasing hydrophobicity (Rivera-Utrilla et al., 2001). In the presence of minerals, the adsorption further increased. The iso-electric point of biochars, however, has been found to be low (pH < 4; Cheng et al., 2008). Adsorption may be facilitated by precipitates forming on carbon surfaces under an electric current (Fig. 4; George and Davies, 1988). The effect of electric currents on microbial adhesion and activity is only beginning to be explored (Bond, 2010). Since some biochars may contain large proportions of minerals, such as those produced from crop residues or animal manures, such processes might be relevant.

Adhesion may also depend on pores sizes (Rivera-Utrilla et al., 2001). Pore sizes for optimum adhesion may need to be 2–5 times larger than cell size if microorganisms are to enter the pores, or about 2–4 μm for *Bacillus mucilaginosus* and *Acinetobacter* sp. (Samonin and Elikova, 2004). Adhesion may be diminished in larger and smaller pores either because pore curvature is too large to enhance adhesion or microorganisms do not fit into the pores, respectively (Samonin and Elikova, 2004). It is therefore likely that the ability of biochars to retain bacteria will vary greatly depending on the biochar properties including the ash content, pore size, and

volatile content that are highly variable (Table 1). Formation of surfactants by microorganisms (Ron and Rosenberg, 2001) may additionally facilitate adhesion to biochars.

The mere increase of colonizable surfaces through biochar may increase the microbial biomass as shown for sediments (Yamamoto and Lopez, 1985). The specific surface area of coarse-textured soil may be increased by additions of those biochars that have large surface areas (Table 1). In how far the shapes of biochar surfaces plays a role (Yamamoto and Lopez, 1985) is not clear.

3.1.4. Biochar protection of microorganisms from other biota

Both bacteria and fungi are hypothesized to be better protected against grazers or competitors by exploring pore habitats in biochars (Ogawa, 1994; Ezawa et al., 2002; Saito and Marumoto, 2002; Thies and Rillig, 2009). No quantitative evidence is currently available for microbial protection in biochar pores, but pore size distribution of microorganisms and biochars, as well as visual investigations (Fig. 2), provide justification for this hypothesis (Thies and Rillig, 2009). As pointed out above, some quantitative

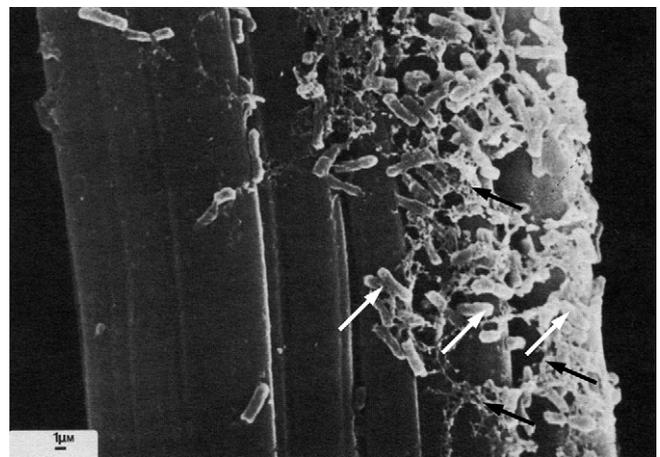


Fig. 4. Adhesion of *Escherichia coli* (white arrows) on activated carbon with the occurrence of precipitates (black arrows) from added Mg^{2+} under application of a negative potential (George and Davies, 1988; with permission).

evidence exists for the importance of pore sizes for the retention of microorganisms (Cassidy et al., 1996; Rivera-Utrilla et al., 2001; Samonin and Elikova, 2004). Targeted experimentation is needed to test whether the physical location within pores or also the adsorption to surfaces confers protection against predators.

3.1.5. Influence of sorption of toxins and chemical signals on microbial abundance

Sorption of compounds to biochar that would otherwise inhibit microbial growth may increase microbial abundance. Compounds such as catechol that are toxic to microorganisms (Chen et al., 2009) were found to be strongly sorbed to comparatively high-temperature biochars produced from ash-rich corn stover (Kasazi et al., 2010). Application of fast-pyrolysis biochar from wood powder increased AM colonization of asparagus in soils that received aromatic acids (e.g., cinnamic, cumaric, and ferulic acids) known to have allelopathic effects. Graphite and activated carbon (which may have properties similar to some high-temperature biochars with the caveats discussed earlier) were shown to increase colonization and germination of *Bacillus* strains under high salt contents on agar media (Matsushashi et al., 1995). For gonococci and meningococci, growth changes varied strongly depending on the type of carbons used and the temperature to which it was heated (Glass and Kennett, 1939). Compounds were desorbed from biochar-type substances used for preparation of an agar growth medium which proved toxic to the bacillus *Bordetella pertussis* (Pollock, 1947), indicating that growth-inhibiting substances were retained by biochars. Cell counts were several fold greater already with 0.05% (w/v) of biochar-type material mixed into the agar and did not increase with greater additions (Ensminger et al., 1953). Microbial cultures were in general shown to grow more profusely with the addition of biochar-type materials (Mishulow et al., 1953); however, the origin and properties of the charcoals used were not sufficiently described to allow further conclusions to be drawn. The authors speculated that sorption of growth-regulating compounds or bacterial cells may have played a role.

DeLuca et al. (2009) speculated whether also adsorption of signaling compounds from legumes, such as flavonoids, to biochar surfaces may render them ineffective for inducing nodule formation (Jain and Nainawatee, 2002). Interference may also occur with flavonoid signaling of AM fungi, wherein flavonoids were shown to be sorbed to activated carbon (Akiyama et al., 2005). Very little is known about how biochar might interfere with or possibly enhance the activity of signaling compounds. Given the strong sorption of organic matter to biochar surfaces (Smernik, 2009), interferences with signaling between roots and rhizosphere microorganisms is very likely. Its quantitative importance has not been explored.

3.1.6. Influence of protection against desiccation on microbial abundance

Periodic drying of soil leads to stress and, ultimately, to dormancy or mortality of microorganisms, with important differences between gram negative and gram positive bacteria, as well as between bacteria and fungi (Schimel et al., 2007). Given the large surface area of biochars (Liang et al., 2006; Downie et al., 2009) and greater water holding capacity after addition to light-textured soil (Kishimoto and Sugiura, 1985; Glaser et al., 2002), biochars may retain moist pore spaces that allow continued hydration of microorganisms in a drying soil. Malik (1990) found that survival capacity and reactivation of several oxygen-sensitive bacteria during freeze-drying was greatly increased in the presence of activated carbon, and attributed the effect partly to reduced surface tension, in addition to pH buffering and sorption of radicals. Given the greatly varying pore structures of different biochars, biochar properties will largely determine whether such processes will occur.

3.1.7. Methodological challenges and recommendations

Several important analytical challenges arise as a result of strong sorption of lysed cells, cell contents, and microbial exoenzymes to biochar. This sorption occurs during bead-beating for DNA extraction, during fumigation–extraction when measuring microbial biomass, and when using fluorometric methods to detect enzyme activity. For fumigation methods, a correction factor needs to be applied to account for the stronger sorption of lysed cell constituents to the biochar than to soil alone. Liang et al. (2010) calculated an extraction factor based on recovery of ^{13}C -labeled microbial biomass added to soil. The recovery of microbial biomass was found to be 21–41% lower in biochar-rich Terra preta soils than in biochar-poor adjacent soils. For temperate soils with additions of corn stover biochar, Jin (2010) used adsorption isotherms to correct for the sorption of dissolved organic C to biochar. After correction for sorption, estimated microbial biomass increased by over 70% at an application rate of 30 t biochar ha^{-1} . The need to correct for sorption significantly increased with greater biochar application rates (Jin, 2010).

Durenkamp et al. (2010) observed no effect on extraction efficiency when biochars were mixed briefly with soil prior to extracting soluble C. This indicates that either short-term exposure to biochars may have limited effects on extractability, or, lysed cells after fumigation are more susceptible to sorption than total soil organic matter. In the same experiment, activated carbon produced at high temperature resulted in significantly decreased extraction efficiency, suggesting that biochars with greater surface area exacerbate the sorption.

Similarly, DNA extracted from biochar-amended soils is reduced by sorption of DNA released from microbial cells to biochar and requires suitable selection of extraction kits to overcome these limitations. O'Neill (2007) found that bacterial abundance was higher in four different Terra preta soils than in adjacent soils when measured by plate counting, whereas, DNA extractions from the same soils indicated the reverse. Indeed, when biochar is added to soil, DNA yield typically decreases (Fig. 5; Jin, 2010). This has been described for various charcoals and activated carbons in the past (Rapaport et al., 1981; Gani et al., 1999). Appropriate selection of an extraction kit with the greatest extraction efficiency as well as purity of the extracted DNA (Fig. 5) is able to address this constraint (O'Neill, 2007; Jin, 2010). Use of different DNA extraction methods did not, however, affect the characterization of the soil microbial community as assessed by terminal restriction fragment length polymorphism (T-RFLP) profiling of both the bacterial and fungal communities (Jin, 2010), which affirmed the robustness of the results for these community analyses despite the challenges of extraction.

It is insufficiently quantified how biochar may interfere with other microbial measurements based on extraction of biomolecules, such as in phospholipid fatty acid (PLFA) analyses, or activity measures, such as determined with dyes during enzyme assays, etc. Enzyme interactions with biochars have been studied by Bailey et al. (2010) who recommended the use of fluorescence studies due to possible sorption making color reactions less reliable. They tested whether the enzyme or the substrate or both were likely to become sorbed to biochar particles and found sorption varied for a range of both enzymes and substrates, making it difficult to draw general conclusions. It is highly likely that the nature of the biochar tested will also alter these results. In contrast to Bailey et al. (2010), Jin (2010) showed that fluorogenic molecules such as 4-methylumbelliferone (MUF) and 7-amino-4-methylcoumarin (MCA) are sorbed strongly to biochar, especially within the first 30 min of incubation with a MUF- or MCA-labeled substrate. Such interferences should be considered more rigorously in the future for all other extraction or staining methods, as most will require

