Nitrogen, Biochar, and Mycorrhizae: Alteration of the Symbiosis and Oxidation of the Char Surface

Chase LeCroy^{1*}, Caroline A. Masiello^{1**}, Jennifer A. Rudgers^{2***}, William C.
Hockaday³, Jonathan J. Silberg⁴

¹Department of Earth Science, Rice University ms 126, 6100 Main St., Houston, TX 77030

²Department of Ecology and Evolutionary Biology, Rice University, ms 170, 6100 Main St., Houston, TX 77030

³Department of Geology, Baylor University, One Bear Place #97354, Waco, TX 76798

⁴Department of Biochemistry and Cell Biology, Rice University, ms 140, 6100 Main St., Houston, TX 77030

*now at Department of Geography, UCLA, 1255 Bunche Hall, Box 951524, Los Angeles, CA 90095

**corresponding author

***now at Department of Biology, University of New Mexico, Castetter Hall 286, MSC03 2020, Albuquerque, NM 87131-0001

KEYWORDS: biochar, mycorrhizae, XPS, charcoal, black carbon

HIGHLIGHTS:

- The presence of both fertilizer and biochar in 4 week sorghum pot trials significantly increased root mycorrhizal infection;
- With both fertilizer and biochar, aboveground biomass production was suppressed, suggesting that the plant-fungal relationship had become parasitic;
- Increased oxidation of the biochar surface could be detected over the 4-week pot trial using x-ray photoelectron spectroscopy;
- The most biochar oxidation occurred in pots with mycorrhizae but without fertilizer, suggesting mycorrhizal oxidation of biochar.

32

33 **Abstract**

34 In some cases amending soil with biochar improves fertility, although the
35 exact mechanisms through which biochar alters soil processes are not well
36 understood. In other cases, however, biochar amendment can have no effect on
37 plant growth, or can have negative effects. When crop benefits occur,
38 simultaneous amendment with biochar and mineral nutrients causes results that
39 are not additive, suggesting that biochar may be capable of improving the
40 efficiency of nutrient uptake by plants, but the mechanisms of this synergy
41 remain unknown. One possible mechanism that has not been fully explored is
42 alterations to the plant-mycorrhizal fungus mutualism, a relationship that occurs
43 in most land plants. In a 4 week greenhouse experiment, we investigated
44 possible effects of the presence of biochar, mycorrhizal fungi, and nitrogen
45 fertilizer on sorghum seedling growth. Results indicated that the combined
46 treatment of biochar, mycorrhizal fungi, and high nitrogen decreased
47 aboveground plant biomass by 42% relative to the mycorrhizae and high nitrogen
48 treatment, while simultaneously promoting mycorrhizal root colonization. This is
49 evidence for an induced parasitism of the mycorrhizal fungus in the presence of
50 nitrogen and biochar within the 4 week timescale of our experiments.

51 Using x-ray photoelectron spectroscopy, we found evidence of increased
52 surface oxidation on biochar particles over the 4 weeks of our trial, consistent
53 with sorption of labile, plant derived dissolved organic matter or char oxidation,
54 either via biotic or abiotic processes. Biochar in soils with mycorrhizae but

55 without sufficient nitrogen showed more surface oxidation than other treatment
56 combinations, and showed a significantly greater fraction of surface carbon
57 present in carbonyl (-C=O) functionalities. Our results suggest that soil nitrogen
58 acts as a switch controlling the ability of char to influence the mycorrhizal
59 symbiosis and, in turn, the degree to which the fungi oxidize the char surface.

60

61

1. Introduction

Of the emerging renewable energy and biofuel technologies, biomass pyrolysis (thermal conversion and/or gasification) stands out as a particularly promising option because of its ancillary benefits, not the least of which is a non-traditional approach to carbon capture and sequestration. The thermal decomposition of biomass under the partial exclusion of oxygen is an exothermic reaction that yields heat, water, combustible gases, and organic liquids (bio-oils) (Bridgwater, 2003). About 30% of the initial biomass carbon can also be retained in the form of charcoal (called biochar if produced intentionally for C sequestration). The critical step in making biomass pyrolysis sustainable and carbon negative is the incorporation of the biochar into the soil, rather than its combustion for energy. The longevity of biochar carbon in soil is such that net carbon emissions for the process are negative over the timescale of centuries to millennia (Lehmann, 2007). Soil emissions in the form of carbon dioxide, methane, and nitrous oxide could be reduced by a theoretical maximum of 1.8 Pg CO₂-C equivalent per year, which would create a meaningful sink in comparison to current fossil fuel emissions of 8.7 Pg C per year (Boden et al., 2011; Woolf et al., 2010).

