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Influences of non-herbaceous biochar on arbuscular mycorrhizal fungal abundances in roots and soils: Results from growth-chamber and field experiments

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ABSTRACT

Biochar holds promise as an amendment for soil quality improvement and sequestration of atmospheric carbon dioxide. However, knowledge of how biochar influences soil properties, especially soil microorganisms, is limited. Three separate studies were conducted, with two studies using *Plantago lanceolata* as the AMF hosting plant, and a third being conducted in the field. Each of the three studies employed a different soil type. Furthermore, a total of five different biochars, and ten different biochar application rates, were used across the three experiments. All experiments had the goal to examine biochar influences on arbuscular mycorrhizal fungal (AMF) abundance in roots and AMF abundance (hyphal lengths) in soils. AMF abundance was either decreased or remained unchanged across all biochar treatments. When AMF abundances decreased, significant changes in soil properties, primarily in soil P availability, were observed. Application of large quantities (2.0% and 4.0%, w/w) of a lodgepole pine biochar, led to significant declines in AMF abundance in roots of 58% and 73% respectively, but not in soils. These declines in AMF abundance were accompanied by significant declines (28% and 34%) in soil P availability. After addition of a peanut shell biochar produced at 360 °C, P increased by 101% while AMF root colonization and extraradical hyphal lengths deceased by 74% and 95% respectively. Field application of mango wood biochar at rates of 23.2 and 116.1 t C ha^{-1} increased P availabilities by 163% and 208% respectively and decreased AMF abundances in soils by 43% and 77%. These findings may have implications for soil management where the goal is to increase the services provided by AMF.

1. Introduction

Biochars, when incorporated into soils, can improve soil quality and may also serve as a means to increase sequestration rates of atmospheric carbon (Lehmann et al., 2006; Lehmann, 2007a). Biochar is produced by thermally degrading (charring or pyrolyzing) biomass-derived feedstocks under oxygen limited conditions. Despite the potential usefulness of biochar for soil management applications, our knowledge of how these materials influence soil physical, chemical and biotic properties is limited compared to other soil amendments (Lehmann, 2007b).

During biomass pyrolysis, the molecular structure of the biochar feedstock changes, yielding highly aromatic, and graphitic C containing biochars (Glaser et al., 1998), which are often highly resistant to microbial decomposition (Preston and Schmidt, 2006). Due to its complex chemical structure, biochar exhibits a long mean residence time in soil, estimated between 1,000 to 10,000 years (e.g. Skjemstad et al., 1998; Swift, 2001; Cheng et al., 2008; Lehmann et al., 2008; Liang et al., 2008; Kuzyakov et al., 2009; Major et al., 2010). Given this recalcitrance, biochar is 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, and bulk density (Tyron, 1948; Glaser et al., 2002; Lehmann et al., 2003; Gundale and DeLuca, 2006; DeLuca et al., 2006). Such alterations may improve soil quality, e.g. by increasing soil nutrient availability

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(see references above); thereby increasing plant biomass production (Lehmann et al., 2003; see review by Blackwell et al., 2009). 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, an increased understanding of how different biochars influence soil physical, chemical and biological properties is critical.

Arbuscular mycorrhizal fungi (AMF) are thought to be one of the most important soil microbial groups in the context of modern organic agricultural practices (Piotrowski and Rillig, 2008) and land reclamation (Renker et al., 2004). AMF form symbioses by colonizing the root tissues of approximately 2/3 of known plant species, including many important crops (Trappe, 1987). AMF are obligate biotrophs, which cannot complete their life cycle without receiving fixed carbon (simple sugars) from their host plant (Smith and Read, 2008). In exchange for these 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). Therefore, 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).

Recent studies indicate that soil biochar amendments can increase AMF percent root colonization among 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). In soils having near neutral pH, where modulation of soil pH would be less pronounced, biochar influences on AMF abundances, e.g. root colonization, are not known.

The physical and chemical properties of biochar are influenced by both the feedstock (Keech et al., 2005; Gundale and DeLuca, 2006) and the maximum temperature attained during pyrolysis (Gundale and DeLuca, 2006; Lehmann, 2007b). 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, or how changes in production temperatures, influence AM fungi. More information is clearly needed about how variations in biochar characteristics influence soil properties, especially in non-acidic soils.

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 biochar effects on AMF have already been evaluated, we examined the effects of different biochar production temperatures, as well as how varying biochar application rates influence the abilities of AMF to colonize both plant roots and surrounding soils. Our underlying hypothesis for each study was that plant biomass and AMF abundances would be significantly affected by treating soils with biochars, which would modify soil properties such as phosphate availability.

