



Adding value to corn and agricultural byproducts through production of biochar and bio-oil: Step Two



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Team Leader:

Kurt Spokas (USDA-ARS; St. Paul, MN)

Co-Principal Investigators:

Roger Ruan and Tim Griffis (University of Minnesota; St. Paul, MN)

Partners:

Minnesota Corn
University of Minnesota

This report highlights the major significant findings of this project. These are presented below in a bulleted list. Overall, this project generated significant public and international interest in the research results, and this is demonstrated with 7 accepted manuscripts and 5 international invited presentations that resulted as a consequence of this research.

Significant Scientific Accomplishments:

- **Physical degradation of biochar:** We completed the initial observations on the physical disintegration of biochar. This can be a major mechanism of biochar disappearance from field plots, and needs further study to fully understand its implication. However, the results from this project provided the first documented observations of this phenomenon. Results were published in *Environmental Science & Technology Letters*. (Appendix A). This work also resulted in invitations for a seminar at Rice University (Houston, TX).

- **Multi-location effort on disintegration of biochar**
As a result of these findings, a multi-location effort was assembled within the USDA-ARS to evaluate the potential impact of physical degradation on biochar's persistence (not part of the initial proposal). Degradation was assessed through the use of litter bags filled with a known amount of biochar, which was then buried at 3 different locations for 2 different time periods (3 and 6 months). This effort was started in the final months of the project, so the final results will be documented in a manuscript that will be submitted early 2016.

As observed in Figure 1, there are significant losses of biochar particles due to fragmentation and passing through the litter bags, which do vary by soil type and climate. This data was for the same oak hardwood biochar (550 °C slow pyrolysis) that was shipped to all the sites and buried in litterbags at 10 cm. This data will be leading to follow-up proposals and additional research into this phenomenon. One potential avenue is to be developed into a "citizen science effort" with biochar litter bags being sent to various volunteers around the US. This work also was invited to be presented at the 5th International Symposium

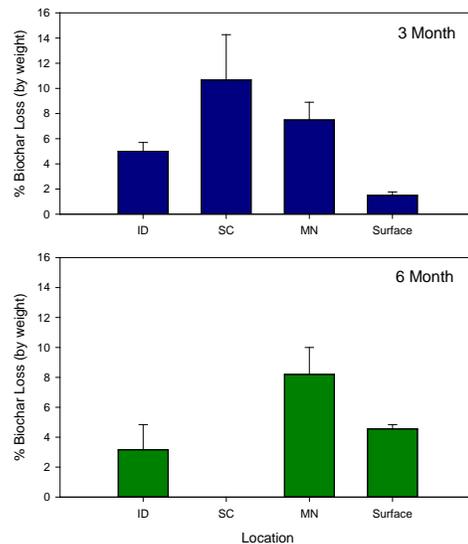


Figure 1. Observed loss of biochar from litter bags buried at 10 cm in Idaho, South Carolina, and Minnesota at (A) 3 and (B) 6 months. The average and standard deviation of 3 replicates are shown.

on Soil Organic Matter in Göttingen, Germany; the Joint International Biochar Symposium 2015 in Geisenheim, Germany; and the Soil Interfaces For Sustainable Development which was a joint meeting of the Commission 2.5 of the International Union of Soil Science, Canadian Society of Soil Science, and Association Québécoise des spécialistes en sciences du sol in Montreal, Canada (July 2015).

- **Predictable impact of biochar addition on hydraulic properties:** Laboratory assessment of the soil saturated conductivity alteration as a result of biochar amendment focusing on the impact of soil texture. The degree of impact does depend on soil texture and biochar particle size. The alteration in the hydraulic permeability was predicted with a spreadsheet model (Excel™) utilizing existing pedotransfer functions for the prediction of soil hydraulic conductivity based on soil texture. This was the first unified determination of the impact on hydraulic conductivity following biochar additions. This work was detailed in two manuscripts, published in *Chemosphere* (Appendix B) and *Horticulture Acta* (Appendix C). This work also resulted in two international invitations: 1) European Geophysical Union Annual Meeting in Vienna, Austria (April 12-17, 2015) and 2) the 3rd International Symposium on Organic Matter Management and Compost Use in Horticulture, held in Murcia, Spain on 20-24th April 2015.
- **Biochar's impact on soil chemical signaling compounds:** In order to assess differences in biochar's impact on corn growth, we first evaluated a series of biochars for their sorption of phenolic acids, since these are important signaling compounds in the root zone and trigger microbial and plant responses. This was the first work to demonstrate non-additive effects of mixing feedstocks prior to pyrolysis. The results are summarized in a manuscript to *Water, Air and Soil Pollution* (Appendix D). This work also was invited to be presented at the 13th IUPAC International Congress of Pesticide Chemistry, and Kathleen Hall (PhD student on this project) was awarded with a poster award in the Environmental Fate and Metabolism section (<http://www.iupac2014.org/>).
- **GHG impacts of biochar additions.** We were able to correlate the original microbial activity to the GHG impact observed following biochar additions. This was the first research that suggested the ability to predict the microbial response in GHG production following biochar addition for a range of soil types, which was a function of the initial microbial activity. This work was summarized in an accepted manuscript to the *Journal of the Total Environment* (Appendix E). This work was also presented at the 2nd Midwest Biochar Conference in Champaign, IL by a visiting PhD student from Brazil on the project.
- **Biochar impacts on corn growth:** Biochar addition did not significantly impact the mass of above or below ground corn biomass produced in greenhouse trails (Table 1; $p < 0.05$). Despite our initial hypothesis that the addition of biochar would have a significant effect on plant growth,

there were no significant differences observed for the biochar treatments across all the soils (Table 1).

However, there were some specific *soil × biochar* combinations that did result in significant differences. Corn seedlings that were grown in with a pine chip derived biochar were shorter than plants grown in the control in the Morris soil and greater than the controls in the Becker soil. The addition of corn cob biochar reduced corn height solely in the Becker soil. The hardwood biochar (RO) reduced total plant height solely in the Morris soil. The AAC resulted in higher plant growth in both the potting soil and Becker soil (Figure 3). Although these differences in height did not amount to a significant difference in dried plant biomass. (Appendix E for more details).

Table 1. Average weight (g) of oven dried above ground [vegetative part (leaf and stem)] and below ground (roots) from corn grown in different soils (RM: Rosemount, MN; PS: Potting soil Sunshine MVP; and UM) and biochar treatments [CS: Corn Stover Biochar (500C); CC: Corn Cob Biochar (500C); PC: Pine chip biochar (500C); RO: Royal Oak hardwood lump charcoal; AAC: Accurel activated charcoal; B: Bamboo and MC: Macadamia nut]. Results represent the average of 3 replicates of seedling growth (2 week period).

SOILS	Plant ¹	Roots ¹
	<i>g pot⁻¹</i>	
UM	2.05	1.61
RM	1.94	1.66
PS	1.83	1.50
BIOCHAR ADDITIONS		
None (Control)	2.00	1.88
CS	1.90	1.41
CC	1.80	1.50
ICM	1.77	1.33
RO	1.88	1.55
MC	1.88	1.77
AAC	2.00	1.44
B	2.11	1.55

¹ The treatments were compared and there are no statistical significant difference between soil and biochar treatment groups (Tukey, P>0.05).

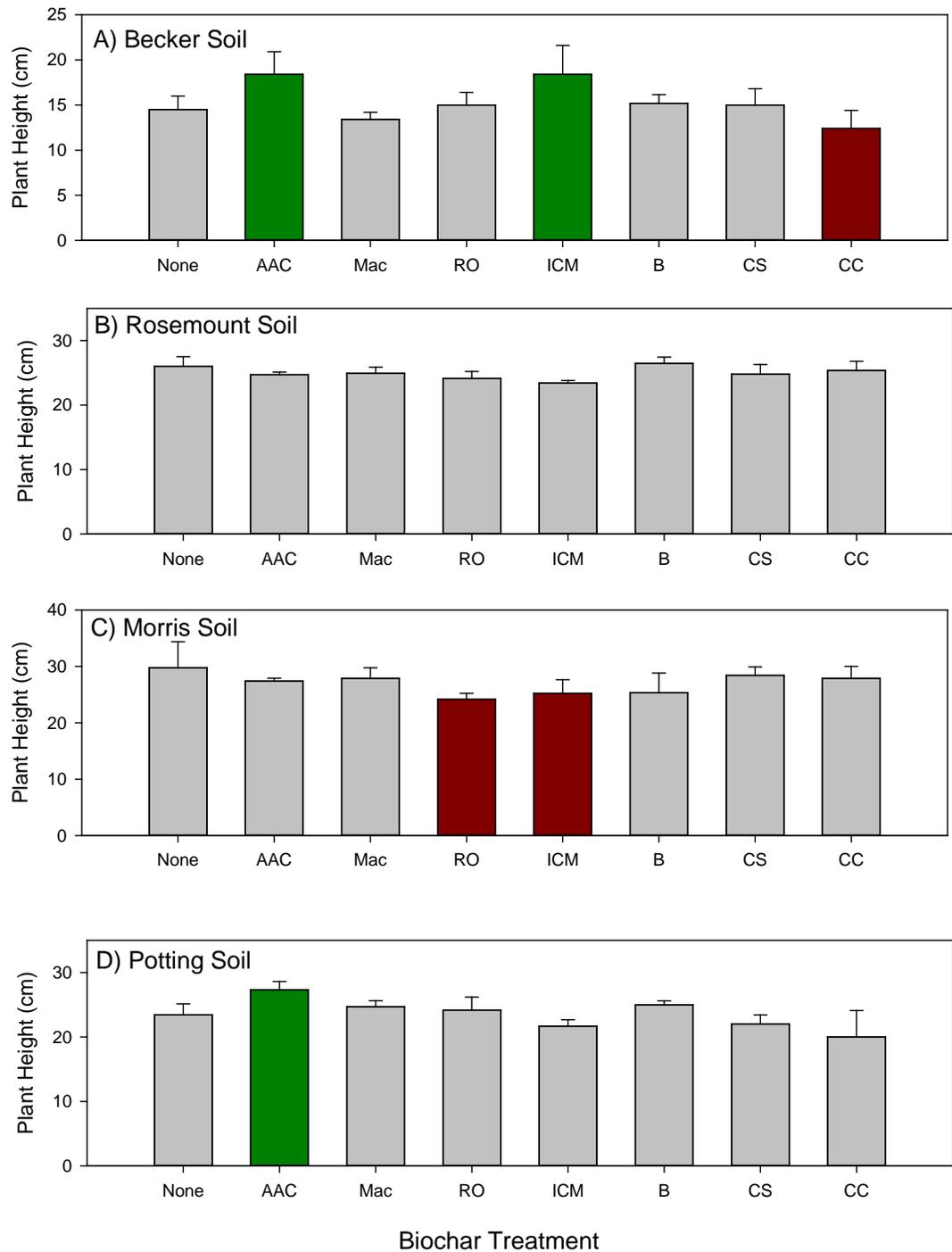


Figure 3. Corn plant height (cm) across the different soils (Becker, Rosemount, Morris, and Potting Soil) and biochar treatment (ICM: Pine chip biochar; RO: Royal Oak hardwood lump charcoal; AAC: Accurel activated charcoal; B: Bamboo; CC: Corn Cob; CS: Corn Stover; and MC: Macadamia nut) after 16 days of planting seeds. Error bars represent the standard deviation from the mean. Bars with different color shading indicate those select treatments within each soil type that are significantly greater (green) or less (red) plant height than the control within each soil type (Tukey, $p < 0.05$).

This work also was linked to the ongoing Minnesota Corn Growers project with Dr. Ken Valentas (UMN) to examine the comparison between hydrochar filtrate and biochar impacts, which also resulted in another accepted publication (Appendix F).

- Evaporation Impacts:** The impact of biochar on evaporation rate was evaluated with and without corn plants. As observed in Figure 4, the rate of evaporation was equivalent for the control and biochar treatments, with the exception of the activated charcoal addition to potting soil, which reduced the total amount of evaporation (Table 2). This can also be observed in the total cumulative evaporation shown in Figure 5 & 6.