Biochar's effect on plants and mycorrhizal fungi is poorly understood. The literature on this interaction is often contradictory: biochar has been shown to increase and decrease plant growth, pH, mycorrhizal colonization, and nutrient availability (Warnock et al., 2007), or simply have no effect (Habte and Antal, 2010). Biochar has been shown to improve crop productivity in some but not all

circumstances (Bell and Worrall, 2011; Chan et al., 2007, 2008; Rajkovich et al., 2012; Schnell et al., 2012; Zhang et al., 2012a; Zhang et al., 2012b). Also confounding is the variety of methods and materials used. For example, the effect of biochar on plant growth can depend on both the feedstock and pyrolysis process (Chan et al., 2008; Rajkovich et al., 2012). Potential mechanisms for generalized biochar improvement in plant growth include a reduction in soil tensile strength (Chan et al., 2007), improved plant access to soil nutrients (Warnock et al., 2007), improved soil water-holding capacity (Atkinson et al., 2010; Briggs et al., 2012; Chan et al., 2007; Glaser et al., 2002; Kinney et al., 2012; Liu et al., in press; Novak et al., 2012; Revell et al., 2012), and/or changes in the efficiency of plant-fungal interactions (Atkinson et al., 2010; Thies and Rillig, 2009; Warnock et al., 2007). However, the mechanisms underlying effects of biochar on plant growth remain unresolved, making it difficult to predict the conditions under which biochar amendments to soil will promote plant productivity.

Other studies have shown that fungi not only respond to the presence of charcoal in soil, but also interact with it, altering it physically and chemically (Hockaday et al., 2006). Long-term environmental alterations of soil charcoal appear to be crucial in the development of an oxidized patina that delivers beneficial ecosystem services, such as high soil cation exchange capacity (Cheng et al., 2006; Cheng et al., 2008). However, the timescale and mechanism of formation of this patina are poorly described.

Based on previous studies, we hypothesized that soil nitrogen availability would act to alter the plant-fungal relationship. We examined biochar-driven changes in both the plant-fungal relationship and the degree of char surface oxidation and tested whether these interactions vary as a function of nitrogen availability in the soil. We sought to determine if biochar alters plant interactions with mycorrhizal fungi in the presence of high versus low nitrogen fertilizer, and whether it is possible to determine how the relationship was altered by measuring (1) plant size, (2) mycorrhizal colonization, and (3) surface oxidation of the biochar particles. We addressed these questions through a greenhouse pot trial of sorghum plants grown with and without biochar, with and without mycorrhizal infection, and at high and low levels of nitrogen fertilization.

2. Methods

2.1 General approach to isolating mechanisms of biochar effects

Our experimental conditions were designed to focus only on biochar, nitrogen, and the plant-mycorrhizal relationship. Therefore, we selected a highly mycorrhizal plant (sorghum) for a growth trial in soil and hydrologic conditions intended to eliminate other hypothesized benefits of biochar. We selected sorghum because it is highly dependent upon fungal symbionts and because of its importance as an agricultural crop used for food and biofuel (Hata et al., 2010). We chose a soil matrix with a very low tensile strength (sand-enriched) to limit any potential tensile-strength reduction from biochar amendment. We also saturated the pots with water to insure that the presence of biochar did not

introduce a bias by enhancing the soil water content. After four weeks, we quantified the levels of root colonization by arbuscular mycorrhizal fungi grown in conjunction with the sorghum and examined changes in the biochar surface chemistry using x-ray photoelectron spectroscopy (XPS).

2.2 Greenhouse Experimental Design

We grew sorghum $\pm$ biochar, $\pm$ ammonium nitrate fertilizer, and $\pm$ mycorrhizae in a sandy soil that was watered to saturation. Each treatment was replicated 30 times. We planted seeds in 115 ml Cone-tainer pots (Stuewe & Sons, model number SC7) filled with a 1:1 ratio of organic potting media (Metro-Mix SB 200, Sun Gro Horticulture Ltd.) and sand (Quikrete, Premium Play Sand) by volume. The Cone-tainers have holes at the bottom, allowing water to drain freely. We inoculated mycorrhizal fungi treatment pots with spores of *Glomus intraradices* (Reforestation Technologies International), an arbuscular mycorrhizal fungus, by adding 5g of inoculum on top of the soil of each pot followed by a cap of sand to prevent contamination by splashing. Nitrogen treatment pots had ammonium nitrate (Aldrich, 99+% purity) added once per week at the rate of 34 kg N/ha in the high nitrogen treatment versus 6.7 kg N/ha in the low nitrogen. Biochar treatment pots had 2.6% biochar by weight added to the growing medium, equivalent to an agricultural amendment rate of 17.6 tons/ha applied at a tillage depth of 20 cm. We planted three sorghum seeds in each pot and at the first sign of growth above soil, we thinned the pots to one plant each. All pots were arranged in a fully randomized array in the greenhouse

at 27-35°C with no artificial lighting. Watering to saturation occurred every day for four weeks until the plants were harvested.

2.2 Biochar Production

We produced biochar from apple wood (*Malus domestica*) sawdust (Allied Kenco, Houston, TX, USA). The wood fragments were 5-10 mm x 0.5-1 mm x 0.5-1 mm and the particle size decreased by 25-50% following pyrolysis. The sawdust was sealed within a 20 liter batch reactor constructed from 306 stainless steel and heated in a propane-fired furnace. Pyrolysis gases were passively vented to a series of heat exchangers at ambient temperature (~30° C) to remove condensable liquids and bio-oils — preventing their condensation into the biochar. Non-condensable gases were combusted in a secondary (venturi-style) burner used to heat the reactor. The reactor used in this experiment produces approximately 2 kg biochar per batch, and all of the biochar used in the pot trial was derived from the same batch. The initial heating rate was approximately 5°C/minute to a reactor temperature of ~400°C. Total heating time was approximately 250 minutes. The reactor was allowed to cool to ambient temperature (overnight) before removing the biochar. When removed from the reactor, the biochar was odorless and visually homogenous. Biochar was manually mixed during removal from the reactor vessel to further ensure homogeneity.