2. Materials and methods

2.1. 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 (48°27′30″N, 113°50′W), formed from flood deposited sediments laid down nine years prior to collection. Piotrowski et al. (2008a) had already established that this soil has high mycorrhizal inoculum potential (MIP), high soil hyphal abundance, and a low soil organic matter (SOM) content of 0.7%. Piotrowski et al. (2008a) also established that this soil had the following properties: pH: 8.1; NO_3 (mg N kg soil^{-1}): 1.8; Olsen P (mg P kg soil^{-1}): 2.0. This soil, with its low SOM content, was selected in an effort to minimize potentially confounding interactions between biochar and SOM. Soil (15 L) was collected (0–20 cm depth) from multiple locations and pooled after sieving (2 mm 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 attained during pyrolysis (600 °C) was maintained for one hour. 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) were filled with 63 g of each treatment soil mixture. The experiment had a completely randomized design with five treatment levels (addition rates), each with 10 replicates, for a total of 50 experimental units (pots).

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 °C, 50–70% relative humidity, 18 h light, at 324 μmol photons m^{-2} s^{-1} PAR). After seven days of growth, the plants were thinned to one individual per pot. Pots were watered to field capacity daily, with tap water. After 30 d of growth, soil and plant materials were collected and examined as described below

2.2. 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). In addition to characterizing the soil from experiment 1, Piotrowski et al. (2008a) also established that this soil exhibited the following properties: % SOM: 0.6; pH: 8.0; NO_3 (mg N kg soil^{-1}): 5.0; Olsen P (mg P kg soil^{-1}): 2.7.

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, GA, USA) by heating 1 kg batches to 360 °C, 400 °C, or 430 °C using a bench scale batch pyrolysis system. Charred materials were removed from the pyrolysis reactor when the temperature had reached the specified maxima and remained stable for five minutes. The resulting biochar pellets were ground to homogenize the material, and we used the 0.20–0.71 mm 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.

Thus, the experiment had a completely randomized design with four treatment levels (control and three different biochars), each replicated eight times, for a total of 32 experimental units (pots). Plant materials, growth conditions, and experimental duration were the same as for Experiment 1; sampling procedures are described below.

2.3. Experiment 3: field study in Colombia

Experimental plots, each 20 m^2, were established at Matazul farm in the Eastern Plains of Colombia (N 04°10′15.2″, W 07°36′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).

Biochar for this experiment was produced from *Mangifera indica* L. (Mango) trunks and branches using methods traditional to Colombia. These materials were stacked, covered with soil and grass and ignited. After pyrolysis the resulting biochar was uncovered and ground to pass through a 0.9-mm sieve. Biochar was incorporated into the top 0.15 m of the soil by two disk harrow passes. Biochar application rates of 0, 11.6, 23.2 and 116.1 t C 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 design, with three replicates; there were thus a total of 12 experimental units (field plots). After biochar incorporation in December 2004, native C4 savannah grasses were allowed to recolonize the plots. Soil samples (0–5 cm depth) were collected in August 2005 and analyzed as described below.

2.4. Biochar characterization

Biochar chemical characteristics, for all biochar types employed here, were examined prior to their use as soil amendments. Biochar pH was estimated from 1:10 slurry (1 g char to 10 mL water or 1N KCl solution) after shaking three times over one hour, using a Symphony gel electrode (VWR, West Chester PA, USA). Percent total carbon and nitrogen contained in biochar materials were determined by combustion on an isotope ratio mass spectrometer (IRMS; PDZ Hydra 20/20, Sercon Ltd., Cheshire, UK) at the University of California Davis Stable Isotope Lab (Davis, California, USA). Soluble P was extracted from biochar materials using the Mehlich-3 extraction procedure (Mehlich, 1984) and analyzed using ICP-MS (Dairy One Labs, Ithaca, New York, USA).

2.5. Soil analysis

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 density was evaluated for correcting biochar dilutions of AMF in Experiments 1 and 2. For these evaluations, we weighed 5 mL of air-dried soil sample, and recorded soil weights for calculations of soil sample density. For these measurements, we analyzed six randomly selected replicates from Experiment 1 and five from Experiment 2.

2.6. Plant and AMF analyses

Root and shoot tissues were separated by cutting above the topmost lateral root during harvest. Any soils still adhering to the root samples were gently washed away with tap water so no soil particles would remain and thus potently influence later measurements of root biomass. Root and shoot biomass for Experiments 1 and 2 were subsequently weighed after drying (60 °C, 24 h).

