Nutrient Losses in Agriculture:
the Role of Biochar and Fungal Associations

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INTRODUCTION

Agriculture is a system of extraction: through it we capture soil nutrients taken up by plants and we channel them away from their local decay for our own consumption. The principles of the system suggest some loss of soil fertility over time, but the practice of agribusiness, with its rigorous tilling and irrigation, leads to a great wash of topsoil and nutrients that, in the United States, most often congregate in the flow of the Mississippi before leaving the continent and mixing with the Gulf waters. While the industrialization of agriculture brought about a huge boom in global food production, it did so by the integration of relatively cheap and abundant energy from fossil fuels to manufacture inorganic fertilizer, apply it with heavy machinery, and power irrigation.[1] In spite of all these efforts to boost productivity, current rates of topsoil runoff lead to a total loss of soil fertility over time.[2] Fertilizer runoff from the same agricultural fields pours excess nitrogen into streams, rivers, and lakes, leading to algal blooms and associated dead zones such as the one found off the Mississippi delta.[3]

Current methods in farming are typically viewed as unsustainable.[4] In this light, and from other perspectives, farmers and scientists are reinvigorating other practices in agriculture. For example, some agriculturalists are invoking our knowledge about soils in the Amazon known as *terra preta,* or 'dark earth', that are still fertile from thousands of years ago when farmers mixed charcoal with them. Today, the material is known as *biochar*, and is loosely defined as organic matter that has been heated in the absence of air. The organic matter ranges from wood chips to rice husks to poultry litter, and heating varies in time and intensity. Biochar itself does not provide a significant amount of nutrients to plants. Rather, it increases soil fertility through four mechanisms: 1) increasing soil pH; 2) increasing soil cation exchange capacity (CEC); 3) improving water holding capacity; and 4) improving the habitat for microorganisms.[5]

The increase in microbial biomass often seen with biochar application has prompted many hypotheses about soil microorganism responses to biochar. One of the more popular hypotheses is that the miniscule pores that cover the surface of a grain of biochar allow for colonization by bacteria and fungi while sheltering them from predatory microbes. The increase in soil CEC caused by biochar also helps to reduce nutrient leaching, but it has been hypothesized that microorganisms, such as mycorrhizae (described below), can help to reduce nutrient leaching even more than the presence of biologically inactive biochar.[6] The potential for interaction between biochar and soil microorganisms, then, appears to be an important area of study if we want to discover effective means of retaining nutrients in our soil systems for plant productivity.

To adequately investigate the hypotheses about biochar function in soils, we must consider specific, key relationships between plants and soil microbes such as those formed with mycorrhizae. Fungi and plants can form mutualistic relationships- that is, when certain fungi and certain plants share resources and serve each other, both benefit. One of the most ubiquitous of such mutualisms exists between a collection of fungi known as mycorrhizal and the roots of plants with which they associate. Fungi infect the roots and then send out filaments into the soil, threads no larger 64 μm that transport nutrients to the plant. In exchange, the plant gives the fungi some of the carbon that it has fixed photosynthetically. Plants that enter into such relationships are often many times more productive than those that do not. Many current agricultural practices, like frequent tilling and the use of fungicides, discourage the growth of such fungi. Because mycorrhizal relationships with plants are so ubiquitous, the interaction between these mutualistic relationships and biochar is likely an important feature of soil nutrient dynamics. Thus, the purpose of this research was to explore the question: How does the interaction of mycorrhizae and biochar in soil influence crop production and soil nutrient retention?

METHODS

Treatments were made up of a fertilized and an unfertilized set, each of which included: pots with char but no mycorrhizal inoculant, with both char and inoculant, and with inoculant but no char. Each treatment as well as a control was replicated four times to give an initial total of 32 pots (in three treatments, three rather than four pots sprouted oats, giving a total of 29 pots). All soils were sterilized. Biochar was ground and homogenized with soils at 5% w/w, with no-char pots totaling 140 g soil and biochar amended pots 147 g. Three oats were seeded per pot, and liquid 46:00:00 fertilizer applied at a rate of 26.4 mg N to fertilized pots. Mycorrhizal inoculant was applied according to distributor recommendations at a rate of 1.3 mL solution per pot (solution =4 cc inoculant/ 1 L H_2O).