2.3 Quantifying Sorghum Biomass

At harvest, we separated the aboveground and belowground portions and dried them in an oven at 45°C for several days. Dry weights were taken for both portions.

2.4 Root Colonization

We stained the roots of each plant using the ink and vinegar technique (Vierheilig et al., 1998). All stained roots were viewed through a Leica DM2500 microscope at 400x magnification. Thirty fields of view were observed for each sample and the number of views in which hyphae were present was recorded and converted into a percentage (McGonigle et al., 1990).

2.5 Changes in Surface Chemistry

We took advantage of the 4 week exposure period in our experiments to determine how the surface chemistry of char was altered by the rhizosphere environment. XPS was used because this method analyzes only the top 10 nm of the surface of particles. Initial reactions between biochar and its soil environment start at the surface, and XPS allows the sensitive detection of the onset of these reactions. We bulked and sieved the growth medium of each treatment to facilitate picking of biochar fragments using tweezers for XPS analysis on individual pieces of biochar. We used XPS to analyze 5 biochar fragments from each treatment as well as from fresh biochar.

We determined the bonding environments of carbon and oxygen atoms on the surface of biochar particles using a PHI Quantera XPS with an Al X-ray source at 1486.6 eV and 40.7 W. The beam diameter was 200.0 µm and the pass energy 26 eV. High resolution, low intensity scans were performed to focus

on the bonding environments. The C1s electron was targeted between 280 and 290 eV with 40 scans. The O1s electron was targeted between 528 and 538 eV with 30 scans. We analyzed and deconvoluted spectra into their component functional groups using MultiPak data analysis software (MultiPak V7.0.1, 04 Mar 16, Ulvac-Phi, Inc., 1994-2004). Images of biochar particles before and after exposure to soil+plants are available in the Supplemental Material (Figure S1 and S2).

2.6 Statistical Analysis

We performed factorial ANOVA using SAS statistical software (SAS v. 9.2, SAS Institute, Inc., Cary, NC). ANOVA models included the response variables of biomass, mycorrhizal colonization rate, and bonding environment percentages and the fixed effects of the biochar treatment (+/-), mycorrhizal fungi treatment (+/-) and nitrogen treatment (high/low) as well as all possible interactions.

3. Results and Discussion

3.1 Above- and Below-ground Biomass: Evidence for Biochar- and Nitrogen-Induced Fungal Parasitism

In the presence of high nitrogen, biochar appeared to promote parasitism by mycorrhizal fungi in the initial stages of plant growth explored by this experiment. This is suggested by the decline in biomass relative to other high nitrogen treatment combinations (Figures 1, 2, and 3). The increase in productivity due to nitrogen addition was immediately recognizable in the bimodal distribution of biomass (Figures 1 and 2). The treatment of biochar, mycorrhizae, and high nitrogen caused an increase in root colonization (Figure 3) and a

220 decrease in aboveground plant biomass with an average that was low enough to
221 approach the level of the unfertilized treatments. We attribute the dramatic
222 decrease in plant biomass to an induced parasitism on the part of the
223 mycorrhizae. If the biochar provides an environment where nitrogen is more
224 readily available to the roots directly, the mycorrhizal hyphae receive the benefit
225 of organic substrates from the plant without providing nitrogen in return.
226 Corroborating this hypothesis is the observed stimulation of mycorrhizal
227 colonization (+B, +M, high N, Figure 3; see next section).

228 For belowground biomass (Figure 2), statistical analysis revealed no
229 significant effect other than the increase due to high nitrogen addition. A marginal
230 reduction in belowground biomass due to the presence of biochar was nearly
231 significant ($p=0.067$). This relationship could be driven by the same parasitism
232 observed in the aboveground biomass, lending support to the idea that there is
233 some mechanism by which biochar induces mycorrhizal parasitism at high levels
234 of mineral nitrogen.

235 Both increases and decreases in mycorrhizal abundance have been
236 observed in conjunction with biochar amendments, although an increase is more
237 common (Lehmann et al., 2011). One of the mechanisms suggested by
238 Lehmann et al. (2011) was a reduced utility of the symbiosis with high nutrient
239 and water availability, but our study shows the opposite pattern: mycorrhizae
240 flourished with high levels of nitrogen fertilizer and water. In another study, Elmer
241 and Pignatello (Elmer and Pignatello, 2011) observed a linear trend of increasing
242 mycorrhizal colonization with biochar amendment rate in asparagus plants. Their

result reflects the colonization levels we observed in inoculated soils without biochar (+M, low N) and with biochar (+M, high N), but does not explain the inflated colonization that occurred with the addition of both high nitrogen and biochar. Nitrogen additions have been observed to increase rates of colonization by mycorrhizal fungi in other studies, including studies on *Glomus intraradices* (e.g. (Hodge and Fitter, 2010; Smith and Smith, 2011)), and high levels of nutrients commonly cause mycorrhizal fungi to shift from plant mutualists to plant parasites (e.g. (Antunes et al., 2012; Johnson et al., 1997)). The novel observation in our study is that biochar accelerated the transition to parasitism in the presence of high nitrogen.

