2009

ARBUSCULAR MYCORRHIZAL RESPONSES TO BIOCHARS IN SOILS - POTENTIAL MECHANISMS OF INTERACTION AND OBSERVED RESPONSES IN CONTROLLED ENVIRONMENTS

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ARBUSCULAR MYCORRHIZAL RESPONSES TO BIOCHARS IN SOILS - POTENTIAL MECHANISMS OF INTERACTION AND OBSERVED RESPONSES IN CONTROLLED ENVIRONMENTS

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Bachelor of Science, University of Oregon, Eugene, Oregon, 2004

Master's Thesis

presented in partial fulfillment of the requirements
for the degree of

Master of Science
in Microbiology, Microbial Ecology

The University of Montana
Missoula, MT

May 2009

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Warnock, Daniel Douglas M.Sc., December 2008 Microbiology

Arbuscular mycorrhizal responses to biochars in soils - potential mechanisms of interaction and observed responses in controlled environments

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Abstract

The following thesis is a two-part study, investigating the influences of biochar (charcoal) on arbuscular mycorrhizal fungi (AMF). The first part of this study is a critical examination and conceptual overview of the literature regarding biochar and AMF available before July 2007. In the second part, I present three experiments all designed to evaluate the influences of biochar applications on AMF abundance in primarily temperate, neutral pH soils. This course of research was selected through an exstensive review of the literature suggesting that biochar presence can strongly affect both soil microbial populations, including mycorrhizal fungi, and biogeochemistry. As both biochar and mycorrhizal associations are subject to management, and because both components are potentially important in various ecosystem services provided by soils (e.g., sustainable plant production) understanding and exploiting interactions between them could be advantageous. After reviewing the experimental evidence for such effects, four mechanisms are proposed by which biochar could influence mycorrhizal abundance and/ or functioning. These mechanisms are: 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; and d) provision of refugia from fungal grazers. Through this overview, a roadmap for research is provided, which is aimed at testing these mechanistic hypotheses. Using this proposed framework as a template, three experiments were designed and implemented, incorporating three different soils, five different biochars, and eight different biochar application rates. Through these experiments, it was illustrated that five different types of biochar are all capable of significantly altering soil orthophosphate availabilities, with four of these biochars not significantly affecting soil pH. Overall, our results indicate that AMF abundances were either unchanged or decreased with biochar amendment across multiple treatments. These results also indicate that biochar, depending on the nature of the feedstock, the temperature attained during pyrolysis and amounts applied can significantly alter soil properties including phosphate availability. These findings may have implications for soil management where the goal is to increase the services provided by AMF.

Acknowledgements

This work was supported by the Division of Biological Sciences and the Microbial Ecology program at the University of Montana, and the Department of Crop and Soil Sciences at Cornell University. We also thank Danny Day and Robert Hawkins from Eprida Inc., for providing the peanut shell biochars used in our experiments.

I would like to thank my advisory committee, Dr. Matthias Rillig, Dr Thomas DeLuca, and Dr. James Gannon for their assistance in my education as a graduate student and for developing this thesis. Many thanks also to Dr. Anna Sala, Dr. William Holben and Dr. Scott Miller, for their help in developing and testing my knowledge in the broader areas of plant physiology and microbial ecology. Many thanks also to Dr. Daniel Mummey for guidance in both conducting my own biochar experiments and for invaluable assistance in composing the resulting manuscripts. I express deep gratitude to members of the Rillig and DeLuca labs, both past and present, for their help, support and tutelage: Carl, Jeff, Andrew, Mary, Annika, Brooke, Rachael, Michael and Derek. Thanks to Andrew, Carl, and Jeff for their extensive help in the laboratory. And last but not least my deepest thanks goes for my family, both immediate and extended, David, Mary, Carlos, Victor, Connie, Thelma and Douglas, for all of their support, wisdom, understanding , advice and patience through this entire process.

Table of contents

Page

List of tables

Page

List of figures

Page

Section A: INTRODUCTION

The first chapter of this thesis serves as a conceptual overview that discusses biochar and AMF research published prior to August 2007. We begin the chapter by providing a definition of what we consider to be biochar, and how this charred, carbon based material is different from other carbon based substances that make up the black carbon continuum. We then discuss much of the available literature regarding the published results centered on how mycorrhizal fungi have responded to biochar presence and/ or additions in previous experiments. We then summarize many of the salient results from this body of research within the body of Table 1 of Chapter 1. Additionally, this overview proposes four potential mechanisms that may at least partly explain the mostly positive responses exhibited by both Ectomycorrhizal fungi (ECM) and AMF. 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. Each of our proposed mechanisms is rooted in results published within currently available literature regarding the responses of plants and ECM or AMF to either biochar or activated charcoal additions or presence in soils. An argument for the existence of each of our mechanisms is presented using available literature discussing results from experiments incorporating either mycorrhizal fungi or biochar into their designs. After each hypothetical mechanism is presented, we make suggestions for how future experiments, especially experiments conducted as part of my master's thesis work, should be designed in order to either support or refute our proposed mechanisms and to better establish how particular

kinds of biochar may be affecting plants, soil and mycorrhizal fungi. Lastly, we propose means for using biochar in future ecosystem restoration, agricultural and climate change mitigation efforts.

The second chapter focuses on the negative aspects of the interactions between biochar and AMF. As both factors are subject to management, understanding and exploiting their interactions may be advantageous. To date, many of the observed positive interactions between charcoal and arbuscular mycorrhizal fungi (AMF) resulted from small to medium additions of herbaceous charcoal to soils. Additionally, many of these experiments have focused almost exclusively on the ability of AMF to colonize plant roots. Results on how non-herbaceous, e.g. wood or nutshell based, charcoals affect the abilities of AMF to both colonize plant roots and soil are scarce. To add to our limited knowledge regarding biochars and their interactions with AM fungi, we designed and implemented three different experiments, incorporating three different soils, five different biochars, and eight different application rates. Through these experiments, we illustrate that five different types of biochar are all capable of significantly altering soil orthophosphate availabilities, with four of these biochars not significantly affecting soil pH. We also show the pressing necessity for increasing research efforts directed at elucidating the range of experimental durations, biochar generation temperatures, in addition to the nature, e.g. herbaceous or woody, of the feed stocks required to simulate the successes already reported in previous experiments. Overall, these findings may have implications for soil management where the goal is to increase the services provided by AMF.

Section B: MYCORRHIZAL RESPONSES TO BIOCHAR IN SOIL – CONCEPTS AND MECHANISMS

Chapter published in Plant and Soil:

Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C., 2007. Mycorrhizal responses to biochar in soil - Concepts and mechanisms. Plant and Soil 300, 9-20.

Key words

Biochar, arbuscular mycorrhiza, ectomycorrhiza, carbon storage, restoration, terra preta

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.

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 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 various 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 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^{o}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^{o}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.

Tyron (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 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 nine-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 unfavorable 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 (Tyron 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 (Tyron 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 Figure 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 act 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. Considering the previously discussed findings of Keech et al. (2005) and Gundale and DeLuca (2006) it seems reasonable to expect that biochar would exhibit similar effects.

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 (>16μm 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 1μm-4μm and 2μm-64μm respectively, with many fungal hypha being smaller than 16μm in diameter (Swift et al. 1979). Additionally, the average body-size of a soil protist is between 8 μm to 100 μm, while the average body size of soil micro-arthropods ranges from 100μm to 2mm (Swift et al. 1979). In contrast, the pore diameters in a biochar particle can often be smaller than 16μm 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, including 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 black carbon particles, including biochar; 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.

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Table 1 Effects of bio-char (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[1]	**Amouont AC[2] or BC[2] present**	**Type(s) of BC[3] or AC[3] applied**	**Response variables[4]**	**Mycorrhiza response[5]**	**Possible functions for ECM, ERM or AMF[6]**	**Source**
			AMF Experiments			
BC Effects on AMF R.C. of *Citrus iyo* in an abandoned orchard (F)	BC: 800g/m^3 in 2, 4.8 m^3 pits	H: R.H.	R.C.	+610%	N.D	Ishii and Kadoya (1994)
Effects of three BC types on AMF (*Glomus fasciculatum*) in river sand (G)	BC: 2.0% B.W.	H: R.H. Citrus Juice Sediment (C.J.) Woody: Western Spruce Bark (W.S.)	R.C.	+540% R.H. +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: 1500g m^{-2}	N.D.	R.C.	+300%	N.D.	Saito (1990)
BC (ground vs. un-ground) effects on AMF infectivity (F)	BC: 33% B.V.	H: R.H.	R.C.	Ground: +100% Un-ground: -20%	N.D.	Ezawa et al. (2002)
BC Effects on AMF (*Glomus sp.*) and *Fusarium oxysporum* R.C. of *Asparagus officinalis* roots. (G)	BC : 10% and 30% B.V.	Woody: Coconut Shell	R.C.	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 10L m^{-2}	Woody: *Acacia mangium* bark	R.C.	+42%	N.D.	Yamato et al. (2006)