AMF percent root colonization was examined for Experiments 1 and 2. Root tissues were stained with trypan blue as described by Brundrett (1994). Mycorrhizal colonization of stained root tissues was then assessed at 200× 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).

Abundance of extraradical AMF hyphae was 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 (200×). Abundances of AMF hyphae were determined by measuring AMF hyphal lengths using a gridline 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.

Potential biochar influences on extraradical hyphae extraction efficiencies were examined 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 re-extraction of typically discarded fractions from the hyphal extraction process, i.e. remaining soil particles and hyphae passing through the 38 μm sieves used to trap fungal hyphae. Materials passing through the sieve were collected by placing a collection tray beneath. A 2 mL aliquot of this collected material was used to quantify lengths of any AMF hyphae. We proceeded in an equivalent fashion with hyphae trapped in the extracted soil residue: we collected 1.0 g of sediments, suspended it in 25 mL of water, and then mounted this material, stained and counted of fungal structures.

Addition of biochars to soil will dilute the amount of AMF inoculum available to infect host plants. Biochar related dilutions of AMF inocula were accounted for by determining the change in soil density due to biochar. Dilution correction factors were generated using the formula, $x = 1 + [1 - (\text{density experimental soil} \times \text{density control soil}^{-1})]$. The resulting correction values were applied to the AMF root colonization and AMF hyphal abundance estimates of Experiments 1 and 2. Amounts of AMF infectious propagules and root colonization rates were assumed to covary 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 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. After employing this correction factor to our AMF abundance data, the adjusted AMF abundance results suggested that only the differences between the AMF percent root colonization in the 400 °C biochar addition treatment and the percent root colonization in the no-biochar treatment of Experiment 2 were

Table 1
Background data for all biochars, with measurement taken prior to biochar incorporation into experimental soils.

Biochar property	Mango wood	Lodgepole	360 °C Peanut shell	400 °C Peanut shell	430 °C Peanut shell
pH (H_2O)	10.14	7.70	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 (%)	0.30	0.13	1.75	1.42	1.65
Soluble P (mg P/g biochar)[a]	0.26	0.02	0.39	0.30	0.42

[a] Previous experiments show that soluble P estimates from the Mehlich-3 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 2
Effects of 600 °C lodgepole 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.

Proportion of biochar in soil (% by weight of soil)	Soil pH[a]	Soil density ($g\,cm^3\,soil^{-1}$)	Soil P availability ($mg\,P\,kg\,soil^{-1}$)[a]	Plant biomass (mg)	Root colonization by AMF (%)[b]	AMF hyphal lengths ($m\,hyphae/cm^3\,soil$)[a,b]
0.0 (control)	7.87 (0.001)a	1.35 (0.017)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	1.39 (0.021)a	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	1.40 (0.013)a	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	1.28 (0.015)b	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	1.12 (0.006)c	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	68.0	5.65	1.30	5.68	14.9
P value	0.024	<0.001	0.002	0.300	0.001	<0.001

Values within a column followed by different letters are significantly different at $P < 0.05$. Column values followed by no letters are not significantly different at $P < 0.05$.
[a] Data from soil pH, soil orthophosphate availability, and AMF hyphal abundance were Log_{10} transformed prior to ANOVA calculations.
[b] AMF abundance results were adjusted to account for soil and/or AMF inoculum dilutions (see Section 2).

Table 3
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.

Biochar generation temperature	Soil pH[a]	Soil density ($g\,cm^3\,soil^{-1}$)	Olsen phosphate availability ($mg\,P\,kg\,soil^{-1}$)[b]	Plant biomass (mg)	Root colonization by AMF (%)[c]	AMF hyphal lengths ($m\,hyphae/cm^3\,soil$)[a,c]
Control (no biochar added)	7.90 (0.131)	1.45 (0.042)	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)	1.40 (0.014)	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)	1.41 (0.017)	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)	1.40 (0.024)	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	0.618	10.7	3.83	4.11	5.58
P value	0.310	0.613	0.002	0.020	0.020	0.006

Values within a column followed by different letters are significantly different at $P < 0.05$. Column values followed by no letters are not significantly different at $P < 0.05$.
[a] For soil pH analyses, we performed a Kruskal–Wallis one-way ANOVA to determine statistical significance of biochar effects on soil pH.
[b] Data from soil orthophosphate were Log_{10} transformed prior to ANOVA calculations.
[c] AMF abundance results were adjusted to account for soil and/or AMF inoculum dilutions (see Section 2).

significantly influenced by the correction factors (Table 1). AMF dilution correction factors for Experiments 1 and 2 are included in Tables 2 and 3, respectively.