3.2 Biochar Stimulates Mycorrhizal Colonization

The treatments with biochar and mycorrhizal inoculation had greater root colonization by the fungus, suggesting that the presence of biochar does stimulate mycorrhizal colonization of roots (Figure 3). Figure 4 shows the microscope view of a normal root (a) and a highly colonized root (b). The blue strands of hyphae show good staining and are easily visible against the cleared root. As expected, the treatments with mycorrhizae inoculation had significantly greater colonization. Contamination within the greenhouse (via spore transport) is likely the cause of the treatments without mycorrhizal inoculation showing some colonization; the growing media were sterilized and the pots were either new or thoroughly washed and bleached.

3.3 Oxidation of the Biochar Surface Measured by XPS

265 Few studies have used XPS to analyze the surface chemistry of biochar,
266 and none have previously been able to control for exposure to mycorrhizal fungi.
267 XPS separates surface carbon functional groups into four categories: -COOR, -
268 C=O, -COR, and a fourth category that includes -CH, C-C, and C=C functional
269 groups (Figure 5). Previous work has shown an increase in the oxidation of
270 biochar with weathering, but causes of the oxidation have not been clear. Time
271 scales and methods in previous studies also cover a wide range. Yao et al. (Yao
272 et al., 2010) performed artificial weathering experiments on biochar produced
273 from sewage sludge, using a modified Soxhlet reactor to repeatedly wet the
274 biochar with water at 30°C and with and without humic acids for 300 hours. They
275 observed an increase in the -COOH and -C=O groups, but it could not be
276 determined whether the oxidation was biotic or abiotic. Cheng et al. (Cheng et
277 al., 2006) and Zimmerman (Zimmerman, 2010) attributed an observed increase
278 in oxidation of biochar to abiotic processes in incubation. Nguyen et al. (Nguyen
279 et al., 2008) recovered biochar ranging in age from 2 to 100 years in soils that
280 had been subjected to slash-and-burn agriculture. Their time series showed a
281 period of rapid oxidation in the form of an increase in the ratio of oxidized to
282 reduced carbon followed by a subsequent constant oxidation level. However, the
283 time period of reaching this static level was poorly constrained due to the 100
284 year range of biochar age. Zimmerman (Zimmerman, 2010) observed a leveling-
285 off of biochar remineralization rates after a one year laboratory incubation, but
286 the oxidative environment was substantially different from that of the char studied
287 by Nguyen et al. (Nguyen et al., 2008).

We observed the largest effect on biochar surface chemistry simply from its addition to the plant-soil environment. All biochar exposed to soil+plants for four weeks showed more surface carbon oxidation in the form of increased percentages of -COR , -C=O , and -COOR groups (Table 1; Fig. 6). It is theoretically possible that the XPS measurements occasionally sampled hyphae fragments on the surface of the biochar rather than the biochar itself, but if this had affected our measurements, it would have been seen in larger standard deviation for the + mycorrhizae treatments (Table 1). This is not the case, so we can be confident that the biochar surface was sampled, not the mycorrhizal hyphae. Our results suggest that the first stage of biochar patina development involves sorption of dissolved organic compounds in soil, leading to a generalized increase in surface carbon oxidation.

Additionally, one treatment showed small, but statistically significant differences in biochar surface oxidation compared to others. The soils with mycorrhizae but low nitrogen caused more oxidation of the biochar surface than the other treatment combinations, and showed a significantly greater fraction of carbon present as carbonyl groups (-C=O , Figure 6). This treatment had significantly less mycorrhizal colonization than the combined treatment of biochar, mycorrhizae, and high nitrogen, suggesting that the greater oxidation is not due to the quantity of mycorrhizae, but perhaps to their behavior. Fungi have been shown to degrade aromatic structures (Ascough et al., 2010; Hedges et al., 1988; Schreiner et al., 2009; Wengel et al., 2006), and the fungal oxidation of lignin produces both aromatic carbonyl (-C=O) and aromatic acid (-COOH)

functionalities, with carbonyl groups preferentially enriched in the early stages of decomposition (Goni et al., 1993; Hedges et al., 1988). Our observed increase in carbonyl (-C=O) and lack of increase in carboxyl carbon (-COOH) detected by XPS is consistent with the early stages of fungal oxidation of aromatic materials. It is possible that our low-nitrogen treatment stimulated the metabolism of mycorrhizal fungi, driving the hyphae to more actively seek nutrients and resulting in more extensive oxidation of biochar via extracellular cometabolism. Likewise, the mycorrhizae could have limited their nutrient-seeking efforts if they were not necessary in the combined biochar, mycorrhizae, and high nitrogen treatment, leading to the observed decreased biochar surface oxidation.

One possible explanation for the transformation of mycorrhizal fungi from symbionts to parasites in the +biochar, +mycorrhizae, high N treatment is biochar sorption of molecules used for cellular signalling, a hypothesis that has been suggested by others (Lehmann et al., 2011; Thies and Rillig, 2009; Warnock et al., 2007). To monitor cell density, fungi release non-polar compounds and use the density-dependent accumulation of these extracellular signalling molecules to coordinate decisions within a population (Albuquerque and Casadevall, 2012; Hogan, 2006), such as spore germination (Macko et al., 1970), the transition between different morphological forms (Oh et al., 2001), and growth rate (Chen et al., 2004). In mycorrhizal fungi, coordinated growth during an infection could represent an evolutionary strategy to maintain the symbiotic relationship with plants and maximize their reproductive fitness in the environment. Unfortunately, the molecular details and fitness consequences of cell-cell signalling are not well