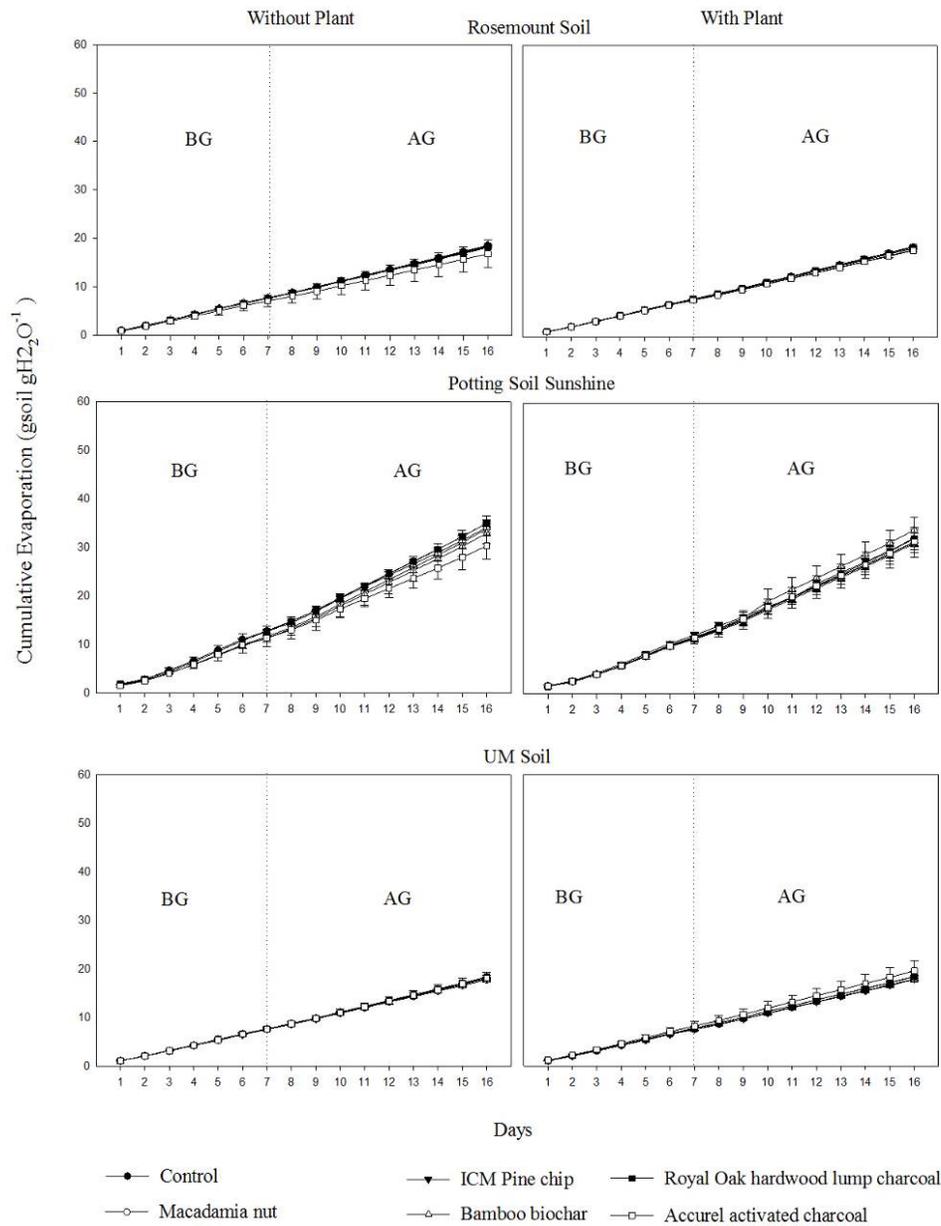


Figure 4. Cumulative evaporation versus days (before germination-BG and after germination-AG) with

different soils and biochars (Table 1). In the figure, error bars represent the standard deviation from the mean. The only significant reduction observed was that of activated charcoal with the potting soil mix without plants.

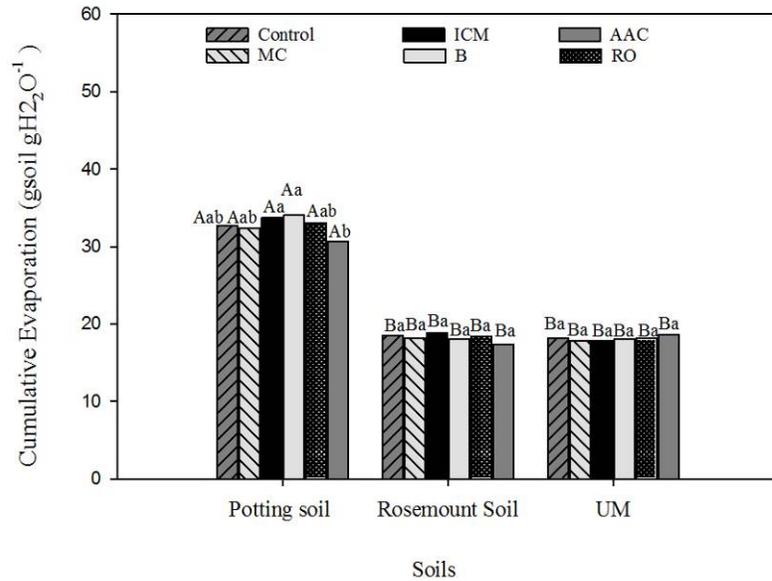


Figure 5. Cumulative evaporation interaction soils and biochar with incubated from different soils and biochars. In the Figure: Bars with uppercase letters compare soils and lowercase compare biochar when distinct letters represent statistically different values (Tukey, $p < 0.05$)

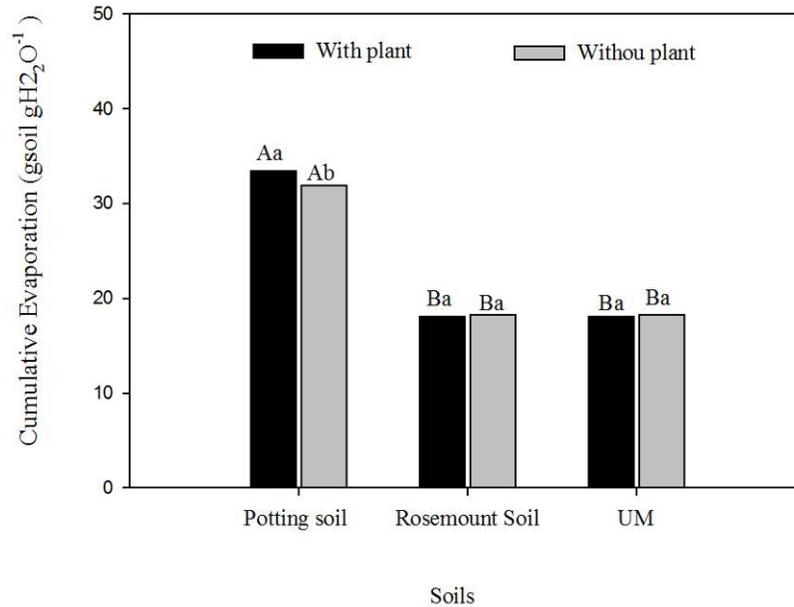


Figure 6. Cumulative evaporation interaction soils and corn plants with incubated from different soils and biochars. In the Figure: Bars with uppercase letters compare soils and lowercase compare plant when distinct letters represent statistically different values (Tukey, $p < 0.05$)

- Biochar from different hardwood species:** We produced a unique series of hardwood biochars created from different species of hardwood trees (Figure 7). These biochars were the first detailed examination into the impact of hardwood species on the resulting biochar properties. This work will be continuing to allow an evaluation of feedstock composition effects on the overall agronomic impacts.



Figure 7. (A) View of the raw hardwoods (maple,, oak, and apple) that were used to create biochar at 3 different temperatures and (B) illustration of the three created biochars in the separated partitions. (Photo from USDA-ARS at Florence, SC).

- Microwave Assisted Pyrolysis:** Different microwave absorbents and their interactions with microwave energy were studied. Heating characteristics and temperature profiles of the microwave absorbents and biomass were analyzed. Product yield and physical and chemical properties as a function of heating characteristics were examined. Figure 8 shows a schematic diagram of the fast microwave assisted biomass conversion system developed and used in the project. The system is composed of: (1) material feeder; (2) inlet quartz connector; (3) microwave oven; (4) quartz reactor; (5) microwave absorbent bed; (6) thermocouple (K-type) to measure the temperature of cavity; (7) thermocouple (K-type) to measure the temperature of bed particles; (8) outlet quartz connectors; (9) liquid fraction collectors; (10) condensers; (11) connection for gas collection. For safety purpose, a microwave detector (MD-2000, Digital Readout) was used to monitor microwave leakage.

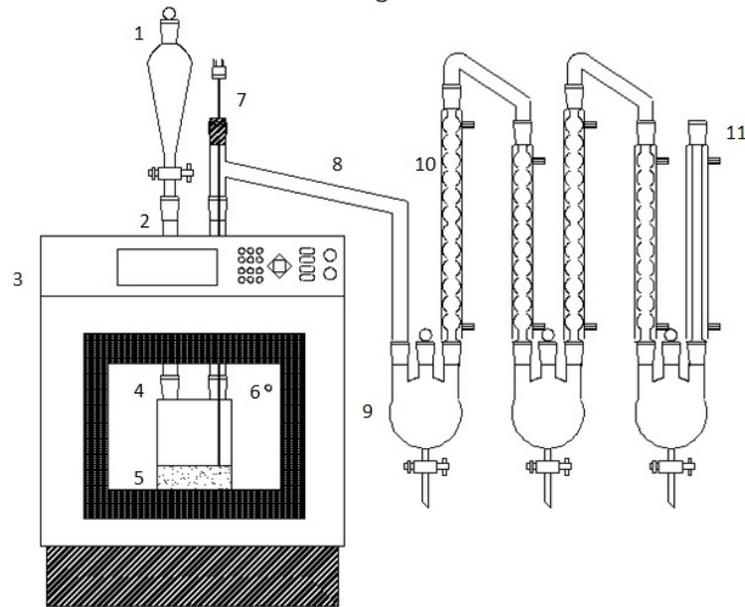


Figure 8. Schematic diagram of microwave-assisted pyrolysis and gasification system.

- **Microwave-assisted pyrolysis (MAP) of turkey wastes:**

- Preparation of materials and experimental setup

Prior to the use, the turkey waste samples were ground and then screened to limit the particle size smaller than 0.5 mm. These ground samples were then dried for more than 24 h at 80 ± 1 °C. 500 g of SiC particles with particle size of 30-grit were put in the quartz reactor as the microwave absorbent bed. For each experiment, 15 g of turkey waste material was used and the temperature for pyrolysis was set at 550 °C. When the bed particles absorbed the microwave and the temperature reached the set value, the prepared sample was dropped through the feeder onto the hot SiC bed, meanwhile the microwave oven was controlled to be on or off in order to keep the temperature of absorbent bed stable. Flowing through the condensers, the gas product was collected into sampling bags for offline analysis, with the condensable components condensed into the liquid collectors as bio-oil. The results showed that the yields of bio-oil, gas and char were 22.6%, 31.0% and 46.4%, respectively. As the ash content of turkey wastes was about 35%, the bio-oil yield on the ash free basis was 34.8%. The GC-MS chromatogram of bio-oil was shown in Fig. 9. The bio-oil was rich in acids, which accounted for 23% based on area percentage of GC-MS chromatogram. Hexadecanoic acid (10.6%) and 9-octadecenoic acid (7.7%) were the most dominant acids in the bio-oil and could be recycled and reused as important laboratory reagents and chemical feedstock. In addition, aliphatic compounds like 2-ethylideneamino-propionitrile, aromatic compounds like pyrimidine and phenols like 2-methoxy-phenol were also found in the bio-oil. The quality of bio-oil obtained could be further improved through addition of catalyst such as zeolite during the pyrolysis process. The carbon content of pyrolysis char was only 29.5% based on elemental analysis, making it unsuitable to be used as adsorbent. However, it is likely that the contents of trace elements such as Mg, Al would be high, but it needs to be further determined.

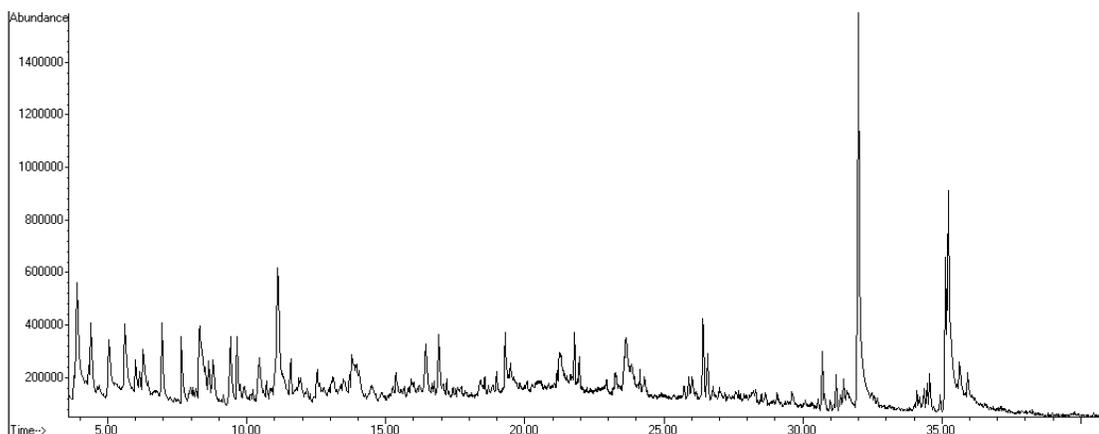


Figure 9. GC-MS chromatogram of bio-oil from MAP of turkey wastes.