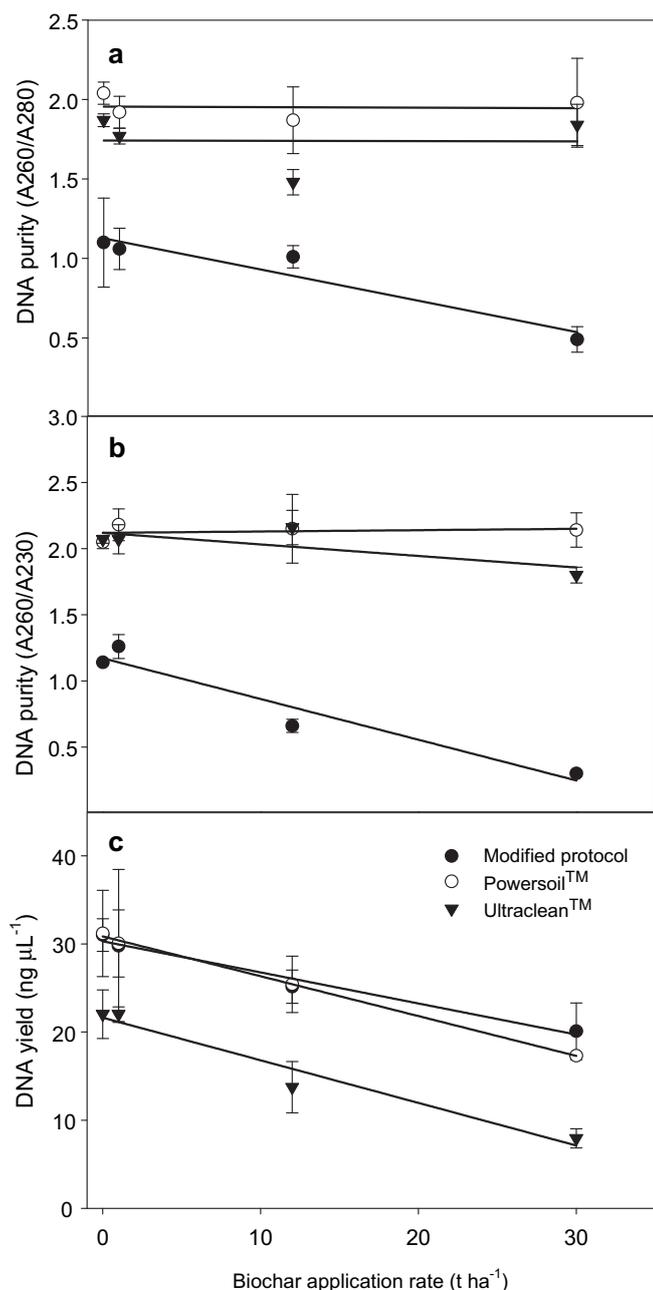


Fig. 5. Purity of DNA extracts with respect to co-extraction of (a) proteins or (b) humic extracts, and (c) efficiency of extraction (yield) using three different protocols (modified from LaMontagne et al., 2002; Powersoil™ and UltraClean™ DNA extraction kits from MoBio Laboratories, Carlsbad, CA, USA) as a function of biochar application rates ($n = 3$; means and standard deviation) (Jin, 2010).

methodological adaptations before their use in characterizing biochar-amended soils and should include recognition of the vastly different properties of different biochars. Failure to recognize that modifications to well-known methods are necessary will propagate erroneous results that will be difficult to rectify a posteriori and may confound the literature in this research area.

3.2. Community structure of microorganisms

Given that biochar induces changes in microbial biomass, it is extremely unlikely that such overall changes in abundance are spread equally across different phylotypes or functional groups.

Instead, the altered soil environment, either in terms of an altered resource base (e.g., available C, nutrients, water), shifts in abiotic factors (e.g., pH, toxic elements), or different habitat as discussed above may cause some microbial groups to become competitively dominant, leading to changes in community composition and structure. Additionally, changes in trophic relationships (as a consequence of changes in soil biota abundances higher up in the soil food web; see Section 3.3) may cause top-down effects that constrain certain microbial groups.

Consequently, studies on (i) Terra preta soils, (ii) soils rich in char from vegetation fires, and (iii) soils amended with biochar have shown significant changes in community composition and diversity of both fungal, bacterial, and archaeal populations (Kim et al., 2007; Otsuka et al., 2008; O'Neill et al., 2009; Graber et al., 2010; Grossman et al., 2010; Jin, 2010; Taketani and Tsai, 2010; Khodadad et al., 2011). Bacterial diversity was greater by as much as 25% in biochar-rich Terra preta soils compared to unmodified soils in both culture-independent (Kim et al., 2007) and culture-dependent (O'Neill et al., 2009) studies, with high diversity reported at both the genus and species (Kim et al., 2007) and at the family (O'Neill et al., 2009) taxonomic levels. However, bacterial diversity was found to be lower in burned and unburned forest soils amended with oak or grass biochar (Khodadad et al., 2011). Lower diversity of archaea (Taketani and Tsai, 2010) and fungi (Jin, 2010) were found in Terra preta and a biochar-amended temperate soil, respectively, compared with unmodified soils, which indicates that different microbial groups respond in different ways. Time since biochar incorporation also differed between all of these studies, with Khodadad et al. (2011) and Jin (2010) being short-duration studies of six months and 2.5 years, respectively, whereas for Terra preta, biochar was incorporated hundreds to thousands of years ago.

3.2.1. Community composition

Bacterial community composition in soils high in black C or biochar differs significantly from that in unmodified soils with the same mineralogy (Kim et al., 2007; O'Neill et al., 2009; Grossman et al., 2010; Jin, 2010). Kim et al. (2007) compared a biochar-enriched Terra preta soil with a pristine forest soil, both sampled from the Western Amazon, by use of oligonucleotide fingerprint grouping. A higher number of unique operational taxonomic units (OTUs) was resolved for the Terra preta (396) as compared to the forest soil (291), with the former displaying a 25% higher taxonomic diversity based on several diversity indices. When the forest soil was compared with Terra preta using overall community similarities at different phylogenetic distances, all of the OTU diversity in the forest soil was found to be represented in the Terra preta. In contrast, when the Terra preta soil was compared to the adjacent forest soil, the Terra preta soil was found to contain additional sequences that did not occur in the forest soil. The greatest differences between the communities in the two soils were found at an evolutionary distance of 5%, suggesting that these differences were primarily at the level of genus and species.

In culture-based studies, O'Neill et al. (2009) observed a higher taxonomic diversity of organisms in the biochar-enriched Terra preta soils from four locations in the Central Amazon as compared to adjacent unmodified soils. The greatest taxonomic differences were at the family level. Grossman et al. (2010) showed that bacterial community composition was most similar among three Terra preta soils formed on Oxisols (divergence 40–70%), which diverged by over 80% from populations in their respective, unmodified, adjacent soils (Fig. 6). The soils differed in land use history, current land use, years since formation and other characteristics. Yet, the historical biochar enrichment of the Terra preta soils, hundreds to thousands of years ago, remained the major driver of bacterial community composition, irrespective of current land use, soil texture, soil

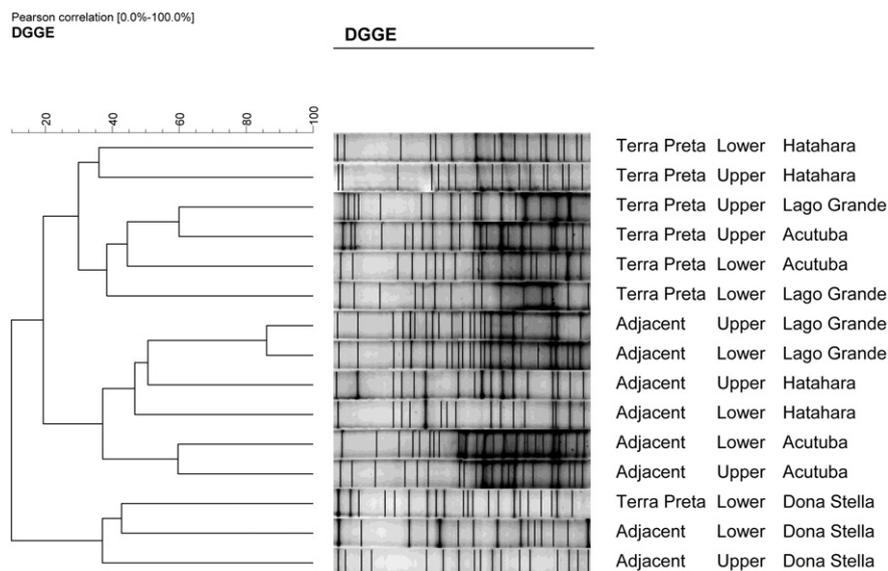


Fig. 6. Cluster analysis of T-RFLP fingerprints, based on PCR amplified bacterial 16S rRNA genes, derived from four paired biochar-enriched anthrosols and adjacent unmodified soils of the same mineralogy (Grossman et al., 2010; with permission).

mineralogy, soil nutrient contents or pH with the notable large difference induced by the Spodosol (Fig. 6). While a few additional OTUs were resolved in T-RFLP fingerprints from some of the adjacent soils, there was no clear evidence from this analysis that the biochar-enriched soils were significantly more or less diverse than their respective adjacent soils; rather, it was the species composition of the communities in the biochar-enriched soils that changed dramatically.

Jin (2010) demonstrated that increasing rates of biochar addition to a temperate soil led to increasing divergence in bacterial community composition, in both corn rhizosphere and bulk soils (Fig. 7). The rhizosphere soils with high biochar application rates (12 and 30 t ha⁻¹) were the most dissimilar to bulk soils with little or no biochar application (0 and 1 t ha⁻¹); conversely, the bulk soils receiving high rates of biochar were most similar to the rhizosphere

soils where no or low biochar was applied. The results suggest that biochar additions change soil properties such that they support communities similar in some respects to the rhizosphere communities examined where biochar was not applied.

3.2.2. Taxonomy

Kim et al. (2007) found two possible new clades of the *Acidobacteria* in Terra preta soils; whereas O'Neill et al. (2009) found isolates from Terra preta soils representing two possibly new clades in the α -*Proteobacteria*. Sequences obtained in the aforementioned studies reveal that the *Acidobacteria* were well-represented in both soil types (Kim et al., 2007; Grossman et al., 2010). Grossman et al. (2010) reported that most of the sequences obtained from the Brazilian soils sampled were novel and matched those in databases at less than 98% similarity. Several sequences obtained only from the biochar-enriched Terra preta soils grouped at 93% similarity with the *Verrucomicrobia*, a genus commonly found in rice paddies in the tropics but increasingly detected in a variety of soils. In this study, however, sequences closely related to *Proteobacteria* and *Cyanobacteria* sp. were recovered only from adjacent soil samples. Sequences related to *Pseudomonas*, *Acidobacteria*, and *Flexibacter* sp. were recovered from both Terra preta and adjacent soils.

In a temperate soil with and without additions of biochar made from corn stover, 70% of the sequences obtained were classified as *Ascomycota*, *Basidiomycota* or *Zygomycota* (Jin, 2010). However, the relative gene frequency of the main phylotypes detected differed between biochar-amended and unamended soils, with a less genetically diverse community found in the biochar-amended soils. Similarly, Taketani and Tsai (2010) found a less diverse archaeal community in Terra preta soils, particularly of ammonia-oxidizing Chrenarcheota. To date, only preliminary information exists about the shifts in population of ammonia-oxidizing bacteria in response to biochar accumulation that may be connected to changes in pH (Ball et al., 2010), which require further experimentation.