understood for any mycorrhizal fungi, including the fungus examined here, *Glomus intraradices*. There is evidence that *Glomus intraradices* is exposed to elevated levels of a signalling molecule, tyrosol, during its infection of plants. Legumes infected with *Glomus intraradices* accumulated tyrosol within the cell walls of their roots, whereas plants lacking an infection do not accumulate this fungal signalling molecule (Schliemann et al., 2008). Tyrosol is a phenolic compound (4-(2-hydroxyethyl)phenol) that has been studied in the human fungal pathogen *Candida albicans*, in which it regulates growth (Chen et al., 2004). Soils containing biochar may deplete tyrosol and other fungal signalling compounds from plant roots, such as farnesol, farnesoic acid, and dimethoxycinnamate. These fungal signalling compounds are non-polar, like compounds previously shown to adsorb to charcoal and activated carbon (Zackrisson et al., 1996). Biochar interference in the chemical communication between sorghum and mycorrhizal fungi could underlie the transition of mycorrhizal fungi from symbionts to parasites, although more research is needed to document and understand this transformation.

4. Conclusion

We showed that biochar induced mycorrhizal parasitism in juvenile sorghum plants in the presence of high levels of nitrogen fertilizer. The increased root colonization rates in the treatment with biochar and nitrogen fertilizer along with the much lower aboveground biomass provide supporting evidence for this conclusion. While this parasitism significantly reduced plant

growth in the first 4 weeks, longer studies will be necessary to determine if an effect can be observed in crop yields.

Along with biochar effects on the plant-fungal relationship, we also explored the changes in biochar after exposure to plants, fertilizer, and mycorrhizal fungi. When compared to fresh biochar, we observed changes in the surface chemistry of biochar that had spent four weeks in soil with sorghum. The percentage of carbon in each of the functional groups -CO , -C=O , and -COOH increased when watered and exposed to the soil environment, regardless of the experimental treatment. We also observed treatment-specific surface chemistry effects. Biochar in the +mycorrhizae, low nitrogen treatment had an increase in the percentage of carbon in the carbonyl fraction versus other soil-exposed biochars. This treatment had significantly less mycorrhizal colonization than the +mycorrhizae, high nitrogen treatment indicating that this form of oxidation is not correlated with the quantity of mycorrhizae. Given that the only difference between the treatments was the presence of mycorrhizae, biotic oxidation is the likely cause for the increase in the carbonyl fraction. It is possible that the mycorrhizae in the +mycorrhizae, low nitrogen treatment were more active in their search for nutrients due to their nitrogen-limited environment and thus oxidized the biochar further.

Acknowledgements

We appreciated the help of Bo Chen with XPS measurements and Prudence Sun and Liz Yin in the greenhouse, and we acknowledge the Rice

380 Shared Equipment authority, which provided access to the XPS. Rebecca
381 Barnes and Krystle Hodge helped with plant harvesting. Reforestation
382 Technologies generously provided the mycorrhizae used here. We acknowledge
383 support from NSF EAR 0911685, NSF EAR 0949337 and from the Rice
384 University Shell Center for Sustainability.

385

386

387

References

- Albuquerque, P., Casadevall, A., 2012. Quorum sensing in fungi - a review. *Medical Mycology* 50, 337-345.
- Antunes, P.M., Lehmann, A., Hart, M.M., Baumecker, M., Rillig, M.C., 2012. Long-term effects of soil nutrient deficiency on arbuscular mycorrhizal communities. *Functional Ecology* 26, 532-540.
- Ascough, P.L., Sturrock, C.J., Bird, M.I., 2010. Investigation of growth responses in saprophytic fungi to charred biomass. *Isotopes in Environmental and Health Studies* 46, 64-77.
- Atkinson, C.J., Fitzgerald, J.D., Hipps, N.A., 2010. Potential mechanisms for achieving agricultural benefits from biochar application to temperate soils: a review. *Plant Soil* 337, 1-18.
- Bell, M.J., Worrall, F., 2011. Charcoal addition to soils in NE England: A carbon sink with environmental co-benefits? *Science of the Total Environment*, The 409, 1704-1714.
- Boden, T.A., Marland, G., Andres, R.J., 2011. Global, Regional, and National Fossil-Fuel CO₂ Emissions, Carbon Dioxide Information and Analysis Center. Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN, USA.
- Bridgwater, A.V., 2003. Renewable fuels and chemicals by thermal processing of biomass. *Chemical Engineering Journal* 91, 87-102.
- Briggs, C., Breiner, J.M., Graham, R.C., 2012. Physical and Chemical Properties of *Pinus ponderosa* Charcoal: Implications for Soil Modification. *Soil Science* 177, 263-268.
- Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2007. Agronomic values of greenwaste biochar as a soil amendment. *Australian Journal of Soil Research* 45, 629-634.
- Chan, K.Y., Van Zwieten, L., Meszaros, I., Downie, A., Joseph, S., 2008. Using poultry litter biochars as soil amendments. *Australian Journal of Soil Research* 46, 437-444.
- Chen, H., Fujita, M., Feng, Q.H., Clardy, J., Fink, G.R., 2004. Tyrosol is a quorum-sensing molecule in *Candida albicans*. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 101, 5048-5052.
- Cheng, C., Lehmann, J., Thies, J., Burton, S., Engelhard, M., 2006. Oxidation of black carbon by biotic and abiotic processes. *Organic Geochemistry* 37, 1477-1488.
- Cheng, C.H., Lehmann, J., Engelhard, M.H., 2008. Natural oxidation of black carbon in soils: Changes in molecular form and surface charge along a climosequence. *Geochimica et Cosmochimica Acta* 72, 1598-1610.
- Elmer, W.H., Pignatello, J.J., 2011. Effect of Biochar Amendments on Mycorrhizal Associations and Fusarium Crown and Root Rot of Asparagus in Replant Soils. *Plant Disease* 95, 960-966.

