Plant Soil (2007) 300:9–20
DOI 10.1007/s11104-007-9391-5

MARSCHNER REVIEW

Mycorrhizal responses to biochar in soil – concepts and mechanisms

Daniel D. Warnock · Johannes Lehmann · Thomas W. Kuyper · Matthias C. Rillig

Received: 19 April 2007 / Accepted: 9 August 2007 / Published online: 19 September 2007

Abstract Experiments suggest that biomass-derived black carbon (biochar) affects microbial populations and soil biogeochemistry. Both biochar and mycorrhizal associations, ubiquitous symbioses in terrestrial ecosystems, are potentially important in various ecosystem services provided by soils, contributing to sustainable plant production, ecosystem restoration, and soil carbon sequestration and hence mitigation of global climate change. As both biochar and mycorrhizal associations are subject to management, understanding and exploiting interactions between them could be advantageous. Here we focus on biochar effects on mycorrhizal associations. After reviewing the experimental evidence for such effects, we critically examine hypotheses pertaining to four mechanisms by which biochar could influence mycorrhizal abundance and/or functioning. These mechanisms are (in decreasing order of currently available evidence supporting them): (a) alteration of soil physico-chemical properties; (b) indirect effects on mycorrhizae through effects on other soil microbes; (c) plant–fungus signaling interference and detoxification of allelochemicals on biochar; and (d) provision of refugia from fungal grazers. We provide a roadmap for research aimed at testing these mechanistic hypotheses.

Keywords Biochar · Arbuscular mycorrhiza · Ectomycorrhiza · Carbon storage · Restoration · Terra preta

Responsible Editor: Hans Lambers.

D. D. Warnock · M. C. Rillig
Microbial Ecology Program,
Division of Biological Sciences, University of Montana,
Missoula, MT 59812, USA

J. Lehmann
Department of Crop and Soil Sciences, Cornell University,
Ithaca, NY 14853, USA

T. W. Kuyper
Department of Soil Quality, Wageningen University,
P.O. Box 47, 6700 AA Wageningen, The Netherlands

M. C. Rillig (✉)
Institut für Biologie, Freie Universität Berlin,
Altensteinstr. 6, 14195 Berlin, Germany
e-mail: rillig@zedat.fu-berlin.de

Introduction

Pioneering studies, conducted primarily in Japan, where biochar application to soil has a long tradition (Ishii and Kadoya 1994), provided evidence that biochar can have positive effects on the abundance of mycorrhizal fungi (Table 1). Soil micro-organisms, especially arbuscular mycorrhizal fungi (AMF), in addition to ectomycorrhizal fungi (ECM) and ericoid mycorrhizal fungi (ERM), have well-recognized roles in terrestrial ecosystems (Zhu and Miller 2003; Rillig

Table 1 Effects of biochar (BC) or activated carbon/charcoal (AC) additions on mycorrhizal fungi, separated by mycorrhizal type (arbuscular mycorrhizal fungi (AMF) ectomycorrhizal fungi (ECM), and ericoid mycorrhizal fungi (ERM), and listed in order of decreasing effect size of the mycorrhizal response variable(s)

Experimental design[a]	Amouont AC[b] or BC[b] present	Type(s) of BC[c] or AC[c] applied	Response variables[d]	Mycorrhiza response[e]	Possible functions for ECM, ERM or AMF[f]	Source
AMF experiments						
BC effects on AMF RC of *Citrus iyo* in an abandoned orchard (F)	BC: 800 g/m^3 in 2, 4.8 m^3 pits	H: RH	RC	+610%	ND	Ishii and Kadoya (1994)
Effects of three BC types on AMF (*Glomus fasciculatum*) in river sand (G)	BC: 2.0% BW	H: RH Citrus Juice Sediment (C.J.) Woody: Western Spruce Bark (W.S.)	RC	+540% RH +88% C.J. +75% W.S.	Enhanced overall plant P nutrition	Ishii and Kadoya (1994)
BC Effects on AMF in soy bean fields (F)	BC: 1,500 g m^{-2}	ND	RC	+300%	ND	Saito (1990)
BC (ground vs un-ground) effects on AMF infectivity (F)	BC: 33% BV	H: RH	RC	Ground: +100% Un-ground: −20%	ND	Ezawa et al. (2002)
BC effects on AMF (*Glomus sp.*) and *Fusarium oxysporum* RC of *Asparagus officinalis* roots. (G)	BC : 10% and 30% BV	Woody: Coconut Shell	RC	10% BC: +50% 30% BC: +69%	Enhanced plant pathogen resistance	Matsubara et al. (2002)
BC effects on infectivity of indigenous AMF (G)	BC: Applied at a rate of 10 l m^{-2}	Woody: *Acacia mangium* bark	RC	+42%	ND	Yamato et al. (2006)
BC effects on AMF RC of non N-fixing, and N-fixing *Phaseolus vulgaris*) roots. (G)	BC: Applied at rates of 0, 30, 60 and 90 g BC kg^{-1} soil	Woody: *Eucalyptus deglupta* logs	RC	Non N-fixing: 30 g, 60 g: −38% 90 g: −20% N-fixing: 30 g, 60 g: NS; 90 g: +16%	ND	Rondon et al. (2007)
BC Effects on AMF RC, and Spore density (S.D.) by *Glomus intraradices* grown in culture with *Zea mays* (G)	BC: 89.8% BV of growth substrate	ND	RC SD in 100 ml^{-1} infectious propagules (IP) in 100 ml^{-1}	RC −21% SD: −5% IP: −38%	ND	Gaur and Adholeya (2000)
ECM experiments						
Quantified ECM RC in different soil fractions of a Montana forest soil (F)	BC: 2% BV	ND	RC, no. ECM root tips 100 cm^3 soil fraction $^{-1}$	+2,900%	ND	Harvey et al. (1976)
Effect of AC on timing of mycorrhizal colonization of *Quercus robur* seedlings by *Piloderma croceum*.(G)	AC: 2% BW	ND	RC Onset of mycorrhiza formation measured in weeks	RC +624% Onset accelerated by 4 weeks	Colonization by *P. croceum* increased drought resistance in *Q. robur*	Herrmann et al. (2004)
AC effects on ability of ECM (*Pisolithus tinctorus*) to colonize *Abies firma* seedlings grown in culture (G)	AC: 0.3% BV	ND	ECM presence or absence of host infection	+200%	ND	Vaario et al. (1999)