Biochar-amended soils had several fold more fungi classified as *Zygomycota* known as glucose and cellulose degraders and *Glomeromycota* being able to form mycorrhizae, while also having 31% lower abundance of *Basidiomycota* and 37% lower abundance of *Ascomycota*, than unamended control soils (Jin, 2010). Some *Ascomycota* are known for their ability to degrade lignin but also include

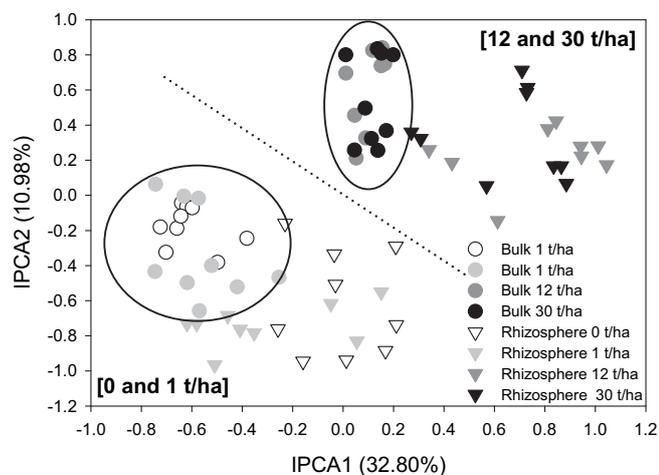


Fig. 7. Multivariate analysis of bacterial 16S rRNA gene T-RFLP profiles using HhaI restriction enzymes. Clear separation of profiles based on the rate of biochar applied (separated by a dashed line; IPCA1 and 2) and bulk vs. rhizosphere (separated by circles, IPCA1) is evident (redrawn after Jin, 2010). Biochar was produced from corn stalks at 550 °C and applied to a loamy Alfisol cropped to corn in May 2007. Soil samples were taken from 0 to 0.15 m depth in October 2008.

many sugar fungi that utilize simple substrates. Lack of available C in the biochar particles themselves may discourage colonization by these latter fungi, whereas dissolved organic C sorbed to the biochar surface may selectively enrich for *Zygomycota*, apparently finding enough easily degradable C sources.

Similarly, bacterial community changed with biochar additions. In response to high-temperature biochars (oak wood and grass pyrolyzed at 650 °C) bacterial diversity increased overall diversity and specific taxa, in contrast to results from biochars made at 250 °C (Khodadad et al., 2011). The relative abundance of *Actinobacteria* and *Gemmatimonadetes* was reported to increase in biochar-treated soils suggesting changes of the community composition in response to the more recalcitrant biochar (Khodadad et al., 2011), consistent with studies on Terra preta (O'Neill et al., 2009) and on char layers after forest fires (Bääth et al., 1995). This may suggest testing for a differential response of fungal and bacterial taxa with respect to their preferred energy sources. Although it is too early to draw definite conclusions, the available data provide ample grounds for interesting hypotheses.

Very little is known about changes in abundance of specific microorganisms (Graber et al., 2010). Ball et al. (2010) found only weak evidence that certain ammonia-oxidizing bacteria are affected by biochar accumulation. Even less information is available on the effects of different biochars, which have shown to result in changing abundance of different microbial taxa (Khodadad et al., 2011).

3.3. Functional ecology of microorganisms with biochar

Many soil processes may be affected by additions of biochar. Denitrification and methane oxidation (Yanai et al., 2007; Van Zwieten et al., 2009), C mineralization (Kuzyakov et al., 2009; Liang et al., 2010) and nutrient transformations (DeLuca et al., 2009) were all found to either increase or decrease in the presence of biochar. The reasons for such responses may be numerous. These include altered C sources or nutrient availability, sorption of inorganic and organic compounds including enzymes, different soil water retention and infiltration properties or changes in pore architecture. Here, we consider changes that are mediated by microorganisms in soil: alterations of soil processes as a result of (i) a changing microbial population structure and abundance, and (ii) a direct change in activity and metabolism induced by an altered physical and chemical environment. In some cases, the distinction between these two types of responses may be blurred or may even influence each other. From the perspective of improving our understanding of the underlying processes, such a differentiated view may be an appropriate starting point.

3.3.1. Mineralization of biochar

As discussed above, the microbial community may show significant responses to biochar additions. A greater microbial abundance may potentially lead to greater mineralization or oxidation of biochar itself as shown for mineralization of non-pyrolyzed organic C which is typically stimulated by a greater microbial biomass (Carney and Matson, 2005). In several reports, however, experimental results have rather indicated the opposite and not only a lower metabolic quotient (the ratio of microbial activity as measured by CO₂ production to microbial biomass; Liang et al., 2010; Jin, 2010), but also a lower absolute amount of respired C or C turnover (Murage et al., 2007; Kuzyakov et al., 2009; Spokas et al., 2009; Liang et al., 2010; Kimetu and Lehmann, 2010; Jin, 2010) or no change (Zackrisson et al., 1996; Haefele et al., 2009; Steiner et al., 2009; Van Zwieten et al., 2010b; for compost, Steiner et al., 2010). This could result from lower amounts of available C sources, either due to the presence of stable biochar or due to the sorption of organic C that would otherwise be easily degraded. In contrast, after additions of

fresh biochar mainly produced from dairy and bull manure, increases in both total respiration and metabolic quotient were observed (Kolb et al., 2009). Possible explanations for this behavior are the high nutrient contents of the manure-based biochar including N and P, and a significant proportion of labile organic C in the biochar as indicated by the low substrate-induced respiration at high biochar additions (Kolb et al., 2009). Deenik et al. (2010) and Zimmerman (2010) found a direct and positive relationship between the amount of volatile, and hence labile, organic matter in biochar and CO₂ evolved in an incubation experiment. Both processes mentioned above, i.e., increases in nutrients and labile C, will likely result from biochar additions to soil, and the net effect on biochar mineralization will depend on the proportion of labile C and the nutrient contents in the biochar applied as well as inorganic nutrients available from the soil.

It is reasonable to expect an influence of an altered microbial community structure on the stability of biochar, as well. An observed shift to a greater abundance of fungi after biochar accumulation in soil may indicate the potential for greater mineralization of biochar itself. White rot fungi are known to degrade lignin in woody biomass and coal (Willmann and Fakoussa, 1997; Hofrichter et al., 1999; Derenne and Largeau, 2001; Hofrichter, 2002). An adaptation of the microbial population to available energy sources is a sensible hypothesis. Interestingly, within the fungi a shift toward taxa that prefer glucose as an energy source may be hypothesized, whereas, the opposite was true for bacteria (Section 3.2.2). It is not clear that the much greater recalcitrance of biochar warrants an adaptation to this food source since more labile organic matter (particulate organic matter, litter, etc.) is likely still abundant in all soil environments. With this background it is understandable that mineralization of biochar did not increase as a result of labile C additions, but rather the mineralization of existing non-pyrolyzed C (Liang et al., 2010).

3.3.2. Effect of biochar on mineralization of other organic matter in soil

A change in microbial abundance and community structure may affect not only biochar mineralization itself, but also mineralization of other soil C. The commonly observed greater microbial biomass has been presented as a reason for a greater decomposition of soil C (also called priming) in the presence of biochar (Wardle et al., 2008). The fact that this has generally not been observed beyond an initial greater mineralization after fresh biochar additions (Hamer et al., 2004; Wardle et al., 2008; Zimmerman et al., 2011) suggests different explanations for the C loss observed in these studies that may instead be related to physical export of C, changes in nutrient contents or pH (Lehmann and Sohi, 2008). Also, labile substances in biochars (such as condensable volatiles as found in smoke) may stimulate microbial activity shortly after biochar application to soil (Fischer and Bienkowski, 1999; Uvarov, 2000; Das et al., 2008; Steiner et al., 2008a), but these are mineralized within a relatively short period of time (Cheng et al., 2006). Longer incubations (beyond one year) and field trials have shown that biochars decrease mineralization of other soil C (Kuzyakov et al., 2009; Kimetu and Lehmann, 2010; Zimmerman et al., 2011). However, the conundrum of greater microbial biomass yet lower soil C respiration still warrants closer examination. Interestingly, similar observations of greater microbial biomass yet lower metabolism have been made in waste water treatment, where biofilms on sand showed greater removal and mineralization rates of dissolved aromatic C than biofilms on activated carbons (Koch et al., 1991) that typically have large surface areas (Downie et al., 2009).

It is possible that CO₂ precipitates as carbonates on biochar surfaces that have high pH and abundant alkaline metals, which would explain reduced detection of CO₂ evolved, despite measured

increases in microbial biomass. This is not further examined here, since it is mainly an abiotic precipitation reaction. What is discussed in more detail here is the possibility that changes in the microbial community composition or in enzyme activities are responsible for lower mineralization of soil C observed with biochar additions. The activity of two carbohydrate-mineralizing enzymes was shown to decrease after biochar additions to soil (Jin, 2010; Fig. 8). Maximum velocity of both glucosidase and cellobiosidase decreased to very low levels with an application rate of 12 t biochar ha⁻¹ or greater. Similar decreases in glucosidase activity were also observed with purified enzymes and fast-pyrolysis biochar produced from switchgrass (Bailey et al., 2010). Given the responses shown in Fig. 8, application rates between 1 and 12 t ha⁻¹ will likely show significant decreases in the activity of some C-mineralizing enzymes. One explanation for such change and the associated decrease in respiration may be a co-location of C and microorganisms on biochar surfaces that may improve efficiency and reduce the need for enzyme production. As seen from greater microbial biomass and the visual assessments of microorganisms colonizing biochars (Fig. 2), soil biota are in close contact with biochar surfaces. A decrease in enzyme activity by mere sorption to biochar is less likely as shown first by Nelson and Griffin (1916) for non-activated charcoal. For example, lipases have been shown to sorb well to activated carbon matrices with long life and high activity (Quirós et al., 2011). So-called “immobilization” of enzymes on materials such as biochar is by now used in many industrial processes that allow stable conditions for optimum enzyme activity (Novick and Rozzell, 2005).

In addition, biochars can sorb large amounts of soil organic C as shown from batch experiments with microbial cells (Liang et al., 2010), plant (Miura et al., 2007) or dissolved organic C (DOC) extracts (Jin, 2010), leaching studies from forest organic horizons watered with birch litter extracts (Pietikäinen et al., 2000), and direct observations of biochar surfaces using high-resolution NEXAFS spectroscopy (Lehmann et al., 2005). These findings are consistent with the large number of observations often showing strong sorption of organic compounds such as polyaromatic hydrocarbons to a variety of black C substrates in soils or sediments (Cornelissen et al., 2005; Koelmans et al., 2006; Smernik, 2009). Such sorption may apply both to C from plant litter as well as to microbial metabolites, may be kinetically limited and therefore increase over time (Kasozi et al., 2010), but may be weaker than that documented for polyaromatic compounds (Pignatello et al.,

2006) or toxins in medical applications (Levy, 1982). This demonstrated co-location of substrate, nutrients (Section 2) and microorganisms may result in greater C use efficiency, and thus less respired C, which is also supported by lower enzyme activity of C-hydrolyzing enzymes along with higher activity of alkaline phosphatase on biochar surfaces (Fig. 9). Biochar particles seem to generate a micro-location in the soil that optimizes resource use for microbial growth. Such a co-location is also well-described in waste water treatment where organic chemicals had lower toxicity effects on microorganisms and were metabolized much quicker when sorbed to activated carbons (Ehrhardt and Rehm, 1985). Similar observations were made using trickling filters with activated carbons which were shown to be more efficient in metabolizing added compounds than the same microbial community in aqueous batch cultures (Kaplan and Kaplan, 1985). Whether the same mechanism also applies to the soil environment and biochars is not certain. It is also possible that such a mechanism leads to opportunities for the well-described syntrophism observed in biofilms (Schink, 1997), where end products from one group of microorganisms are readily utilized by another.