430 Glaser, B., Lehmann, J., Zech, W., 2002. Ameliorating physical and chemical
 431 properties of highly weathered soils in the tropics with charcoal -a review.
 432 *Biology and Fertility of Soils* 35, 219-230.
 433 Goni, M.A., Nelson, B., Blanchette, R.A., Hedges, J.I., 1993. Fungal degradation
 434 of wood lignins - geochemical perspectives from CuO-derived phenolic dimers
 435 and monomers. *Geochimica et Cosmochimica Acta* 57, 3985-4002.
 436 Habte, M., Antal, M.J., 2010. Reaction of Mycorrhizal and Nonmycorrhizal
 437 *Leucaena leucocephala* to Charcoal Amendment of Mansand and Soil. *Comm Soil*
 438 *Sci Plant An* 41, 540-552.
 439 Hata, S., Kobae, Y., Banba, M., 2010. Interactions Between Plants and
 440 Arbuscular Mycorrhizal Fungi, *International Review of Cell and Molecular Biology*,
 441 Vol 281. Elsevier Academic Press Inc, San Diego, pp. 1-48.
 442 Hedges, J., Blanchette, R., Weliky, K., K., Devol, A., 1988. Effects of fungal
 443 degradation on the CuO oxidation products of lignin - a controlled laboratory
 444 study. *Geochim Cosmochim Ac* 52, 2717-2726.
 445 Hockaday, W.C., Grannas, A.M., Kim, S., Hatcher, P.G., 2006. Direct molecular
 446 evidence for the degradation and mobility of black carbon in soils from ultrahigh-
 447 resolution mass spectral analysis of dissolved organic matter from a fire-
 448 impacted forest soil. *Organic Geochemistry* 37, 501-510.
 449 Hodge, A., Fitter, A.H., 2010. Substantial nitrogen acquisition by arbuscular
 450 mycorrhizal fungi from organic material has implications for N cycling.
 451 *Proceedings Of The National Academy Of Sciences Of The United States Of*
 452 *America* 107, 13754-13759.
 453 Hogan, D.A., 2006. Talking to themselves: Autoregulation and quorum sensing in
 454 fungi. *Eukaryotic Cell* 5, 613-619.
 455 Johnson, N.C., Graham, J.H., Smith, F.A., 1997. Functioning of mycorrhizal
 456 associations along the mutualism-parasitism continuum. *New Phytologist* 135,
 457 575-586.
 458 Kinney, T.J., Masiello, C.A., Dugan, B., Hockaday, W.C., Dean, M.R., Zygourakis,
 459 K., Barnes, R.T., 2012. Hydrologic properties of biochars produced at different
 460 temperatures. *Biomass & Bioenergy* 41, 34-43.
 461 Lehmann, J., 2007. Bio-energy in the black. *Frontiers in Ecology and the*
 462 *Environment* 5, 381-387.
 463 Lehmann, J., Rillig, M.C., Thies, J., Masiello, C.A., Hockaday, W.C., Crowley, D.,
 464 2011. Biochar effects on soil biota - A review. *Soil Biology and Biochemistry* In
 465 Press.
 466 Liu, J., Schulz, H., Brandl, S., Miehtke, H., Huwe, B., Glaser, B., in press. Short-
 467 term effect of biochar and compost on soil fertility and water status of a Dystric
 468 Cambisol in NE Germany under field conditions. *Journal of Plant Nutrition and*
 469 *Soil Science*.
 470 Macko, V., Staples, R.C., Gershon, H., Renwick, J.A.A., 1970. Self-inhibitor of
 471 bean rust uredospores - methyl 3,4-dimethoxycinnamate. *Science* 170, 539-&.

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 Phytologist* 115, 495-501.

Nguyen, B.T., Lehmann, J., Kinyangi, J., Smernik, R., Riha, S.J., Engelhard, M.H., 2008. Long-term black carbon dynamics in cultivated soil. *Biogeochemistry* 89, 295-308.

Novak, J.M., Busscher, W.J., Watts, D.W., Amonette, J.E., Ippolito, J.A., Lima, I.M., Gaskin, J., Das, K.C., Steiner, C., Ahmedna, M., Rehrich, D., Schomberg, H., 2012. Biochars Impact on Soil-Moisture Storage in an Ultisol and Two Aridisols. *Soil Science* 177, 310-320.

Oh, K.B., Miyazawa, H., Naito, T., Matsuoka, H., 2001. Purification and characterization of an autoregulatory substance capable of regulating the morphological transition in *Candida albicans*. *Proceedings Of The National Academy Of Sciences Of The United States Of America* 98, 4664-4668.

Rajkovich, S., Enders, A., Hanley, K., Hyland, C., Zimmerman, A.R., Lehmann, J., 2012. Corn growth and nitrogen nutrition after additions of biochars with varying properties to a temperate soil. *Biology and Fertility of Soils* 48, 271-284.