Effectiveness of RH BC/forest top soil mix as ECM inoculum source for *Shorea smithiana* trees grown in degraded forest soil. (F)	BC: 300 cm^3 BC mixed with 1 l soil. BC/soil mix placed in potting hole 25-cm deep × 25-cm diameter	H: RH	Presence or absence of host infection by ECM fungi	+80%	ND	Mori and Marjenah (1994)
Effects of AC slurry on dissolved phenol concentration and *Picea mariana* seedling growth (G)	AC: Applied to soil as slurry, (250 g AC 3 l^{-1} water) microcosm surface area=1,890 cm^2	ND	RC	−38% in type B fungi	ND	Wellstedt et al. (2002)
ERM experiments						
Effect of AC only, or AC and carbon source (0.5 g l^{-1} glucose or pectin) additions on ERM RC of *Vaccinium angustifolium*	AC: Added to solid agar medium at 1 g l^{-1}	Darcco G60, Fisher	RC	+95% AC +128% AC + Glucose, or AC + Pectin	ND	Duclos and Fortin (1983)

[a] *G*, Greenhouse; *F*, Field

[b] *BV*, By volume; *BW*, By weight

[c] AC is produced via one of the following activation procedures, CO_2, steam, or chemical (e.g. phosphoric acid). All three processes remove remaining organic compounds and nutrients from previously pyrolyzed biomass while greatly increasing carbonyl content, yielding a porous material with an extremely high surface area and a very high sorptive capacity. Because the AC activation process begins with charred biomass, until further evidence is provided to the contrary, it is assumed that BC and AC will both act similarly as adsorbents, in the soil environment. However, AC will likely have a much greater surface area than BC (Pan and van Staden 1998). *H*, Herbaceous biochar; *RH*, Rice husk biochar

[d] *RC*, root colonization; *SD*, spore density

[e] *NS*, non significant difference; effect size for response variables was calculated as $((X_{\text{treatment}}-X_{\text{control}})/X_{\text{control}})\times 100$

[f] *ND*, Not determined

2004; Read et al. 2004; Rillig and Mummey 2006). Mycorrhizal fungi are frequently included in management, since they are widely used as soil inoculum additives (Schwartz et al. 2006). With both biochar additions and mycorrhizal abundance subject to management practices, there clearly are opportunities for exploiting a potential synergism that could positively affect soil quality.

While data on biochar effects on mycorrhiza are accumulating, there are several important gaps in our knowledge on these interactions. The most important gap concerns the mechanisms by which biochar might affect the abundance and functioning of mycorrhizal fungi. Therefore, the goals of this paper are to first evaluate the evidence of biochar effects on mycorrhizal associations thus far, and then to propose mechanisms for these biochar effects on mycorrhizae (primarily using examples of arbuscular mycorrhiza and ectomycorrhiza). In doing so, we also point out future research priorities (Fig. 1). To clarify the nomenclature used throughout this discussion we first provide a brief overview of biochar properties.

Biochar definition and properties

Biochar is a term reserved for the plant biomass-derived materials contained within the black carbon (BC) continuum. This definition includes chars and charcoal, and excludes fossil fuel products or geogenic carbon (Lehmann et al. 2006). Materials forming the BC continuum are produced by partially combusting (charring) carbonaceous source materials, e.g. plant tissues (Schmidt and Noack 2000; Preston and Schmidt 2006; Knicker 2007), and have both natural as well as anthropogenic sources. Restricting the oxygen supply during combustion can prevent complete combustion (e.g., carbon volatilization and ash production) of the source materials. When plant tissues are used as raw materials for biochar production, heat produced during combustion volatilizes a significant portion of the hydrogen and oxygen, along with some of the carbon contained within the plant's tissues (Antal and Gronli 2003; Preston and Schmidt 2006). The remaining carbonaceous materials contain many poly-aromatic (cyclic) hydrocarbons, some of

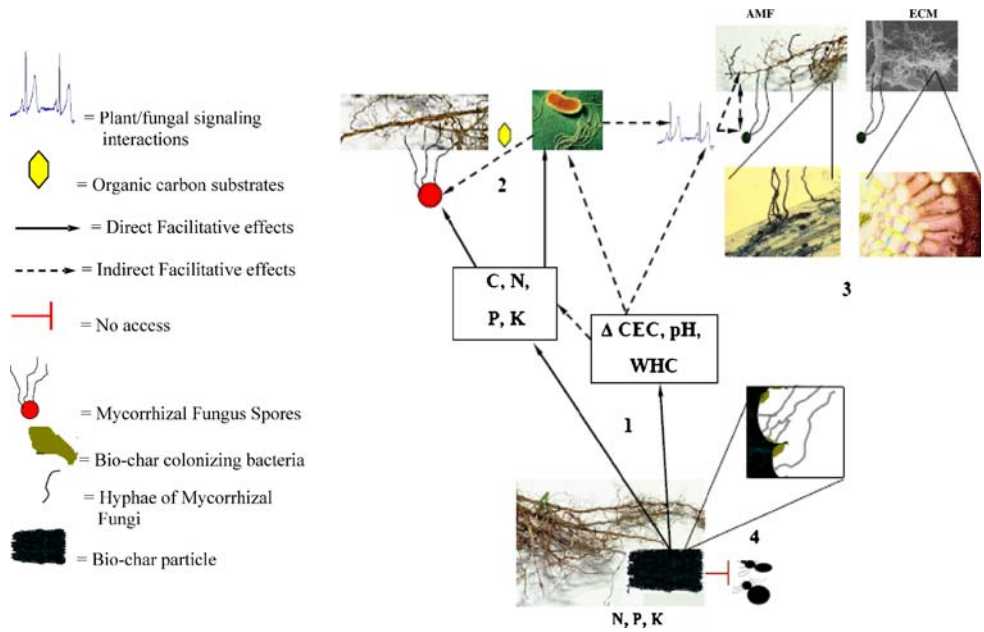

Fig. 1 Schematic representation of bio-char and its direct and indirect effects on mycorrhizal fungi abundance/functioning, emphasizing the hierarchical nature of effects. The numbers included in figure body correspond to mechanisms discussed in text: (*1*) effects on soil physio-chemical properties; (*2*) effects through influences on other soil microbes; (*3*) interactions with plant–fungus signaling; and (*4*) provision of refugia from fungal grazers. *Solid arrows* indicate direct facilitative effects; *dashed arrows* indicate indirect facilitative effects