2.7. Statistical analyses

When the data fulfilled the assumptions of normality, a one-way ANOVA was used 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 statistical software (Version 6. SAS Institute Inc., Cary, NC, 1989–2005). When normality assumptions of ANOVA were not met, a Kruskal–Wallis one-way ANOVA, a non-parametric ranking procedure, was performed using NCSS statistical software (NCSS, Kaysville, UT, USA). A one-way randomized block ANOVA was performed to analyze all data generated in Experiment 3 using CoStat statistical software (ver 6.311; CoHort Software, Monterey, CA, USA). Data points more than two stan-

Table 4
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.

Biochar addition rate (Tons biochar-C ha^{-1})	Soil pH	Soil density ($g\,cm^3\,soil^{-1}$)	Soil carbon ($mg\,C\,g\,soil^{-1}$)	Soil P availability ($mg\,P\,kg\,soil^{-1}$)	Plant biomass[b] ($t\,dry\,matter\,ha^{-1}$)	AMF hyphal abundance ($m\,hyphae/cm^3\,soil$)[a]
0	5.60 (0.100)c	1.29 (0.06)a	6.47 (0.767)b	6.43 (0.700)c	1.64 (0.218)	19.2 (1.91)a
11.6	5.72 (0.083)c	1.09 (0.07)a	11.9 (0.973)b	7.72 (1.00)bc	Data not available	17.6 (1.87)a
23.2	6.08 (0.044)b	1.13 (0.11)a	15.2 (2.45)b	10.5 (0.263)ab	4.74 (0.505)	10.9 (2.56)b
116.1	6.91 (0.085)a	0.69 (0.01)b	59.6 (6.23)a	13.4 (0.736)a	Data not available	4.45 (0.687)c
F ratio	55.7	13.1	51.7	18.3	32.0	8.40
P value	<0.001	0.002	<0.001	<0(.001)	<(0.01)	0.014

Values within a column followed by different letters are significantly different at $P < 0.05$. Column values followed by no letters are not significantly different at $P < 0.05$.
[a] AMF hyphal abundance results were not adjusted to account for biochar additions in these treatments.
[b] Plant biomass results first presented in Major et al. (2010).

dard deviations away from the mean were considered outliers and omitted from all statistical analyses.

3. Results

3.1. Chemical properties of biochars

Biochars produced from peanut shells were found to contain substantially greater soluble P than biochar produced from lodgepole pine (Table 1). Peanut shell biochar also contained greater percent total N. All 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 (Table 1).

3.2. Soil density and hyphal extraction efficiencies

Both of the greater lodgepole biochar addition rates, 2.0% (w/w) and 4.0% (w/w) employed in Experiment 1 significantly affected soil densities (Table 2). Treating the nine year old soils with the peanut shell biochars did not significantly affect soil densities in Experiment 2 (Table 3). Lastly, as with Experiment 1, results from Experiment 3 indicate that large additions of biochar significantly decreased soil density (Table 4). Respective hyphal extraction efficiencies were estimated at 92.5, 96.1, 94.0, 96, and 98.3%, for the 0, 0.5, 1, 2, and 4% lodgepole pine biochar addition treatments. Differences between treatments were not significant ($F = 1.00$, $P = 0.435$).

3.3. Experiment 1: multiple addition rates

Plant biomass production was not significantly affected by any of the five biochar addition treatments (Table 2). Both 2.0% and 4.0% biochar addition treatments resulted in significantly reduced AMF hyphal lengths and root colonization compared to non-amended soils, while lower application rates did not result in a significant change (Table 2). Soil P availability was significantly lower for 1.0% and 4.0% biochar addition treatments (Table 2).

3.4. Experiment 2: multiple biochar generation temperatures

Plant biomass production was significantly greater in the 430 °C biochar treatment than in all other treatments (Table 3). AMF root colonization was found to be significantly lower for the 360 °C and 400 °C biochar treatments compared to the control (Table 3). AMF extraradical hyphal lengths were found to be significantly lower in soils of the 360 °C biochar treatment than in all other treatments (Table 3). While soil pH was not significantly influenced by any of the peanut shell biochars, all significantly increased soil P availability (Table 3).