Oat seeds sprouted within one week, at which time pots with multiple sprouts were thinned down to one plant. For the next six weeks, 5 mL water were applied to all plots 5-6 times weekly to avoid excess water stress on the oats. These water additions did not produce leachate. Once weekly, pots were inundated with either 40 or 60 mL water, and leachate was collected in scintillation vials, which were then stored at 4° C. At the end of six weeks all whole oat plants were harvested, their live biomass recorded, and root samples taken for mycorrhizal testing. These roots were stored in a 1:1 solution on ethanol and water at 4° C. Mycorrhizal infection was assessed by staining roots segments with lactophenol cotton blue, mounting on slides, and examining roots at 10x-40x magnification. Dry oat biomass was collected after one week at 60° C. Nitrate and ammonium content were then measured from leachate samples on a Lachat Quickchem Autoanalyzer. Total N leached, both at individual time points and cumulative across the experiment, were analyzed with SAS using repeated measures ANOVA (PROC MIXED).

RESULTS

Fertilized pots without biochar leached 35-50% of N applied at the first week, which was more than any other treatment ($p < 0.05$, Figure 1). Biochar addition to fertilized pots reduced N loss substantially in the first week, with pots leaching 10% of total N applied. The effect of both fertilizer and biochar on nutrient leaching was variable in following weeks: fertilized pots with biochar often did not loose significantly more N in later weeks than did control plots, and the effect of biochar to reduce N loss significantly in fertilized pots (below levels of fertilized pots without biochar) was intermittent. The role of mycorrhizal inoculant in altering N loss was sporadic, appearing only in weeks three and four. The strongest effect, significant across all six weeks, was the large N loss in fertilizer-only pots compared to unfertilized biochar pots with or without mycorrhizae ($p<0.05$).

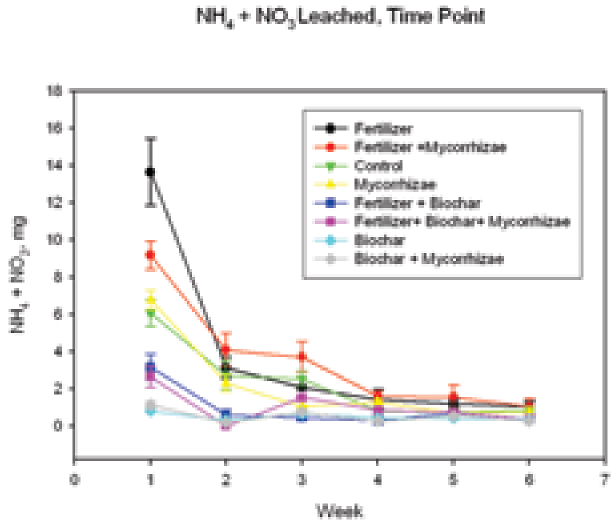

Figure 1. Nitrogen leached at individual time points from greenhouse oats and pot soil subjected to the indicated treatments over a six week period. Each point is the mean of four leachates except in Fertilizer, Control, and Fertilizer + Biochar + Mycorrhizae treatments when $n=3$; error bars represent standard errors.

Trends of cumulative N lost (Figure 2) were similar to those revealed via individual time points (Figure 1), with the only exception being on week one, when fertilized additions of mycorrhizal inoculant reduced N loss in fertilized pots without biochar ($p<0.05$). In subsequent weeks, mycorrhizal inoculation had no effect on cumulative N loss. Pots receiving fertilizer alone exceeded all others in cumulative N leaching, followed by control and mycorrhizae pots. The addition of biochar to fertilized pots reduced cumulative N leaching to a level below that of control pots, and pots receiving neither fertilizer nor biochar leached significantly less total N than any other treatment.

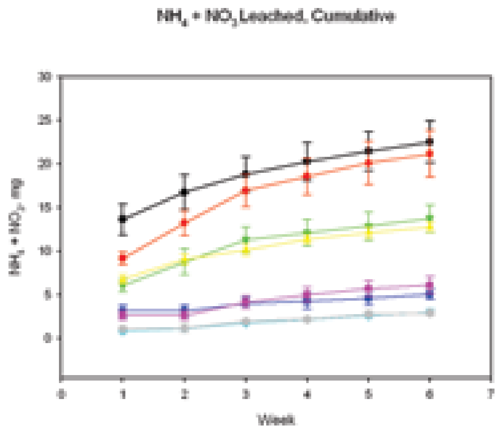

Figure 2. Nitrogen leached, cumulative over the experiment, from greenhouse oats and pot soil subjected to the indicated treatments over a six week period. Each point is the mean of four leachates except in Fertilizer, Control, and Fertilizer + Biochar + Mycorrhizae treatments when n=3; error bars represent standard errors.