which may contain functional groups with oxygen or hydrogen (Schmidt and Noack 2000; Preston and Schmidt 2006). Depending on the temperatures reached during combustion and the species identity of the source material, a biochar's chemical and physical properties may vary (Keech et al. 2005; Gundale and DeLuca 2006). For example, coniferous biochars generated at lower temperatures, e.g. 350°C, can contain larger amounts of available nutrients, while having a smaller sorptive capacity for cations than biochars generated at higher temperatures, e.g. 800°C (Gundale and DeLuca 2006). Furthermore, plant species with many large diameter cells in their stem tissues can lead to greater quantities of macropores in biochar particles. Larger numbers of macropores can for example enhance the ability of biochar to adsorb larger molecules such as phenolic compounds (Keech et al. 2005).

Because of its macromolecular structure dominated by aromatic C, biochar is more recalcitrant to microbial decomposition than uncharred organic matter (Baldock and Smernik, 2002). Biochar is believed to have long mean residence times in soil, ranging from 1,000 to 10,000 years, with 5,000 years being a common estimate (Skjemstad et al. 1998; Swift 2001; Krull et al. 2003). However, its recalcitrance and physical nature represent significant obstacles to the quantification of long-term stability (Lehmann 2007).

Evidence for biochar effects on mycorrhizal fungi

From the experiments summarized in Table 1, it appears that the addition of biochar materials to soil often results in significant responses by both plants and mycorrhizal fungi.

Tryon (1948), Matsubara et al. (2002), DeLuca et al. (2006), and Gundale and DeLuca (2006) demonstrated that biochar additions can change soil nutrient availability by affecting soil physico-chemical properties. Increases in soil nutrient availability may result in enhanced host plant performance and elevated tissue nutrient concentrations in addition to higher colonization rates of the host plant roots by AMF (Ishii and Kadoya 1994). Lastly, experiments by Matsubara et al. (2002) suggested that biochar can also increase the ability of AMF to assist their host in resisting infection by plant pathogens.

In three of the six ECM studies and the single ERM study represented in Table 1, experiments demonstrated the effects of adding biochar in growth media on both the ability of the ECM and ERM fungi to colonize the host plant seedlings, and the overall effects on seedling growth. Additionally, the experiment conducted by Herrmann et al. (2004) showed that activated carbon (AC), which may in many cases have similar properties as biochar, affected the timing of host plant colonization by ECMF, which occurred 4 weeks earlier in the AC treatment than in the control. The other ECM related experiments evaluated the effects of biochar presence on host tree colonization rates (Harvey et al. 1976; Mori and Marjenah 1994). In these two cases, the presence of biochar corresponded with significant increases in plant root colonization by ECM. Observations made by Harvey et al. (1978, 1979) also support these results.

In contrast to those experiments in Table 1 showing positive effects of biochar or AC additions on abundance of mycorrhizal fungi, a few studies observed negative effects. In these cases, it appears that the negative effects of the biochar or AC additions on AMF were largely due to nutrient effects. For example, Gaur and Adholeya (2000) found that the biochar media limited the amount of P taken up by host plants, compared to rates from plants grown in river sand or clay-brick granules, suggesting that P was less available. Additionally, Wallstedt et al. (2002) reported decreases in both bio-available organic carbon and nitrogen in their ectomycorrhizal system.

An important consideration pertains to the study design of the experiments reported in Table 1. The first issue deals with the soils used in the experiments, e.g. river sand or OM-rich field soil; the other issue concerns the materials added to these soils as controls, e.g. organic matter vs biochar. Are soil biota, including mycorrhizal fungi, responding to an experimental addition of biochar simply because carbon is being added or are they responding to biochar's unique properties? In at least two cases where data from field soils were presented, it appears that mycorrhizal fungi responded more positively to biochar additions than to additions of other types of organic material added as control (Harvey et al. 1976; Ishii and Kadoya 1994). The experiment by Matsubara et al. (2002) showed that a fresh organic amendment had fairly similar effects as biochar in increasing AMF-mediated host plant resistance against *Fusarium* and

that the asparagus plants reached similar mycorrhizal colonization levels with both additions. But the 9-week gap between inoculation with AMF and with *Fusarium* makes this aspect of the experiment somewhat difficult to evaluate. However, it is still possible that these positive responses shown by mycorrhizal fungi are determined in part by the amount of carbon in the material being added to the soil, with the expectation that the biochar is more carbon-rich than the organic matter. We may not be able to answer this question satisfactorily until experiments control for C amendment effects in the biochar treatment(s) and/or take into account the relative addition of C to soils.

Work on *terra preta de índio* (TP) soil, the fertile Amazonian Dark Earths, has served as a major inspiration for the use of biochar as a promising soil additive promoting crop growth and carbon storage (Glaser et al. 2002; Glaser and Woods 2004; Lehmann et al. 2006; Glaser 2007). However, no published data are available on the impact of TP soils on mycorrhizal functioning. For that reason, the studies discussed above refer to short-term experiments and not to the historical, pre-Columbian Amazonian soils. TP soils are not only much richer in biochar than the surrounding soils, but also in non-pyrogenic carbon and nutrients, especially phosphorus and calcium; therefore it is likely that TP effects on mycorrhizal functioning could be beyond those of biochar addition alone.

Mechanisms

At least four mechanisms could explain how biochar can lead to altered total abundance and/or activity of mycorrhizal fungi in soils and plant roots: (1) Biochar additions to soil result in altered levels of nutrient availability and/or other alterations in soil physico-chemical parameters that have effects on both plants and mycorrhizal fungi. (2) Additions of biochar to soils result in alterations with effects that are beneficial or detrimental to other soil microbes, for instance mycorrhization helper bacteria (MHB) or phosphate solubilizing bacteria (PBS). (3) Biochar in soils alters plant–mycorrhizal fungi signaling processes or detoxifies allelochemicals leading to altered root colonization by mycorrhizal fungi. (4) Biochar serves as a refuge from hyphal grazers. Since a primary goal of this discussion is identifying mechanisms explaining the effects of biochar on mycorrhizae, with the intention of guiding attempts for developing methods to exploit them as soil management tools, and because many of the biochar effects included in Table 1 appear positive, we primarily present arguments explaining why biochar generally appears beneficial to mycorrhizae.