3.5. Experiment 3: colombian field experiment

Treatments in which biochar was incorporated into soils at higher rates (23.2 t and 116.1 t biochar-C ha^{-1}) exhibited significantly decreased AMF hyphal abundance (Table 4). In contrast, application of both 23.2 t and 116.1 t biochar-C ha^{-1} resulted in significantly increased soil P availability (Table 4) and in the case of the 23.2 t biochar-C ha^{-1} addition treatment, plant biomass production was increased by 189% (Major et al., 2010). Furthermore, grasses, forbs and legumes in the 23.2 t biochar-C ha^{-1} amended plots produced 93, 292 and 1916% more biomass than in the 0 t biochar-C control plots, respectively (Major et al., 2010). Additionally, soil pH was found to increase significantly in soils treated with increasingly larger quantities of biochar (Table 4). Lastly, regression analysis indicates a strong, linear relationship between a decrease in AMF hyphal abundances in soils and greater phosphate availabilities in soils treated with biochar (Fig. 1).

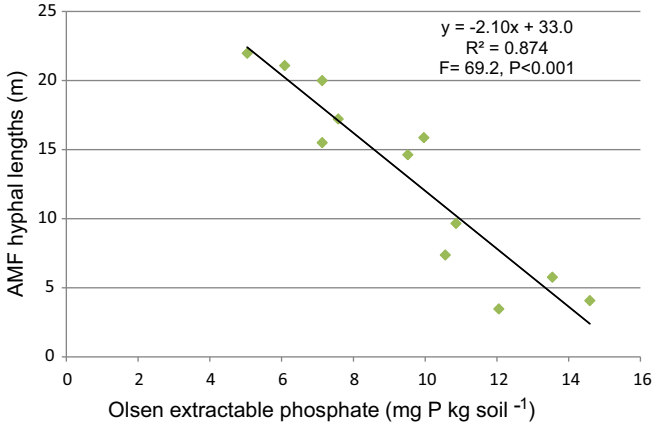

Fig. 1. The relationship between Olsen extractable P available in soils and the abundance of AMF hyphae, measured as hyphal lengths (m), in biochar treated soils from experiment 3.

4. Discussion

All three of our experiments, encompassing a range of biochars and soils, indicate neutral to decreased AMF abundance with biochar additions as measured by percent root colonization and/or extraradical hyphal lengths (Tables 2–4). In multiple cases, decreases in AMF abundances were accompanied by changes in soil properties, including both decreases (Table 2) and increases (Tables 3 and 4) in soil P availability, as well as changes in soil pH (Table 4). Moreover, these are the first results to show significant reductions in AMF hyphal abundances after biochar application to soils. Lastly, the AMF-hosting plant *P. lanceolata* showed significantly increased biomass production in response to biochar additions in only a single treatment (Table 3). However, the underlying mechanisms behind these observations remain unclear.

Some longer-duration studies, where biochar aging processes (weathering) within soil environments likely occurred (Cheng et al., 2006, 2008; Lehmann, 2007b), have reported increased AMF abundance in response to biochar additions to soils in Japan (Matsubara et al., 2002; Yamato et al., 2006). In these studies, soil pH increased after biochar addition to soils, suggesting that pH modulation may, in part, be a mechanism influencing AMF abundance (Matsubara et al., 2002; Yamato et al., 2006). In the present study, only the pH of the Colombian field soil (Experiment 3) was significantly influenced by biochar additions (Table 4). However, in contrast to what was observed for the soils in Japan, AMF abundance decreased in this soil with increased biochar application rates and soil pH. This suggests that other mechanisms besides pH modulation are responsible for altered AMF abundance in this soil.

Phosphate is central to interactions between plants and AMF (Smith and Read, 2008). Multiple sources suggest that either low (Allen et al., 2003; Drew et al., 2006) or high (Corbin et al., 2003; Covacevich et al., 2006; Gryndler et al., 2006) soil P availabilities can adversely affect AMF abundance in roots and soils. Results from Experiment 1, which used lodgepole pine biochar generated at high temperature and containing relatively low amounts of soluble P (Table 1), indicate decreased soil P availability in the presence of biochar (Table 2). In a recent review from Lehmann (2007a), results are reported from an experiment also featuring a wood based, lab generated biochar. This study provides evidence that these particular biochar particles likely had the capacity to adsorb measurable quantities of phosphate ions from a soil-free solution. However, results from experiments with treatments com-

bining both biochars and soils, showing a similar trend, are not currently available within the literature. Another possibility, suggested by Kuzyakov et al. (2009), is that biochar sorption of labile organic C could serve 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, all three peanut shell biochars and the mango-wood biochar contained greater soluble P than biochar derived from lodgepole pine (Table 1). This adds to results of other studies indicating that biochars can contain available 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 Experiments 2 and 3 indicate significantly increased P availability after addition of peanut shell and mango-wood biochars (Tables 3 and 4).