Alternatively, the sorption of soil organic C by various processes to biochar surfaces may be strong enough to reduce its availability as speculated by Liang et al. (2010) and, hence, to decrease the ability of exoenzymes to contact, assume proper spatial orientation with and break down the sorbed C. The lower mineralization of herbicides and pesticides sorbed to biochars (Yang et al., 2006; Yu et al., 2009) and activated carbons (Yang et al., 2009) in soils may

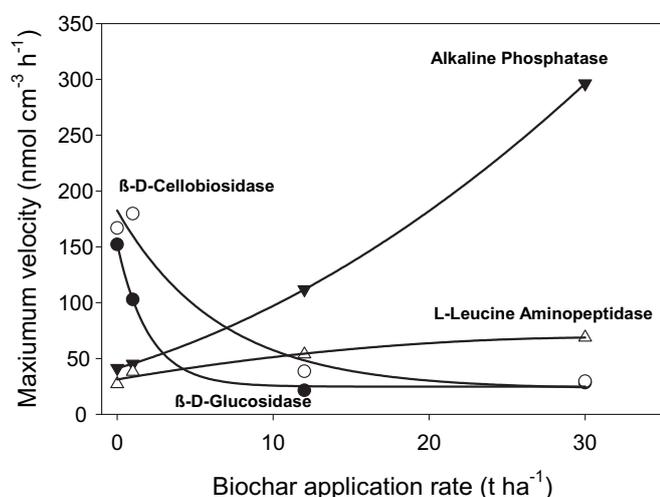


Fig. 8. Activity of different soil enzymes (0–0.15 m) one year after application of corn stalk biochar (slow pyrolysis at 550 °C) at rates of 0, 1, 12, 30 t ha⁻¹ to a loamy Alfisol cropped to corn (drawn after Jin, 2010).

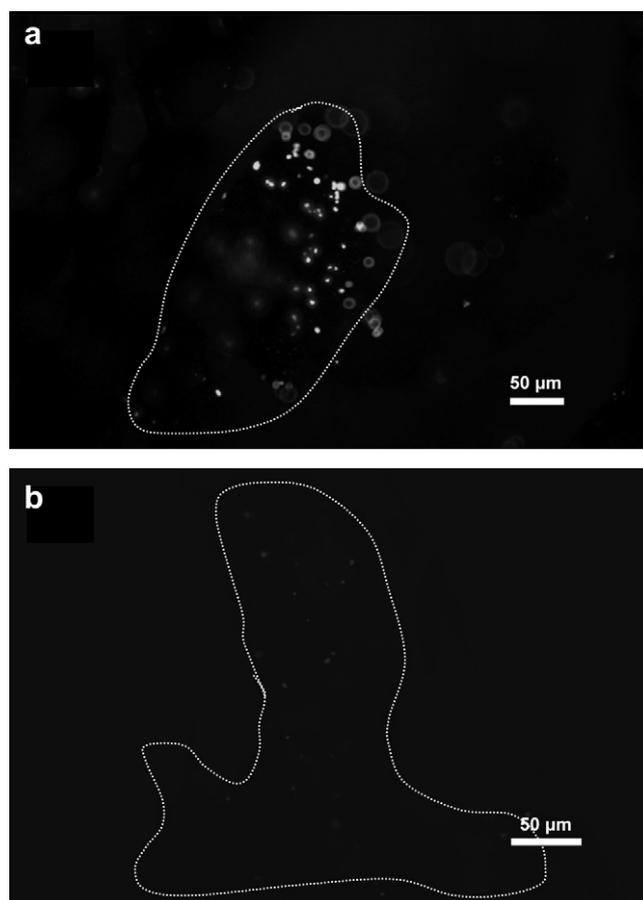


Fig. 9. Activity of the enzyme alkaline phosphatase on biochar particle surfaces (corn stalk biochar from slow pyrolysis at 550 °C), visualized by fluorescence microscopy; (a) soil with biochar, (b) soil without biochar (Jin, 2010).

provide supporting information for a decreased substrate bioavailability in general. Even a hardening of microbial biofilms on activated C surfaces may need to be considered, as a result of microbial polysaccharides production (Andrews and Tien, 1981). However, given the increase in microbial biomass after biochar additions to soil and the more recent evidence of co-location of enzymes, organic matter and microorganisms on biochar surfaces as discussed above, the microbiologically mediated process of improved resource use as discussed above, seems a more likely explanation for observed decreases in CO₂ evolution.

Substrate use patterns of microorganisms may also change through biochar additions to soil as shown with Biolog[®] assays of a forest organic horizon (Pietikäinen et al., 2000). Whether this is due to changes in microbial populations or sorption of substrate or enzymes, is not entirely clear, but may additionally suggest an effect of a changing population. A dominance of certain groups of microorganisms, such as coenocytic fungi degrading simple C compounds (e.g., *Zygomycota*) was observed when corn biochar was added to a temperate Alfisol, whereas, abundance of septate fungi (such as *Basidiomycota* (known lignin degraders) and *Ascomycota*) decreased (Jin, 2010). An increase in fungi that metabolize simpler sugars would be in accordance with greater microbial biomass and sorption of labile C compounds on biochar surfaces, rather than the inaccessibility of sorbed organic matter. Possibly, a lower abundance of degraders of more complex compounds could result in lower decomposition of lignin or aromatic structures of the biochar itself, increasing its stability, which should be tested in future experiments.

3.3.3. Effect of biochar on nutrient transformation

Biochar can have significant effects on microbially-mediated transformation of nutrients in soil. In forest soils, nitrification was increased by biochar additions to soil (DeLuca et al., 2002, 2006; Berglund et al., 2004; Gundale and DeLuca, 2006; MacKenzie and DeLuca, 2006; Ball et al., 2010) and explained by sorption of phenolics that would otherwise inhibit nitrification (Zackrisson et al., 1996; Wardle et al., 1998; Wallstedt et al., 2002; DeLuca et al., 2006) and an increase in ammonia-oxidizing bacteria (Ball et al., 2010). Whether the observed change in ammonia-oxidizing community composition (Ball et al., 2010) played a role, it not clear. Changes in pH that may trigger similar responses in soil were not able to explain the observed changes in nitrification (DeLuca et al., 2006). On the other hand, biochar additions to agricultural and grassland soils have shown no changes or even decreases in net N mineralization (DeLuca et al., 2006; Rondon et al., 2007) and lower N availability for plants (Lehmann et al., 2003), likely as a result of N immobilization during mineralization of a labile fraction of the biochar bearing a high C/N ratio (Deenik et al., 2010). In fact, the greater the mineralizable fraction of biochar (often quantified and described as volatile matter), the greater the N immobilization with resultant decreases in N uptake and growth of crops (Deenik et al., 2010). A larger microbial biomass observed with biochar additions will certainly contribute to both effects.

In addition, activity of alkaline phosphatase, aminopeptidase and N-acetylglucosaminidase was found to increase with biochar applications (Bailey et al., 2010; Jin, 2010). Alkaline phosphatase increased by 615% and aminopeptidase by 15% with increasing rates of corn biochar application to an Alfisol (Fig. 8; Jin, 2010). This is in contrast to the decreases in cellobiosidase and glucosidase discussed earlier. Possibly, plant uptake of N and P and growth of fine roots and root hairs into biochar pores (as discussed below) stimulate the production of organic N and P mineralizing enzymes. However, N-acetylglucosaminidase activity was also decreased in the absence of plant roots (Bailey et al., 2010). The observation that biochar induces changes in the bacterial community similar to

rhizosphere effects (Fig. 7) indeed suggests a broader effect than merely N and P limitation due to plant nutrient uptake as an explanation for the greater enzyme activity.

3.3.4. Nitrous oxide and methane production

The observed varying effects of biochar on N₂O and CH₄ production (Rondon et al., 2005; Yanai et al., 2007; Spokas and Reicosky, 2009; Clough et al., 2010; Singh et al., 2010; Van Zwieten et al., 2010b; Zhang et al., 2010; Knoblauch et al., 2011; Scheer et al., 2011; Taghizadeh-Toosi et al., 2011) could at least partially be explained by a changing microenvironment for the microbial population. Biochar may change water relations (Ayodele et al., 2009; Hidetoshi et al., 2009; Busscher et al., 2010), which could conceivably decrease or increase O₂ availability, thereby modifying non-CO₂ GHG emissions (Singh et al., 2010; Van Zwieten et al., 2010b; Zhang et al., 2010). Also N availability may increase or decrease as discussed above. Evidence for reduced nitrous oxide emissions from urine patches was found by isotope tracing to be at least partially caused by lower N availability after biochar additions to a New Zealand pasture (Taghizadeh-Toosi et al., 2011).

Similarly, C availability for microorganisms may change, which depends on the net effects of C sorption (Miura et al., 2007; Liang et al., 2010), litter production (Major et al., 2010) and the interactions between water and N availability. In addition to a range of possible abiotic effects including catalytic reduction with minerals or radicals (as discussed by Van Zwieten et al., 2009) and adsorption of NH₃ (Asada et al., 2006), changes in the dominance of either bacterial or fungal communities may play a role in greenhouse gas production, but no concrete evidence has yet been presented.

In biodigesters, the abundance of anaerobic bacteria was shown to increase and, as a result, enhance biogas formation when biochar (commercial charcoal) was added (Kumar et al., 1987). This result was also obtained with activated carbon (Hunsicker and Almeida, 1976; Kumar et al., 1987), but not with graphite, carbon black (fossil fuel soot) or petroleum coke (Kumar et al., 1987). The reason for this increased abundance of anaerobic bacteria under anaerobic conditions in slurries may be similar to the effects discussed for microbial abundance in general, including greater resource supply of C substrates and nutrients, more stable physical conditions, better pH buffering and possibly sorption and neutralization of harmful substances.