BC Effects on AMF R.C. of non N-fixing, and N-fixing *Phaseolus vulgaris*) roots. (G)	BC: Applied at rates of 0, 30, 60 and 90g BC kg^{-1} soil	Woody: *Eucalyptus deglupta* logs	R.C.	Non N-fixing: 30g, 60g: -38% 90g: -20% N-fixing: 30g, 60g and N.S. 90 g +16%	N.D.	Rondon et al. (2007)
BC Effects on AMF R.C., and Spore density (S.D.) by *Glomus intraradices* grown in culture with *Zea mays* (G)	BC: 89.8% B.V. of growth substrate	N.D	R.C. S.D. in 100ml^{-1} Infectious propagules (IP) in 100ml^{-1}	R.C. -21% S.D: -5% I.P: -38%	N.D.	Gaur and Adholeya (2000)
ECM experiments						
Quantified ECM R.C. in different soil fractions of a Montana forest soil (F)	BC: 2% B.V.	N.D.	R.C., # ECM root tips 100 cm^3 soil fraction $^{-1}$	+2900%	N.D.	Harvey et al. (1976)

Effect of AC on timing of mycorrhizal colonization of *Quercus robur* seedlings by *Piloderma croceum*. (G)	AC: 2% B.W.	N.D.	R.C. Onset of mycorrhiza formation measured in weeks	R.C. +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% B.V.	N.D.	ECM presence or absence of host infection	+200%	N.D.	Vaario et al. (1999)
Effectiveness of R.H. BC/forest top soil mix as ECM inoculum source for *Shorea smithiana* trees grown in degraded forest soil. (F)	BC: 300cm^3 BC mixed with 1L soil. BC/Soil mix placed in potting hole 25cm deep x 25cm diameter	H: R.H.	Presence or absence of host infection by ECM fungi	+80%	N.D.	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 = 1890 cm^2	N.D.	R.C.	-38% in type B fungi	N.D.	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 R.C. of *Vaccinium angustifolium.*	AC: Added to solid agar medium at 1g l^{-1}	Darcco G60, Fisher	R.C.	+95% AC +128% AC + Glucose, or AC + Pectin	N.D.	Duclos and Fortin (1983)

[1] G = Greenhouse, F= Field

[2] B.V. = By volume, B.W. = By weight

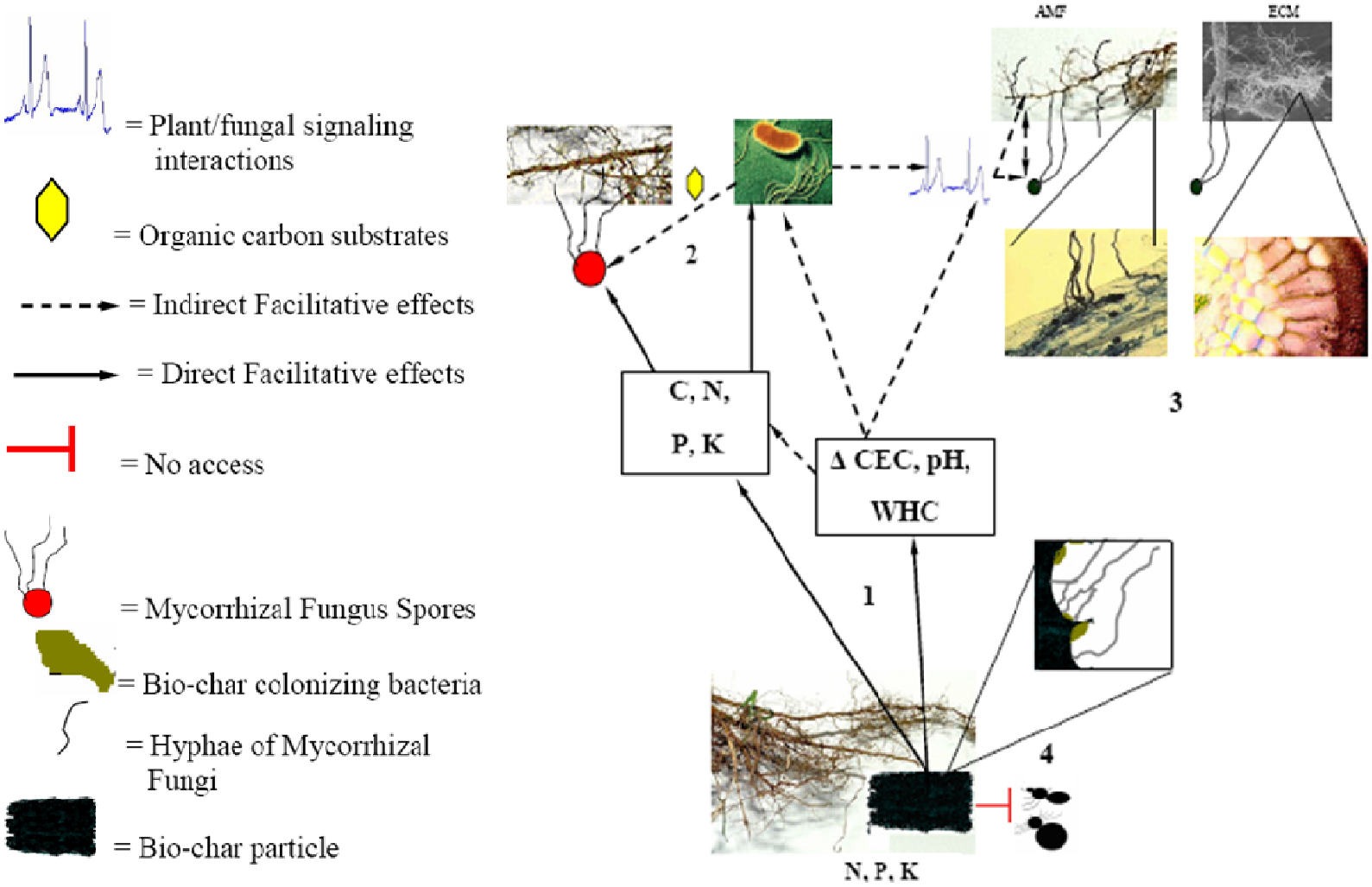
[3] AC is produced via one of the following activation procedures, CO_2, steam, or chemical (e.g. phosphoric acid). All three processes remove remaining organic compounds and nutrients from previously pyrolyzed biomass while greatly increasing carbonyl content, yielding a porous material with an extremely high surface area and a very high sorptive capacity. Because the AC activation process begins with charred biomass, it is reasonable to expect that BC and AC will both act similarly as adsorbents, in the soil environment. However, AC will likely be a much stronger adsorbent than BC because of its enhanced surface area and carbonyl content (Pan and van Staden 1998). H. = Herbaceous bio-char, R.H. = Rice Husk bio-char

[4] R.C. = Root colonization, S.D. = Spore density

[5] N.S. = Non significant difference, Effect size for response variables was calculated as ((mean $X_{treatment}$ mean - $X_{control}$/ $X_{control}$) *100.

[6] N.D. = Not determined

Figure 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.*



Section C: NON-HERBACEOUS BIOCHAR AMENDMENTS CAN DECREASE ARBUSCULAR MYCORRHIZAL FUNGI ABUNDANCE IN ROOTS AND SOIL

Abstract

Biochar shows potential as a soil amendment for improvement of soil quality and for carbon sequestration. However, knowledge of how biochar amendments can influence various soil properties and populations of soil microorganisms is limited. We conducted three experiments employing three different soils and five different biochars to examine biochar influences on arbuscular mycorrhizal fungal (AMF) abundance in roots and soil. Our results indicate that AMF abundance either remained unchanged or decreased with biochar amendment across all treatments. Our results also indicate that biochar, depending on the nature of the feedstock, the temperature attained during pyrolysis and amounts applied can significantly alter soil properties including phosphate availability. These findings may have implications for soil management where the goal is to increase the services provided by AMF.

Key words

Arbuscular mycorrhizal fungi; root colonization; extraradical hyphae; Biochar; Black carbon

Introduction

Biochars can improve soil quality and have been proposed as a potential means to sequester atmospheric carbon (Lehmann et al., 2006; Lehmann, 2007a, b). Despite the

potential usefulness of biochar for soil management applications, our knowledge of how these materials influence soil physical, e.g. bulk density or water holding capacity, chemical and biotic properties is limited compared to other soil supplements.

Biochars, or charcoals, and other black carbon materials are produced by partially combusting (charring or pyrolyzing) biomass-derived feedstocks. Ash production during pyrolysis is largely prevented via oxygen gas limitation, producing biochar. During pyrolysis, the molecular structure of the feedstock changes, yielding polyaromatic hydrocarbon rich biochars (Schmidt and Noack, 2000; Preston and Schmidt, 2006) which are typically highly resistant to microbial decomposition (Baldock and Smernik, 2002). Due to its complex chemical structure, biochar is believed to typically have a long mean residence time in soil, with estimates of between 1,000 to 10,000 years being common (e.g. Skjemstad et al., 1998; Swift, 2001; DeLuca & Aplet 2008; Kuzyakov et al., 2009). Given these residence times, biochars are beginning to receive attention as a potential means for delivering and storing C in soils on a stable and long-term basis (Lehmann, 2007a, b).