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 3 and 4), but significant for Experiment 3 (Table 4). However, the addition of a biochar with a high pH of 8 (in water) as in Experiment 1 to a soil that already has a high pH of 8, could have induced lower P availability without a measurable change in total soil pH, e.g., through inducing decreases in P mineralization rates. In addition to any direct effects of adding P with the biochar, it seems plausible that large applications, e.g., 23.2 t and 116.1 t biochar-C, of high pH mango-wood biochar (Table 1), contributed to the increased soil P by increasing soil pH levels (Table 4). The relationship of cause and effect for these observations is not clear, but could involve a lower reliance of plants on AMF for resource acquisition. With greater P availability and improved soil pH, plants may not rely to the same extent on their AMF associates (Smith and Read, 2008).

Another explanation may be 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. These biochar properties and, hence, how biochars influence AMF abundance may change with biochar production process conditions and equilibration, i.e. weathering, in the soil environment (Cheng et al., 2006, 2008; Lehmann, 2007b). 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 3).

Although further work is needed to elucidate longer-term biochar influences on AMF, i.e., in experiments where biochars are given more time to equilibrate with surrounding soils, 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 biochar application. With further optimization, biochar applications to soils could ultimately increase both AMF abundances (Matsubara et al., 2002; Yamato et al., 2006) and crop yields (Lehmann et al., 2006). Lastly, our results illustrate that biochar properties/effects can differ with feedstock identities and temperatures reached during pyrolysis.

In conclusion, our results show the potential for some biochars to significantly decrease AMF abundance. The functional relationship between biochar application, improved soil fertility and AMF abundance and colonization is not clear. However, it is clear from our study that a wide parameter space (feedstock properties, production conditions, and technically feasible application rates) is necessary to cover potential effects on AM fungi.

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References

Allen, M.F., Swenson, W., Querejeta, J.I., Egerton-Warburton, L.M., Treseder, K.K., 2003. Ecology of mycorrhizae: a conceptual framework for complex interactions among plants and fungi. Annu. Rev. Phytopathol. 41, 271–303.

Antal Jr., M.J., Grønli, M., 2003. The art, science, and technology of charcoal production. Ind. Eng. Chem. Res. 42, 1619–1640.

Blackwell, P., Riethmuller, G., Collins, M., 2009. Biochar application to soil. In: Lehmann, J., Stephen Joseph, J. (Eds.), Biochar for Environmental Management. Science and Technology, Earthscan, London, UK, pp. 207–226.

Brundrett, M., 1994. Estimation of root length and colonization by mycorrhizal fungi. In: Brundrett, M., Melville, L., Peterson (Eds.), Practical Methods in Mycorrhiza Research. Mycologue Publications, Waterloo, USA, pp. 51–59.

Cheng, C.H., Lehmann, J., Thies, J.E., Burton, S.D., Engelhard, M.H., 2006. Oxidation of blackcarbon by biotic and abiotic processes. Org. Geochem. 37, 1477–1488.

Cheng, C.H., Lehmann, J., Engelhard, M., 2008. Natural oxidation of black carbon in soils: changes in molecular form and surface charge along a climosequence. Geochim. Cosmochim. Acta 72, 1598–1610.

Corbin, J.D., Avis, P.G., Wilbur, R.B., 2003. The role of phosphorus availability in the response of soil nitrogen cycling, understory vegetation and arbuscular mycorrhizal inoculum potential to elevated nitrogen inputs. Water Air Soil Poll. 147, 141–161.

Covacevich, F., Marino, M.A., Echeverriá, H.E., 2006. The phosphorus source determines the arbuscular mycorrhizal potential and the native mycorrhizal colonization of tall fescue and wheatgrass. Eur. J. Soil. Biol. 42, 127–138.

DeLuca, T.H., MacKenzie, M.D., Gundale, M.J., Holben, W.E., 2006. Wildfire-produced charcoal directly influences nitrogen cycling in ponderosa pine forests. Soil. Sci. Soc. Am. J. 70, 448–453.

Drew, E.A., Murray, R.S., Smith, S.E., 2006. Functional diversity of external hyphae of AM fungi: ability to colonize new hosts is influenced by fungal species, distance and soil conditions. Appl. Soil. Ecol. 32, 350–365.