However, as discussed previously, biochar applications do not always benefit mycorrhizal fungi (see Table 1). In these situations, one could argue that biochar, via any of our proposed mechanisms, reduces formation of mycorrhiza, e.g. by decreasing nutrient availability or creating unfavourable nutrient ratios in soils (Wallstedt et al. 2002). This negative effect could be especially prominent in cases where the biochar has a very high C/N ratio and a portion of the biochar is decomposable, leading to N-immobilization. Under such conditions, biochar could also negatively affect plant growth, e.g. as seen in Gaur and Adholeya (2000). Given the above possibilities for negative responses by both plants and mycorrhizal fungi to biochar amendments, and plants to mycorrhizal fungi (Johnson 1993), it cannot be assumed that biochar amendments will always result in a net benefit to plant productivity even though few such cases have been reported so far.

A conceptual overview of the mechanisms and hypothesized pathways discussed in the following sections is provided in Fig. 1, emphasizing the hierarchical nature of contributing factors. In the following discussion it should be kept in mind that (a) mechanisms are not mutually exclusive but likely several contribute to the outcome, perhaps even with opposite effects; (b) there is little information available on which mechanism is likely the most important in any given environmental situation; and finally that (c) many mechanisms are hypothetical with most support for mechanism 1 at this time (we are presenting mechanisms below in decreasing amount of evidence). This figure therefore also serves as a roadmap for future research.

Mechanism 1: Biochar changes soil nutrient availability

Modifications of nutrient availability would clearly be a mechanism of primary importance for mycorrhizal fungal abundance. For example, nutrient additions might alleviate growth limitations of the fungi

themselves in nutrient-poor soils (Treseder and Allen 2002). Additionally, altering the balance of nutrients can exert strong control over fungal root colonization, as for example known for shifts in soil N/P ratios for AMF (Miller et al. 2002).

Biochar addition can result in elevated quantities of bio-available nutrients such as N, P and metal ions, in the affected soils (Tryon 1948; Lehmann et al. 2003; Gundale and DeLuca 2006; DeLuca et al. 2006), but has also been shown to lead to decreases particularly of N availability (Lehmann et al. 2003). These changes in soil nutrient availabilities, may be explained by some of the following observations. Additions of biochar to soil alters important soil chemical and physical (see below) properties such as pH (has caused both increases and decreases), and typically increase soil cation exchange capacity (CEC), and can lead to greater water holding capacity (WHC), while generally decreasing bulk density (Tryon 1948). Increases in soil pH towards neutral values (Lucas and Davis 1961), in addition to increased CEC (Glaser et al. 2002), may result in increases in bio-available P and base cations in biochar influenced soils. Additionally, Lehmann et al. (2003), Topoliantz et al. (2005), Gundale and DeLuca (2006) and Yamato et al. (2006) showed that biochar itself contained small amounts of nutrients that would be available to both soil biota (including mycorrhizal fungi) and plant roots. Lastly, DeLuca et al. (2006) showed that biochar from forest wildfire stimulated gross and net nitrification rates, most likely mediated by biochar adsorbing inhibitory phenols. This mechanism is likely specific to soils with ectomycorrhizal trees and/or ericaceous shrubs with an abundance of phenolic compounds, whereas in agricultural soils biochar may in the short term reduce ammonification and nitrification by a reduction either in N availability due to immobilization during initial decomposition of the N-poor biochar (Lehmann et al. 2006) or by a reduction in C cycling.

Some of the experiments conducted to evaluate the effects of biochar upon mycorrhizae (Table 1) lend support to mechanism 1. These experiments show that additions of biochar materials generally result in the alteration of soil physico-chemical properties that may lead to increases in soil nutrient availability (measurements taken from both soil samples and plant tissues) and/or increases in root colonization by mycorrhizal fungi (Ishii and Kadoya 1994; Matsubara et al. 2002; Yamato et al. 2006). In a greenhouse experiment by Matsubara et al. (2002), the soil pH of treatments receiving biochar increased from 5.4 to 6.2 (10% biochar by volume) and 6.3 (30% biochar by volume). According to Lucas and Davis (1961), these pH values fall within the pH range (5.5 to 7.0) where plant nutrients are near their maximum availability in agricultural soils. Many of these alterations in soil characteristics probably occur at a micro-scale (Gundale and DeLuca 2006), and thus may only affect hyphae that are in the immediate vicinity of biochar particles.

Mechanism 2: Biochar alters the activity of other micro-organisms that have effects on mycorrhizae

Mycorrhization Helper Bacteria (MHB; Garbaye 1994) are capable, under specific conditions, of secreting metabolites, e.g. flavonoids (AMF) and furans (ECM), that facilitate the growth of fungal hyphae and the subsequent colonization of plant roots by ECM (Founoune et al. 2002; Duponnois and Plenchette 2003; Aspray et al. 2006; Riedlinger et al. 2006) and AM fungi (Duponnois and Plenchette 2003; Hildebrandt et al. 2002, 2006). Hildebrandt et al. (2002, 2006) have demonstrated that certain compounds (including raffinose and other unidentified metabolites) produced by strains of *Paenibacillus* can directly enhance the growth of AMF extraradical mycelium. Additionally, Kothamasi et al. (2006) demonstrated that other species of bacteria, such as *Pseudomonas aeruginosa*, can solubilize important plant nutrients, especially phosphate, making them part of a group of bacteria called phosphate solubilizing bacteria (PSB). These mineralized nutrients are then accessible to mycorrhizal fungi and eventually to the host plant. Furthermore, Xie et al. (1995) and Cohn et al. (1998) state that *Rhizobium sp.* and *Bradyrhizobium sp.* can produce compounds that induce flavonoid production in nearby plants (legumes) that may ultimately increase root colonization of plant roots by AM fungi.

Biochar may serve as a source of reduced carbon compounds (either the biochar particle itself, or organic molecules adsorbed to the particle's matrix), and/or nutrients, and as a refuge (see mechanism 4) for any biochar colonizing soil bacteria, including MHB and PSBs (Pietikäinen et al. 2000; Samonin and Elikova 2004). Increased populations of PSB and/or MHB might then indirectly benefit mycorrhizal fungi (Fig. 1).