The procedure for microwave-assisted gasification (MAG) was similar as that for MAP, but the temperature was set at 900 °C and air was used as the gasifying agent. The results showed that the gas yield was 50.5%, which was much lower than that from gasification of lignocellulosic biomass due to the high ash content of turkey wastes. However, the H₂ and CO contents in the gas product were 32.4% and 24.3%, respectively. Therefore, the syngas content was 56.7% and H₂ to CO ratio reached 1.33, which were better than those from lignocellulosic biomass gasification. The tar yield was 18.2% and could be reduced through application of catalyst such as Ni-based catalyst. Steam addition would further increase H₂ to CO ratio in syngas by water-gas shift reactions and steam reforming reactions of tar, methane, and other light hydrocarbons. The syngas can be directly burned to provide heat and electricity, or further converted to other chemicals through subsequent processes such as Fischer-Tropsch synthesis.

The results on process development have been published in peer-reviewed journals (3 papers) and presented in a conference. The results were also cited in grant proposals recently submitted to state and federal agencies.

Appendix A

Spokas, K. A., J. M. Novak, C. A. Masiello, M. G. Johnson, E. C. Colosky, J. A. Ippolito and C. Trigo (2014). "Physical Disintegration of Biochar: An Overlooked Process." Environmental Science & Technology Letters 1(8): 326-332.

Title:

Physical disintegration of biochar: An overlooked process

Authors: K. A. Spokas^{*1,2}, J. M. Novak³, C.A. Masiello⁴, M. G. Johnson⁵, E. C. Colosky², J.A. Ippolito², and C. Trigo¹

Affiliations:

1 - United States Department of Agriculture – Agriculture Research Service, 1991 Upper Buford Circle, 439 Borlaug Hall, St. Paul, MN, 55018

2 - University of Minnesota, Department of Soil, Water and Climate, 1991 Upper Buford Circle, 439 Borlaug Hall, St. Paul, MN, 55108

3 - United States Department of Agriculture – Agriculture Research Service, 2611 West Lucas St., Florence, SC, 29501

4 –Departments of Earth Science and Chemistry, Rice University, Rice University, 6100 Main St., Houston, TX, 77005

5 - United States Environmental Protection Agency, 200 SW 35th St, Corvallis, OR, 97333

6- United States Department of Agriculture – Agriculture Research Service, 3793 North 3600 East, Kimberly, ID, 83341

*Correspondence to: kurt.spokas@ars.usda.gov

USDA-ARS; 1991 Upper Buford Circle, 439 Borlaug Hall, St. Paul, MN 55108

Abstract:

Data collected from both artificially and field (naturally) weathered biochar suggest that a potentially significant pathway of biochar disappearance is through physical breakdown of the biochar structure. Through scanning electron microscopy (SEM) we characterized this physical weathering which increased structural fractures and possessed higher numbers of liberated biochar fragments. This was hypothesized to be due to the graphitic sheet expansion accompanying water sorption coupled with comminution. These fragments can be on the micro and nano-scale, but are still carbon-rich particles with no detectable alteration in the oxygen to carbon ratio of the original biochar. However, these particles are now easily dissolved and could be moved by infiltration. There is a need to understand how to produce biochars that are resistant to physical degradation in order to maximize long-term biochar C-sequestration potential within soil systems.

Introduction:

Black carbon (BC) is the continuum of solid residuals resulting from the chemical-thermal conversion of carbon-containing materials, and includes soot, char, and charcoal^{1, 2}. Due to its economic, soil fertility, and archeological importance, BC have been examined over the last century for susceptibility to microbial and chemical oxidation^{3, 4}. Biochar is intentionally created BC for soil carbon sequestration and soil fertility improvement⁴. Therefore, biochar is chemically a BC, but not all BC is biochar.

The degradability of BC in soils is a function of its chemical composition, physical incorporation, and host soil microbial community structure⁵⁻⁷, but with an overall consensus that BC does represent a carbon pool with increased resistance to microbial degradation^{8, 9}. Since BC has extrapolated mean residence times from centuries to thousands of years in soils^{6, 8}, it should be a major constituent in soils. Nevertheless, comparisons of the estimated BC generation rates with the measured soil BC pool require losses of BC to maintain mass balance: this is referred to as the “black carbon paradox”¹. Some potential solutions to this paradox include transportation of BC with surface run-off¹⁰⁻¹², explaining surface and hill slope losses¹³. In addition, vertical movement in the soil profile also occurs and will be a function of BC particle size or its protective incorporation into the soil matrix^{14, 15}. However, BC does not maintain its original physical size following soil incorporation¹⁶. Physical deterioration has been hypothesized to impact the longevity of BC in soils as well as its potential input into fluvial systems^{12, 17}. It is our contention that the physical disintegration of BC is an important yet overlooked process in current biochar research, dramatically reducing BC longevity in soils.

Physical degradation of biochar occurs via several mechanisms. High oxygen:carbon (O:C) ratio BC materials (e.g., brown coals) are known to dissolve rapidly when exposed to desiccation and rewetting/saturation cycles (i.e. slacking)¹⁸. Sorption of water and water vapor can stress the physical structure of BC due to exothermic graphitic sheet swelling¹⁹. These mechanisms result in swelling and

expanding the physical biochar structure which increases opportunities for further physical weathering²⁰. Furthermore, fresh exposures of new biochar surfaces and fissures could accelerate microbial mineralization²¹, abiotic reactions²², or surface sorption phenomenon²³. BC typically is thought to be mechanically stronger than the original biomass, but is subject to structural fracturing at lower strains than the original biomass²⁴. Furthermore, with aging (weathering) this mechanical strength is reduced²⁰. These structural defects will eventually lead to the formation of fragments, when BC is exposed to additional mechanical stresses²⁵. Ultimately, the comminution of BC particles leads to the creation of small liberated fragments, termed dissolved black carbon (DBC)²⁶.

The fate of DBC is an especially uncertain aspect of global BC cycles. The mobilization of DBC from biochar-amended soils to wetlands and riparian areas could provide a source of DBC to ground and surface waters¹⁷. It is also possible that DBC production is a major loss process for biochar-amended soils, reducing biochar's climate mitigation potential. However, the converse scenario is at least as plausible: it may be essential to break BC into smaller, more easily extractable fragments to increase the opportunity for these molecular pieces to react with soil minerals, creating stable organo-mineral complexes^{27, 28}. These complexes are known to increase native soil organic carbon residence times²⁹.

Here we present data confirming the physical disintegration of biochar over short time periods (24 hr), a result that has implications for this material as a soil carbon sink. Despite its documented recalcitrant nature to microbial reactions, biochar may be very susceptible to physical deterioration, abrasion, and subsequent transport by fluvial or alluvial processes. We suggest that physical comminution is a previously overlooked loss mechanism of biochar degradation and needs to be understood for accurate extrapolation of biochar's soil C sequestration potential and the interpretation of charcoal's presence in the archeological/geologic record³⁰.

Materials and Methods:

In order to determine whether biochar physical stability is a control on its carbon residence time, we added various biochars (5 g oven dried weight) to distilled water (1:20 w/w) in triplicate 125 mL polyethylene bottles and placed in a reciprocating shaker (60 cycle min⁻¹) for 24 hr. Even though this artificial weathering does not fully mimic field weathering conditions³¹, this methodology is also used for estimating water dispersible clays³², batch sorption experiments³³, and water extractable nutrients from biochar³⁴. Following this agitation period, the solution was filtered (20-25 µm; Whatman No. 40). The bottle was triple rinsed (20 mL DI water) to remove BC particles, which was also filtered. The solid residue collected on the filter paper was oven-dried (105 °C) for 24 hr and weighed to assess the overall biochar mass loss (Table 1). Due to the errors of manually rinsing and difficulty removing adsorbed biochar particles from the polyethylene bottle, this method may not be 100% accurate, but is used to assess the order of magnitude mass loss through physical fragmentation of the various biochars³⁵. We also conducted inductively coupled plasma–optical emission spectrometry (ICP–OES) and dissolved carbon analysis (DOC) analyses of the filtrate to evaluate the dissolved content.

We analyzed pre- and post-rinsed biochars using scanning electron microscopy-electron

dispersion spectroscopy (SEM-EDS). These biochars were mounted with a carbon conductive adhesive pad (PELCO Tabs™, Ted Pella, Inc; Redding, CA). In addition to the solid biochars, we also analyzed the dissolved residuals in the rinse water by direct evaporation of 100 µL directly on the aluminum SEM mount. In addition to these artificially laboratory weathered biochars, two biochars were included that had been aged for 5 years in agricultural field plots in Rosemount, MN¹⁶ and compared to the laboratory stored counterparts (Table 1). These biochars were applied to an agricultural soil (Waukegan silt loam; 1% w/w) under continuous corn production, with annual rototilling. Biochar particles located at the soil surface were collected, rinsed with DI water attempting to dislodge the entrapped soil, and then dried at 105 °C for analysis. These biochars were also attached to the SEM mounts by carbon conductive adhesive pads (PELCO Tabs™, Ted Pella, Inc; Redding, CA). Due to the conductivity of the charcoal, there was no surface coatings (i.e. gold, or carbon) used during this SEM imaging. The elemental composition was acquired using the point EDS analysis method, averaging a total of 10 different representative particles and locations³⁶. Unfortunately, EDS data is semi-quantitative measure of elemental concentration, and relative amounts can be inferred from differences in peak heights³⁷.

Results and Discussion:

Fresh biochar had various salts and organic oils coating their surfaces (Figure 1). After 24 hr water rinsing, these coatings were reduced revealing further structural details not immediately visible on the “fresh” biochar (Figure 1). A majority of these surface deposits disappeared with water rinsing. In many cases, the EDS data indicates higher carbon content in the post rinsed biochar (Table 1). Some of the deposits were inorganic salts due to the presence of inorganic elements (e.g., K, Cl, Ca, Mg, P, Ca, N, and O) visualized with EDS point data analyses, which was also confirmed in the ICP-OES analysis of the rinse waters (Table S1). From these analyses, it was concluded that a majority of these deposits were precipitated surface salts, which upon water shaking were removed from the surface. The inorganic elements evaluated contained from 0.1 to 90% of the total mass loss observed from the biochar rinsing (Table S1), which suggests that some of the mass lost from the biochar was DBC (see Figure 3). It is clear that these surface precipitates conceal the actual biochar surfaces and some of these salts are actually precipitated in pores limiting their immediate availability (Figure S1). Thereby, the removal of these surface coatings through dissolution opens additional porosity. However, under field conditions the release of these surface inorganic salts and organics would vary with climatic conditions and soil hydrology.

In addition, water rinsed biochars showed some interesting physical surface features, including occasional microscopic erosion features (Figure S2). These features suggest that the water shaking did remove material from the biochar surface leaving these relic erosion structures. In addition, the biochar surfaces had smaller micron and sub-micron size pieces of biochar that were structurally freed from the biochar particle (Figure S3). The results show water rinsing not only removed the fine biochar particles which are loosely attached to the biochar particle surface (via physical forces, see Figure 1A), but also modifies the surface morphology of the biochar particle itself removing material by physical forces. This exfoliation and structural friability of BC has been noted in other studies with exposure to water, particularly in an alkaline environment²². Biochar physical breakdown is more pronounced in lower temperature biochars (<500 °C), where >50% of mass loss could be attributed to this physical

fragmentation process³⁵. This increased friability could be responsible for its quicker transport through laboratory columns³⁸. Therefore, biochar particle size should not be regarded as a static property.