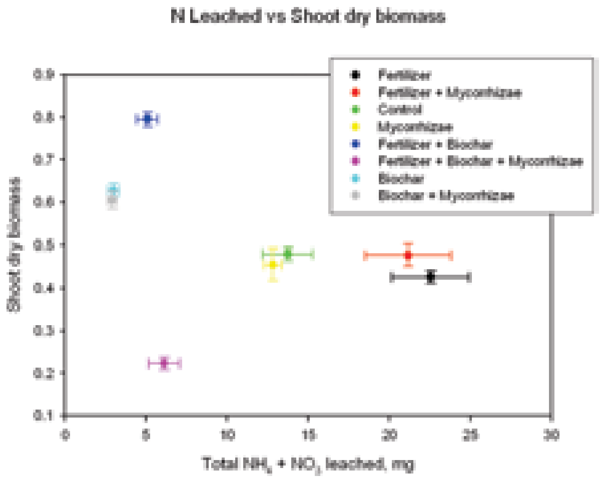

Figure 3. Relationship between total N leached and dry shoot biomass production. Regression: y = 0.6956 - 0.0123; r^2= 0.6023 when point representing Fertilizer + Biochar +Mycorrhizae is removed. Each point represents data from four oat plants, except in Fertilizer, Control, and Fertilizer + Biochar + Mycorrhizae treatments when n=3; error bars represent standard errors.

With all treatments included in a regression of total N leached versus shoot dry biomass, N leached did not explain variation in biomass (Figure 3, p=0.36). Excluding data representing pots that received fertilization, biochar, and mycorrhizae the relationship between total N leached and shoot biomass is significant ($p<0.05$, r^2=0.6023). The relationship between water loss and shoot dry biomass was similar: with all treatments, average weekly percentage loss of water did not explain variation in biomass (Figure 4, p=0.22). Excluding data representing pots that received fertilization, biochar, and mycorrhizae the relationship between water leached and shoot biomass is significant (r^2=0.7383).

Mycorrhizal Colonization

In spite of the periodic, significant effects of mycorrhizal inoculation described above, mycorrhizal infection of sub-samples of roots from all of the inoculated pots was not observed.

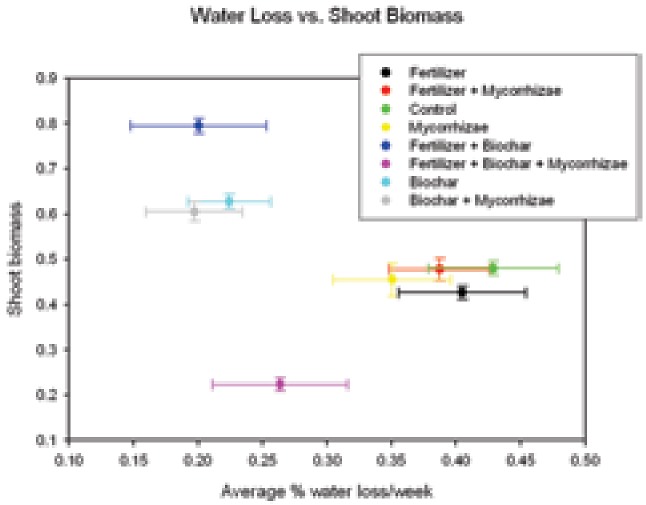

Figure 4. Relationship between average percent water loss each week and shoot dry biomass production. Regression: y= 0.8983 – 1.104; r^2= 0.7383 when point representing Fertilizer + Biochar + Mycorrhizae is removed. Each point represents data from four oat plants, except in Fertilizer, Control, and Fertilizer + Biochar + Mycorrhizae treatments when n=3; error bars represent standard errors.

DISCUSSION

Nitrogen losses from soil were regulated by fertilizer inputs and biochar application. Though mycorrhizal treatment significantly influenced N loss on two sampling dates, overall, mycorrhizal inoculant had no significant effect on N loss. Taken in conjunction with weekly water percolation, time point data give us a valuable insight into the dynamics of N retention and loss over this six week period, while cumulative data provide a more time-integrated perspective, which is important for plant production. Water loss and N lost both described a portion of shoot productivity.