Recently, ethylene found to be generated by fresh biochars may be linked to decreases in N₂O production (Spokas et al., 2010). Ethylene is both part of the remaining non-aromatic compounds in fresh biochars and is produced by microorganisms in the presence of biochar, which may also partly explain observed decreases in CO₂ production (Spokas et al., 2010). Non-woody biochar materials produced at lower temperatures were found to generate ethylene at significantly greater rates than soil alone, whereas woody biochars and activated carbons may have sorbed the generated ethylene. In the presence of microorganisms ethylene production was 215% greater than in sterilized soil (Spokas et al., 2010). Ethylene may regulate a series of soil processes (Abeles et al., 1992; Frankenberger and Arshad, 1995) which need to be investigated further.

3.3.5. Microorganisms, biochar and plant growth

Changes in microorganism occurrence and resulting direct effects on plant growth are only beginning to be explored. Graber et al. (2010) demonstrated through phylogenetic characterization of bacterial isolates based on 16S rRNA gene analysis that of the 20 unique identified isolates from the biochar-amended growing media cropped to pepper and onion, 16 were affiliated with previously described plant-growth-promoting and biocontrol agents. The genus *Trichoderma*, known for including plant-growth-promoting species, was only isolated from the rhizosphere of pepper when

biochar had been added. A possible explanation for the observed greater crop growth observed by Graber et al. (2010) was therefore the promotion of beneficial microorganisms in the rhizosphere.

3.3.6. Electrochemical reactions and biochar

Carbon materials that are mostly produced at high temperatures above 1200 °C can have a range of electrochemical properties (Portet et al., 2007). The extent to which these properties may be selected for by choice of different feedstocks and pyrolysis temperatures is largely unknown (Joseph et al., 2010). However, there is a wealth of knowledge on the electrochemical properties of carbon (McCreary, 1999). There is also considerable new work on carbon nanotubes and carbon-based nanomaterials that demonstrates the types of reactions that can be catalyzed on carbon surfaces. Liu et al. (2005) demonstrated direct electron transfer to glucose oxidase by carbon nanotubes. In another process of relevance to extracellular redox reactions, studies with the bacterium *Shewanella oneidensis* have shown facilitated electron transfer rates from cytochromes located in the bacterial outer membrane via carbon nanotubes to extracellular electrodes (Peng et al., 2010). Similar processes could be envisioned with biochar.

Within individual particles of biochar, electrochemical properties could be expected to be highly variable across microsite locations as the local surface properties within a biochar particle will vary depending on the chemical structures that were pyrolyzed (Amonette and Joseph, 2009). The different functional groups, binding of metals, and metal oxide precipitates will further change the electrical conductivity of carbon surfaces. When carefully controlled, carbon surfaces can be generated with different surface oxides and electrochemical behavior. Many properties such as those conveyed by different metal oxides may be largely irreversible. These properties ultimately lead to electro-catalytic surfaces that can promote electron transfer (Joseph et al., 2010). The extent to which this would affect various biologically driven redox processes on biochar surfaces is a promising research area.

3.4. Faunal population and biochar

The soil fauna are among the least well-studied components of the soil biota with respect to biochar effects. This is unfortunate, since soil fauna may be important in at least three ways. First, soil animals are part of the fungal and bacterial energy channels in the soil food web (Cragg and Bardgett, 2001), and as such, they may provide top-down control that is important in order to understand microbial responses to biochar additions. Second, geophagous organisms, such as earthworms, could be important modifiers of microbial effects to biochar, could modify the biochar material themselves, or could be agents of transport of biochar within the soil profile. Finally, soil fauna may react to potentially toxic components of biochar in ways that are not reflected in the study of microorganisms.

3.4.1. Earthworms

The interaction of earthworms with biochar appears to be the best-studied among all soil fauna effects. Earthworms clearly ingest biochar particles. Using a peregrine tropical endogeic earthworm species, *Pontoscolex corethrurus*, Topoliantz and Ponge (2003, 2005) demonstrated the ingestion of biochar particles in microcosm experiments. The earthworms evidently could grind the material and mix it into the soil, in fact, preferring soil with biochar over soil alone. The authors even propose that populations of this species may be adapted to consumption of charred material and point to the potential to include this earthworm in management practices involving soils with charred material (Ponge et al., 2006). Using a behavioral experiment, Van Zwieten et al. (2010a) showed for an

Australian Ferrosol that earthworms clearly preferred biochar-amended soil over the controls; however, this preference was not present in a different soil type (Calcarosol) included in the same experiment. In contrast, Gomez-Eyles et al. (2011) observed a significant weight loss of earthworms that had been given hardwood biochar in a soil contaminated with PAH relative to the same soil without biochar. It is not obvious, however, whether the sorbed PAHs were the root cause of the negative effects of biochars on earthworms, since at the same time, PAH bioavailability was also reduced.

It is not clear what earthworms gain from ingesting biochar. Biochar may serve to grind organic matter in their gizzard similar to what has been observed for sand (Marhan and Scheu, 2005). Geophagous earthworms may feed on microbes and microbial metabolites (Lavelle, 1988) which are more abundant on biochar surfaces as often shown for soil amended with biochar (discussed above). Topoliantz and Ponge (2003) also proposed that its ingestion may favor microbes on which earthworms depend for enzymatic digestion, or that they profit from detoxifying or pH-ameliorating effects of the material.

Irrespective of the advantage to the earthworm, bioturbation by this group of organisms, perhaps mostly by anecic earthworms, is likely responsible for vertical mixing of biochar within the soil profile (Gouveia and Pessenda, 2000; Carcaillet, 2001). Major et al. (2010), while not quantifying contribution of earthworms to the downward migration of applied biochar in experimental plots, made the observation that earthworms were active in the sites, and that the inside of earthworm burrows was stained darker than surrounding soil. Eckmeier et al. (2007) observed natural char particles in earthworm feces at 0.08 m depth 6.5 years after an experimental fire, clearly indicating that earthworms may contribute to the movement of biochar within the soil profile.

Inorganic N concentrations increased to a greater extent when biochar and earthworms were added together to soil, than if either earthworms or biochar were added alone (Noguera et al., 2010). At the same time, growth and yield of a rice crop also increased the most if earthworms and biochar were used together. Possibly, microorganisms in the guts of earthworms are equally more abundant and N-processing enzymes more active in the presence of biochar as was discussed above for soil. A greater microbial biomass and enzyme activity would then increase N release from organic matter in the gut. Alternatively, or in addition, substances inhibitory to N mineralization and nitrification may have been sorbed to biochars similar to observations made in forest soils (DeLuca et al., 2009).

3.4.2. Nematodes

Data on the response of nematodes to biochar is very limited. Matlack (2001) carried out an observational study at the landscape scale, but could not detect a relationship between nematode populations and charred material in the soil. Direct experimental evidence for the effects of biochar on nematodes is not yet available. Soils exposed to smoke from charcoal production increased density of soil nematodes, increased density and diversity of collembolans, and diversity of oribatid mites (unpublished data cited in Uvarov, 2000), indicative of the effects of pyrolysis condensates present in biochars on soil fauna. Evidence for a positive relationship between nematodes and biochar is still very weak and warrants further study.

3.4.3. Microarthropods

As for nematodes, there is a dearth of information on the response of microarthropods to biochar in soil. Using a micromorphological approach, Bunting and Lundberg (1987) and Phillips et al. (2000) provided evidence that fecal pellets from microarthropods were deposited within a charcoal-rich layer in forest soil, indicating that this material can be ingested and processed by

these organisms. However, it is unclear if charcoal ingestion is incidental or what the microarthropods may gain from its consumption; perhaps they are consuming fungal hyphae colonizing the biochar. Since microarthropods are part of the fungal energy channel in the soil food web (Moore et al., 1988), one would expect increased populations, following a stimulation of fungal biomass. However, to our knowledge there is no evidence in support of this.

Bioavailability of pollutants (such as polychlorinated biphenyls, polyaromatic hydrocarbons) or organic agrochemicals (such as herbicides and pesticides) to soil fauna may be reduced due to the strong sorption of non-polar and semi-polar compounds to biochars (Smernik, 2009). There is no information available from biochar-enriched soils, but ample research with activated carbons indicates that remediation of pollution in sediments is possible even at large scale (Cho et al., 2009). Activated carbon has been shown to decrease availability of pollutants to diverse sets of fauna such as clams (McLeod et al., 2007), polychaetes (*Neanthes arenaeodentata*) and an amphipod (*Leptocheirus plumulosus*) (Millward et al., 2005).

3.5. Biochar and plant roots

Biochar-type materials have been reported to stimulate root growth for some time (Breazeale, 1906; Nutman, 1952). The very different properties of biochar in comparison to surrounding soil in most known cases improved root growth (Table 3). In fact, roots may even grow into biochar pores (Lehmann et al., 2003; Joseph et al., 2010). Makoto et al. (2010) showed not only a significant increase in root biomass (47%) but also root tip number (64%) increased within a layer of char from a forest fire with larch twigs, birch twigs, and shoots of dwarf bamboo buried in a dystic Cambisol. The number of storage roots of asparagus also increased with coconut biochar additions to a tropical soil (Matsubara et al., 2002). Also, root length of rice was shown to increase with biochar additions (Noguera et al., 2010). Germination and rooting of fir embryos (*Abies numidica*) significantly increased from 10 to 20% without additions to 32–80% of embryos when activated carbon was added to various growth media (Vookova and Kormutak, 2001). Therefore, not only abundance, but also growth behavior of roots may change in response to the presence of biochar.

3.5.1. Reasons for changes in root growth

The reasons for changes in root growth are rarely well identified in existing studies, and will likely vary depending on biochar properties and the conditions that restrict root and shoot growth in different soil environments. Biochar with properties that improve the chemical and physical characteristics of a given soil such as

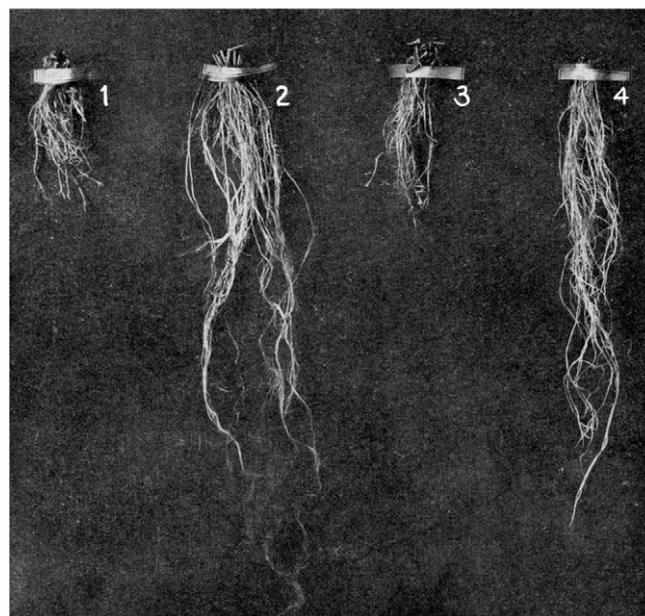


Fig. 10. Root system of wheat grown for 15 days in water culture made from various soil extracts with and without filtering by soot material: (1) fertile clay soil, (2) fertile clay soil filtered, (3) poor clay soil, (4) poor clay soil filtered (48 wheat plants grown in bottles with aqueous extract for 15 days; changed after Breazeale, 1906).

nutrient or water availability, pH, or aeration will likely improve root growth. In several cases, not only root and shoot biomass increased after biochar additions, but the shoot-to-root ratio increased, as well (Table 3). Such an increase in the shoot-to-root ratio may indicate improved resource supply that requires fewer roots to sustain the same above-ground biomass production (Wilson, 1988). Conversely, lower shoot-to-root ratios at lower growth rate may indicate lower resource supply. Given the effects biochars can have on nutrient and water availability mentioned before, changes in resource supply are likely to play a role in root dynamics. However, decreasing shoot-to-root ratios have also been reported at increased shoot growth (Table 3). These observations are likely unrelated to resource supply but may need to be explained by neutralization of a mechanism inhibiting root growth. Already a century ago, Breazeale (1906) and Dachnowski (1908) have explained the pronounced increase in root growth after additions of carbon black (soot) to soil with sorption of allelopathic compounds that were phytotoxic (Fig. 10). Later experimentation including biochar-type material found similar behavior (Skinner and Beattie, 1916). The effects observed in the former experiment

Table 3
Changes in root mass and shoot-to-root ratios as a result of biochar additions to soil from all available studies (positive values indicating an increase, negative ones a decrease).