A number of studies indicate that biochar can alter soil physicochemical properties, including pH, cation exchange capacity (CEC), and bulk density (BD) (Tyron, 1948; Glaser et al., 2002; Lehmann et al., 2003; Gundale and DeLuca, 2006; DeLuca et al., 2006). Such alterations may improve soil quality; thereby increasing plant biomass production (Lehmann et al., 2003; Oguntunde et al., 2004). Thus, biochar may constitute an important soil management tool in the context of sustainable agriculture and land reclamation. However, to fully realize the potential of biochar as a soil amendment,

further knowledge of how different biochars influence soil physical, chemical and biological characteristics is required.

Arbuscular mycorrhizal fungi (AMF) are suggested to be one of the most important soil microbial groups in the context of modern organic agricultural practices (see reviews by Gosling et al., 2006 and Piotrowski et al., 2008c) and land reclamation (Renker et al., 2004). AMF form symbioses with approximately 2/3 of known plant species including many important crops (Trappe, 1987). These obligate biotrophs cannot complete their life cycle without receiving fixed C from their host plant (Smith and Read, 2008). In exchange for sugars, AMF provide their hosts with benefits including increased access to immobile nutrients, especially phosphorus, improved water relations, and greater pathogen resistance (Newsham et al., 1995; Smith and Read, 2008). Soil amendments which increase AMF abundance and/ or functionality could be beneficial to plant hosts and result in improved soil quality via influences on soil structure (Rillig and Mummey, 2006).

A few studies indicate that soil biochar amendments can increase AMF percent root colonization in plants growing in acidic soils (Ezawa et al., 2002; Matsubara et al., 2002; Yamato et al., 2006). Although the mechanisms responsible are poorly understood, modulation of soil pH likely plays a role (Warnock et al., 2007). Less is known about biochar influences on AMF abundance in non Iron oxide rich soils. Moreover, biochar influences on production of AMF extraradical hyphae, the fungal structures that actually explore the soil environment and facilitate plant nutrient uptake, are unknown.

Both biochar feedstock (Keech et al., 2005; Gundale and DeLuca, 2006) and the maximum temperature attained during combustion influences biochar physical and

chemical properties (Gundale and DeLuca, 2006; Lehmann 2007a). In terms of feedstocks, approximately half of the studies reporting positive interactions between biochar and AMF also reported using biochars derived from herbaceous plant materials, most commonly rice husks (Warnock et al., 2007). Much less is known about how biochars derived from non-herbaceous materials, such as nutshell or wood, influence AM fungi. More information is clearly needed about how variations in biochar characteristics influence soil properties, especially in non-acidic soils.

This study was conducted to evaluate whether biochar amendment enhances mycorrhizal fungal abundance, both in terms of root colonization and extraradical hyphae production. Given the increased interest in use of biochar as a soil amendment, we aimed to broaden the information base concerning how biochar amendments initially influence AMF abundance after application. In order to increase the parameter space for which effects on AMF are examined we used biochars produced at different temperatures and also biochars applied at different rates.

Materials and Methods

Experiment 1: Multiple application rates

Soil, including its constituent AMF inoculum, was collected from a well characterized, site on the Nyack floodplain adjacent to Glacier National Park (48º 27' 30" N, 113º 50' W) (Table 2). This soil was formed through deposition of flood sediments 9 years prior to collection. Piotrowski et al., (2008a) established that this soil has a

relatively low soil organic matter (SOM) content, a high mycorrhizal inoculum potential (MIP), and soil hyphal abundance, in addition to having a low. A soil with a low SOM was selected so we could minimize interactions between biochar and SOM. Soil (15 L) was collected using a spade (0 to 20 cm depth) from multiple locations and pooled after sieving (2mm mesh).

Biochar used for this experiment was derived from *Pinus contorta* Douglas ex Louden (lodgepole pine) wood. Wood chips were tightly packed into 250 cm^3 metal canisters and heated in a muffle furnace. The maximum temperature reached during charring (600^oC) was stabilized for one hour the after feedstock materials were placed in the furnace. The resulting biochar was ground through a 1 mm sieve, and subsequently mixed with soil at the following rates (w/w): 0.0% (control), 0.5%, 1.0%, 2.0%, and 4.0%. Pots (50 mL; n = 10) were filled with 63g of each treatment soil mixture.

Plantago lanceolata L. (narrowleaf plantain) served as the AMF host plant. Each pot was planted with two seedlings and placed in a growth chamber (21^oC, 50-70% relative humidity, 18h light, at 324 μmol photons m^{-2} s^{-1} PAR). After 7 d growth, the plants were thinned to one individual per pot. Pots were watered to field capacity daily, with tap water. After 30 days of growth, soil and plant materials were collected and examined as described below.

Experiment 2: Multiple biochar production temperatures

Soil for this experiment was also collected from the Nyack floodplain using a similar sampling protocol as Experiment 1. However, the flood sediments that form this

soil were laid down only two years prior to collection and, in contrast to soil used in Experiment 1, AMF abundance and MIP are known to be relatively low (Piotrowski et al., 2008b). Like the soils from experiment 1, theses soils were also shown to have a low SOM content (Table 2)

Three different biochars, varying only in the maximum temperature attained during pyrolysis, were used in this experiment. These biochars were commercially produced from peanut shell pellets (Eprida Inc., Athens, Georgia, USA) by heating 1 kg batches to 360^{o}C, 400^{o}C or 430^{o}C using a bench scale batch pyrolysis system. Charred materials were removed from the muffle furnace when the temperature had reached the specified maxima and remained stable for 5 minutes. We ground the resulting biochar pellets to homogenize the material, and used the 0.20 mm to 0.71mm size fraction for the experiment. Biochar materials were mixed with soil (10% v/v) and 100 mL of the mixture placed in pots (Cone-tainers^{tm}; 120 ml; Stuewe and Sons, Canby OR, USA). A non-amended soil served as the control treatment. All treatments were replicated 8 times. Plant materials, growth conditions, and experimental duration were the same as for Experiment 1; sampling procedures are described below.

Experiment 3: field study in Colombia

Experimental plots were established at Matazul farm in the Eastern Plains of Colombia (N 04º10’15.2”, W 07 º36’12.9”), a region of non-flooded savannas that receive an average of 2200 mm rainfall annually, with 95% falling between April and December.

Soils of the area (Tropeptic Haplustox) were developed from alluvial sediments (Rippstein et al., 2001), and like soils from experiments 1 and 2, our analyses showed that these soils, when not treated with biochars also had a relatively low C content (Table 6).

Biochar for this experiment was produced from *Mangifera indica* L. (Mango) trunks and branches. These materials were stacked, covered with soil and grass and combusted. After pyrolysis the resulting biochar was uncovered and ground to pass through a 0.9mm sieve. Biochar was incorporated into the top 15cm of the soils by two disk harrow passes. Biochar application rates of 0, 13, 26 and 130 Mg ha^{-1} were used to increase soil carbon pools by 0%, 50%, 100% and 500%, respectively. Biochar was applied to soils in a randomized, complete block arrangement, with a total of three blocks, so that each treatment was replicated 3 times. After biochar incorporation in December 2004, native C4 savannah grasses were allowed to re-colonize the plots. Soil samples (0-5cm depth) were collected in August 2005 and analyzed as below.

Biochar

Biochar chemical characteristics were examined prior to their use as soil amendments. Biochar pH was estimated from 1:10 slurry (1g char to 10mL water or 1N KCl solution) after shaking 3 times over 1 hour using a Symphony gel electrode (VWR, West Chester PA, USA). Percent total C and N contained in biochar materials was examined using a CN analyzer (UC Davis Stable Isotope Lab, Davis, California, USA). Soluble P was

extracted from char materials using the Mehlich-3 extraction procedure (Mehlich, 1984) and analyzed using ICP-MS (Dairy One Labs, Ithaca, New York, USA).

Soil

Soil pH and plant available P was measured for soils from all three experiments. Soil pH was measured in deionized water (Peech, 1965). Sodium bicarbonate extractable P was examined using an ascorbic acid method as described by Murphy and Riley, (1962).

Soil densities were evaluated for both Experiments 1 and 2. Air-dried soil samples were placed in a container with known weight and volume. Soil weight and volume were recorded for calculations of sample density. For these measurements we analyzed six randomly selected replicates from Experiment 1 and five from Experiment 2.

Plants and AMF

Root and shoot biomass for Experiments 1 and 2 was determined after drying (60^{o}C, 24h).

AMF percent root colonization was examined for Experiments 1 and 2 as described by Brundrett, (1994). We assessed mycorrhizal colonization at 200X using a gridline intersect method (McGonigle et al., 1990) scoring AMF hyphae, vesicles and arbuscules. AMF were differentiated from other root colonizing fungi based on morphological characteristics, including: dark melanization, clamp connections, regularly

septate hyphae, or frequent non-dichotomous branching, which are considered traits indicative of non-AM fungi (Rillig et al., 1999).

Extraradical AMF hyphae were examined for all experiments. Hyphae were extracted from soil samples (5 cm^3) using an aqueous membrane filtration method (Rillig et al., 1999) and analyzed using microscopy (200X). Hyphal length was measured using a grid-line intersect method as described in Jakobsen et al., (1992). AMF hyphae were distinguished from hyphae of other soil fungi based on morphological criteria as above for AMF percent root colonization.