Fertilization was associated with enhanced N losses, a large proportion of which occurred in the short term (up to 50% of N applied on the first week), while biochar reduced N losses across all time points. Though the ability of biochar to reduce nutrient loss is rarely explored in the literature, Lehmann *et al* (2003) suggest that reductions in N loss from biochar-amended soils may occur as a result of “the creation of sites for electrostatic adsorption [or] the retention of soil water and therefore nutrients contained in it”. These sites for electrostatic adsorption, also referred as the cation exchange capacity, are lower in fresh biochar (as used in this study) compared to aged biochar, a difference attributed to their incomplete oxidation. It is through oxidation that biochar acquires the negative surface charges that can bind positively charged molecules such as nitrate.[7] The use of sterilized soil may have played a role in nutrient transformations, as the normal compliment of nitrogen-fixing, ammonifiying, and denitrifying bacteria were exterminated, with unknown rates of re-colonization. Biochar’s high water holding capacity also likely contributed to reduction in N loss.

Both N lost and water loss had some explanatory power on shoot biomass production. Fertilized pots without biochar, while leaching significantly more than other treatments, did not encourage greater shoot production than control or mycorrhizae pots, possibly because such a large percentage of total N applied had been leached from the soils in the first weeks (35-50%). It is likely that the greater shoot production in biochar pots, fertilized and unfertilized, was influenced by their greater capacity to retain nutrients (but see exception below). N losses in later weeks may have been limited by the ability of soil, biochar-amended or otherwise, to retain nutrients, and/or by the diminished N remaining in soils, resulting from either leaching or plant uptake.

Differences in water retention across treatments may be explained either directly, by the porosity of biochar, or indirectly, by the growth of the oats. The interactions between water loss and plant vigor are difficult to untangle: larger root systems and bigger leaves provide greater surface areas to both absorb and transpire water, preventing its loss, while greater water retention by biochar may have allowed greater plant growth. The anomalous behavior of fertilized pots with mycorrhizae and biochar, which lost moderate amounts of water but produced the most diminutive shoots, may be attributed to their expansive root systems. Their average root: shoot ratio of 2.55 (n=3, s= 0.311) was higher than any other treatment. Greater proportional allocation of carbon in roots has been associated with mycorrhizal symbioses,[8] and also offers explanation for their low shoot production compared to other treatments with similar N losses.

In this oat-soil system, biochar appears more influential in reducing N loss than mycorrhizae. In this study, mycorrhizal infection may not have occurred, but their presence in the soil, and their possible function as heterotrophic organisms, suggests that inoculant can influence N losses. Furthermore, mycorrhizal additions appear to have had a strong positive influence on root: shoot ratios in fertilized biochar pots, influencing shoot production and water loss. The low shoot production in these pot receiving fertilizer, biochar, and mycorrhizae was the exception in a pattern of greater shoot production associated with biochar amended pots compared to treatments without biochar. These reductions in water loss and N loss associated with biochar amended soils are important in their potential positive influence on soil fertility, water quality, and crop production.

ENDNOTES

[1] Pfeiffer, David Alan, <u>Eating Fossil Fuels: Oil, Food and the Coming Crisis in Agriculture</u>,
(New Society Publishers, Canada, 2006).

[2] Ibid.
[3] Ibid.
[4] Jackson, Wes and Jon Piper, "The Necessary Marriage Between Ecology and Agriculture.", <u>Ecology</u>, vol. 70 (1989): 1591-1593.

[5] Lehmann, Johannes and Stephen Joseph, editors, <u>Biochar for Environmental Management: Science and Technology</u>, Earthscan, London, 2009.

[6] Ibid.
[7] Ibid.
[8] Nadian H., Hashemi M. and Herbert, S. J., "Soil Aggregate Size and Mycorrhizal Colonization Effect on Root Growth and Phosphorus Accumulation

by Berseem Clover", Soil Science & Plant Analysis, vol. 40 Issue 15/16 (2009): 2413-2425.

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Nadian H., Hashemi M. and Herbert, S. J. "Soil Aggregate Size and Mycorrhizal Colonization Effect on Root Growth and Phosphorus Accumulation by Berseem Clover." Soil Science & Plant Analysis, vol. 40 Issue 15/16 (2009): 2413-2425.

Warnock D. et al. "Mycorrhizal responses to biochar in soil- concepts and mechanisms", Plant Soil, vol. 300 (2007): 6-20.