In addition to these comminution processes, there was also evidence of cracking and fracturing of the biochar surface both with water and soil exposure (Figure 2 & S3). The SEM images present a suggestion of weaker layers of BC in the biochar matrix that are preferentially broken-down during water extraction (Figure 2), analogous to geologic sediment layer and geologic outcrop weathering³⁹. More importantly, there are visible fragments from the biochar that have broken off from the parent BC physical structure (Figure S3). These disassociated BC fragments are estimated to range in size from nanoscale to over 100 μm as estimated through measurement with the SEM software tools. This fragmentation occurs more readily in sandy textured soils (Figure S4). From our observations, wood and high lignin feedstocks appear to disintegrate into smaller particles more readily than the corresponding feedstocks with higher cellulose contents (e.g., manures, grasses, corn stalks). Higher pyrolysis temperature leads to smaller fragment formation, consequentially lower physical mass loss rates. This temperature dependency has already been noted for archeological reconstructions³⁵ and the biochar particle size dependency agrees with observations of biochar particle movement in laboratory column³⁸ and field studies¹¹.

Despite being dislodged from the original biochar particle, these biochar pieces are chemically equivalent to the original biochar as confirmed by SEM-EDS data (Table 1). In other words, these fragments do not show signs of oxidative or other chemical weathering, just physical comminution. In the evaporated portion of the water extraction, we observed $<20\ \mu\text{m}$ and nanoscale particles of BC that were not removed by filtration (Figure 3). The presence of nanoscale particles have been previously demonstrated for pyrolyzed BC materials⁴⁰ and could alter the mobility of sorbed organic compounds on these fragments⁴¹. The presence of this DBC is important, since the typical dissolved organic carbon (DOC) analysis via persulfate-UV might not adequately detect these fragments of DBC without more intense chemical oxidation conditions⁴² (Table S2). This lack of quantification might further account for the “black carbon paradox” and confirms the suggestion by Jaffe et al¹⁷. To put this rapid mass loss in perspective, a recent study observed less than 5% of the carbon in biochar was mineralized over a 8.5 yr laboratory incubation⁵.

Others have observed that once biochar is exposed to soils, soil particles can fill exposed cavities and fissures¹⁶ (Figure S4). These sealing processes could be accelerated by exothermic water sorption onto BC surfaces¹⁹ and accelerate desiccation drying. It is conceivable that the physical accumulation of colloidal, dissolved and particulate material, including soluble inorganic salts and/or alumino-silicates would rapidly infill fractures and pores⁴³ (Figure S4). This infilling could potentially stabilize the BC particle from further physical degradation, analogous to the soil mineral protection of native soil organic material⁴⁴. Soil particle stabilization of biochar does require further scrutiny, but could be an essential mechanism for extending biochar’s longevity, particularly in clay-rich soils.

It is well known that natural physical processes cause abrasion on geologic materials and shape their external morphology. We hypothesize that once charcoal is placed in the soil environment, it is subject to similar weathering and aging processes that act upon all geologic materials. While a majority

of the current research has focused on surface chemical and microbial reactions, our observations stress the overwhelming importance of the physical friability of biochar and the need to account for the corresponding protection mechanisms when predicting long-term soil behavior.

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The authors declare no competing financial interest.

Supporting Information Available

Additional SEM images of biochar structural and physical alterations and dissolved concentrations of the rinse waters are provided in Table S1. This information is available free of charge via the Internet at <http://pubs.acs.org/journal/estlcu>.

Table 1. Summary of biochar characteristics and mass loss from physical dissolution for various biochars.

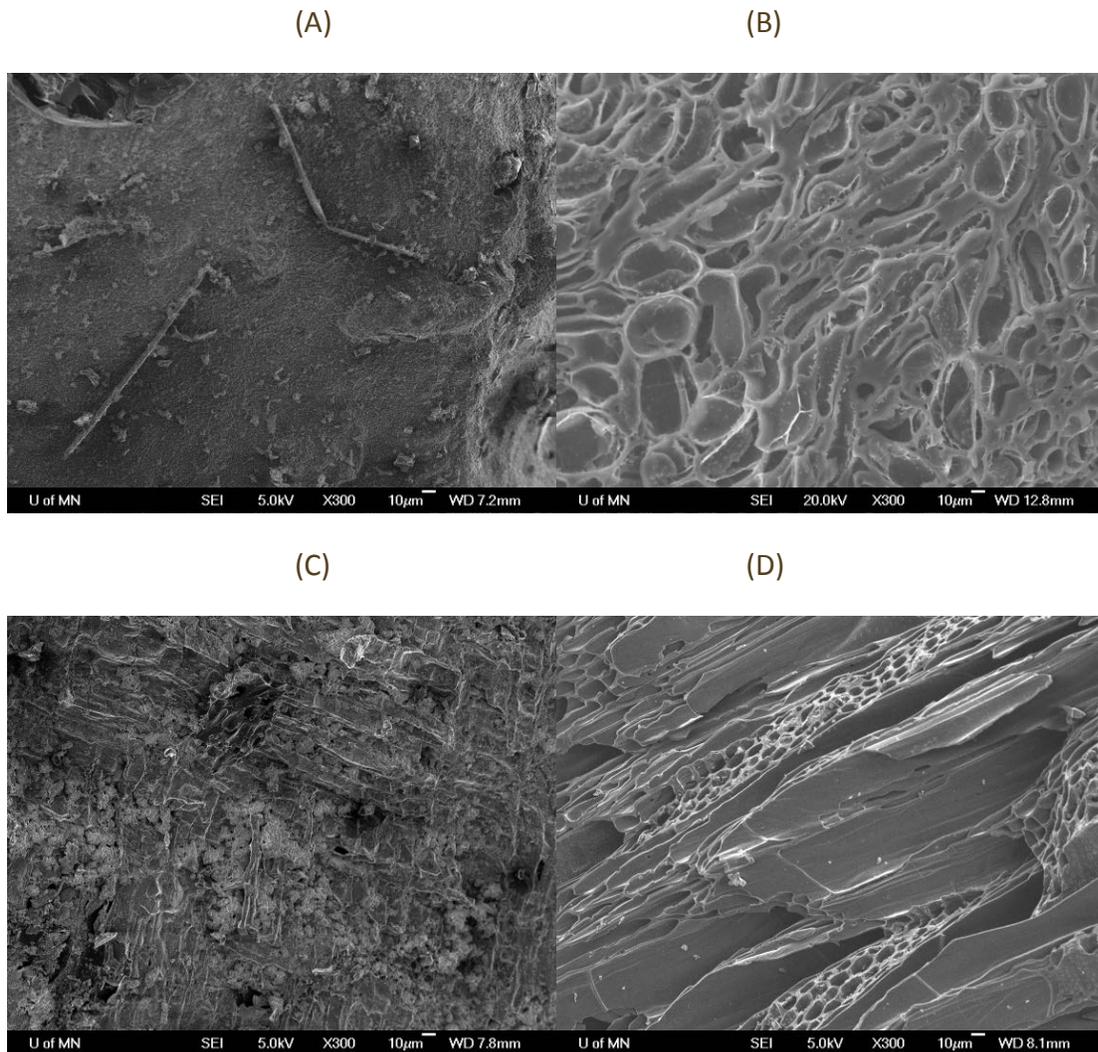
BC#	Feedstock	Pyrolysis Temperature (°C)	From EDS Point Analysis		% Mass Loss	
			C (%)	O (%)		
1	Switch grass	500			5.7 (1.4)	
			<i>Original</i>	85.6 (0.9)	12.3 (0.9)	
			<i>24 hr Rinsed</i>	81.8 (0.1)	12.5 (0.1)	
			<i>Fragments</i>	83.1 (0.1)	12.6 (0.2)	
2	Poultry Litter	350			47.0 (2.1)	
			<i>Original</i>	47.9 (0.2)	20.6 (0.4)	
			<i>24 hr Rinsed</i>	80.1 (0.3)	14.8 (0.5)	
			<i>Fragments</i>	83.4 (0.5)	10.2 (0.1)	
3	Coconut Shell (2 pyrolysis steps)	500 then 900			1.0 (0.3)	
			<i>Original</i>	94.7 (0.2)	5.0 (0.2)	
			<i>24 hr Rinsed</i>	95.8 (0.4)	3.9 (0.2)	
			<i>Fragments</i>	96.4 (0.2)	3.2 (0.1)	
4	Pine Chip	350			16.9 (0.9)	
			<i>Original</i>	76.4 (0.2)	15.7 (0.1)	
			<i>24 hr Rinsed</i>	86.7 (0.2)	10.7 (0.3)	
			<i>Fragments</i>	85.4 (0.5)	9.8 (0.7)	
5	Pine Chip:Poultry litter (50:50)	350			27.9 (0.9)	
			<i>Original</i>	53.3 (0.4)	15.9 (0.5)	
			<i>24 hr Rinsed</i>	78.4 (0.6)	11.0 (0.2)	
			<i>Fragments</i>	82.4 (0.5)	12.0 (0.9)	
6	Pine Chip	700			9.7 (0.3)	
			<i>Original</i>	84.7 (1.2)	13.5 (0.5)	
			<i>24 hr Rinsed</i>	90.5 (0.2)	8.4 (0.2)	
			<i>Fragments</i>	91.7 (1.0)	7.9 (0.6)	
7	Hardwood	500			12.9 (1.6)	
			<i>Original</i>	86.4 (0.2)	20.4 (0.8)	

			24 hr Rinsed	92.4 (0.3)	6.3 (0.3)
			Fragments	93.0 (0.5)	5.4 (0.2)
8	Macadamia nut shell	500			18.7 (2.0)
			Original	68.4 (1.2)	26.4 (1.8)
			24 hr Rinsed	87.4 (3.1)	14.9 (1.2)
			Fragments	89.7 (3.2)	11.4 (4.1)
<hr/> FIELD EXPOSED BIOCHARS <hr/>					
F1	Macadamia nut shell	500			24.9 (2.3)
			Original	55.4 (2.1)	36.4 (2.8)
			24 hr Rinsed	65.7 (1.1)	11.0 (2.1)
			Fragments	75.3 (2.1)	10.2 (1.9)
F2	Hardwood Charcoal	550			34.9 (4.5)
			Original	92.4 (1.1)	19.4 (1.8)
			24 hr Rinsed	95.1 (1.8)	9.6 (1.2)
			Fragments	95.8 (3.1)	10.2 (1.4)

Note: Processing and characterization of biochars are outlined elsewhere^{16, 45}.

Figure Captions:

Figure 1. Representative SEM images of the (A) fresh fast pyrolysis macadamia nut biochar (BC# 8), (B) rinsed fast pyrolysis macadamia nut biochar (BC# 8), (C) fresh slow pyrolysis hardwood biochar (BC# 7), (D) rinsed slow pyrolysis hardwood biochar (BC# 7), (E) fresh slow pyrolysis hardwood biochar (BC# F2), and (F) a 5-yr field exposed biochar (BC# F2). All images were collected at 5.0 kV probe current, with each pair at identical magnification and the scale bar is shown in each panel.



(E)

(F)