Crop	Fertilization	Soil type	Type of biochar (feedstock/pyrolysis temperature/application rate)	Root biomass (% change from control)	Above-ground biomass (% change from control)	Shoot-to-root ratio (% change from control)	References
Pea	PK	Compost and peat	Unidentified wood/NA/5% w/v	–24	–37	–17	Devonald (1982)
Birch	No fertilizer	Organic horizons	<i>Empetrum hermaphroditum</i> /450/3 t ha ⁻¹	–13	+29	+34	Wardle et al. (1998) ^b
Pine	No fertilizer	Organic horizons	<i>Empetrum hermaphroditum</i> /450/3 t ha ⁻¹	+300	+350	+58	Wardle et al. (1998) ^b
Cowpea	NPK + lime or no fertilizer	Oxisol	Unidentified wood/NA/20% w/w	+17 to +28	+68 to +83	+44	Lehmann et al. (2003)
Maize	NPK or no fertilizer	NA ^a	<i>Acacia</i> bark/260–350/10 L m ⁻²	+88 to +92	+28 to +48	–23 to –49	Yamato et al. (2006)
Common bean	NP + lime	Oxisol	<i>Eucalyptus deglupta</i> /350/9% w/w	–9.9 to +9.3	+3.5 to +77.4	+29 to +37	Rondon et al. (2007)
Rice	No fertilizer	Inceptisol and Oxisol	<i>Eucalyptus deglupta</i> /350/2.6% w/w and unidentified wood/NA/4.6% w/w	+1 to +10	+1 to +152	+2 to +200	Noguera et al. (2010)
Wheat	NP fertilizer	Sandy clay loam	<i>Eucalyptus</i> /open pan method/1.6–6 t ha ⁻¹	–5 to +110	–25 to +73	–33 to +58	Solaiman et al. (2010)

^a NA, not available.

^b only Ericaceous site.

could not be explained by additions of nutrients, and such additions of activated carbon have been used to neutralize phytotoxic compounds up to now (Inderjit and Callaway, 2003). However, these results have been criticized by Lau et al. (2008), who point out that they could be due to the creation of artifacts such as nutrients leaching from the activated carbons and that the addition of carbonaceous adsorbents may have multiple effects on soil. In addition, many of the studies lacked a control of only activated carbon additions. No studies have been published where shoot-to-root ratios increased while plant growth decreased, which would indicate a direct toxic effect of biochars on plant roots through the presence of organic or inorganic (heavy metals) compounds.

3.5.2. Interactions with phytotoxic compounds

Phytotoxic compounds may originate from different sources. These may, for example, play a role in interactions between different root systems. Mahall and Callaway (1992) observed a greater rate of root elongation in the presence of activated carbon, partially overcoming intraspecific competition of creosote bush (*Larrea tridentata*) root systems (Fig. 11). The results were explained by a sorption of allelopathic compounds onto the activated carbon, making these substances ineffective in suppressing neighboring plants of the same or other species. Phytotoxicity may also result from phenolic compounds contained in leaf biomass used as mulches. Some plants are particularly rich in such phenolics. The evidence of a suppression of the phytotoxic effects from these compounds by activated carbon is weak, and both alleviation of growth suppression as well as no significant effects were found (Rutto and Mizutani, 2006; Sampietro and Vattuone, 2006). Some evidence can be gleaned from improved growth of birch (*Betula pendula*) after addition of fire-derived char to ericaceous-rich organic horizons (Wardle et al., 1998). It is not clear whether this effect was caused by stimulation of nitrification or direct root effects.

Activated carbons have been used for in vitro tissue culture (Klein and Bopp, 1971) and were found both to inhibit or promote growth, which was variously attributed to (i) a darkened environment; (ii) the sorption of undesirable or inhibitory substances; (iii) sorption of growth regulators and other signaling compounds; or (iv) the release of growth-promoting substances present in or sorbed by activated carbon (Pan and Van Staden, 1998). Specifically, the improvement of orchid germination in the presence of activated carbon has a long history (Yam et al., 1990), including those cultivated for medicinal purposes (Hossain et al., 2009), but has also found application for other plant cultivation as diverse as oak

(Pintos et al., 2010) or sorghum (Nguyen et al., 2007). Fridborg et al. (1978) showed that both embryogenesis and germination of wild carrot (*Daucus carota*) and onion (*Allium cepa*) were significantly enhanced in the presence of 1% activated carbon. The authors explained the increased growth with a sorption and inactivation of benzoic and phenylacetic acid assumed to be excreted by growing cells. Sorption of hydroxymethylfurfural was also identified as a possible growth inhibitor that could be made largely ineffective through additions of activated carbon (Weatherhead et al., 1978).

However, decreased growth of cultures of soybean (*Glycine max*) and goldenweed (*Haplopappus gracilis*) (Fridborg and Eriksson, 1975), as well as root organogenesis of tobacco (*Nicotiana tabacum*) has also been observed (Constantin et al., 1977). Constantin et al. (1977) showed that plant hormones were simultaneously removed from the culture by the activated carbon. The detrimental effect of plant hormone sorption by activated carbons has since been noted numerous times in culture media (Weatherhead et al., 1978). Still, a clear functional relationship appears to be absent in many of these studies, and its transferability to biochars in soil is unclear, yet merits further investigation.

Pan and Van Staden (1998) stated a need to match specific activated carbons to plants or plant growth stages. But systematic studies have rarely been conducted beyond comparisons of different brands of activated carbons without information about their production conditions or properties. The state of knowledge on biochars is even more disappointing in this respect. Similarly, biochars may have multiple and different effects on root growth that may also occur simultaneously, and their outcome will depend on biochar properties, soil type and crop species. Notably, there is a scarcity of studies that have investigated sorption properties as well as effects of biochars on microbial function in the rhizosphere. It is not clear whether single explanations of the observed phenomena are sufficient to capture the complex rhizosphere effects of biochars.

In cases where biochar is added in ecosystems harboring plant communities (e.g., agricultural fields containing crops and weeds, in a restoration context, etc.), such additions may also trigger changes in the species composition of plant communities (see Section 4.1), and thus also of root biomass, with resulting changes in overall rooting features (e.g., depth, length, architecture). It is also worth noting that changes in roots could be important for understanding changes in microbial community composition in the affected soils. Some functions of roots have not yet been examined in this context. For example it is unknown if there are changes in rhizodeposition in response to biochar.

4. Management and risks of biochar for soil biota

4.1. Changing the native soil biota

Biochar is likely to be applied to agroecosystems, in a restoration context, or in other situations in which the soil biota are either directly or indirectly already being managed. Perhaps as a consequence, concern about altering the indigenous soil biota has not been a primary consideration, although perhaps it should be. Biochar, when produced, is devoid of biota. However, during storage or transport inoculation with microbes could occur, which would then be added – potentially inadvertently – to the target ecosystem. This could be a particularly important consideration in hydrothermal carbonization, because in this case the carbonization product is wet as a consequence of the production process, and therefore particularly prone to colonization by microbes, for example molds, during storage (Rillig, unpubl. observation).

Biochar has not always been beneficial to soil biota abundance. For example, even though positive effects have frequently been observed for mycorrhizae and total microbial biomass (see sections

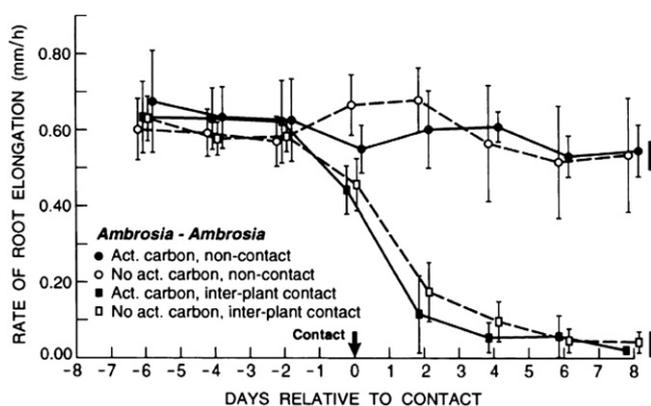


Fig. 11. Root elongation of creosote (*Larrea tridentate*) test roots before and after contact (Day 0) with roots from another creosote plant, either in the presence or absence of activated carbon (Mahall and Callaway, 1992; with permission); root elongation remained high after contact only in the presence of activated carbon, even when roots were in contact with each other.

above), there are examples of negative impacts as well (Warnock et al., 2007). It is not valid to conclude from positive effects on one organism group that a particular biochar will also have similar positive effects on others. For example, hydrochar can have positive effects on arbuscular mycorrhizae, but negative effects on plant growth (Rillig et al., 2010).

Many of the primary concerns of negative effects of biochar on soil biota are associated with a mineralizable or labile fraction often quantified and described as the volatile matter, as well as with salts such as Na or Cl. These may be short-term effects that need to be taken seriously in consideration and be evaluated for their suitability as a soil amendment. In previous research, Brown et al. (1951), Turner (1955) and Gibson and Nutman (1960) used extensive washing procedures of biochar-type charcoals to remove both organic and inorganic substances before application to soil. Without pretreatment, Turner (1955) reported withering of the petioles and discoloration of the leaves of clover plants. In addition, Devonald (1982) speculated whether the observed decrease in nodule size and abundance could be attributed to some properties of the biochar that was applied to peas.