Mechanism 3: Biochar alters the signaling dynamics between plants and mycorrhizal fungi or detoxifies allelochemicals

The rhizosphere is a zone of intense signaling between microbes, including mycorrhizal fungi, and plant roots (Bais et al. 2004; Harrison 2005; Bais et al. 2006; Paszkowski 2006). For example, experiments conducted using both field soils and *in vitro* cultures show that compounds (e.g. CO_2, flavonoids, sesquiterpenes and strigolactones) secreted by plant roots lead to both increased colonization of plant roots by AMF (Bécard and Piché 1989; Nair et al. 1991; Xie et al. 1995) and increased spore germination and AMF hyphal branching (Gianinazzi-Pearson et al. 1989; Akiyama et al. 2005). Additions of biochar could alter the exchange of signals in several ways, as shown in Fig. 1; however, we emphasize that most of the pertinent evidence stems from sterile *in vitro* culture studies with uncertain relevance to conditions in the soil.

Angelini et al. (2003) demonstrated that some flavonoid signaling compounds could be either inhibitory or stimulatory to specific groups of soil biota as a function of pH. As discussed under mechanism 1, biochar additions usually increase soil pH. Hence, it is possible that these pH changes alone can lead to stimulatory effects, causing increases in fungal abundance.

Sorptive properties of biochar (e.g. for hydrophobic substances), particularly higher temperature (e.g., 800°C) biochar, could also cause signaling interference in the rhizosphere: biochar could serve as signal reservoirs or as a sink, both for signaling compounds and for inhibitory compounds (allelochemicals). Recently, Akiyama et al. (2005) demonstrated that AC was capable of adsorbing AMF signaling (strigolactones) compounds from a hydroponic solution that were subsequently desorbable with acetone. Once desorbed, these compounds retained their activity and stimulate hyphal branching and growth of *Gigaspora margarita*. Biochar particles could adsorb signal molecules not immediately intercepted by AMF hyphae or spores, or consumed by other soil biota. Later on, these stored signal molecules could be desorbed by soil water reaching the biochar particles. After being re-dissolved into soil water, they would again be available to stimulate mycorrhizal colonization of plant roots. By functioning in this manner, biochar particles would be serving as secondary sources of signal molecules, acting concomitantly with MHB and plant roots.

However, biochar's capacity to adsorb signaling compounds and add as a sink could also decrease the ability of mycorrhizal fungi to colonize plant roots. If biochar permanently rather than temporarily removes signal molecules from soils, this signal sorption activity results in a net decrease in the number of signal molecules reaching mycorrhizal hyphae and spores. As a result, hyphal growth and spore germination, and ultimately fungal abundance, could actually decrease because of biochar activity.

In addition to chemical signals, biochar may also adsorb compounds toxic to mycorrhizal fungi. For example, Wallstedt et al. (2002) showed that the addition of an AC slurry to an experimental soil resulted in a decreased amount of water-soluble phenols. Herrmann et al. (2004) and Vaario et al. (1999) related their results of stimulated ECM fungus colonization of roots in the presence of AC to toxin sorption.

Mechanism 4: Biochar serves as a refuge for colonizing fungi and bacteria

This mechanism is purely physical in nature, and therefore could function in a similar fashion for ECM, ERM, AMF and bacteria. Hyphae and bacteria that colonize biochar particles (or other porous materials) may be protected from soil predators (Saito 1990; Pietikäinen et al. 2000; Ezawa et al. 2002), which includes mites, collembola and larger (>16 μm in diameter) protozoans and nematodes. The documented physical parameters of the biochar particles themselves make this mechanism plausible. The average sizes of soil bacteria and fungal hyphae range from 1 to 4 μm and 2 to 64 μm, respectively, with many fungal hypha being smaller than 16 μm in diameter (Swift et al. 1979). Additionally, the average body-size of a soil protist is between 8 to 100 μm, while the average body size of soil micro-arthropods ranges from 100 μm to 2 mm (Swift et al. 1979). In contrast, the pore diameters in a biochar particle can often be smaller than 16 μm in diameter (Kawamoto et al. 2005; Glaser 2007; Hockaday et al. 2007). Based on the differences in the body sizes across these different organisms, it is clearly possible that many of the pores within a biochar particle are large enough to accommodate soil microorganisms, includ-

ing most bacteria and many fungi, to the exclusion of their larger predators. Thus, the biochar would be acting as a refuge for MHB, PSB and mycorrhizal fungi. Supporting evidence for this hypothesis comes from Saito (1990), Gaur and Adholeya (2000) and Ezawa et al. (2002) who all showed that AMF readily colonize porous materials and were capable of heavily colonizing biochar particles in the soil. Lastly, Pietikäinen et al. (2000) and Samonin and Elikova (2004) showed that bacteria readily colonized biochar particles; these may include MHB and/or PSB.

An important factor controlling pore size distribution is the charring temperature with higher temperatures yielding finer pores. Another major factor in determining the degree to which biochar may serve as a refuge is the anatomical structure of the biological tissues pyrolyzed to yield the biochar. Considering the effects that cell diameter alone can have on the sorptive capability of a given biochar material (Keech et al. 2005; Gundale and DeLuca 2006), it stands to reason that the cell types contained within the original plant tissues (e.g., tracheids, vessel elements or sieve cells) determine the pore sizes of the biochar. Not only the charring conditions and source material, but also subsequent interactions of biochar with soil can change porosity and pore sizes. For example, adsorption of organic matter to biochar surfaces can decrease porosity by blocking pores (Kwon and Pignatello 2005).

While it seems clear that mycorrhizal fungi can use biochar as a habitat, the quantitative importance to the extraradical mycelium is not evident. This will highly depend on the biochar properties and the biochar addition rates. Nevertheless, the finer parts of the mycelium, generally the absorptive hyphae, are more vulnerable to fungal grazers (Klironomos and Kendrick 1996), and it is primarily these architectural elements that could be effectively protected within biochar particles. It would depend, then, on the extent to which these 'protected' fine hyphae make a substantial contribution towards nutrient uptake compared to the relatively 'unprotected' hyphae in the mineral and organic soil, whether this hypothesized mechanism is quantitatively important.