We examined potential biochar influences on extraradical hyphae extraction efficiencies in soil samples from Experiment 1 amended with 0, 0.5, 1, 2 and 4% lodgepole pine biochar (w/w). Extraction efficiencies were estimated by collection and examination of hyphae passing through sieves and associated with soil sediments after hyphal extraction (Rillig et al., 2000).

Addition of biochar to soil dilutes the amount of AMF inoculum available to infect host plants. We accounted for these biochar related dilutions by determining the change in soil density due to biochar. Dilution correction factors were generated using the formula, $x = 1 + [1 - (\text{density experimental soil} * \text{density control soil}^{-1})]$. We applied the resulting values to the AMF root colonization and AMF hyphal abundance estimates of Experiments 1 and 2. We assumed that amounts of AMF infectious propagules and root colonization rates covaried linearly, as shown in previous short-term pot experiments (Moorman and Reeves, 1979; Tarbell and Koske, 2007). Conversely, results of a number of experiments suggest that for some AMF inoculum sources changing the concentration of AMF inocula does not significantly alter root colonization rates in short-term

mycorrhizal inoculation potential experiments (Perner et al., 2006; Rowe et al., 2007; Tarbell and Koske, 2007). Therefore our 'dilution' correction was likely conservative. Because of its longer duration, we felt such a correction was unwarranted for the field study, Experiment 3, as secondary colonization events would have occurred.

Statistical analyses

When the data fulfilled the assumptions of normality, we used a one-way ANOVA in Experiments 1 and 2 to compare the effects of biochars on AMF root colonization, plant growth, as well as both soil parameters. ANOVA tests were followed by Tukey-Kramer multiple comparisons analyses using JMP (Version 6. SAS Institute Inc., Cary, NC, 1989-2005). When normality assumptions of ANOVA were not meet, we performed Kruskal-Wallis one-way ANOVA, a non-parametric ranking procedure, using NCSS (NCSS, Kaysville, Utah, USA). One-way randomized block ANOVA was used to analyze all data generated in Experiment 3. CoStat software (ver 6.311; CoHort Software, Monterey CA, USA) was used for these analyses. Data points more than two standard deviations away from the mean, were considered outliers and omitted from analyses.

Results

Chemical properties of biochars

The peanut shell biochars from all three generation temperatures, 360°C, 400°C and 430°C, were found to contain substantially more soluble P and N, than the lodge pole wood pine biochar (Table 3). All five biochars, examined exhibited basic pH (> 7.7), with the mango biochar pH (measured in H_2O) being at least 1.7 units greater than the other biochars.

Soil bulk density

Lodgepole biochar amendments in Experiment 1 significantly affected soil densities ($F=68.0$, $P<0.001$). While unamended soil had a bulk density of 1.35 g cm^{-3}, addition of 2.0% and 4.0% biochar decreased soil density to 1.28 g cm^{-3} and 1.12 g cm^{-3}, respectively. In contrast, peanut shell biochar did not significantly affect soil densities in Experiment 2 ($F=0.618$, $P=0.613$), which averaged 1.40 g cm^{-3}.

Hyphal extraction efficiency

Our biochar addition rates (w/w) showed no effects on the hyphal extraction efficiencies in any of our lodge pole pine biochar treatments ($F= 1.00$, $P= 0.435$). Respective hyphal extraction efficiencies were estimated at 92.5%, 96.1%, 94.0%, 96.7% and 98.3%, for the 0.0%, 0.5%, 1.0%, 2.0% and 4.0% biochar addition treatments. These efficiencies are reflected in the data we present.

AMF inoculum dilution

Only differences between the 400^{o}C biochar addition treatment and the no-biochar treatment of Experiment 2 were influenced by applying correction factors to account for AMF inoculum dilution (Table 5). AMF dilution correction factors for Experiments 1 and 2 are included in Tables 4 and 5, respectively.

Experiment 1: Multiple addition rates

Plant mortality reduced the number of replicates to nine in the 1.0% biochar addition treatment, and eight in the 4.0% biochar addition treatment. Also, because root or soil samples were unavailable at the time that slides were made, the number of replicates for AMF root colonization and hyphal abundance were reduced in the following treatments: nine total replicates, for both measurements in the control, eight total replicates in the 4.0% treatment, with seven replicates for hyphal length measurements in the 0.5% addition treatment.

Both 2.0% and 4.0% biochar addition treatments resulted in significantly reduced AMF hyphal lengths compared to unamended soils (Table 4). Soil P availability was significantly lower for 1.0% and 4.0% biochar addition treatments (Table 4).

Experiment 2: Multiple generation temperatures

Plant biomass production was significantly greater in the 430^{o}C biochar treatment than in all other treatments (Table 5). No other significant differences for this measure were

found between treatments. AMF root colonization was found to be significantly less for the 360°C and 400°C biochar treatments compared to the control (Table 5). AMF extraradical hyphal lengths were found to be significantly less in soils of the 360°C biochar treatment than in all other treatments. No other significant differences in this measure were found between treatments. While soil pH was not significantly influenced by any of the peanut shell biochars, all significantly increased soil P availability (Table 5).

Experiment 3: Colombian field experiment

Treatments in which biochar was incorporated into soils at higher rates (26 t and 130 t biochar ha^{-1}) exhibited significantly decreased AMF hyphal abundance (Table 5). Application of both 26 t and 130 t biochar ha^{-1} resulted in significantly increased P availability (Table 6). Soil pH was found to increase significantly with increased biochar application rate (Table 6).

Discussion

All three of our experiments, encompassing a range of biochars and soils, indicate neutral to decreased AMF abundance as measured by percent root colonization and/or extraradical hyphae production. Furthermore, the results from experiments 1 and 2 are the first to show significant reductions in AMF abundance after biochar application to

temperate, non-acidic soils. However, the underlying mechanisms behind these observations remain unclear.

At least two studies thus far, have reported increased AMF abundance in response to biochar addition treatments to acidic soils in Japan (Matsubara et al., 2002; Yamato et al., 2006). In these studies pH was shown to increase after addition of biochar to soil, suggesting that pH modulation might be a mechanism responsible influencing AMF abundance. In the present study, only the pH of the acidic Colombian field soil (Experiment 3) was significantly influenced by biochar addition. However, in contrast to what was observed for acidic soils in Japan, AMF abundance decreased in this soil with increased biochar application rate and soil pH. This suggests that other treatment effects besides pH are responsible for altered AMF abundance in this soil.

Phosphate is central to interactions between plants and AMF (Smith and Read, 2008), with multiple sources suggesting that either extremely low (e.g. Allen et al., 2003; Drew et al., 2006) or high (Corbin et al., 2003; Covacevich et al., 2006; Gryndler et al., 2006) soil P availability can adversely affect AMF abundance in roots and soils.

Results from Experiment 1, which used Lodgepole pine biochar containing relatively low amounts of soluble P, indicate decreased soil P availability in the presence of biochar (Table 3). Compared to peanut shell biochars used in Experiment 2, this biochar was produced at relatively high temperatures, which is known to increase sorptivity of resulting chars for different molecules (Antal and Grønli, 2003; Gundale and DeLuca, 2006; Smernik et al., 2006; Lehmann 2007a), potentially including phosphorus. Kuzyakov et al. (2009), suggested biochar sorption of nutrients and available organic C as a mechanism for decreased SOM decomposition. Although we have no data regarding

OM mineralization in the present study, decreased OM mineralization, and concurrent P mineralization, could result in decreased P availability.

In contrast, peanut shell biochars contained greater soluble P than biochar derived from lodge pole pine (Table 3). This adds to results of other studies indicating that biochars can contain P (Topoliantz et al., 2005; Gundale and DeLuca, 2006; Yamato et al., 2006), which may be desorbed into the soil solution. Although not constituting direct evidence for P desorption from biochar, results from Experiment 2 indicate significantly increased P availability after addition of peanut shell biochar (Table 5).

Biochar applications can alter soil P availability via modulation of soil pH (Tyron, 1948; Matsubara et al., 2002; Glaser et al., 2002). Our results show that soil alterations of pH due to biochar application were minimal for Experiments 1 and 2 (Tables 4 and 5), but significant for Experiment 3 (Table 6). Given our results, it seems plausible that large applications, e.g. 26 Mg ha^{-1} and 130 Mg ha^{-1}, of high pH mango-wood biochar and accompanying ash (Table 3), contributed to the increased soil P, potentially by increasing soil pH levels toward circum neutral values (Table 6).

Our results indicate that AMF abundance can significantly decrease in the presence of newly applied biochar may have important implications for its use as a soil amendment. However, biochar properties and, hence, how biochars influence AMF abundance may change with equilibration to the soil environment (Cheng et al., 2006, 2008; Lehmann, 2007a).

For example, a number of studies indicate that biochars can contain organic pyrolytic byproducts, including phenolics and polyphenolics, which may be inhibitory to soil organisms, including AMF. Generated from the condensates of cellulose, tannins,

and lignin polymers originally contained in the feedstock materials prior to charring (Antal and Grønli, 2003; Gundale and DeLuca, 2006), these substances are most typically associated with low temperature pyrolysis which serves to limit volatilization. Phenolics would be expected to be relatively labile in the soil environment, especially in relation to other biochar constituents, and the potential for microbial inhibition may therefore be transient. Although data pertaining to potential inhibitory substances associated with biochars used in our experiments are not available, biochars generated at lower temperatures resulted in the greatest decreases in both intra and extraradical AMF abundance (Table 5).