Ebeling, A.M., Bundy, L.G., Kittell, A.W., Ebeling, D.D., 2008. Evaluating the Bray P1 test on alkaline, calcareous soils. Soil. Sci. Soc. Am. J. 72, 985–991.

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

Glaser, B., Haumaier, L., Guggenberger, G., Zech, W., 1998. Black carbon in soils: the use of benzenecarboxylic acids as specific markers. Org. Geochem. 29, 811–819.

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

Gryndler, M., Larsen, J., Hrselová, H., Rezácová, V., Gryndlerová, H., Kubát, J., 2006. Organic and mineral fertilization, respectively, increase and decrease the development of external mycelium of arbuscular mycorrhizal fungi in a long-term field experiment. Mycorrhiza 16, 159–166.

Gundale, M.J., DeLuca, T.H., 2006. Temperature and source material influence ecological attributes of Ponderosa pine and Douglas-fir charcoal. For. Ecol. Manage. 231, 86–93.

Jakobsen, I., Abbott, L.K., Robson, A.D., 1992. External hyphae of vesicular-arbuscular mycorrhizal fungi associated with *Trifolium ubterraneum* L. spread of hyphae and phosphorus inflow into roots. New. Phytol. 120, 371–380.

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

Kuzyakov, Y., Subbotina, I., Chen, H., Bogomolova, I., Xu, X., 2009. Black carbon decomposition and incorporation into soil microbial biomass estimated by 14C labeling. Soil Biol. Biochem. 41, 210–219.
Lehmann, J., Da Silva Jr., J.P., Steiner, C., Nehls, T., Zech, W., Glaser, B., 2003. Nutrient availability and leaching in an archaeological Anthrosol and a Ferralsol of the Central Amazon basin, fertilizer, manure and charcoal amendments. Plant Soil 249, 343–357.
Lehmann, J., Gaunt, J., Rondon, M., 2006. Biochar sequestration in terrestrial ecosystems—a review. Mitigation and Adaptation Strategies for Global Change 11, 403-427.
Lehmann, J., 2007a. Bio-energy in the black. Front. Ecol. Environ. 5, 381–387.
Lehmann, J., 2007b. A handful of carbon. Nature 447, 143–144.
Lehmann, J., Skjemstad, J.O., Sohi, S., Carter, J., Barson, M., Falloon, P., Coleman, K., Woodbury, P., Krull, E., 2008. Australian climate-carbon cycle feedback reduced by soil black carbon. Nat. Geosci. 1, 832–835.
Liang, B., Lehmann, J., Solomon, D., Sohi, S., Thies, J.E., Skjemstad, J.O., Luizão, F.J., Engelhard, M.H., Neves, E.G., Wirick, S., 2008. Stability of biomass-derived black carbon in soils. Geochim. Cosmochim. Acta 72, 6078–6096.
Major, J., Lehmann, J., Rondon, M., Goodale, C., 2010. Fate of soil-applied black carbon: downward migration, leaching and soil respiration. Glob. Change Biol. 16, 1366–1379.
Matsubara, Y.-I., Hasegawa, N., Fukui, H., 2002. Incidence of *Fusarium* root rot in asparagus seedlings infected with arbuscular mycorrhizal fungus as affected by several soil amendments. J. Jpn. Soc. Hortic. Sci. 71, 370–374.
McGonigle, T.P., Miller, M.H., Evans, D.G., Fairchild, G.L., Swan, J.A., 1990. A new method which gives an objective measure of colonization of roots by vesicular-arbuscular mycorrhizal fungi. New Phytol. 115, 495–501.
Mehlich, A., 1984. Mehlich 3 Soil Test Extractant: a modification of Mehlich 2 Extractant. Commun. Soil. Sci. Plan. 15, 1409–1416.
Moorman, T., Reeves, F.B., 1979. The role of endomycorrhizae in revegetation practices in the semiarid west-II. A bioassay to determine the effect of land disturbance on endomycorrhizal populations. Am. J. Bot. 66, 14–18.
Murphy, J., Riley, J.P., 1962. A modified single solution method characterization of available P for determination of P in natural waters. Anal. Chim. Acta 27, 31–36.
Newsham, K.K., Fitter, A.H., Watkinson, A.R., 1995. Arbuscular mycorrhiza protect an annual grass from root pathogenic fungi in the field. J. Ecol. 83, 991–1000.
Peech, M., 1965. Hydrogen-ion activity. In: Black, C.A. (Ed.), Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties #9. American Society of Agronomy, Madison, WI, pp. 914–925.