Conclusions and research recommendations

Experimental results (Table 1) point to exciting possibilities regarding biochar and its possible synergy with arbuscular, ericoid, and ectomycorrhizal symbioses. We have synthesized available data into several potential mechanisms of biochar effects on mycorrhizae (Fig. 1). This should serve as a springboard for testing the occurrence and relative importance of these factors/mechanisms in the soil. Based on this discussion we derive the following research recommendations:

(a) *Methods reporting*. In many cases it is helpful to know as much detail about the experimental biochar application as possible. This should include: source material, production temperature, application rate, application method, and what material was used in the control application to account for C addition effects (and the amounts of available nutrients for both). This would facilitate comparisons among studies and help distinguish among the different mechanistic pathways; frequently these pieces of information are incomplete.

(b) *Management implications*. None of the studies to date have examined the management context of biochar application on AMF, and this would also be an important research need, since application practices could have overriding effects on soil biota.

(c) *Fungal communities*. Studies to date have focused on quantifying potential responses in fungal abundance measures, primarily root colonization and spore numbers (see Table 1). However, mycorrhizal fungi occur as species assemblages in ecosystems and in roots of individual plants (Johnson et al. 1992; Husband et al. 2002; Vandenkoornhuyse et al. 2003; Mummey et al. 2005). The species composition of a mycorrhizal fungal assemblage can be important to mycorrhizal functioning (e.g., van der Heijden et al. 1998). Data on this important aspect of the response of mycorrhizal fungi to biochar are not yet available, but represent an important priority for future studies. Here, we limited our discussion to mechanisms affecting abundance; however, many of the arguments presented could also be applied to explain potential shifts in mycorrhizal fungal species composition, because fungal life history strategies and responsiveness to changing soil environments vary between fungal taxa (e.g., Hart and Reader 2002; Escudero and Mendoza 2005; Drew et al. 2006).

(d) *Negative effects*. There is a potential for negative effects on mycorrhizal fungi, as discussed above;

it is therefore clearly also a research priority to define the environmental circumstances (e.g., soil nutrient content, plants species) and biochar parameters (e.g., quality and application rate) that lead to such effects. It is possible that negative or neutral effects have been under-reported.

Increasing atmospheric concentrations of carbon dioxide have prompted the search for avenues of long-term sequestration of carbon, particularly in the soil (Lal 2004; Schiermeier 2006). Work on *terra preta de índio* soil has inspired the use of biochar as a promising soil additive promoting carbon storage (Day et al. 2005; Lehmann et al. 2006; Marris 2006; Glaser 2007). Biochar can add value to non-harvested agricultural products (Major et al. 2005; Topoliantz et al. 2005), and can promote plant growth (Lehmann et al. 2003; Oguntunde et al. 2004). Lehmann et al. (2006) estimated that a total of 9.5 billion tons of carbon could potentially be stored in soils by the year 2100 using a wide variety of biochar application programs. Once equipped with a better understanding of this potential synergism and the mechanisms that drive it, we could utilize biochar/mycorrhizae interactions for sequestration of carbon in soils to contribute to climate change mitigation. This interaction could also be harnessed for the restoration of disturbed ecosystems, the reclamation of sites contaminated by industrial pollution and mine wastes, increasing fertilizer use efficiencies (with all associated economic and environmental benefits) and the development of methods for attaining increased crop yields from sustainable agricultural activities.

References

Akiyama K, Matsuzaki K-I, Hayashi H (2005) Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. Nature 435:824–827

Angelini J, Castro S, Fabra A (2003) Alterations in root colonization and nodC gene induction in the peanut-rhizobia interaction under acidic conditions. Plant Physiol Biochem 41:289–294

Antal MJ Jr, Grønli M (2003) The art, science, and technology of charcoal production. Indust Engin Chem Res 42:1619–1640

Aspray TJ, Eirian Jones E, Whipps JM, Bending GD (2006) Importance of mycorrhization helper bacteria cell density and metabolite localization for the *Pinus sylvestris–Lactarius rufus* symbiosis. FEMS Microbiol Ecol 56:25–33

Bais HP, Park SW, Weir TL, Callaway RM, Vivanco JM (2004) How plants communicate using the underground information superhighway. Trends Plant Sci 9:26–32

Bais HP, Weir TL, Perry LG, Gilroy S, Vivanco JM (2006) The role of root exudates in rhizosphere interactions with plants and other organisms. Annu Rev Plant Biol 57:233–266

Baldock JA, Smernik RJ (2002) Chemical composition and bioavailability of thermally altered *Pinus resinosa* (Red pine) wood. Organic Geochem 33:1093–1109

Bécard G, Piché Y (1989) Fungal growth stimulation by CO_2 and root exudates in vesicular–arbuscular mycorrhizal symbiosis. Appl Environ Microb 55:2320–2325

Cohn J, Bradley D, Stacey G (1998) Legume nodule organogenesis. Trends Plant Sci 3:105–110

Day D, Evans RJ, Lee JW, Reicosky D (2005) Economical CO_2, SO_x, and NO_x capture from fossil-fuel utilization with combined renewable hydrogen production and large-scale carbon sequestration. Energy 30:2558–2579

DeLuca TH, MacKenzie MD, Gundale MJ, Holben WE (2006) Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa pine forests. Soil Sci Soc Am J 70: 448–453

Drew EA, Murray RS, Smith SE (2006) Functional diversity of external hyphae of AM fungi: ability to colonize new hosts is influenced by fungal species, distance and soil conditions. Appl Soil Ecol 32:350–365

Duclos JL, Fortin JA (1983) Effect of glucose and active charcoal on *in-vitro* synthesis of ericoid mycorrhiza with *Vaccinium spp*. New Phytol 94:95–102

Duponnois R, Plenchette C (2003) A mycorrhiza helper bacterium enhances ectomycorrhizal and endomycorrhizal symbiosis of Australian *Acacia* species. Mycorrhiza 13: 85–91

Escudero V, Mendoza RE (2005) Seasonal variation of arbuscular mycorrhizal fungi in temperate grasslands along a wide hydrologic gradient. Mycorrhiza 15:291–299

Ezawa T, Yamamoto K, Yoshida S (2002) Enhancement of the effectiveness of indigenous arbuscular mycorrhizal fungi by inorganic soil amendments. Soil Sci Plant Nutr 48:897–900