Although further work is needed to elucidate long-term biochar influences on AMF, our results are at least relevant to annual production systems and the initial stages of land restoration or reclamation in the first few months after biochars application. Our results also illustrate that biochar properties can differ with feedstock and temperature achieved during pyrolysis. This highlights the need for reporting biochar feedstock, generation temperature and chemical properties in studies where biochar is used as a soil amendment.

Conclusion

Our results show the potential for some biochars to significantly affect AMF shortly after incorporation; if a goal of biochar application is the improvement of soil fertility, then our results send a strong cautionary note that materials should be thoroughly tested for potential adverse (micro-)biological effects prior to large scale field-application. It is

clear from our study that a wide parameter space (feedstock properties, production conditions, and application rates) is necessary to cover potential effects on AM fungi, and likely on other soil biota as well.

Acknowledgements

We thank Danny Day and Robert Hawkins (Eprida, Inc., Athens, Georgia, USA) for providing the peanut shell biochars for this study. We would like to acknowledge financial support from the Integrative Microbiology/Biochemistry department at the University of Montana. We would also like to acknowledge support from the Department of Crop and Soil Sciences at Cornell University.

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Table 2: Preliminary, abiotic measurements of soil characteristics for young 1-13yr old, Nyack soils. These results are published in Piotrowski et al., (2008b). Characteristics from the soils employed in our experiment one correspond to those from the 7yr soil, with the soil characteristics of the 1 yr old soil corresponding to the soil used in our experiment 2. Numbers in parenthesis are equal to one standard error.

Site Age	pH	% OM	NO3 (mg/kg)	Olsen P (mg/kg)
1	8.0 (0.0)	0.7 (0.8)	5.0 (2.6)	2.7 (0.3)
4	8.1 (0.0)	0.4 (0.0)	1.5 (0.3)	2.0 (0.0)
7	8.1 (1.0)	0.6 (0.2)	1.8 (0.6)	2.0 (0.0)
13	8.1 (0.0)	0.7 (0.0)	1.0 (0.5)	2.0 (0.0)

Table 3: Background data for all biochars, with measurement taken prior to biochar incorporation into experimental soils.

Biochar property	**Field produced Mango wood**	**600°C Lodgepole pine**	**360°C Peanut shell**	**400°C Peanut shell**	**430°C Peanut shell**
pH (H_2O)	10.14	7.7	8.35	8.34	8.23
pH (1 N KCl)	8.92	8.2	6.72	6.72	6.70
Total C (%)	71.7	67.8	60.0	65.7	64.7
Total N (%)	0.3	0.13	1.75	1.42	1.65
Soluble P[1] (mg P g^{-1} char)	Not available	0.02	0.39	0.30	0.42

[1]Previous experiments show that soluble P estimates from the Mehlich3 extraction procedure correlate well with those estimates from either Olsen P, or Bray P1 tests for soluble P respectively, in either basic or acidic soils (Schmisek et al., 1998; Ebeling et al., 2008).

Table 4: Effects of 600°C Lodge pole pine biochar addition rates on soil pH, P availability, plant biomass and AMF. Numbers in parentheses represent standard error of the mean; numbers in brackets represent the biochar correction factor applied to the AMF response data from each biochar addition treatment.

Treatment: Percent biochar in soil mixture (w/w)	**Soil pH[1]**	**Soil P availability (mg P kg soil^{-1})[1]**	**Plant Biomass (mg)**	**Root colonization by AMF (%)[2]**	**AMF hyphal lengths (m hyphae/ cm^3 soil)[1,2]**
0.0% (Control)	7.87 (0.001)^{a}	3.43 (0.032)^{a}	16.2 (1.70)	80.9 (4.08)^{ab} {1.00}	16.7 (0.071)^{a} {1.00}
0.5%	7.72 (0.003)^{b}	3.26 (0.022)^{ab}	15.4 (1.20)	83.2 (2.11)^{ab} {0.97}	19.9 (0.090)^{a} {0.97}
1.0%	7.84 (0.001)^{ab}	2.34 (0.037)^{bc}	18.4 (1.70)	92.3 (3.24)^{a} {0.96}	12.6 (0.070)^{ab} {0.96}
2.0%	7.76 (0.003)^{ab}	2.46 (0.036)^{abc}	16.0 (1.20)	77.3 (3.20)^{b} {1.05}	7.09 (0.057)^{b} {1.05}
4.0%	7.83 (0.001)^{ab}	2.28 (0.054)^{c}	14.0 (0.700)	70.8 (3.17)^{b} {1.17}	4.50 (0.084)^{b} {1.17}
F ratio	3.43	5.65	1.30	5.68	14.9
P value	0.024	0.002	0.300	< 0.001	< 0.001

[1]Data from soil pH, soil orthophosphate availability, and AMF hyphal abundance data were Log_{10} transformed prior to ANOVA calculations.
[2]AMF abundance results were adjusted to account for soil and/ or AMF inoculum dilutions (see Methods).

Table 5: Effects of peanut shell biochar generation temperature on soil pH, Olsen P availability, plant biomass and AMF. Numbers in parentheses represent standard error of the mean; numbers in brackets represent the biochar correction factor applied to the AMF abundance data from each biochar addition treatment.

Treatment: biochar generation temperature	Soil pH[1]	Olsen phosphate availability (μg g^{-1} soil)[2]	Plant Biomass (mg)	Percent Root colonization by AMF[3]	AMF hyphal lengths (m hyphae cm^{-3} soil)[1,3]
Control (no biochar)	7.90 (0.131)[a]	4.19 (0.036)[a]	22.9 (2.56)[a]	15.9 (4.74)[a] {1.00}	2.12 (0.198)[a] {1.00}
360°C	7.97 (0.018)[a]	8.44 (0.026)[b]	24.4 (1.48)[a]	4.18 (1.95)[b] {1.03}	0.124 (0.225)[b] {1.03}
400°C	7.90 (0.070)[a]	11.6 (0.065)[b]	22.8 (2.41)[a]	5.03 (1.49)[b] {1.03}	0.904 (0.139)[a] {1.03}
430°C	7.86 (0.322)[a]	8.74 (.078)[b]	33.5 (2.44)[b]	5.61 (1.49)[ab] {1.03}	1.33 (0.120)[a] {1.03}
F ratio	3.61	10.7	3.83	4.11	5.58
P value	0.310	0.002	0.020	0.020	0.006

[1] For soil pH analyses, we performed a Kruskal-Wallis one-way ANOVA to determine statistical significance of biochar effects on soil pH.
[2]Data from soil orthophosphate were Log_{10} transformed prior to ANOVA calculations.
[3] AMF abundance results were adjusted to account for soil and AMF inoculum dilutions (see Methods).

Table 6: Effects of mango wood biochar addition rates on soil pH, P availability, AMF; numbers in parentheses are equal to one standard error of the mean.

Treatment: biochar addition rate (Tons biochar hectare^{-1})	**Soil pH**	**Soil Carbon (mg C g soil^{-1})**	**Olsen P availability (mg P kg soil^{-1})**	**AMF hyphal abundance (m hyphae/cm^3 soil)[1]**
0	5.60 (0.100)^{a}	6.47 (0.767)^{a}	6.43 (0.700)^{a}	19.2 (1.91)^{a}
13	5.72 (0.083)^{a}	11.9 (0.973)^{a}	7.72 (1.00)^{ab}	17.6 (1.87)^{a}
26	6.08 (0.044)^{b}	15.2 (2.45)^{a}	10.5 (0.263)^{bc}	10.9 (2.56)^{b}
130	6.91 (0.085)^{c}	59.6 (6.23)^{b}	13.4 (0.736)^{c}	4.45 (0.687)^{c}
F ratio	55.7	51.7	18.3	8.40
P value	< 0.001	< 0.001	< 0.001	0.014

[1]AMF hyphal abundance results were not adjusted to account for biochar additions in these treatments.

Section D: GENERAL CONCLUSIONS

Through the discussion and evaluation of multiple sets of experimental results, this thesis illustrates the ability of multiple biochars to significantly influence total AMF abundance and total plant biomass production. Furthermore, based on these results, the biochar related influences on AMF abundance abundances varied from neutral to strongly negative. It is also possible that this variation occurs over multiple time scales. Therefore, if a goal of particular biochar application is the improvement of soil fertility, then the results from our non-herbaceous biochar experiments should send a strong cautionary note that all biochar parent materials should be thoroughly tested for potential adverse (micro-)biological effects prior to large scale field-application.

Based on our experimental results, it appears increasingly vital that we attempt to bolster our understanding how biochar treatments could affect different aspects of AM fungal biology, e.g. total AMF abundance and community composition, by encompassing an increasingly wide parameter space in future biochar and AMF experiments, As mentioned in our literature review, we still seem to lack any understanding of how biochar applications may ultimately affect overall AMF community composition. Considering the already discussed relationships between AMF community composition, plant community diversity and productivity, in addition to overall ecosystem functioning (Section A), this may be another critical aspect of biochar and AMF research, likely requiring further evaluation as we endeavor to scale-up our biochar application projects to the whole-field level.

Once equipped with a better understanding of this potential synergism and the mechanisms that drive it, we could exploit 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.