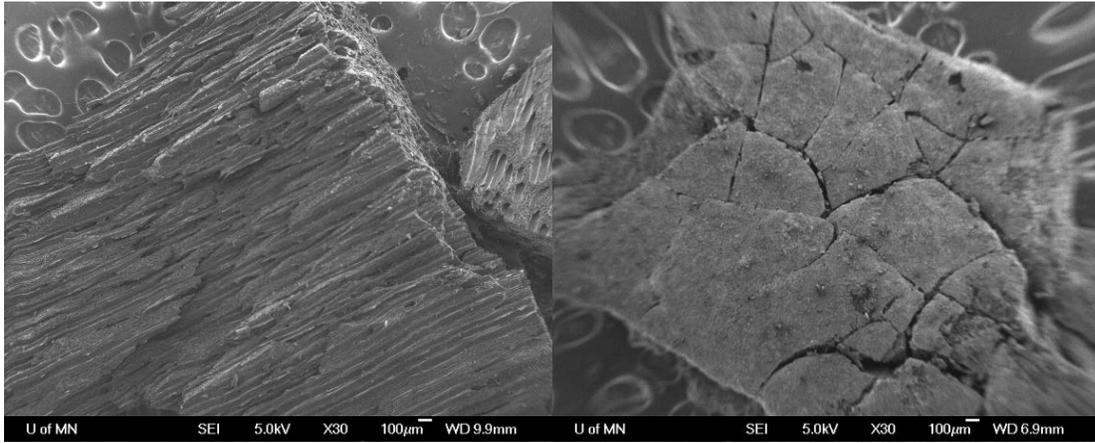


Fig. 1

Fig 2

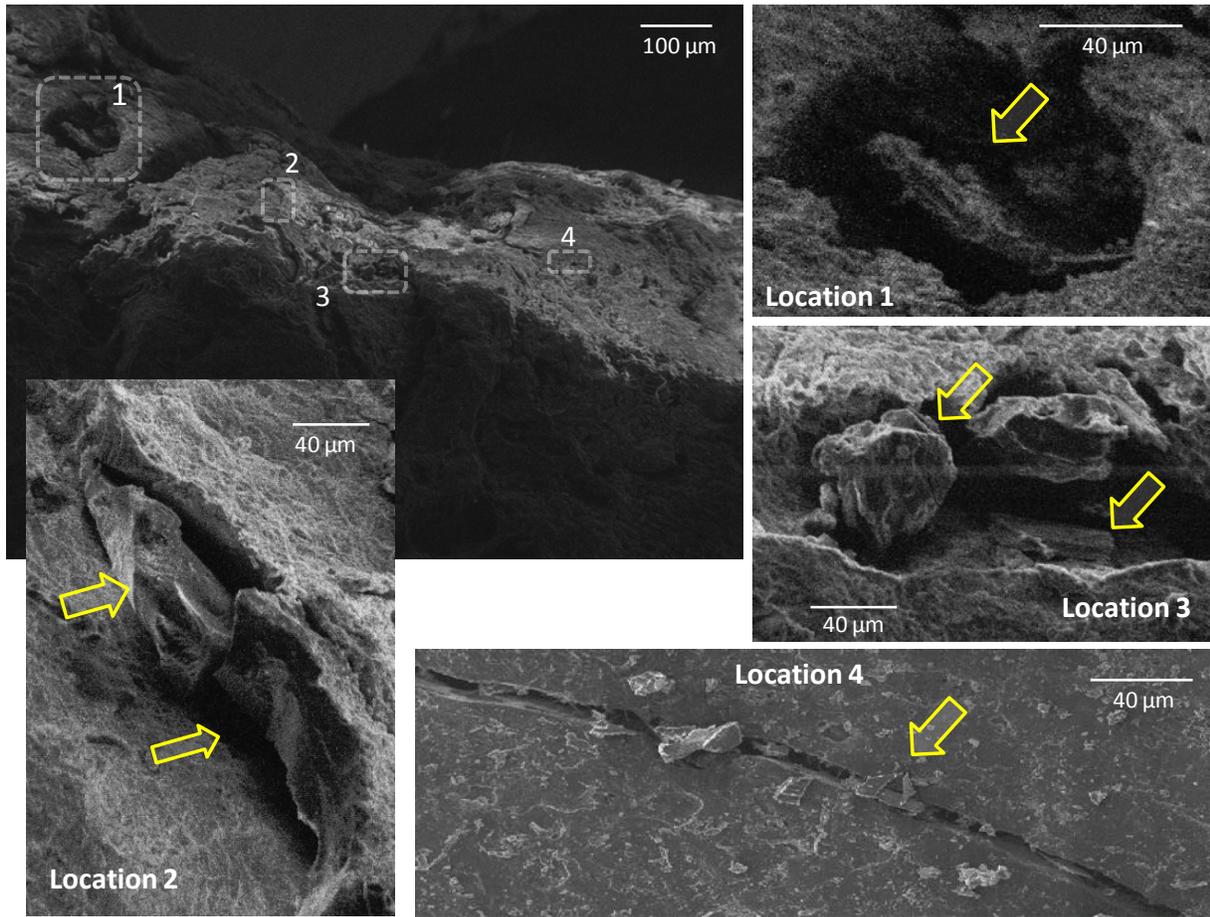


Figure 2. SEM images after 24 hr rinsing of a pine chip:poultry litter biochar (BC# 5). Location 1 illustrates a local collapse in the BC structure (i.e. sink hole) with a liberated BC particle approximately 100 microns being formed. Location 2 illustrates the expansion of the intrasheet spacing between the graphitic layers resulting in the structural failure (fragment designated by arrow). Location 3 illustrates the preferential erosion by water of the weaker BC layers, leading to the fragmentation of the top layer as support is removed. Location 4 illustrates a developing fracture in the biochar particle. Original biochar is shown in Figure S5. Arrows highlight described features.

Fig. 3

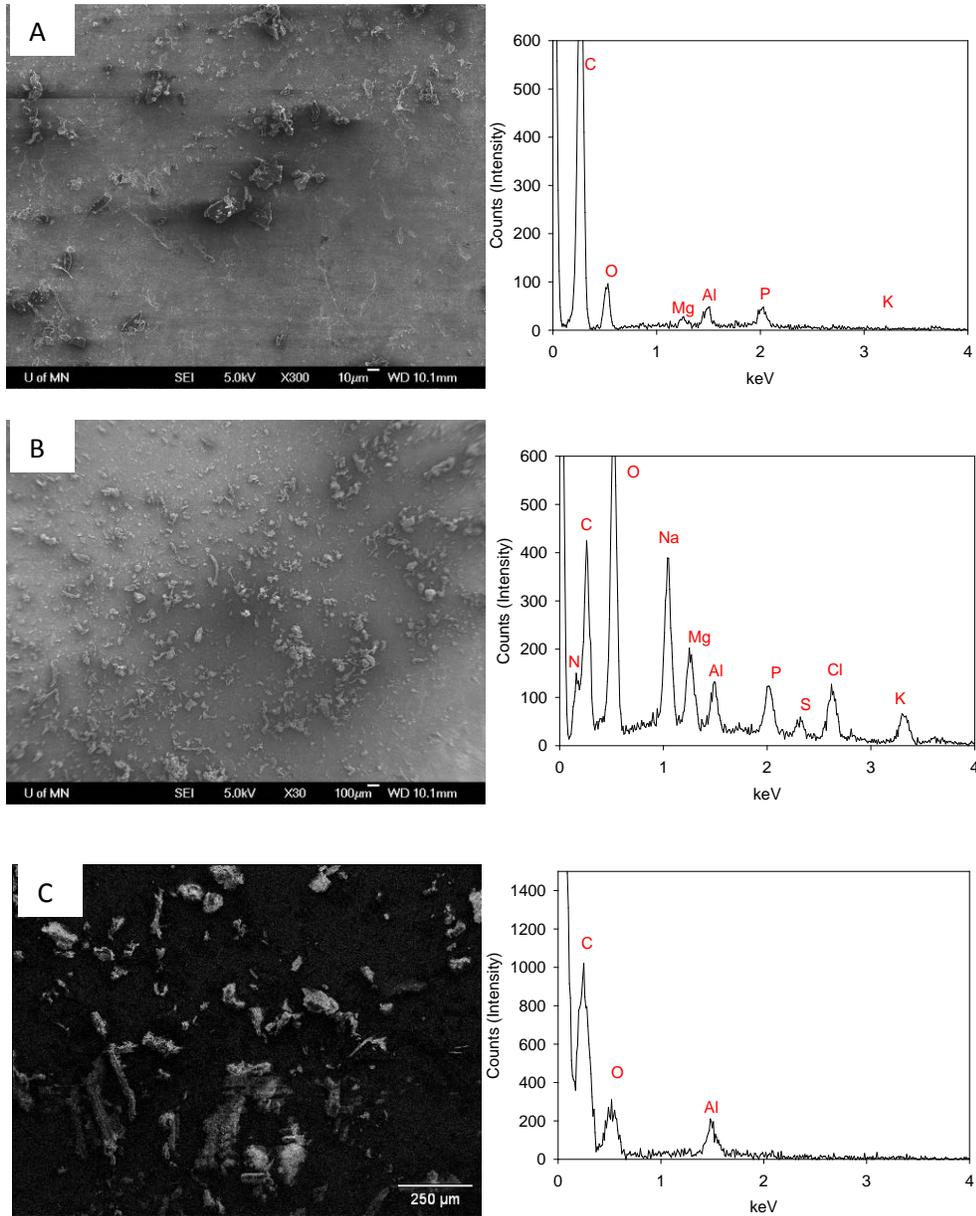


Figure 3. Illustration of observed particles in 100 μ L of rinse water evaporated on a SEM mount for A) hardwood biochar (BC # 7), B) poultry litter biochar (BC# 9), and C) switchgrass biochar (BC# 1). The corresponding spectral scan of the view areas with EDS is shown immediately to the right of each panel. The presence of an Al peak could be due to the SEM mount itself and not conclusive evidence for its presence in the biochar rinse water (Table S1). There is evidence of a peak for carbon, but its exact amount cannot accurately be determined from this analysis.

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Appendix B

Lim, T. J., K. A. Spokas, G. Feyereisen and J. M. Novak (2016). "Predicting the impact of biochar additions on soil hydraulic properties." Chemosphere **142**: 136-144.

Predicting the Impact of Biochar Additions on Soil Hydraulic Properties

Lim, T.J.¹, Spokas, K.A.^{2,3*}, Feyereisen, G.^{2,3}, and Novak, J.M.⁴

- 1- Rural Development Administration, Horticultural & Herbal Crop Environment Division, Suwon, South Korea.
- 2- USDA-ARS, Soil and Water Management Unit, St. Paul, MN
- 3- University of Minnesota, Department of Soil, Water and Climate, St. Paul, MN
- 4- USDA-ARS, Coastal Plain Soil, Water and Plant Conservation Research Center, Florence, SC

* - Corresponding author (kurt.spokas@ars.usda.gov)

ABSTRACT

Different physical and chemical properties of biochar, which is made out of a variety of biomass materials, can impact water movement through amended soil. The objective of this research was to develop a decision support tool predicting the impact of biochar additions on soil saturated hydraulic conductivity (K_{sat}). Four different kinds of biochar were added to four different textured soils (coarse sand, fine sand, loam, and clay texture) to assess these effects at the rates of 0, 1, 2, and 5 % (w/w). The K_{sat} of the biochar amended soils were significantly influenced by the rate and type of biochar, as well as the original particle size of soil. The K_{sat} decreased when biochar was added to coarse and fine sands. Biochar with larger particles sizes (60%; >1 mm) decreased K_{sat} to a larger degree than the smaller particle size biochar (60%; <1 mm) in the two sandy textured soils. Increasing tortuosity in the biochar amended sandy soil could explain this behavior. On the other hand, for the clay loam 1% and 2% biochar additions universally increased the K_{sat} with higher biochar amounts providing no further alterations. The developed model utilizes soil texture pedotransfer functions for predicting agricultural soil K_{sat} as a function of soil texture. The model accurately predicted the direction of the K_{sat} influence, even though the exact magnitude still requires further refinement. This represents the first step to a unified theory behind the impact of biochar additions on soil saturated conductivity.

Key words: Biochar, Saturated hydraulic conductivity, soil texture.

1. INTRODUCTION

The saturated hydraulic conductivity (K_{sat}) of soil is a function of soil texture, soil particle packing, clay content, organic matter content, soil aggregation, bioturbation, shrink-swelling, and overall soil structure (Hillel, 1998; Moutier et al., 2000; West et al., 2008). The K_{sat} is one of the main physical properties that aids in predicting complex water movement and retention pathways through the soil profile (Keller et al., 2012; Quin et al., 2014), and it is also widely used as a metric of soil physical quality (Reynolds et al., 2000). Sandy soils provide high K_{sat} values, which leads to rapid water infiltration and drainage (Abel et al., 2013; Bieglow et al.,

2004). This fast infiltration is advantageous for reducing run-off and field storm event flooding, but it is also an environmental risk since rapid infiltration rates decrease the time and opportunities for attenuation of dissolved nutrients and agrochemicals before reaching groundwater resources (Li et al., 2013). Conversely, clay-rich soils need to be remediated to improve water drainage/infiltration for enhanced crop productivity (Anikwe, 2000; Benson and Trast, 1995). Since the dawn of agriculture, we have been using crop residues/organic amendments to accomplish these hydraulic improvements; however, since organic additions are typically mineralized, the achieved benefits are of finite duration (i.e., Schneider et al., 2009). However, biochar provides the opportunity for a material that is more resistant to microbial mineralization than biomass (Zimmerman, 2010).

The impact of biochar on the soil hydraulic properties is a complex interaction of soil and biochar physical properties. Several studies have reported that the incorporation of biochar to soil increased the K_{sat} (Herath et al., 2013; Moutier et al., 2000; Oguntunde et al., 2008), but other studies have observed decreased K_{sat} following biochar additions (Brockhoff et al., 2010; Githinji et al., 2014; Uzoma et al., 2011b). The effect of different biomass sources and the particle size of biochar and soil additions have not been exhaustively studied, despite the fact that hydraulic impacts have been known to be soil texture dependent (Tryon, 1948).