Founoune H, Duponnois R, Bâ AM, Sall S, Branget I, Lorquin J, Neyra M, Chotte JL (2002) Mycorrhiza Helper Bacteria stimulate ectomycorrhizal symbiosis of *Acacia holosericea* with *Pisolithus*. New Phytol 153:81–89

Garbaye J (1994) Helper bacteria: a new dimension to the mycorrhizal symbiosis. New Phytol 128:197–210

Gaur A, Adholeya A (2000) Effects of the particle size of soilless substrates upon AM fungus inoculum production. Mycorrhiza 10:43–48

Gianinazzi-Pearson V, Branzanti B, Gianinazzi S (1989) In vitro enhancement of spore germination and early hyphal growth of a vesicular–arbuscular mycorrhizal fungus by host root exudates and plant flavonoids. Symbiosis 7: 243–255

Glaser B (2007) Prehistorically modified soils of central Amazonia: a model for sustainable agriculture in the twenty-first century. Phil Trans R Soc B 362:187–196

Glaser B, Lehmann J, Zech W (2002) Ameliorating physical and chemical properties of highly weathered soils in the tropics with charcoal – a review. Biol Fert Soils 35: 219–230

Glaser B, Woods W (2004) Towards an understanding of amazon dark earths. In: B Glaser, W Woods (eds)Amazon dark earths: explorations in space and time. Springer, Berlin, pp 1–8

Gundale MJ, DeLuca TH (2006) Temperature and source material influence ecological attributes of Ponderosa pine and Douglas-fir charcoal. For Ecol Manag 231:86–93

Harrison MJ (2005) Signaling in the arbuscular mycorrhizal symbiosis. Annu Rev Microbiol 59:19–42

Hart MM, Reader RJ (2002) Taxonomic basis for variation in the colonization strategy of arbuscular mycorrhizal fungi. New Phytol 135:335–344

Harvey AE, Jurgensen MF, Larsen MJ (1976) Comparative distribution of ectomycorrhizae in a mature Douglas-fir/Larch forest soil in western Montana. Forest Sci 22:350–358

Harvey AE, Jurgensen MF, Larsen MJ (1978) Seasonal distribution in a mature Douglas-fir/Larch forest soil in western Montana. Forest Sci 22:203–208

Harvey AE, Larsen MF, Jurgensen MF (1979) Comparative distribution of ectomycorrhizae in soils of three western Montana forest habitat types. Forest Sci 25:350–358

Herrmann S, Oelmuller R, Buscot F (2004) Manipulation of the onset of ectomycorrhiza formation by indole-3-acetic acid, activated charcoal or relative humidity in the association between oak micro-cuttings and *Piloderma croceum*: influence on plant development and photosynthesis. J Plant Physiol 161:509–517

Hildebrandt U, Janetta, K, Bothe H (2002) Towards growth of arbuscular mycorrhizal fungi independent of a plant host. Appl Environ Microb 68:1919–1924

Hildebrandt U, Ouziad F, Marner F-J, Bothe H (2006) The bacterium *Paenibacillus validus* stimulates growth of the arbuscular mycorrhizal fungus *Glomus intraradices* up to the formation of fertile spores. FEMS Microbiol Lett 254: 258–267

Hockaday WC, Grannas AM, Kim S, Hatcher PG (2007) The transformation and mobility of charcoal in a fire-impacted watershed. Geochim Cosmochim Ac 71:3432–3445

Husband R, Herre EA, Turner SL, Gallery R, Young JPW (2002) Molecular diversity of arbuscular mycorrhizal fungi and patterns of host association over time and space in a tropical forest. Mol Ecol 11:2669–2678

Ishii T, Kadoya K (1994) Effects of charcoal as a soil conditioner on citrus growth and vesicular–arbuscular mycorrhizal development. J Jpn Soc Hortic Sci 63:529–535

Johnson NC, Tilman D, Wedin D (1992) Plant and soil controls on mycorrhizal fungal communities. Ecology 73:2034–2042

Johnson NC (1993) Can fertilization of soil select less mutualistic mycorrhizae?. Ecol Appl 3:749–757

Kawamoto K, Ishimaru K, Imamura Y (2005) Reactivity of wood charcoal with ozone. Wood Sci 51:66–72

Keech O, Carcaillet C, Nilsson MC (2005) Adsorption of allelopathic compounds by wood-derived charcoal: the role of wood porosity. Plant Soil 272:291–300

Klironomos JN, Kendrick WB (1996) Palatability of microfungi to soil arthropods in relation to the functioning of arbuscular mycorrhizae. Biol Fert Soils 21:43–52

Knicker H (2007) How does fire affect the nature and stability of soil organic nitrogen and carbon? A review. Biogeochemistry 85:91–118

Kothamasi D, Kothamasi S, Bhattacharyya A, Kuhad RC, Babu CR (2006) Arbuscular mycorrhizae and phosphate solubilising bacteria of the rhizosphere of the mangrove ecosystem of Great Nicobar island, India. Biol Fert Soils 42:358–361

Krull ES, Skjemstad JO, Graetz D, Grice K, Dunning W, Cook G, Parr JF (2003) ^{13}C-depleted charcoal from C4 grasses and the role of occluded carbon in phytoliths. Org Geochem 34:1337–1352

Kwon S, Pignatello JJ (2005) Effect of natural organic substances on the surface and adsorptive properties of environmental black carbon (char): pseudo pore blockage by model lipid components and its implications for N_2-probed surface properties of natural sorbents. Env Sci Technol 39:7932–7939

Lal R (2004) Soil carbon sequestration to mitigate climate change. Geoderma 123:1–22

Lehmann J (2007) Bio-energy in the black. Frontiers in Ecology and the Environment 5:381–387

Lehmann J, Da Silva JP Jr, Steiner C, Nehls T, Zech W, Glaser B (2003) Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin: fertilizer, manure and charcoal amendments. Plant Soil 249:343–357

Lehmann J, Gaunt J, Rondon M (2006) Biochar sequestration in terrestrial ecosystems – a review. Mitig Adapt Strat Global Change 11:403–427

Lucas RE, Davis JF (1961) Relationships between pH values of organic soils and availabilities of 12 plant nutrients. Soil Sci 92:177–182