Appendix A: BIOCHAR INFLUENCES ON SPECEIS INVASIVENESS VIA INFLUENCES ON ARBUSCULAR MYCORRHIZAL FUNGAL (AMF)-HOST PLANT DYNAMICS

A peer-reviewed publication based on the results discussed below is currently in preparation and I expect to submit the manuscript for publication within the calendar year; the following text is an excerpt of the draft currently in preparation for eventual publication.

My overall goal for this experiment was to determine if applications of a high temperature biochars could adsorb allelopathic compounds secreted by spotted knapweed plants and thus gain more insight into the role of AM symbioses in knapweed invasion dynamics.

Materials and Methods

Biochar production procedures

Doug-fir wood chunks were immersed in a sand bath, for oxygen limitation, and were charred at 350^{o}C or 650^{o}C in a muffle oven for two hours. The resulting biochar was ground through a 1 mm sieve, and mixed in with the soil.

We selected biochar generation times, temperatures, and source materials based on results published in Gundale and DeLuca (2006), who also used Doug-fir wood, and a

two hour generation time, however, they selected generation temperatures of 350°C and 800°C. For our experiment, we expected the 350°C biochar to have reduced chemical effects on the soil, i.e. it would not be a strong sorbent of root exudates but, it would still have similar effects and bulk density as the 650°C biochar. Additionally, based on their analyses of 800°C biochar in Gundale and DeLuca (2006), we did expect that the higher generation temperature of 650°C would act as a stronger sorbent of root exudates, in comparison the 350°C biochar, and thus partially neutralize their effects on soils and therefore reveal the influences of native AMF (Gundale and Deluca 2006).

Experimental design and harvesting procedures

This experiment consisted of 9 different treatments, and 12 replicates per treatment. The treatments consisted of soils amended with the following components: ±350°C char, or ±650°C char (10% v/v), ± spotted knapweed, and ± Idaho fescue. A total of 450mL biochar and soil mixture was placed in each pot. We first planted four pre-germinated Idaho fescue seeds per pot. All seeds for both plant species were pre-germinated by placing seeds on wet filter paper, inside of separate, closed Petri-plates. Petri-plates were placed on a lab bench-top until germination. After one week, we thinned to two seedlings per pot in the intraspecific Festuca only pots, and one seedling per pot in the intraspecific Idaho fescue/ spotted knapweed pots. After six weeks, we planted four pre-germinated spotted knapweed seeds per pot. After one week, the pots planted with spotted knapweed were thinned following the same procedures as with the Fescue pots. All plants were allowed to grow and additional six weeks. All plants were grown in a

growth chamber (16h light/8h dark, at 324 μmol photons m^{-2} s^{-1} PAR, with 50-70% humidity, and 20°C).

At harvest, we carefully separated the plants from the soils and rinsed the root systems with tap water. When dealing with the competition pots, we carefully separated each the root system from each plant species prior to root and shoot separation. Soils were placed in paper bags and air-dried at room temperature. After drying, we placed all soil samples in plastic bags for storage. Once we separated the plants from the soils, we then separated the plant's roots and shoots from each other. After separation, we place the roots and shoots dried (60°C for 24 hours).

Plant analyses

We quantified both root and shoot mass as dry weight. After quantifying shoot dry mass, leaves of each plant were separated from stems and foliar nutrients analyzed using ICP (Spectrum Analytical, Washington Court House, OH, USA).

AMF analyses

AMF percent root colonization was examined as described by Brundrett et al. (1994). We assessed mycorrhizal colonization at 200X by the gridline intersect method (McGonigle et al. 1990) at ~100 randomly selected locations covering the entire slide, scoring any AMF structures as positive for colonization (hyphae, vesicles, arbuscules). AMF were differentiated from other root colonizing fungi based on morphological

characteristics: melanization, clamp connections, regularly septate hyphae, or non-dichotomous branching (Rillig et al. 1999).

Extraradical hyphae were examined for all experiments. Soil hyphae were extracted from soil samples (5 cm^3) according to Rillig et al. (1999), employing an aqueous membrane filtration with subsequent microscopic examination at 200X. Hyphal length was measured using the grid-line intersect method as described in Jakobsen et al. (1992) and Tennant (1975). The AMF hyphae were distinguished from hyphae of other soil fungi based on morphological criteria as above.

Soil analyses

We determined soil pH and extracted soil Olsen-P for all treatments. Soil pH was estimated using a 1:1 (w/v) slurry (15g soil to 15mL deionized water) (Peech 1965). Available soil orthophosphate, e.g. sodium bicarobonate extractable phosphate, was examined using an ascorbic acid method as described by Murphy and Riley (1962). Neither our biochar addition treatments, nor our plant completion scenarios significantly affected soil pH ($H= 14.3$, $P= 0.072$). The mean soil pH among all nine treatments was approximately 7.5.

Data Analyses

When the data met the assumptions of normality, we used we used a two-way ANOVAs to compare the effects of both biochars and plant competition scenarios, on root biomass, shoot biomass, root to shoot ratio, tissue nutrient contents AMF root colonization, AMF

hyphal lengths in soils, as well as soil pH and orthophosphate available. In addition to the two-way ANOVAs, we also performed a series of one-way ANOVAs to determine significance of differences within intraspecific and interspecific treatments. Our one-way ANOVA procedures were coupled with Tukey-Kramer analyses where appropriate, when the data fulfilled the assumptions of normality. All ANOVA and Tukey-Kramers analyses were performed using 6.411 (CoHort Software, Monterey, CA, U.S.A, 1996-2008). If data did not meet the assumptions of ANOVA, we performed a Kruskal-Wallis test using NCSS (NCSS, Kaysville, Utah, USA). Identification and removal of outling datapoints if they met Pierce's criteria (Pierce 1852) for outliers, as discussed in Ross (2003).

Results and Discussion

Shoot production of spotted knapweed was greatly increased when grown in the presence of the 650^{o}C Doug-fir biochar, and was nearly doubled when grown in the presence of both the biochar and Idaho fescue (Figures 2A and 2F). Furthermore, based our root and shoot biomass results from the (+)350^{o}C biochar treatments (Figures 2A, 2B, 2D and 2E), it does not appear that the 350^{o}C biochar treatments exerted any appreciable influences upon the competitive relationships between plant species. Laslty, when combined with the positive shoot responses from in the interspecific 650^{o}C biochar treatment, both our AMF root colonization (Figures 3A and 3B), and our AMF hyphal length (Figure 5B) results, indicate that spotted knapweed's overall response may have been caused by factors beyond AMF, and allelopathic root exudates

While showing no apparent effects on competitive relationships between these two plant species, the results from our intrapsecific treaements do illustrate the importance of how increases in biochar generation temperatures can alter the capacity for resultant biochars to influence the relationships between a plant host and its AMF symbionts. In this experiment the root and shoot biomasses produced from both species, were each significantly greater in the intraspecific 650^{o}C biochar treatment, without showing a similarly significant response to the 350oC biochar treatments (Figures 2A-2D). Also, from the intraspecific 350^{o}C biochar treatments, we observed significant increases in AMF root colonization within Idaho fescue plants, without seeing a similar response in the Idaho fescue (+) 650^{o}C biochar treatment (Figure 3A). Lastly, from the spotted knapweed pots, we observed a significant decline in AMF root colonization rates when comparing the treatment mean from the 350^{o}C treatment to that of 650^{o}C treatment (Figure 3B). However, we should note that neither of these two treatment means were significantly different from the mean from the (–) biochar treatment.

Biochar related influences on soils, plants, and AMF: Intraspecific treatments

As stated in the paragraph discussing the different treatment effects tied to various biochar generation temperatures, both plant species showed significantly positive responses to the 650^{o}C biochar for all of our plant biomass measures (Figures 2A, 2B, 2D and 2E). Interestingly, we also observed a significant decline in root biomass production when knapweed plants were in soils treated with the 350^{o}C biochar (Figure 2D). However, we observed no other significant plant responses in response to soils treated

with this biochar. Lastly, based on results from both tissue P analyses (Figures 4A and 4B), and soil Olsen-P extractions (Figure 5B), it appears that variables other than changes in P are driving these largely positive responses to our biochar treatments.

When looking at other components of our study, e.g. AMF and soil P, we see some other interesting responses to our biochar treatments. First, none of our six intraspecific treatment combinations yielded any significant results in our AMF hyphal abundance measures (Figure 5A), despite the results discussed in the previous paragraph. Second, AMF root colonization increased significantly in only the Fescue (+) 350^{o}C biochar treatment (Figure 3A), even though root and shoot biomasses changed significantly in multiple treatments. Third, in contrast to results from Lehmann et al. (2003), and Oguntunde et al. (2004), our results show multiple instances of significant declines in soil P availability in biochar treated soils (Figure 5B). In this experiment, two of these instances were in soils treated with either 350^{o}C or 650^{o}C biochar and intraspecific spotted knapweed (Figure 5B). Based on suggestions from Gundale and DeLuca (2006) and Gundale and DeLuca (2007), both of our Douglas-fir biochars had a large capacity to sorb and thus remove multiple phenolic compounds from soil solutions, including catechin (Gundale and DeLuca 2007). Furthermore, Thorpe et al. (2006), discusses the possibility that one of the catechin isomers secreted by knapweed roots, i.e., (+)-catechin, is capable of complexing with metals including, Fe, Al, and Ca. Because the soils surrounding Missoula are Ca rich, a decrease in the quantity of available catechin in our biochar treated soils could have reduced the amount of metal chelation in our experimental soils, though we have no evidence that these sorption events occurred, thus contributing to the decreases in P availability seen in figure 5B (Thorpe et al. (2006).