A variety of agronomic effects of soil biochar additions on crop yields have been shown in many studies (Chan et al., 2007; Feng et al., 2014; Glaser et al., 2002; Steiner et al., 2007). Even though the exact mechanism is not fully known, the improvement of crop productivity have been attributed to the increase in soil available nutrients (Asai et al. 2009; Uzoma et al., 2011a) and enhanced soil physical properties (e.g., decrease in soil bulk density, increase in water holding capacity) after the incorporation of biochar (Brockhoff et al., 2010; Akhtar et al., 2014). However, despite the critical importance of saturated hydraulic conductivity to agricultural soil water dynamics, there are a limited number of studies addressing the direct impacts of biochar on K_{sat} effects (Asai et al., 2009; Atkinson et al., 2010; Laird et al., 2010; Kameyama et al., 2012a). These studies have observed differing impacts from no effect, increases and decreases with no conclusive guidelines for improving soil hydraulic properties with biochar additions; primarily resulting in the same conclusions since the 1950's where the impact depends on soil and biochar properties (Tryon, 1948).

The objectives of this research were to (1) evaluate the K_{sat} when wood or plant based biochar is added to four different soil texture classes (coarse sand, fine sand, loam, and clay) and (2) develop a prediction tool to aid in forecasting biochar impacts on the biochar amended soil K_{sat} value.

2.0 MATERIALS AND METHODS

2.1 Soils

Soils that were evaluated here were based on overall soil textures: coarse sand, fine sand, silt loam, and a clay loam texture soil. The silt loam was collected from the 0-5 cm depth

interval from the University of Minnesota's Research and Outreach Station in Rosemount, MN (44°45' N, 93°04' W) from a Waukegan silt loam (Fine-silty sandy-skeletal, mixed, superactive, mesic Typic Hapludoll) and the Webster clay loam (Fine loamy, mixed, superactive, mesic Typic Endoaquoll) was collected from the 0-5 cm interval from a poorly drained site at the University of Minnesota Southern Research and Outreach Center in Waseca, MN (44°04' N, 93°31' W). The two sands were commercial mixes of a high purity washed and kiln dried silica sand (Quikrete Companies, Atlanta, GA USA). A coarse and fine sand were selected to span different particle sizes. All soils were air-dried, sieved to < 2 mm, and stored at room temperature before use.

Particle size distribution of the soils was determined by manual dry sieving of a 150 g subsample of soil. There were five different sized sieves used arranged in decreasing sizes from 2.0, 1.0, 0.5, 0.1, and 0.05 mm. Dry sieving was used with 20 minute agitation. The mass of soil retained on each sieve was measured to generate the cumulative particle size distribution.

2.2 Biochars

The four biochars used for experiments were selected primarily due to the different particle sizes that existed in these biochars (Figure 1, Table 1). These biochars were derived from the following feedstock materials: Hardwood wood pellets (*Quercus robur*; PelletKing Amherst, NH USA), pine wood chips (50:50; *Pinus ponderosa* & *Pinus banksiana*; KD Landscape Supply & Recycling, Medina, MN USA), hardwood chip (~33:33:34; *Quercus robur*; *Acer saccharum*; *Fraxinus Americana*; KD Landscape Supply & Recycling, Medina, MN USA), and oat hulls (*Avena sativa*; General Mills, Fridley, MN USA). A programmable furnace equipped with a retort (model #5116HR; Lindberg, Watertown, WI), an inert atmosphere (N₂; 4 L min⁻¹) during heating and cooling, and a final temperature of 500 °C with a 4 hr hold time was used to produce biochar. Proximate and ultimate analysis data are also shown for these biochars which were conducted according to ASTM D3172 and D3176, respectively (Hazen Research; Golden, CO USA) (Table 1). For this study, we did not grind or further process the biochar due to the potential chemical alteration of the biochar surface with grinding (e.g., Solomon and Mains, 1977).

Particle size distribution of biochar was determined by manual dry sieving of a 150 g subsample of homogenized biochar. There were seven different sized sieves used arranged in decreasing sizes from 8.0, 4.0, 2.0, 1.0, 0.5, 0.1, and 0.05 mm. Dry sieving was used with 20 minute agitation. The mass of biochar retained on each sieve was measured to generate the cumulative particle size distribution.

2.3 Preparation of columns

The four different biochars were each combined at 1, 2, and 5% by weight with four different soils (coarse-, fine-, loam, and clay) and thoroughly mixed to provide a homogeneous mixture. To determine the hydraulic conductivity, the soil, biochar, or soil mixtures were gently repacked into a soil column (polyvinylchloride; 6 cm diameter x 20 cm high) to approximately a 5 cm height with light tamping and vibration of the column to eliminate any gaps and voids during

packing. The targeted density was 1.2 g cm^{-3} . Four independent replicates of each potential soil treatment were implemented.

2.4 Saturated hydraulic conductivity

Saturated hydraulic conductivity (K_{sat}) was measured using a falling head method (Klute and Dirksen, 1986). A piece of filter paper was placed on the soil surface to minimize soil disturbance when filling with water. Tap water was gently poured into column until it was full (20 cm height of column) and hydraulic testing was performed after steady flow conditions were attained, usually after 3-4 repetitive flushing of the entire column. The average drop in hydraulic head over a known time period was used to calculate the K_{sat} value for each sample by the following equation (Klute and Dirksen, 1986):

$$k = \frac{L}{t} \ln \left(\frac{h_o}{h_f} \right),$$

where L is the length of the soil sample (5 cm), t is the time period (sec), h_o is the initial height of water in the column referenced to the soil column outflow (cm), and h_f is the final height of water also referenced to the soil outflow (cm). Since the diameters of the column and water column were equivalent these factors cancelled out from the equation.

2.5 Bulk density

The bulk density of each individual column was determined by dividing the known mass of the oven dried sample added to the columns by the measured sample volume. This soil volume measurement occurred immediately after the hydraulic conductivity assessments.

2.6 Statistical analysis

Averages and standard deviations of the quadruplicates were calculated. The statistical interactions between biochar type, biochar amendment rates, and soil type were evaluated by a 3-way analysis of variance (ANOVA). Fisher protected least significant differences were used to compare treatment means at the 95% ($p=0.05$) significance level.

3.0 RESULTS AND DISCUSSION

3.1 Particle size distributions

Images of the four biochars are shown in Figure 1, and the corresponding particle size distributions of the soil and biochars are in Figure 2. Hardwood chip biochar possessed the largest particle size fraction with >88% of the total particles being >1 mm and then pine chip was next with 44% of particles >1 mm (Figure 2). The oat husk (44%; < 0.5 mm) and wood pellet (57%; < 0.5mm) biochars possessed smaller particle sizes (Table 1; Figure 2). This data suggests that the particle size of the biochar can be controlled by pre- and post-treatment of the biomass or biochar, with one example of this being larger wood chip sizes (Figure 1). These observations

support the possibility of developing specific particle sizes for targeted hydraulic improvements. However, biochar particle size is not a static property, as the particle themselves can physically disintegrate (Spokas et al., 2014).

3.2 Bulk density

The soil type, amendment rate of biochar, and biochar additions had a statistically significant influence on the soil bulk density after application ($P < 0.05$; Table 2). The incorporation of biochar lowered the bulk density by increasing total soil pore volume (Jones et al., 2010; Oguntunde et al., 2008). This decrease in bulk density following biochar incorporation has also been observed in other studies (e.g., Mukherjee et al., 2014; Pathan et al., 2003; Laird et al., 2010) and is expected due to the lower particle density of the biochar materials compared to soils (Laird et al., 2010; Brewer et al., 2014; Rogovska et al., 2014). Interestingly, the difference between the weighted averaged of the two materials and the measured bulk density was the largest for the clay textured soil, with ranges from 14-20% lower bulk densities (Table S1). For the sandy texture soils, the differences were not as large, ranging from 1-16% and the differences for the loam textured soil were even further reduced (-2 to 6 %; Table S1). This suggests that biochar does alter the packing of soil particles, thereby creating additional external soil porosity.

The hardwood chip biochar (the largest particle size) resulted in the lowest bulk density among the biochars (Table 2). However, this was expected since it also contained the lowest bulk density of the biochar evaluated here (0.32 g cm^{-3} ; Table 1). In other words, for the equivalent mass addition, the lower bulk density results in a higher total volume being added to the soil. The hardwood chip biochar at 1% and 5% additions reduced the bulk density by 4 and 20% in coarse sand, respectively (Table 2). In the fine sand, a similar decrease was observed, with the 1% and 5% biochar lowering the bulk density by 4 and 20%. The reductions were greater in the clay loam soil, with decreases observed of 18 and 26%, respectively (Table 2). The soil type that was impacted the least by the range of biochar additions was the loam textured soil. We attribute this lack of alteration in the bulk density to the diversity of soil particle sizes already present in the soil providing buffering to these particle size additions (Figure 2).

3.3 Hydraulic conductivity

The K_{sat} of the amended soils was significantly influenced by particle size and rates of biochar application, as well as the particle size of soil (Table 2). The K_{sat} of the un-amended coarse sand, fine sand, loam, and clay textured soil was 248.9, 107.7, 30.8, and 10.3 mm h^{-1} , respectively (Figure 3). Particle size distribution strongly controls the resulting pore geometry and thereby the K_{sat} ($P < 0.001$), as already noted (Vereecken, 1995).

Figure 3 illustrates the K_{sat} values of the soils and biochar materials when sieved to a particular size class. The significant observation is that similar sized materials have the same K_{sat} when examined by particle size divisions, which is similar to the impact of soil particles of differing mineralogy (McKeague et al., 1982). This strongly suggests that the impact of biochar

additions on K_{sat} can be modeled as a particle size effect.

Soil amendment with biochar possessing a larger particles sizes (60%; >1 mm) had a more significant impact on decreasing K_{sat} than the smaller particle size biochar (60%; <1 mm) (Figure 3 and 4). For example, application of 5% wood pellet decreased K_{sat} 53% in coarse sand and 75% in fine sand, whereas the application 5% of hardwood chip biochar reduced K_{sat} by 96 and 86 % in the coarse and fine sand, respectively (Fig. 3 and 4). In addition, the increase in the application rates of biochar sharply decreased the K_{sat} in coarse sand, manifesting the highest absolute drop in the K_{sat} observed in this experiment (Table 2). This drop in K_{sat} can be advantageous in sandy textured soils, since the plant roots would be in contact with the infiltration front for a longer duration. This could lead to higher biomass yields due to the reduced infiltration rates.

For instance, K_{sat} values acquired by incorporation of 1, 2, and 5% hardwood chip decreased the K_{sat} to 68.8, 31.9, and 10.5 mm h⁻¹ from 249 mm h⁻¹ in coarse sand and 69.1, 55.8, and 15.4 mm h⁻¹ from 108 mm h⁻¹ in fine sand, respectively. For a 50 cm thick root zone, this would equate to a difference of 2 days for the coarse sand 5% hardwood chip compared to the control soil for movement of the infiltration front. These results are in agreement with earlier studies that also confirmed that K_{sat} in sandy soils typically decreased after biochar addition (Brockhoff et al., 2010; Pathan et al., 2003), particular with biochar of small particle sizes (<1 mm). This dependency on amendment particle size has also been observed for zeolite (Huang and Petrovic, 1994) and gypsum (Keren et al., 1980) additions to soils.

There has been research into the macro- and micro-porosity of biochar (e.g., Yu et al., 2006; Joseph et al., 2010; Kinney et al., 2012), since the overall assumption has been that biochar will lead to an improved water holding capacity due to the numerous micro- and nano-scale pores that are observed within the biochar particles (Atkinson et al., 2010). From soil capillary forces, a given height of water rise in a capillary column can be related to the pore radius by the following equation:

$$h = \frac{2 \gamma \cos(\theta_{contact})}{g r (\rho_{water})}$$

where h is the height of rise in the capillary column (pore) (m), γ is the surface tension of water [@ 25°C = 71.97 kg sec⁻²], $\theta_{contact}$ is the contact angle (assumed = 0° rad), g is the acceleration due to gravity (9.8 m sec⁻²), ρ_{water} is the density of water (999.97 kg m⁻³), and r is the radius of the pore (m). Therefore, the largest pore that will be holding water at a soil moisture potential of -1500 kPa (~150 m water column) is 0.2 μm (Gardner et al., 1999). In other words, soil pores <0.2 μm are not of agronomic significance, since this soil moisture will not be plant available as well as not significantly to saturated soil water flow. The biochar particles would effectively behave as a solid particle and their resulting impact on K_{sat} would be soil texture and biochar particle size dependent (Figure 2). However, for clay loam soils, 1% and 2% (w/w) biochar additions increased K_{sat} , with 5% of biochar addition providing no further increases or decreases

(Figure 4). In the clay textured soil, the incorporation of small amounts of biochar (with particle sizes larger than 1 mm) increased K_{sat} , which is contrary to the impact observed in the coarser textured soils.