In the long term, these effects may be of lesser concern as labile organic matter is mineralized and salts are leached from the soil. Biochar-type substances such as chars produced by vegetation fires are found in almost all soils as already pointed out by Schreiner and Brown (1912), who identified chars in all studied soils from various parts of the U.S. under agriculture as well as forests. This first regional assessment of biochar-type materials concentrated on larger particulate chars separated by density (Fig. 12). Using spectroscopic techniques rather than physical separation, there is now ample evidence of a ubiquitous distribution of char in soils, black C being found on all major continents (Schmidt et al., 1999; Skjemstad et al., 2002; Krull et al., 2008; Lehmann et al., 2008a).

Nonetheless, effects on soil biota, rhizosphere ecology and plant communities are also likely in the long term. While information about soil microorganisms and fauna is only emerging, as reviewed

above, more long-term studies are available that report changes in plant communities. On 100-year-old charcoal hearths with 25% or more charcoal-containing soil, growth of oak was suppressed (Mikan and Abrams, 1996) and plant composition on charcoal deposits changed to a greater abundance of yellow poplar (Mikan and Abrams, 1995). These changes were explained by changes in the nutrient availability in the soil. In some instances, changes can also be explained by enhanced activity of microorganisms, such as rhizobia (Vantsis and Bond, 1950; Rondon et al., 2007), which may lead to an increased abundance of legumes (Anderson and Spencer, 1948; Major et al., 2005, 2010). Effects on root signaling have also been observed after additions of activated carbon that removed phytotoxic root exudates (Mahall and Callaway, 1992; Callaway and Aschehoug, 2000). As a result, abundance of invasive weeds was reduced (Ridenour and Callaway, 2001). Recent research is starting to identify individual compounds that are released by the plant and subsequently sorbed by biochar. One of the phytotoxic compounds that have been observed to be neutralized was catechin (Bais et al., 2003). Future research may target such compounds directly to identify biochar properties that optimize inactivation of phytotoxic root exudates. Possibly, application of specifically designed biochars may be used as a selective tool in ecological restoration (Kulmatiski and Beard, 2006; Kulmatiski, 2010).

4.2. Biochar as inoculant carrier

Soil additives and inoculant carriers have been used for, for example, *Azotobacter*, *Bacillus*, *Clostridium*, *Frankia*, *Pseudomonas*, or *Rhizobium* (van Elsas and Heijnen, 1991), but little is understood in terms of their mode of action, even as far as the relatively well-studied rhizobia are concerned (Deaker et al., 2004). Biochar-type materials have been suggested as inoculant carriers substituting for the increasingly expensive, rare, greenhouse gas-releasing and non-renewable peat for some time (Gukova and Bukevich, 1941; Wu, 1958, 1960; Wu and Kuo, 1969; Tilak and Subba Rao, 1978; Ogawa, 1989; Beck, 1991). Given the previously documented positive effects of biochar on microorganism abundance and reproduction rates, use of biochar as an additive to commercial mycorrhizal inoculum, or even as a carrier material seems promising. But comprehensive discussions of the mechanisms by which biochar properties influence inoculant efficiency and survival have not yet occurred. This is partly because the ability to manipulate biochar properties to potentially optimize its use as an inoculant carrier has not been fully recognized in the past. Three aspects play a role for evaluating the suitability of the carrier material: (i) the survival of the inoculants during storage; (ii) the survival in the soil; and (iii) the inoculation efficiency. Typically, past research has focused on survival during storage, since carrier materials such as peat are rapidly decomposed in soil and would not improve survival once added to soil. Biochar, on the other hand, will remain in the soil and may positively influence abundance of the inoculant organisms as shown for microbial biomass in general and possibly inoculation efficiency.

Adding biochar-type residues from vegetation fires to soil significantly increased nodulation of subterranean clover in a yellow podzolic soil in Australia (Hely et al., 1957), providing some of the first evidence for positive effects of biochar on rhizobia. When biochar was mixed with peat, press mud, compost or farm yard manure, these inoculant carriers were observed to increase rhizobial cell counts, viability from 30 days to three months and nodulation in soybean and pigeon pea (Tilak and Subba Rao, 1978). Kremer and Peterson (1983) compared different inoculant carriers for peanut *Rhizobium* including the commonly used peat material, and found biochar materials to perform as well if not better than peat (Fig. 13). Similarly, Sparrow and Ham (1983) reported that

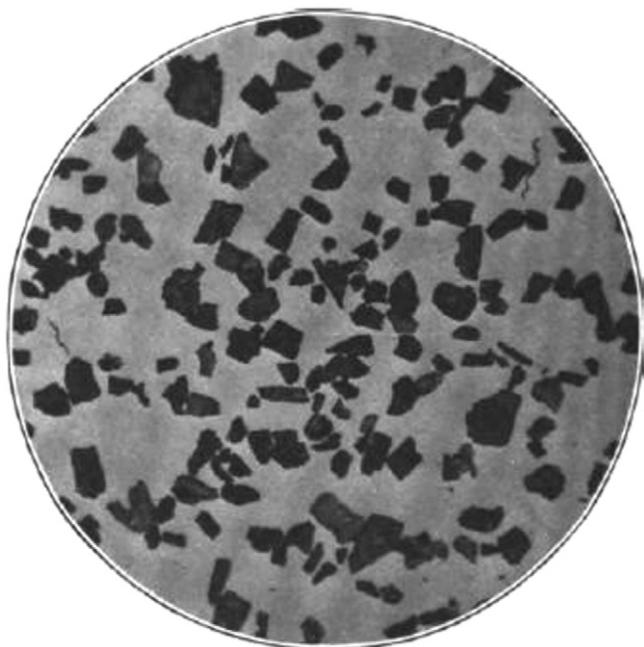


Fig. 1.—SPECIMENS OF CHARCOAL AND CHARCOALLIKE PARTICLES FOUND IN THE SOILS EXAMINED.

Fig. 12. Black C or char particles isolated from soils in the United States (Schreiner and Brown, 1912; with permission).

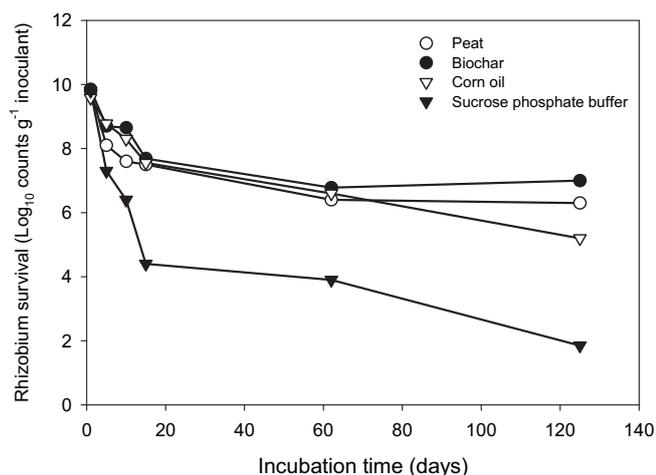


Fig. 13. Survival of peanut *Rhizobium* strain CA001 with different inoculant carrier materials at 35 °C; pH of biochar 8.7 ($n = 2$; only sucrose phosphate buffer is significantly different at $P < 0.05$; determine by the most-probable-number technique on yeast extract-mannitol agar; redrawn after Kremer and Peterson, 1983).

biochar made from hardwood (pH 8.1) could be used as an inoculant carrier for *Rhizobium phaseoli* and was superior to peanut hulls, corn cobs or polyacrylamide gel. The results varied significantly between peat, vermiculite and biochar, depending on the different strains of *Rhizobia* used, indicating that matching carrier properties and microorganisms even on the sub-species level is worth examining.

Khavazi et al. (2007) used biochar to adjust the pH of the inoculant carrier material, which was as effective as other carrier materials and ensured survival of *Bradyrhizobium japonicum* for more than six months at an acceptable level. Beck (1991) showed that biochars can be mixed with soil and provide a carrier material that was equally effective as a high-quality peat. Similar results were obtained by mixing biochar with composts (Wu, 1960), and 10% w/w biochar additions to peat was either similar or increased inoculant survival compared to peat alone (Newbould, 1951). Clearly, some properties of biochars offer advantages over using the very effective peat to improve inoculant survival under some conditions. Mixing soot into peat outperformed any other mixture with peat (Hedlin and Newton, 1948), suggesting that adsorptive properties may play an important role for survival of rhizobia.

With respect to mycorrhizae, Ogawa (1989) mentioned preliminary data showing that spores of *Gigaspora margarita* could be preserved for more than 180 days at room temperature, if mixed into balls of biochars made from bark or saw dust. On the other hand, Rutto and Mizutani (2006) found reduced mycorrhizal symbiosis in peach roots when activated carbon was added, which may provide grounds for the hypothesis that high surface area alone may not provide the properties appropriate for use as an inoculant carrier material.

Some results, however, also showed that peat was clearly superior to biochar, if the pH of the peat was properly adjusted, as shown for *E. coli* as a test organism (Lochhead and Thexton, 1947). In all studies reported here without exception, only one single type of biochar or activated carbon from unknown origin and unknown production conditions was used. Given the dramatically varying properties of biochars described earlier (Table 1), it can be expected that different biochar properties will have significantly different effects on inoculant organisms. It is reasonable to assume that biochars may be designed specifically for certain inoculants and possibly soil conditions.

Rhizobia inoculant carriers are intended to protect against desiccation, adverse pH or toxic substances in soil, be environmentally safe and non-toxic to the target organisms themselves, release the organisms and be abundant in supply (Stephens and Rask, 2000; Deaker et al., 2004), all of which may theoretically be achieved with appropriately designed biochars. However, Cassidy et al. (1996) suggested that encapsulation may be preferable to biochar-type materials because such beads may support inoculants for a longer period of time due to better protection from environmental stress, greater metabolic activity, and reduced contamination with other microorganisms. A combination of approaches may prove beneficial.

4.3. Pathogens and biochar

Circumstantial evidence for the beneficial effect of biochar-type materials on suppressing plant diseases, such as potato rot (Allen, 1846a: 382) or rust and mildew (Allen, 1846b: 45) were reported by farmers more than a century ago, and isolated studies have observed reduced damping off (caused by various pathogens) after additions of charcoal (Retan, 1915). However, little direct experimentation has been conducted so far.

Biochar may act in a similar way in suppressing plant diseases as is described for other organic amendments such as composts. Several principal mechanisms have been proposed and partly proven for composts (Hoitink and Fahy, 1986; Noble and Coventry, 2005), including (1) a direct release of inhibitors of plant pathogens; (2) the promotion of microorganisms that act antagonistic to pathogens, such as parasites, through production of antibiotics, or by successful competition for nutrients; (3) improved plant nutrition and vigor, leading to enhanced disease resistance; and (4) activation of plant defense mechanisms (induced systemic resistance) by enhancing certain microorganisms. Any and all of these four mechanisms may also be applicable to biochar. In addition, (5) the known strong sorption of organic compounds onto biochar may modify signaling between plant and pathogens, or (6) affect the mobility and activity of the pathogen itself. The following section examines the evidence for effects of biochar on pathogens and possible mechanisms.