Considering similar soil Olsen extractable P results were not seen in our two biochar (+) fescue treatments, this plant species apparently employes another type of P solubilization mechanism that is not vulnerable to sorption of soluble phenolics by biochars.

Biochar related influences on nutrient acquisition in different plant species: Intraspecific treatments

Collectively, considering all of the plant, soil and AMF results shown in figures 2 through 5, it seems possible that there were some overall changes in the spotted knapweed – AMF relationships with regard to P acquisition strategies and allocation of photosynthates. In the knapweed pots, where biochar addition treatments lead to decreases in soil Olsen-P (Figure 5B), we also see significant changes in root biomasses, where root mass decresed in response to 350C biochar and increased in response to the 650^{o}C biochar (Figure 2E). Interestingly, when looking at AMF root colonization rates, we observe the opposite response when the means of these two experimental treatments are compared against each other (Figure 3B). However, we should note that neither of these two treatment means were significantly different from the mean from the (–) biochar treatment. Perhaps in the 350^{o}C biochar treatments, knapweed is receiving a larger percentage of its P supply from AMF, while in the 650^{o}C a larger quantity of P is being supplied by its own root system. This ability to compensate for decreased soil Olsen-P availability via increased associations with AMF or through increased root production, may explain why our tissue P content results show no significant changes in plant P nutrition in these soils.

Based on our AMF root colonization results from the Intraspecific Idaho fescue treatments, with the exception of the 350^{o}C biochar treatment, as well as results from our analyses of fescue tissue P contents, soil Olsen-P availabilities, root biomasses and shoot biomasses, it does not appear that either of our biochar addition treatments significantly affected the Idaho fescue – AMF relationship within the intraspecific Idaho fescue treatments in this experiment. This potentially means that biochar effects on soil properties, and plant physiology, outside of those measured here, are the major reasons behind the increases fescue biomass seen if figures 2A and 2B. One such possibility would be if our biochars, especially the 650oC biochar affected the availability of mineral N in treated soils. Although we have no supporting results this possibilty from the soils in our experiment, it is plausible that increases in N mineralization in response to the presence of 650^{o}C biochar in our soils occured, as discussed in both DeLuca et al. (2006) and Gundale and DeLuca (2007), and thus increased N uptake by Idaho fescue roots in the 650^{o}C biochar treatment explains the increases in shoot and root biomasses (Figures 2A and 2B)..

Biochar related influences on plants, AMF, and soils: Interspecific treatments

Based on our results from the shoot biomass production exhibited by spotted knapweed it seems clear that this plant species significantly increases its shoot biomass production when in the presence of 650^{o}C biochar and a native perennial bunchgrass competitor (Figure 2D). We should also note that it was only through this combination of treatment factors that we were able to observe knapweed biomass production results similar to

those from Marler et al (1999), Zabinski et al. (2002), and Carey et al. (2004). Lastly, based on our results, this increase in shoot production came without any significant changes in AMF root colonizations (Figure 3B), soil Olsen-P availability (Figure 5A), tissue P content (Figure 4C), or AMF hyphal abundances in soils used in interspecific competition treatments (Figure 5A). Collectively, this suggests that results from response variables other than those analyzed in our experiment, e.g. changes N cycling rates, and/ or alterations in overall AMF community composition favoring knapweed competitiveness, are likely responsible for this response exhibited by spotted knapweed in association with Idaho fescue and soils treated with 650^{o}C Doug-fir biochar.

Interestingly, both discussions from Marler et al (1999), and results from Carey et al. (2004), point to at least one mechanism for how spotted knapweed individuals are able to out-compete their Idaho fescue neighbors, especially in the presence of 650^{o}C Doug-fir biochar. At the core of this mechanism is the relationship that each plant species forms with its AMF symbionts. Results from Marler et al (1999) and Carey et al. (2004) suggest that AMF species that colonize spotted knapweed plants are capable of siphoning resources via their extraradical mycelium (ERM), e.g. parasitizing, one of their hosts, Idaho fescue, to the benefit of the spotted knapweed plants. Thus, through 650^{o}C biochar induced changes in the relationships between soils, plants and possibly even AMF communities, it is possible that the capacity for the AMF to transfer carbon from fescue to knapweed, as described in Carey et al. (2004), was only really in effect within this one treatment of our experiment. Ultimatley, this greater resource subsidy could benefit the spotted knapweed plants directly (Carey et al. 2004), the AMF network either associated

with its root system (Fitter et al. 1998) or perhaps even both symbionts, along a source-sink type relationship.

Conclusions and Future directions

Future analyses from this experiment and others like it should include an analysis on a broader range of biochar affected soil variables, in addition to analyzing the biochar particles themselves, as featured in section B. This should include an analysis of a broader range of soil nutrient availabilities, beyond just Olsen-P and pH, and should also include analyses of the soil's organic matter content, ion exchange capacity, water holding capacity, bulk density, aggregate stability and overall texture, in both the control, and biochar treated soils. In addition to these analyses of soil properties, experiments should also include analyses of the bacterial and AMF communities assembled within the roots of each plant species, and the soils used in each treatment. Such analytical procedures could help inform us if there are biochar-facilitated shifts in the community of AMF and soil bacteria associated with each plant species. If not a shift in overall community composition, these analyses would also inform us if there are particular species of organisms that simultaneously interact with each plant species, which become more numerically dominant in the system, and are then better exploited by one plant species, more so than any of the others, when in the presence of biochar. Lastly, based on our experience, it seems that analyses of all the soil properties discussed should also be performed on a subset of soils collected for, but not acutally used in any of the treatments in the experiments. When provided with such data, we woudld be able to better interpret a soil's quality prior to any biochar addition treatment, and therefore

better understand if a particular soil would acutally benefit from a biochar-centric management regime. Ultimately, it appears that the 650^{o}C Doug-fir biochar, via currently unknown influences on the AMF community in spotted knapweed, potentially increased the quantity of carbon transferred away from Idaho fescue and to spotted knapweed, ultimately increasing the shoot biomass production of,spotted knapweed in this experiment.

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Figure 2. *The overall effects of both biochar types on biomass production in: A) Shoot dry mass in Festuca idahoensis, B) Root dry mass in F. idahoensis, C) Root to Shoot ratio in F. idahoensis, D) Shoot dry mass in Centaurea maculosa, E) Root dry mass in C. maculosa, and F) Root to Shoot ratio in C. maculosa. In all panels, black bars represent means from intraspecific competition treatments; grey bars represent means from interspecific competition treatments. Bars in graph are equivalent to the mean ± one standard error.*