Soil pores larger than 30 μm will increase water holding capacity from saturated ($\Psi=0$ kPa) to gravity drained (field capacity) conditions ($\Psi=-33$ to 100 kPa), but this water quickly drains and typically is not counted as part of the plant available water (Hillel, 1998). Herath et al. (2013) reported the biochar particles (>0.5 mm) were associated with the increase of macroporosity in soil. Therefore, biochar additions do alter the saturated conductivity, but these alterations are largely due to particle packing differences (tortuosity) and not due to the internal porosity of the biochar. These differences in particle packing may (Novak et al., 2012) or may not (Chang et al., 1977) change the total soil moisture holding capacity. For coarse textured soils, small particle sized amendments (e.g., wood ash, zeolites, diatomaceous earth) have typically improved overall water holding capacity of the soil, but typically do not alter the agronomic plant available water (Bigelow et al., 2004), which is the moisture held between field capacity and the wilting point. On the other hand, organic material addition (i.e. peat, compost) typically do lead to improved plant available water due to the larger particle sizes and added hydrophilic surfaces (Aggelides and Londra, 2000).

Despite the lack of uniform alteration in the net water holding capacity from biochar additions, the differences in saturated hydraulic conductivity could impact the overall field water balance between infiltration, evaporation, and run-off. In addition, the differences in infiltration rate of biochar amended soils could change with time (Novak et al., 2015). This data also suggests that the critical factor for K_{sat} improvement is particle size versus hydrophobicity or biochar's intra-porosity (e.g., Jeffery et al., 2015).

3.4 Model development

An initial tool developed in Microsoft ExcelTM was used to calculate the impacts of biochar additions on K_{sat} . Barnes et al (2014) utilized the d_{50} of biochar addition to attempt to predict K_{sat} of the mixtures. However, this method was not successful due to the impact of biochar on soil particle packing and bulk density (Table S1). Based on the lessons learned in that study, we decided to use a simplified model for the biochar: either it was a large (>1 mm) or small (<1 mm) particle size amendment. Despite the fact that this technique is not the traditional sand particle size boundaries, this might account for some of the physical disintegration potential of the biochar as well (Parr and Mitchell, 1930; Naisse et al., 2014; Spokas et al., 2014).

The biochar addition was assumed to impact a particular particle size fraction: sand or clay. The reason for this separation was the fact that the soil pedotransfer functions (PTF) utilized were based on the clay and sand size fractions (Table S1). Overall, these particular models were selected since they included the two textural classes and have been shown to be good estimators for overall soil K_{sat} prediction (Ferrer Julià et al., 2004), even though the specific

accuracy can be questioned (Duan et al., 2011). The spreadsheet averaged results from these 4 different PTFs to arrive at the estimation of the K_{sat} for the biochar amended sample. Since this was the first attempt at a universal tool for hydraulic impacts from biochar application, we focused initially on predicting the direction and order of magnitude impacts on the saturated hydraulic conductivity as a function of the biochar addition. This tool was validated using the data collected in this experiment, as well as other existing literature studies on the impact of biochar additions on K_{sat} (Table 3).

From this model, we see that the complex interactions of the biochar particle size and soil texture were predicted from this tool (Table 3). This model correctly predicted for the sandy textured soils a decrease in K_{sat} due to the obstructions in the soil matrix from the biochar particles, increasing the tortuosity of the soil (Kameyama et al., 2012a). These decreases in K_{sat} occur even though one might expect the lower bulk density to result in higher K_{sat} values. The impact of biochar on K_{sat} can be solely predicted from the size classification of biochar particles, versus the d_{50} and bulk density attempted previously (Barnes et al., 2014). Biochar particles are also subject to physical fragmentation (Spokas et al., 2014), which could clog conductive pores in the soil matrix (Reddi et al., 2005; Dikinya et al., 2008).

For loam soils, which already have a diverse and well balanced particle size distribution, a 1-5% biochar addition will not significantly alter the hydraulic conductivity (Table 3). Therefore, this results in biochar additions having minimal alteration on hydraulic properties for loam textured soils. These trivial impacts have already been documented in the published studies (Table 3). As seen in the modeling (Figure S1) and substantiated by the existing studies with high amendment rates (Ghodrati et al., 1995), extremely high amendment rates would be needed to alter loamy textured soils (Shelley and Daniel, 1993).

This model represents the first tool for predicting biochar use for soil hydraulic alteration projects. The model predicts the direction of saturated hydraulic conductivity alterations following biochar additions for a particular soil texture. Despite not always matching the absolute magnitude of the hydraulic conductivity (Table 3), this model presents a means of justifying biochar use to remediate hydraulic deficiencies. This model permits the forecasting of whether the biochar addition will increase or decrease the K_{sat} as a function of the biochar particle size and the original soil texture, thereby demystifying this physical interaction.

4.0 CONCLUSIONS

Saturated hydraulic conductivity (K_{sat}) is influenced by the particle size distribution of biochar, the application rate, and the original soil textures. In coarse and fine sand, the increase of biochar application rates decreased the K_{sat} value showing larger particles sizes (60%; >2 mm) had a more significant impact on decreasing K_{sat} . The incorporation of biochar in the poorly drained clay based soil conversely increased the K_{sat} value. These effects are a function of the original soil texture and the biochar particle size distribution, which was accurately predicted with a simple soil texture based PTF model. This model universally applies to all biochars,

despite differences in surface chemistry and porosity, if the particle size of the biochar and soil are known. We envision that this tool begins to answer the engineering questions of how much biochar would need to be added to ameliorate water movement for both well drained sandy soils and poorly drained clay rich soils. However, further research is needed to understand the duration of these effects, particularly with the friable nature of biochar particles.

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Table 1. Chemical and physical properties of the four different biochars.

Biochar	ρ_{BD}	d_{50}	C	N	O	H	S	Ash	% Moisture (air dried)
	(g/cm ³)	(mm)							
Wood pellet	0.50	0.7	77.6	0.4	11.3	3	<0.1	7.7	4.8
Pine chip	0.54	3.8	64.3	3.1	6.2	1.2	<0.1	25.2	11.3
Hardwood chip	0.32	1.6	71	0.2	22	4	0.1	2.7	6.7
Oat husk	0.34	2.1	32	2.5	43	8	<0.1	14.5	55.4

Table 2. The change of bulk density and saturated hydraulic conductivity (K_{sat}) after four rates of different biochar were added to coarse sand, fine sand, and clay soil.

Soil Texture	Biochar Addition	Incorporation Rate ($w w^{-1}$)	Bulk Density ($g cm^{-3}$)	K_{sat} ($mm h^{-1}$)	
Coarse sand	Control	0	1.67 (0.02)	248.9 (19.4)	
		1	1.64 (0.04)	193.7 (12.2)	
		2	1.58 (0.05)	156.9 (11.9)	
	Wood pellet	5	1.47 (0.04)	117.7 (18.2)	
		Pine chip	1	1.61 (0.02)	109.8 (5.1)
			2	1.53 (0.04)	70.9 (4.3)
	5		1.34 (0.03)	35.9 (4.0)	
	Hardwood chip	1	1.59 (0.02)	68.8 (5.1)	
		2	1.53 (0.04)	31.9 (4.2)	
		5	1.36 (0.06)	10.5 (0.9)	
	Oat husk	1	1.60 (0.02)	112.3 (8.3)	
		2	1.53 (0.03)	45.1 (3.2)	
		5	1.35 (0.04)	30.2 (2.9)	
	Fine sand	Control	0	1.63 (0.05)	107.7 (9.8)
		Wood pellet	1	1.60 (0.03)	86.9 (1.9)
2			1.57 (0.02)	65.5 (5.4)	
5			1.40 (0.04)	26.6 (1.2)	
Pine chip		1	1.56 (0.06)	77.7 (0.8)	
		2	1.45 (0.03)	63.9 (0.9)	
		5	1.22 (0.04)	28.5 (1.4)	
Hardwood chip		1	1.56 (0.05)	69.1 (0.9)	

		2	1.49 (0.06)	55.8 (1.0)
		5	1.31 (0.04)	15.4 (0.3)
	Oat husk	1	1.57 (0.03)	64.2 (0.8)
		2	1.49 (0.04)	52.6 (1.2)
		5	1.34 (0.05)	34.2 (5.1)
Clay	Control	0	1.36 (0.06)	10.3 (0.9)
	Wood pellet	1	1.16 (0.04)	16.5 (1.0)
		2	1.13 (0.02)	18.5 (1.0)
		5	1.08 (0.05)	18.2 (0.9)
	Pine chip	1	1.11 (0.02)	17.6 (0.8)
		2	1.05 (0.04)	18.9 (1.3)
		5	1.00 (0.03)	13.2 (2.8)
	Hardwood chip	1	1.13 (0.05)	14.4 (0.3)
		2	1.06 (0.06)	18.5 (0.3)
		5	0.98 (0.04)	10.2 (2.2)
	Oat husk	1	1.15 (0.04)	18.5 (0.4)
		2	1.11 (0.02)	19.9 (0.5)
		5	1.05 (0.05)	20.2 (3.5)

Table 2. (Continued)

Soil Texture	Biochar Addition	Incorporation Rate ($w w^{-1}$)	Bulk Density ($g cm^{-3}$)	K_{sat} ($mm h^{-1}$)
Loam	Control	0	1.15 (0.02)	30.8 (2.1)

Wood pellet	1	-	-
	2	1.16 (0.02)	29.8 (3.2)
	5	1.10 (0.04)	28.4 (1.9)
Pine chip	1	-	-
	2	1.14 (0.02)	30.2 (1.8)
	5	1.12 (0.02)	31.3 (2.5)
Hardwood chip	1	-	-
	2	1.16 (0.03)	28.1 (2.9)
	5	1.12 (0.03)	27.8 (2.8)
Oat husk	1	-	-
	2	1.12 (0.03)	31.1 (2.3)
	5	1.18 (0.04)	29.7 (3.9)

Source of variation

Particle size (S)	***	***
Biochar (B)	ns †	***
Incorporation Rate (R)	** *	***
S × B	ns	***
S × R	** *	***
B × R	ns	ns

** and *** represent significant at 1% and 0.1% probability levels, respectively.

† ns represent non significant.

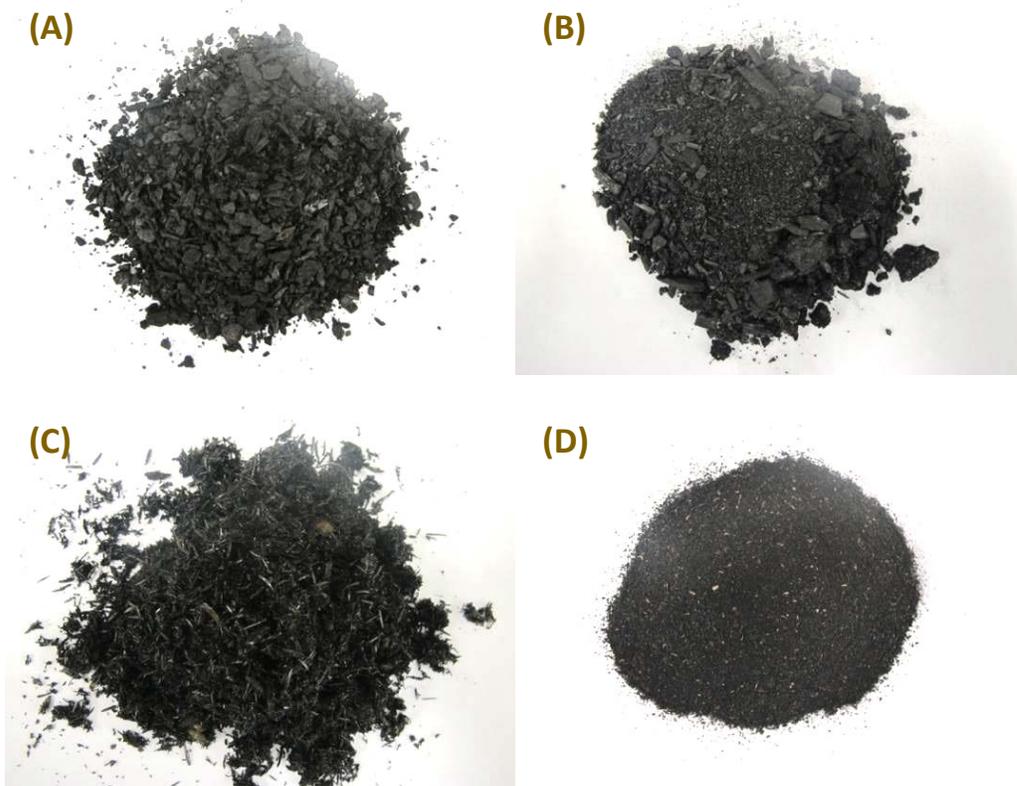
Table 3. Comparison of literature results with model results.