Perner, H., Schwarz, D., George, E., 2006. Effect of mycorrhizal inoculum and compost supply on growth and nutrient uptake of young leek plants grown on peat based substrates. Hort. Sci. 41, 628–632.
Piotrowski, J.S., Morford, S.L., Rillig, M.C., 2008a. Inhibition of colonization by a native arbuscular mycorrhizal fungal community via Populus trichocarpa litter, litter extract, and soluble phenolic compounds. Soil Biol. Biochem. 40, 709–717.
Piotrowski, J.P., Lekberg, Y., Harner, M.J., Ramsey, P.W., Rillig, M.C., 2008b. Dynamics of mycorrhizae during development of riparian forests along an unregulated river. Ecography 31, 245–253.
Piotrowski, J.S., Rillig, M.C., 2008. Succession of arbuscular mycorrhizal fungi: patterns, causes, and considerations for organic agriculture. Adv. Agron. 97, 111–130.
Preston, C.M., Schmidt, M.W., 2006. Black (pyrogenic) carbon: A synthesis of current knowledge ad uncertainties with special consideration of boreal regions. Biogeosciences 3, 397–420.
Renker, C., Zobel, M., Öpik, M., Allen, M.F., Allen, E.B., Vosátka, M., Rydlová, J., Buscot, F., 2004. Structure, dynamics, and restoration of plant communities: do arbuscular mycorrhizae matter? In: Temperton, V.M., Hobbs, R.J., Nuttle, T., Halle, S. (Eds.), Assembly Rules and Restoration Ecology—Bridging the Gap Between Theory and Practice. Island, Washington, DC, pp. 189–229.
Rillig, M.C., Field, C.B., Allen, M.F., 1999. Soil biota responses to log-term atmospheric CO_2 enrichment in two California annual grasslands. Oecologia 119, 572–577.
Rillig, M.C., Mummey, D.L., 2006. Mycorrhizas and soil structure. New Phytol. 171, 41–53.
Rippstein, G.E., Amezquita, E., Escobar, G., Grollier, C., 2001. Condiciones naturales de la sabana. In: Rippstein, G., Escobar, G., Motta, F. (Eds.), Agroecologia y Biodiversidad de las Sabanas en los Llanos Orientales de Colombia. Centro Internacional de Agricultura Tropical (CIAT), Cali, Colombia, pp. 1–21.
Rowe, H.I., Brown, C.S., Claassen, V.P., 2007. Comparisons of mycorrhizal responsiveness with field soil and commercial inoculum for six native montane species and *Bromus tectorum*. Restor. Ecol. 15, 44–52.
Schmisek, M.E., Cihacek, L.J., Swenson, L.J., 1998. Relationships between the Mehlich-III soil test extraction procedure and standard soil test methods in North Dakota. Commun. Soil Sci. Plan. 29, 1719–1729.
Skjemstad, J.O., Janik, L.J., Taylor, J.A., 1998. Non-living soil organic matter: what do we know about it? Aus. J. Exp. Agric. 38, 667–680.
Smith, S.E., Read, D.J., 2008. Mycorrhizal Symbiosis. Springer, Berlin.
Swift, R.S., 2001. Sequestration of carbon by soil. Soil Sci. 166, 858–871.
Tarbell, T.J., Koske, R.E., 2007. Evaluation of commercial arbuscular mycorrhizal inocula in a sand/peat medium. Mycorrhiza 18, 51–56.
Topoliantz, S., Ponge, J.F., Ballof, S., 2005. Manioc peel and charcoal: a potential organic amendment for sustainable soil fertility in the tropics. Biol. Fert. Soils 41, 15–21.
Trappe, J.M., 1987. Phylogenetic and ecologic aspects of mycotrophy in the angiosperms from an evolutionary standpoint. In: Safir, G.R. (Ed.), Ecophysiology of VA Mycorrhizal Plants. CRC Press, Boca Raton, USA, pp. 5–25.
Tyron, E.H., 1948. Effect of charcoal on certain physical, chemical, and biological properties of forest soils. Ecol. Monogr. 18, 81–115.
Warnock, D.D., Lehmann, J., Kuyper, T.W., Rillig, M.C., 2007. Mycorrhizal responses to biochar in soil–concepts and mechanisms. Plant Soil 300, 9–20.
Yamato, M., Okimori, Y., Wibowo, I.F., Anshiori, S., Ogawa, M., 2006. Effects of the application of charred bark of *Acacia mangium* on the yield of maize, cowpea and peanut, and soil chemical properties in South Sumatra, Indonesia. Soil Sci. Plant Nutr. 52, 458–489.