Revell, K.T., Maguire, R.O., Agblevor, F.A., 2012. Influence of Poultry Litter Biochar on Soil Properties and Plant Growth. *Soil Science* 177, 402-408.

Schliemann, W., Ammer, C., Strack, D., 2008. Metabolite profiling of mycorrhizal roots of *Medicago truncatula*. *Phytochemistry* 69, 112-146.

Schnell, R.W., Vietor, D.M., Provin, T.L., Munster, C.L., Capareda, S., 2012. Capacity of Biochar Application to Maintain Energy Crop Productivity: Soil Chemistry, Sorghum Growth, and Runoff Water Quality Effects. *Journal of Environmental Quality* 41, 1044-1051.

Schreiner, K.M., Filley, T.R., Blanchette, R.A., Bowen, B.B., Bolskar, R.D., Hockaday, W.C., Masiello, C.A., Raebiger, J.W., 2009. White-Rot Basidiomycete-Mediated Decomposition of C 60 Fullerol. *Environ. Sci. Technol.* 43, 3162-3168.

Smith, S.E., Smith, F.A., 2011. Roles of Arbuscular Mycorrhizas in Plant Nutrition and Growth: New Paradigms from Cellular to Ecosystem Scales, *Annual Review of Plant Biology*, Vol 62. Annual Reviews, Palo Alto, pp. 227-250.

Thies, J.E., Rillig, M.C., 2009. Characteristics of Biochar: Biological Properties, In: Lehmann, J., Joseph, S. (Eds.), *Biochar for Environmental Management*. Earthscan, London, pp. 85-102.

Vierheilig, H., Coughlan, A.P., Wyss, U., Piche, Y., 1998. Ink and vinegar, a simple staining technique for arbuscular-mycorrhizal fungi. *Applied and Environmental Microbiology* 64, 5004-5007.

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

Wengel, M., Kothe, E., Schmidt, C.M., Heide, K., Gleixner, G., 2006. Degradation of organic matter from black shales and charcoal by the wood-rotting fungus *Schizophyllum commune* and release of DOC and heavy metals in the aqueous phase. *Science of the Total Environment* 367, 383-393.

Woolf, D., Amonette, J.E., Street-Perrott, F.A., Lehmann, J., Joseph, S., 2010.
 Sustainable biochar to mitigate global climate change, *Nature Communications*,
 pp. 1-9.
 Yao, F.X., Arbestain, M.C., Virgel, S., Blanco, F., Arostegui, J., Macia-Agullo, J.A.,
 Macias, F., 2010. Simulated geochemical weathering of a mineral ash-rich
 biochar in a modified Soxhlet reactor. *Chemosphere* 80, 724-732.
 Zackrisson, O., Nilsson, M., Wardle, D., 1996. Key ecological function of charcoal
 from wildfire in the Boreal forest. *Oikos* 77, 10-19.
 Zhang, A.F., Bian, R.J., Pan, G.X., Cui, L.Q., Hussain, Q., Li, L.Q., Zheng, J.W.,
 Zheng, J.F., Zhang, X.H., Han, X.J., Yu, X.Y., 2012a. Effects of biochar
 amendment on soil quality, crop yield and greenhouse gas emission in a Chinese
 rice paddy: A field study of 2 consecutive rice growing cycles. *Field Crops
 Research* 127, 153-160.
 Zhang, A.F., Liu, Y.M., Pan, G.X., Hussain, Q., Li, L.Q., Zheng, J.W., Zhang, X.H.,
 2012b. Effect of biochar amendment on maize yield and greenhouse gas
 emissions from a soil organic carbon poor calcareous loamy soil from Central
 China Plain. *Plant And Soil* 351, 263-275.
 Zimmerman, A.R., 2010. Abiotic and Microbial Oxidation of Laboratory-Produced
 Black Carbon (Biochar). *Environmental Science & Technology* 44, 1295-1301.

Nitrogen, Biochar, and Mycorrhizae: Alteration of the Symbiosis and Oxidation of the Char Surface

LeCroy, Masiello, Rudgers, Hockaday, Silberg, in review

Table Caption

Table 1. Bonding environment percentages averaged for 5 replicates in each group. Data from analysis using MultiPak software.

Group	-COOR Average	Standard Deviation	-C=O Average	Standard Deviation	-C-OR Average	Standard Deviation	-CH, C- C, C=C Average	Standard Deviation
Fresh								
Biochar	1.96	1.17	3.69	1.03	13.52	0.88	80.83	2.66
-M, -N	3.36	1.58	7.35	3.29	22.83	4.48	66.46	8.75
+M, -N	4.60	0.81	9.73	1.72	24.93	4.80	60.73	6.52
-M, +N	3.75	0.36	6.45	0.88	22.37	1.45	67.42	2.30
+M, +N	1.82	3.15	9.03	2.80	21.07	2.21	66.75	4.90

Nitrogen, Biochar, and Mycorrhizae: Alteration of the Symbiosis and Oxidation of the Char Surface

LeCroy, Masiello, Rudgers, Hockaday, Silberg, in review

Figure Captions

Figure 1. Average mass of dried aboveground biomass from 30 plants per group. Different letters indicate values that are significantly different as determined by factorial ANOVA. Error bars represent standard error. ([+B]: Biochar added to soil, [+M]: Mycorrhizal inoculum added to soil, [low N]: fertilized with ammonium nitrate at 6.7 kg N/ha, [high N]: fertilized with ammonium nitrate at 34 kg N/ha.)