Major J, Steiner C, Ditommaso A, Falcão NP, Lehmann J (2005) Weed composition and cover after three years of soil fertility management in the central Brazilian Amazon: compost, fertilizer, manure and charcoal applications. Weed Biol Manag 5:69–76

Marris E (2006) Black is the new green. Nature 442:624–626

Matsubara Y-I, Hasegawa N, Fukui H (2002) Incidence of Fusarium root rot in asparagus seedlings infected with arbuscular mycorrhizal fungus as affected by several soil amendments. J Jpn Soc Hortic Sci 71:370–374

Miller RM, Miller SP, Jastrow JD, Rivetta CB (2002) Mycorrhizal mediated feedbacks influence net carbon gain and nutrient uptake in *Andropogon gerardii*. New Phytol 155:149–162

Mori S, Marjenah (1994) Effect of charcoaled rice husks on the growth of Dipterocarpaceae seedlings in East Kalimantan with special reference to ectomycorrhiza formation. J Jap Forestry Soc 76:462–464

Mummey DL, Rillig MC, Holben WE (2005) Neighboring plant influences on arbuscular mycorrhizal fungal community composition as assessed by T-RFLP analysis. Plant Soil 271:83–90

Nair MG, Safir GR, Siqueira JO (1991) Isolation and identification of vesicular–arbuscular mycorrhiza-stimulatory compounds from clover (*Trifolium repens*) roots. Appl Environ Microb 57:434–439

Oguntunde PG, Fosu M, Ajayi AE, Van De Giesen ND (2004) Effects of charcoal production on maize yield, chemical properties and texture of soil. Biol Fert Soils 39:295–299

Pan MJ, Van Staden J (1998) The use of charcoal in *in-vitro* culture – A review. Plant Growth Regul 26:155–163

Paszkowski U (2006) A journey through signaling in arbuscular mycorrhizal symbioses. New Phytol 172:35–46

Pietikäinen J, Kiikkilä O, Fritze H (2000) Charcoal as a habitat for microbes and its effect on the microbial community of the underlying humus. Oikos 89:231–242

Preston CM, Schmidt MWI (2006) Black (pyrogenic) carbon: A synthesis of current knowledge and uncertainties with special consideration of boreal regions. Biogeosciences 3:397–420

Read DJ, Leake JR, Perez-Moreno J (2004) Mycorrhizal fungi as drivers of ecosystem processes in heathland and boreal forest biomes. Can J Bot 82:1243–1263

Riedlinger J, Schrey SD, Tarkka MT, Hampp R, Kapur M, Fiedler H-P (2006) Auxofuran, a novel metabolite that stimulates the growth of fly agaric, is produced by the mycorrhiza helper bacterium *Streptomyces* strain AcH 505. Appl Environ Microb 72:3550–3557

Rillig MC (2004) Arbuscular mycorrhizae and terrestrial ecosystem processes. Ecol Lett 7:740–754

Rillig MC, Mummey DL (2006) Mycorrhizas and soil structure. New Phytol 171:41–53

Rondon M, Lehmann J, Ramírez J, Hurtado MP (2007) Biological nitrogen fixation by common beans (*Phaseolus vulgaris* L.) increases with biochar additions. Biol Fert Soils 43:699–708

Saito M (1990) Charcoal as a micro habitat for VA mycorrhizal fungi, and its practical application. Agric Ecosyst Environ 29:341–344

Samonin VV, Elikova EE (2004) A study of the adsorption of bacterial cells on porous materials. Microbiology 73:810–816

Schiermeier Q (2006) Putting the carbon back. Nature 442:620–623

Schmidt MWI, Noack AG (2000) Black carbon in soils and sediments: Analysis, distribution, implications and current challenges. Global Biogeochem Cy 14:777–793

Schwartz MW, Hoeksema JD, Gehring CA, Johnson NC, Klironomos JN, Abbott LK, Pringle A (2006) The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. Ecol Lett 9:501–515

Skjemstad JO, Janik LJ, Taylor JA (1998) Non-living soil organic matter: What do we know about it? Aust. J Exp Agr 38:667–680

Swift RS (2001) Sequestration of carbon by soil. Soil Sci 166:858–871

Swift MJ, Heal OW, Anderson JW (1979) Decomposition in terrestrial ecosystems. University of California Press, Berkeley

Topoliantz S, Ponge J-F, Ballof S (2005) Manioc peel and charcoal: a potential organic amendment for sustainable soil fertility in the tropics. Biol Fert Soils 41:15–21

Treseder KK, Allen MF (2002) Direct nitrogen and phosphorus limitation of arbuscular mycorrhizal fungi: a model and field test. New Phytol 155:507–515

Tryon EH (1948) Effect of charcoal on certain physical, chemical, and biological properties of forest soils. Ecol Monogr 18:81–115

Vaario LM, Tanaka M, Ide Y, Gill WM, Suzuki K (1999) In vitro ectomycorrhiza formation between *Abies firma* and *Pisolithus tinctorius*. Mycorrhiza 9:177–183

Vandenkoornhuyse P, Ridgway KP, Watson IJ, Fitter AH, Young JPW (2003) Co-existing grass species have distinctive arbuscular mycorrhizal communities. Mol Ecol 12:3085–3095

Van der Heijden MG, Klironomos JN, Ursic M, Moutoglis P, Streitwolf-Engel R, Boller T, Wiemken A, Sanders IR (1998) Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity. Nature 396:69–72

Wallstedt A, Coughlan A, Munson AD, Nilsson MC, Margolis HA (2002) Mechanisms of interaction between *Kalmia angustifolia* cover and *Picea mariana* seedlings. Can J For Res 32:2022–2031

Xie Z-P, Staehelin C, Vierheilig H, Wiemken A, Jabbouri S, Broughton WJ, Vogeli-Lange R, Boller T (1995) Rhizobial nodulation factors stimulate mycorrhizal colonization of nodulating and nonnodulating soybeans. Plant Physiol 108:1519–1525

Yamato M, Okimori Y, Wibowo IF, Anshiori S, Ogawa M (2006) Effects of the application of charred bark of *Acacia mangium* on the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia. Soil Sci Plant Nutr 52:489–495

Zhu YG, Miller RM (2003) Carbon cycling by arbuscular mycorrhizal fungi in soil–plant systems. Trends Plant Sci 8:407–409