Fusarium infection of asparagus was found to decrease after addition of coconut biochar and was similar to the benefits derived from manure made from coffee residue (Matsubara et al., 2002). A decrease in *Fusarium* infection of asparagus was also reported after addition of biochar made by fast pyrolysis of wood powder (Elmer and Pignatello, 2011). Also, infection of tomato with another soil-borne disease, bacterial wilt (*Ralstonia solanacearum*), was significantly reduced by adding wood biochar in some experiments and consistently by adding biochar made from municipal biowaste (Nerome et al., 2005). The disease suppression improved with greater application rates of up to 40% (v/v), with benefits persisting beyond 90 days after planting.

Disease severity of powdery mildew (*Leveillula taurica*) significantly decreased after biochar was added to both a sandy soil and an organic potting mix (Fig. 14; Elad et al., 2010), suggesting that biochar acts differently from other organic matter with respect to the studied disease. The studied biochar produced from citrus wood using traditional charcoal-making techniques was found to induce systemic resistance also to another foliar fungal pathogen, *Botrytis cinerea* (gray mold) on pepper and tomato and to the broad mite pest (*Polyphagotarsonemus latus*) on pepper (Elad et al., 2010). Since all three disease agents are spatially separate from the soil-applied biochar (but on above-ground plant parts), since plant nutrition did not differ as a result of biochar additions, and since all pots were watered equally, induced systemic resistance was suggested to be the most likely explanation. This resistance was presumed to result from either low-level stress exerted by

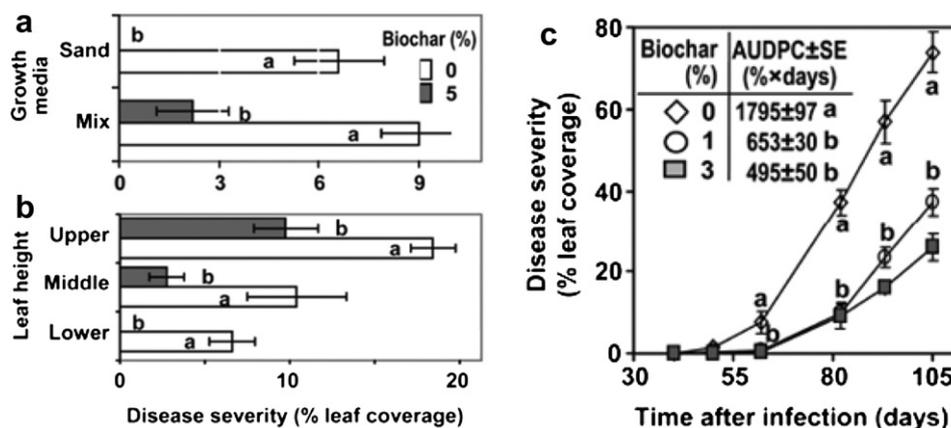


Fig. 14. Disease severity of powdery mildew (*Leveillula taurica*) on tomato as affected by biochar additions (a) in both a sand and a coconut fiber-tuff soilless potting medium (mix); (b) in sand; (c) in the potting medium over 105 days (Elad et al., 2010; with permission).

phytotoxic compounds contained in the biochar (e.g., ethylene and propylene glycol) or through larger populations of microorganisms isolated from the biochar-treated soils that are known to induce resistance, such as *Trichoderma* spp. (Graber et al., 2010). The disease suppression was apparent with the lowest tested application rate of 1% (by weight) and was most strongly expressed early in the development of disease symptoms, with a calculated delay period of 20 days slowing the disease epidemic (Fig. 14).

Elmer and Pignatello (2011) proposed the following explanation for their observed decline in *Fusarium* infection of asparagus: biochar may have adsorbed allelopathic compounds in replant soil such as coumaric, caffeic and ferulic acids which led to a measurable increase in mycorrhizal infection. Greater AM abundance may then have led to suppression of the disease.

For soil-borne root diseases, it is also conceivable that biochars reduce compounds in the soil solution that would otherwise facilitate the ability of pathogens to detect and infect roots. Root exudates are known to act as chemoattractants for a range of pathogens such as *Pythium* (Jones et al., 1991), and to elicit germination of *Pythium* spores through linoleic and oleic acids in root exudates (Windstam and Nelson, 2008). Under greenhouse (Callaway and Aschehoug, 2000) and field conditions (Kulmatiski and Beard, 2006; Kulmatiski, 2010), activated carbon was shown to sorb allelopathic compounds produced by plants as discussed above. This was hypothesized and empirically shown already much earlier using soot from fossil fuel combustion (Breazeale, 1906; Schreiner and Reed, 1907). It is reasonable to hypothesize similar interferences with the interactions between roots and soil-borne diseases when using fresh biochar. Such an interference may possibly include microbial volatile organic compounds which have been shown to sorb to biochar-type materials and are being explored for monitoring indoor air quality (Matysik et al., 2009). In addition, the mentioned ethylene production after biochar is added to soil is known to have a significant impact on a range of soil and plant metabolic activities (Spokas et al., 2010). Whether similar effects apply to root and foliar pathogens and pests will need to be proven directly.

However, disease symptoms, plant mortality of *Arabidopsis* and root colonization by *Pseudomonas syringae* has also been observed to increase in the presence of activated charcoal (Bais et al., 2005). The reason for the greater infection was the sorption of antimicrobial compounds exuded by the roots onto the surfaces of the activated charcoals. These antimicrobial exudates are effective in suppressing a range of pathogens (Walker et al., 2003). But the relevance of results from the activated carbons with high surface areas and low functional surface groups for biochars is yet to be

shown. It may be desirable to investigate sorption properties of different biochars for model substances that are associated with antimicrobial and phytotoxic effects, bearing in mind that these sorption properties change over time in soil (Cheng et al., 2008; Cheng and Lehmann, 2009).

An additional consideration for the management of diseases is the effect of biochar on pesticide efficiency. Pesticides as well as herbicides may be sorbed to biochars (Zheng et al., 2010) and therefore be less effective (Andersen, 1968; Jordan and Smith, 1971; Yang et al., 2006). On the other hand, uptake into crops and leaching of these substances may be reduced (Yu et al., 2009, 2010) which could improve environmental health and food safety. This should be considered when managing plant diseases and weeds with biochar.

5. Future research

The available literature provides ample justification for further investigation into the effects of biochar on microbial, faunal and root abundance, community composition of various biota and their functions. A greater abundance of microorganisms after biochar additions to soil is relatively well established (Table 4). The effects of biochar on soil faunal abundance, however, is barely investigated apart from a few studies on earthworms, and resulting community composition of the entire soil biota has only been studied to a limited extent for microorganisms. Little information is available for purposeful use of biochars to manage roots, pathogens or

Table 4
Relative levels of existing knowledge on biochar effects on soil biota and our opinions on suggestions for research priorities.

Research area	Level of existing knowledge	Research priority
Microbial abundance	+++	+
Faunal abundance	+	+++
Root abundance	++	+
Microbial community	++	++
Faunal community	+	++
Microbial function	+	+++
Faunal function		+++
Root function	++	+++
Biochar inoculants	+	+++
Biochar enzyme interaction	+	+++
Biochar pathogen control	+	+++
Environmental risk	+	+++

+, ++, +++, low, medium and high level of existing knowledge or priority for future research.

microorganisms apart from biochars as inoculant carriers. As for the example of biochar inoculants, none of the available studies uses more than one type of biochar or gives sufficient information about their properties or production conditions. This is symptomatic for much of the available literature on biochar as a whole. Given the greatly varying properties of different biochars, few advances in biochar design or insights into processes responsible for observed changes in soil ecology and biogeochemistry can be expected from such studies. Therefore, the feedstock types and production procedures must be systematically varied and investigated for their effects on soil biota.

Often, preliminary information can be gleaned from studies using activated carbons, which provide some guidance about biochars from woody material with high surface area that are produced at higher temperatures (including those from gasification), and about what is presumably a fraction of surface properties of many biochars even if they are produced at lower temperatures. Nonetheless, direct evidence is still to be gathered for biochars. In the future, biochar studies on soil biota must include characterization of a minimum set of properties of the specific biochars. Some general guidance for biochar characterization was provided by Joseph et al. (2009), even though it is not clear what biochar properties are important for the control, for example, of microbial abundance at any given location. Studies specifically on soil biota may at a minimum need to document microbially available C, surface area, pore size distribution, pH, ash content, and elemental analyses as well as production conditions (temperature and time at highest temperature) and feedstock type. In addition, contrasting biochars have to be compared rather than one biochar studied on its own.

Knowledge gaps needing urgent attention include biochar effects on faunal abundance (especially micro- and meso-fauna), on the ecology of biota including environmental risk, on electrochemical properties as well as on the utility as inoculant carriers, on interactions with enzymes and for managing plant pathogens (Table 4). Soil fauna may serve as a useful indicator for environmental risks associated with certain biochar types and could help in constraining biochar properties for assessment of its bio-safety. In all such studies, appropriate characterization and contrasting of different biochars is critical.

Biochars may influence chemical and physical properties of the entire soil, such as water content, aeration or pH. However, this has been insufficiently clarified whether changes in soil properties after biochar additions are merely an average of soil and biochar properties or whether biochar confers distinct changes to surrounding soil. In any event, all changes induced by biochars will be most pronounced close to the surfaces of biochars. Some effects that may only occur around biochar particles, such as sorption of nutrients and organic matter, create micro-locations in soil, which we call the “biochar-sphere”. Such co-location of energy and other resources may promote abundance and efficiency of the soil biota. Our traditional view typically focuses on bulk soil properties; this perspective is chiefly a result of analytical limitations. Such a view is increasingly deemed inappropriate when studying soil biota (Young and Crawford, 2004), and inadequate for fostering a better understanding of biochar effects in soil, since biochars are mainly particulate (Skjemstad et al., 1996; Nguyen et al., 2008; Lehmann et al., 2008b) and may rather emulate aggregate properties. Microorganisms may directly interact with biochar surfaces and pores as demonstrated throughout this paper. Important questions emerge from a biochar-sphere perspective: How far does the influence of biochar reach into the bulk soil? What are the critical soil components and characteristics influenced by its surface properties?

There will likely not be one single answer to these or other questions about the net effects of biochars, but answers will vary between biochars, soil and plant conditions which should be

studied in combination. On the short term, characterization standards need to be developed that adequately capture the most important differences in biochar properties starting with those mentioned above. Availability of standard biochar materials to the research community would accelerate the knowledge gain and ensure comparability between research methods. On the medium term, an international research network may prove critical to address concerns over biochar effects on soil health more expeditiously under a variety of soil and environmental conditions. A long-term research vision building on such a network effort, should include the development of a comprehensive dynamic model that builds on a thorough understanding of biochar properties and their interactions with soil biota.

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