Figure 2. Average mass of dried belowground biomass from 30 plants per group. Different letters indicate values that are significantly different as determined by factorial ANOVA. Error bars represent standard error.

Figure 3. Root colonization rates for ~25 plants per treatment. Different letters indicate values that are significantly different as determined by factorial ANOVA. Error bars represent one standard error. The [+B, +M, high N] treatment significantly increased colonization while decreasing aboveground and total plant biomass relative to other high N treatments.

Figure 4. Sorghum roots at 400x magnification. 4a) uninfected root. 4b) highly colonized root; the blue ribbons are mycorrhizae growing into and around the root.

Figure 5. XPS spectra of the C1s electron for biochar. Above, fresh biochar; below, biochar grown with fertilizer and mycorrhizal inoculation. The fresh biochar has one dominant peak representing C-C bonds. After being in soil, the biochar becomes more oxidized as seen by the extra peaks at higher binding energies.

Figure 6. XPS spectra of Carbon 1s electron, resolved into bonding environment using MultiPak data analysis software. Each group is averaged from five replicate samples that were picked from their respective group of bulked soil. Different letters indicate values that are significantly different as determined by factorial ANOVA. Error bars represent one standard deviation; instrument error is <5%. Increased oxidation is shown by all the biochars that were added to soil. The treatment with mycorrhizae but low nitrogen fertilizer shows an increase in the percentage of carbon in the carbonyl fraction.

Figure 1. Average mass of dried aboveground biomass from 30 plants per group. Different letters indicate values that are significantly different as determined by factorial ANOVA. Error bars represent one standard error. ([+B]: Biochar added to soil, [+M]: Mycorrhizal inoculum added to soil, [low N]: fertilized with ammonium nitrate at 6.7 kg N/ha, [high N]: fertilized with ammonium nitrate at 34 kg N/ha.)

Figure 2. Average mass of dried belowground biomass from 30 plants per group. Different letters indicate values that are significantly different as determined by factorial ANOVA. Error bars represent standard error.

Figure 3. Root colonization rates for ~25 plants per treatment. Different letters indicate values that are significantly different as determined by factorial ANOVA. Error bars represent one standard error. The [+B, +M, high N] treatment significantly increased colonization while decreasing aboveground and total plant biomass relative to other high N treatments.

Figure 4. Sorghum roots at 400x magnification. 4a) uninfected root. 4b) highly colonized root; the blue ribbons are mycorrhizae growing into and around the root.

Figure 5. XPS spectra of the C1s electron for biochar. Above, fresh biochar; below, biochar grown with fertilizer and mycorrhizal inoculation. The fresh biochar has one dominant peak representing mostly C-C bonds, with the other

peaks indistinct and overlapping. After being in soil, the biochar becomes more oxidized as seen by the extra peaks at higher binding energies.

Figure 6. XPS spectra of Carbon 1s electron, resolved into bonding environment using MultiPak data analysis software. Each bar is averaged from five replicate samples that were picked from their respective group of bulked soil. Different letters indicate values that are significantly different as determined by factorial ANOVA. Error bars represent one standard error; instrument error is <5%. Increased oxidation is shown by all the biochars that were added to soil. The treatment with mycorrhizae but low nitrogen fertilizer shows an increase in the percentage of carbon in the carbonyl fraction.

Nitrogen, Biochar, and Mycorrhizae: Alteration of the Symbiosis and Oxidation of the Char Surface

Chase LeCroy^{1*}, Caroline A. Masiello^{1**}, Jennifer A. Rudgers², William C.
Hockaday³, Jonathan J. Silberg⁴

¹Department of Earth Science, Rice University ms 126, 6100 Main St., Houston, TX 77030

²Department of Ecology and Evolutionary Biology, Rice University, ms 170, 6100 Main St., Houston, TX 77030

³Department of Geology, Baylor University, One Bear Place #97354, Waco, TX 76798

⁴Department of Biochemistry and Cell Biology, Rice University, ms 140, 6100 Main St., Houston, TX 77030

Supplemental Material

17

18

19 Figure S1. Above, an applewood biochar fragment that was in a pot with an
20 inoculated Sorghum plant for 4 weeks. Various particles can be seen on the
21 surface, including soil particles such as vermiculite and perlite along with
22 mycorrhizae. Below, a biochar fragment not exposed to soil.

23

24 Figure S2. Insets of the biochar fragments from figure S1. The biochar that was
25 in soil, above, has visible minerals and possibly hyphae on its surface while the
26 control biochar, below, has a uniform and plain surface.

27

28

29

30

31

32

33 Figure S1. Above, an applewood biochar fragment that was in a pot with
34 an inoculated Sorghum plant for 4 weeks. Magnification is 16x. Various particles
35 can be seen on the surface, including soil particles such as vermiculite and

36 perlite along with mycorrhizae. Below, a biochar fragment that was not present
37 in soil.

38

39

40

41

42

43

44

45

46 Figure S2. Insets of the biochar fragments from figure 5. Magnification is

47 100x. The biochar that was in soil, above, has visible minerals and possibly

48 hyphae on its surface while the control biochar, below, has a uniform and plain

49 surface.

50