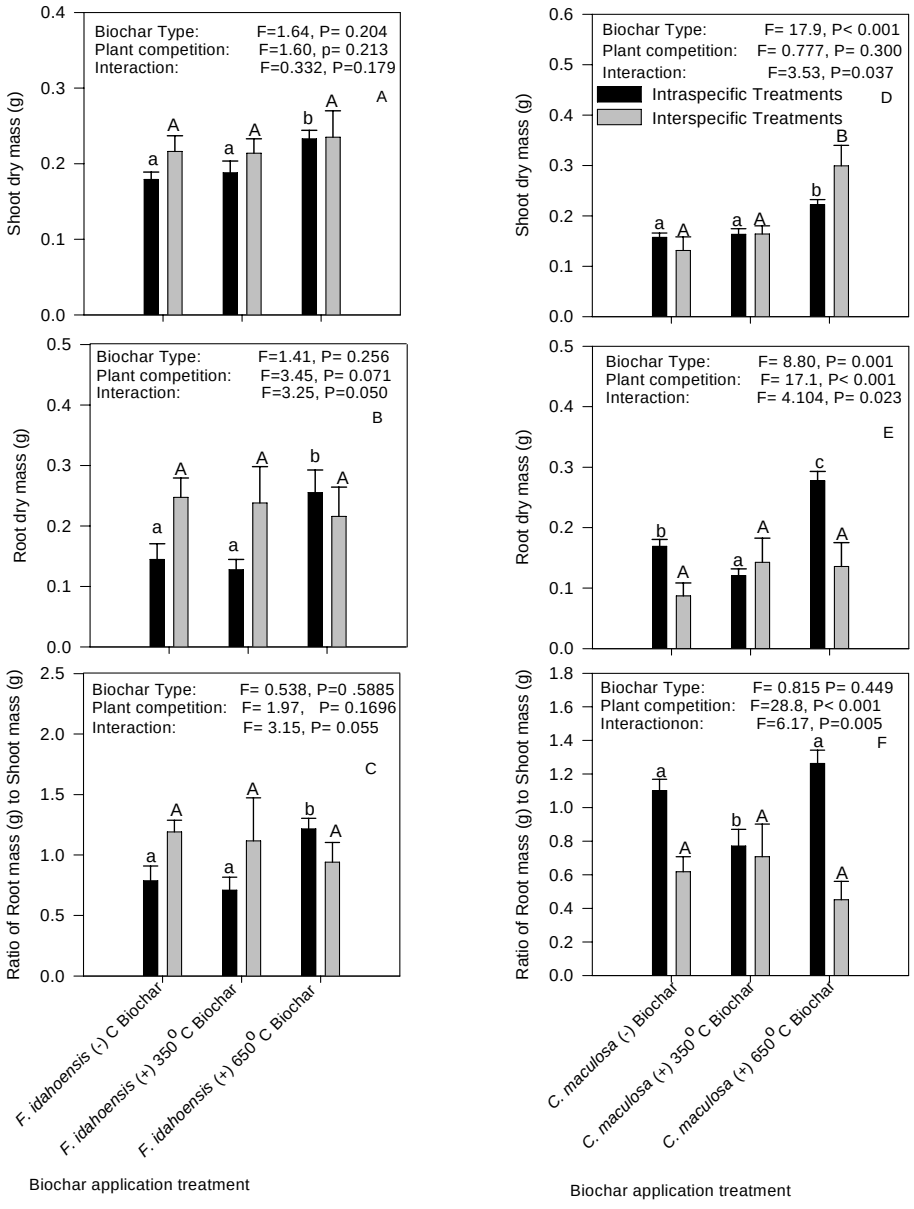

Figure 3*. The overall effects of both biochar types on root colonization by AMF in: A) Festuca idahoensis and B) C. maculosa. In both panels, black bars represent means from intraspecific competition treatments; grey bars represent means from interspecific competition treatments. Bars in graph are equivalent to the mean ± one standard error.*

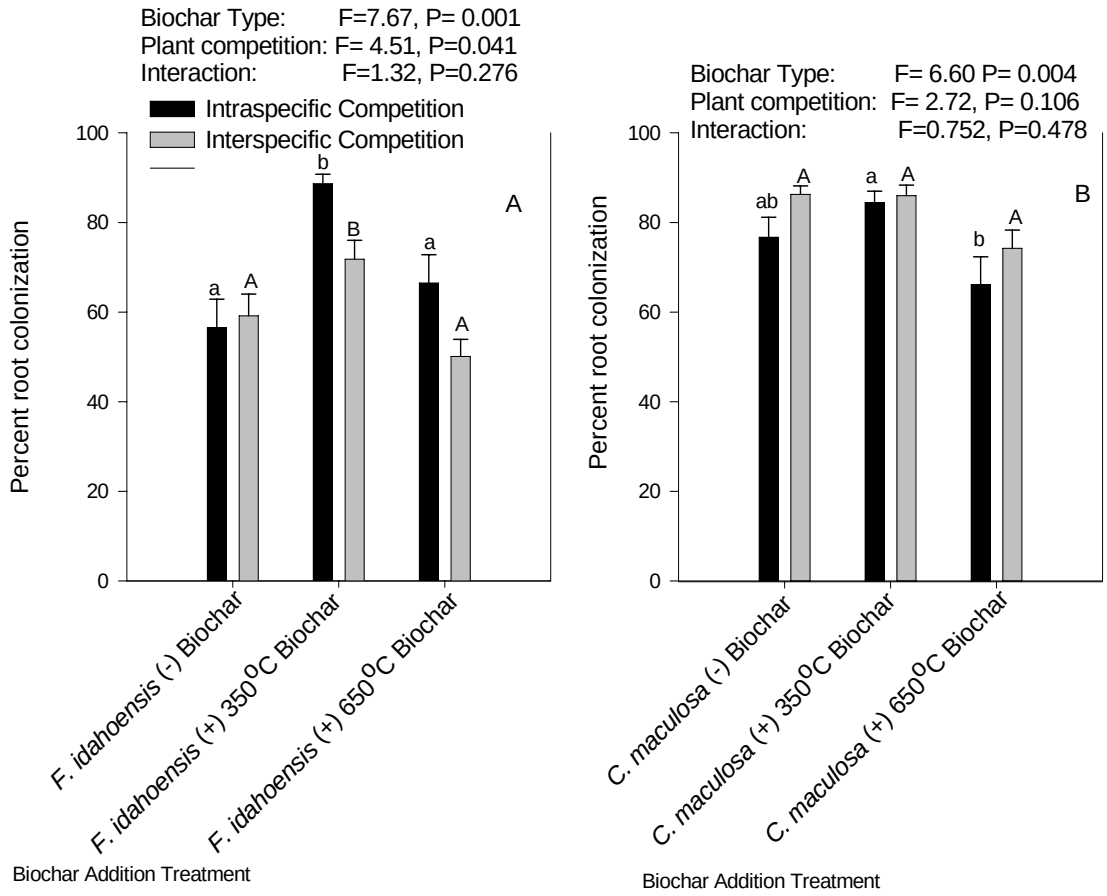

Figure 4. *The overall effects of both biochar types on shoot tissue quality as measured by A) P quantitiy in Festuca idahoensis (µg) and C) P quantity in Centaurea. maculosa (µg), as well as tissue concentrations of B) Cu and Zn in F. idahoensis (PPM), and D) Cu and Zn in C. maculosa (PPM). Bars in graph are equivalent to the mean ± one standard error.*

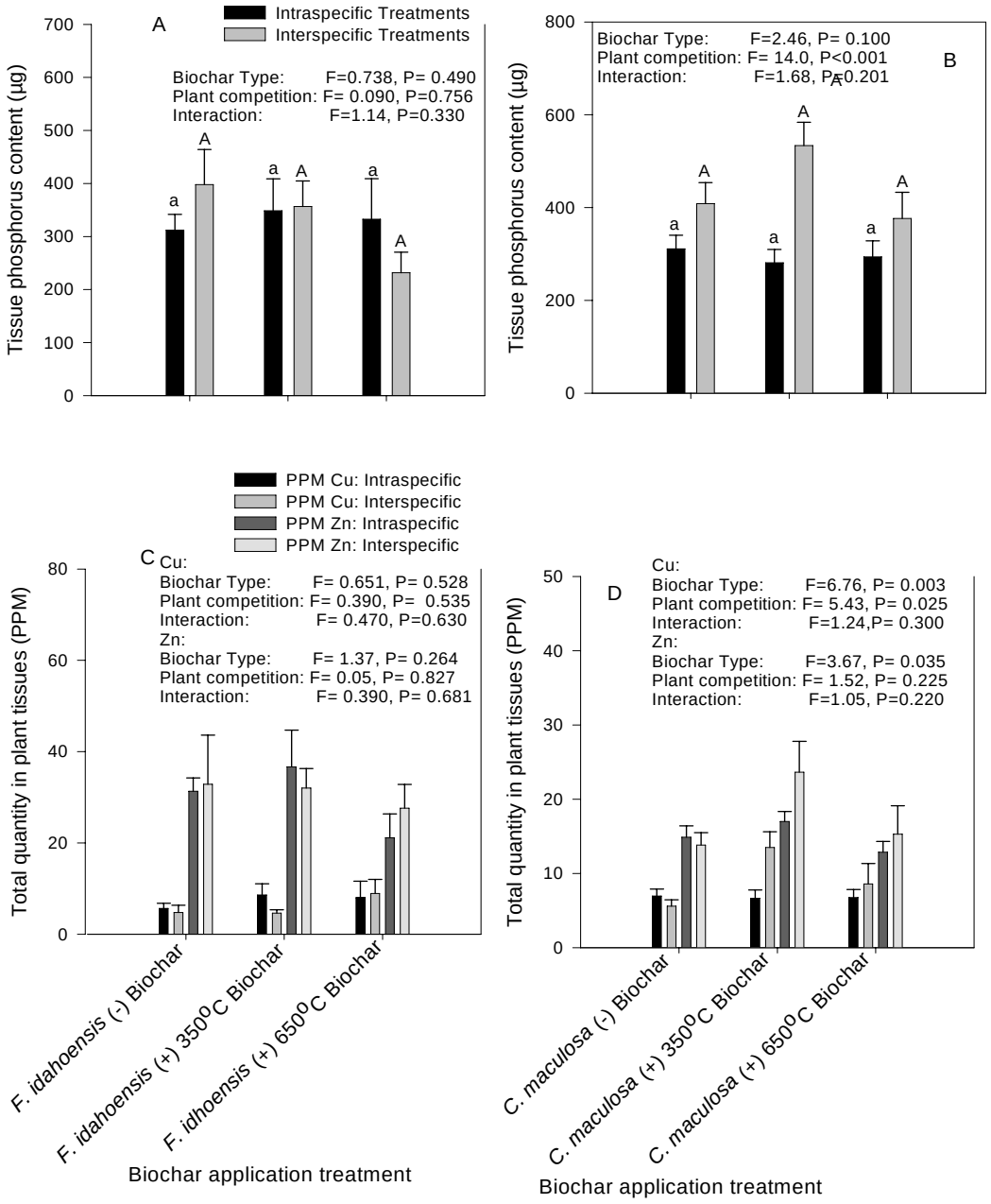

Figure 5. *The influence of Doug-fir biochar additions and plant species competition type on A) Soil hyphal lengths in AM fungi and B) Soil orthophosphate availabilities. Bars in graph are equivalent to the mean ± one standard error.*