Author & Year	Soil Texture (%)			Biochar Particle Size (mm)	Application rate of Biochar (w/w)	Reported Results (cm d ⁻¹)	Model Prediction (cm d ⁻¹)			
	Sand	Silt	Clay				0%	1%	3%	5%
(Asai et al., 2009)	18	34	48	< 2	~1, 2, and 3%	<input type="checkbox"/>	7	7	7.5	7.87
	27	45	28	< 2	~1, 2, and 3%	<input type="checkbox"/> 20→36	14.3	14.6	15.3	15.95
(Brockhoff et al., 2010)	99.8	0.1	0.1	NA	0-5%	<input type="checkbox"/> 2035→700	368	344	263	211
(Hardie et al., 2014)	72.8	16.8	10.5	3.84	~5%	<input type="checkbox"/>	156	157	159	161
(Herath et al., 2013)	Silt loam (Typic Fragiaqualf)			1.06	~1%	<input type="checkbox"/> 242→320	21.7	22.2	23.1	24.0
	Silt loam (Typic Hapludand)			1.10	~1%	<input type="checkbox"/> 242→579	21.7	22.2	23.1	24.0
(Laird et al., 2010)	Fine loamy (Typic Hapludolls)			< 0.5	0-2%	<input type="checkbox"/>	12.0	12.1	12.1	12.2
(Lei and Zhang, 2013)	40	35	25	< 2	5%	<input type="checkbox"/>	2.0	2.0	2.2	2.3
(Pathan et al., 2003)	94	2	4	< 0.20	0-10%	<input type="checkbox"/>	222	199	165	140
	96	1	3			<input type="checkbox"/>	257	229	186	156
(Rogovska et al.,	Loam			< 1.0	~0-3%	<input type="checkbox"/>	102	98	91	86

2014)	(Typic Hapludolls)										
(Uzoma et al., 2011)	95	1.3	3.7	< 0.18	~0 to 3%	2822→1888	<input type="checkbox"/>	240	211	177	150
(Ghodrati et al., 1995)	Hammonton loamy sand			<0.10 76-79% silt	30%		<input type="checkbox"/>	76.9	30%=>19.9		

Figure Captions:

Figure 1. Photos of the various biochar used in this experiment: A) Pine chip, B) Hardwood chip, C) Oat husk, and D) Hardwood pellet (sieved to <4 mm).



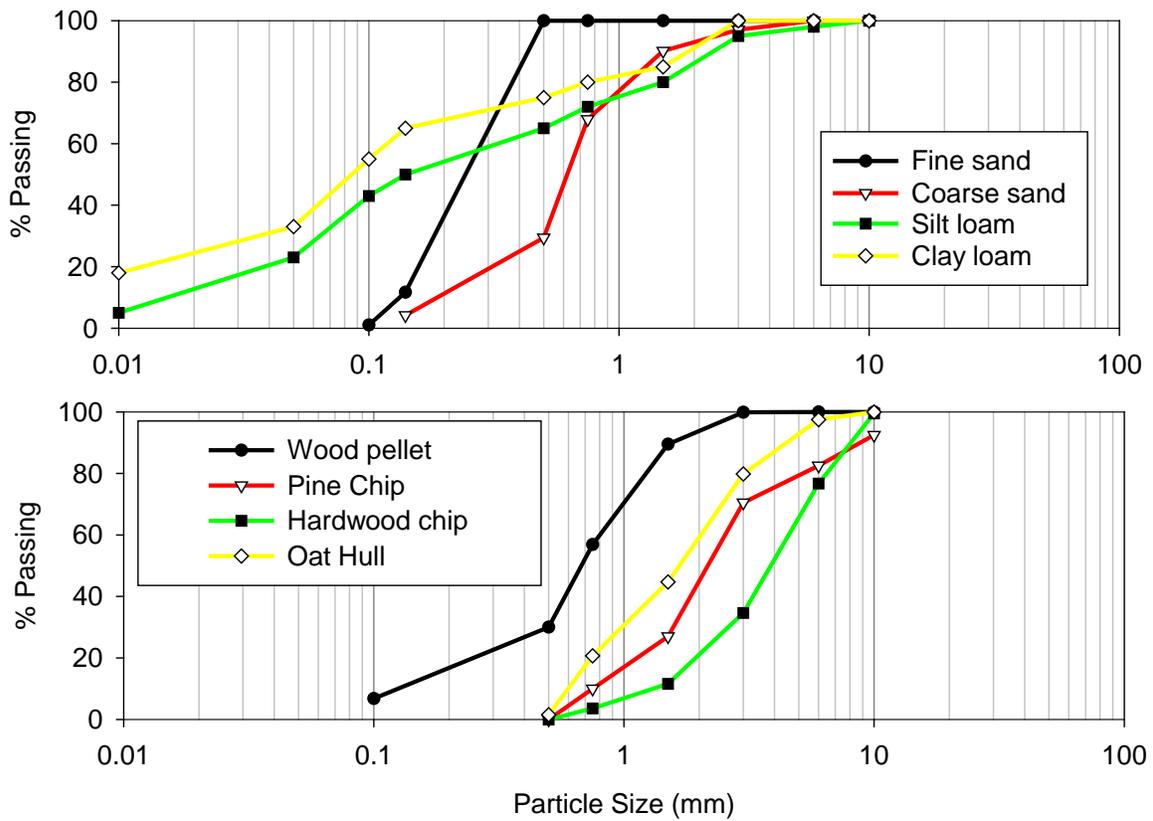


Figure 2. Particle size distribution for (A) the original four soil materials and (B) the four biochars.

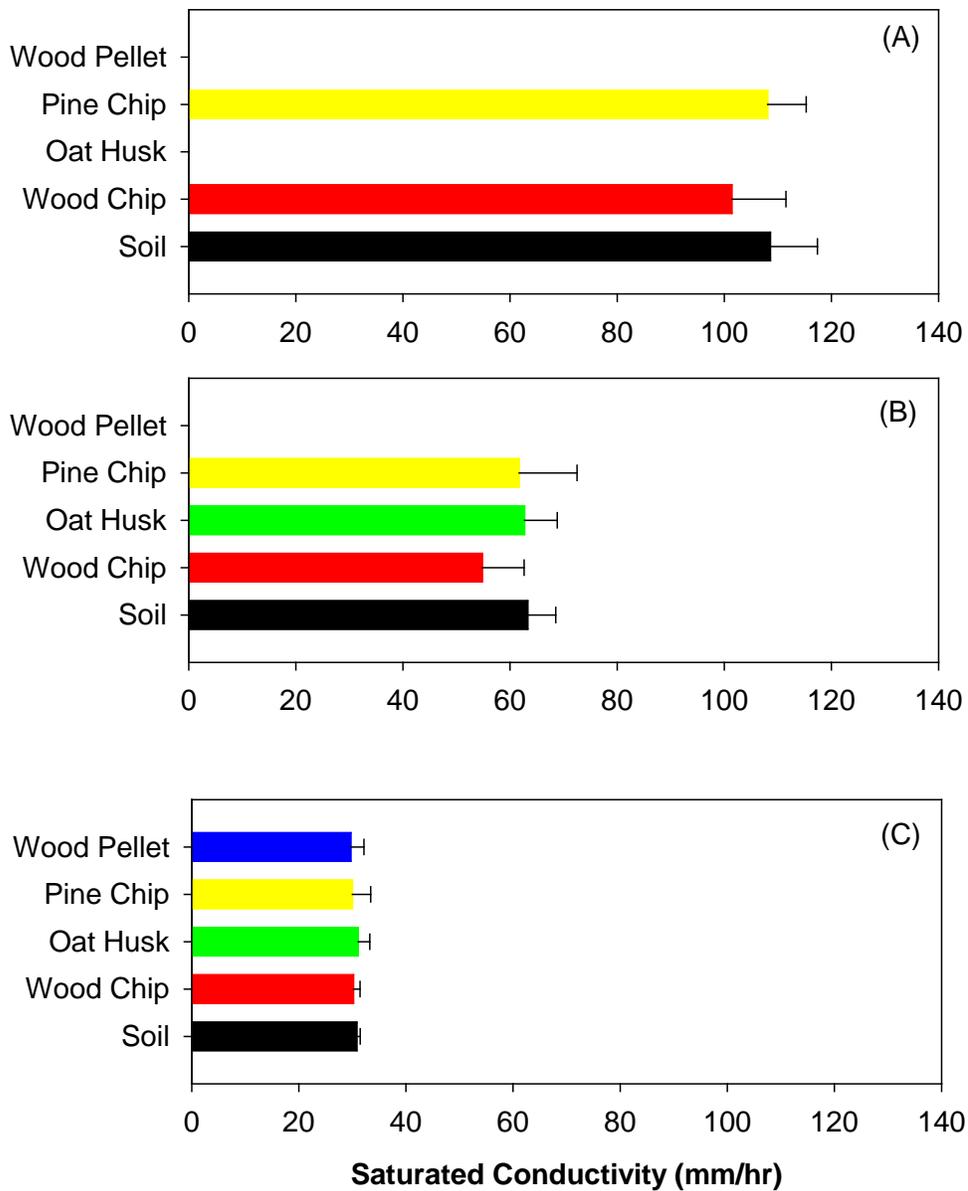
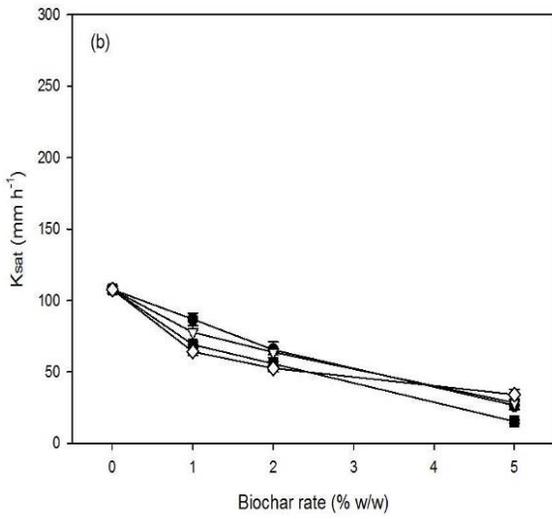
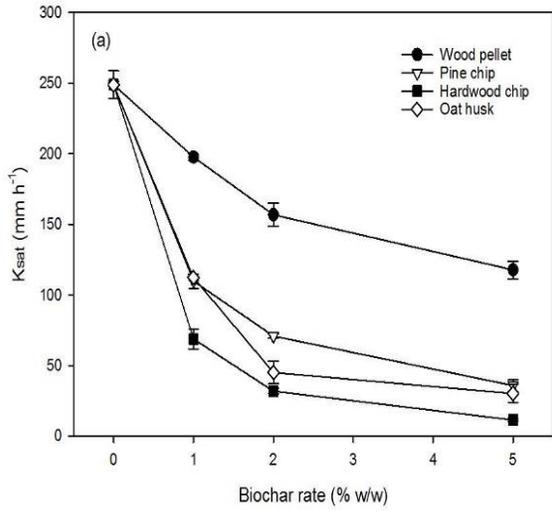


Fig. 3. Comparing saturated conductivity of particle size fractions for the various materials from (A) 7-8 mm, (B) 4-6 mm, and (C) 1-2 mm. Note there are no values for >2 mm for the wood pellet biochar and the 7-8 mm for the oat husk biochar, due to the lack of those particle size

classes in the respective biochar. Note there are no statistically significant differences in the saturated conductivity of each particle size class of these materials.



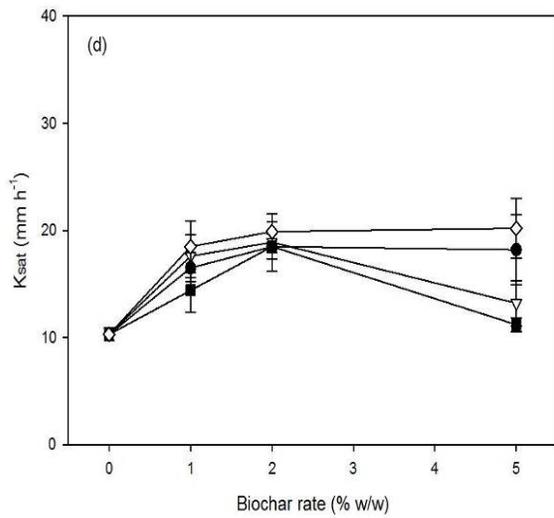
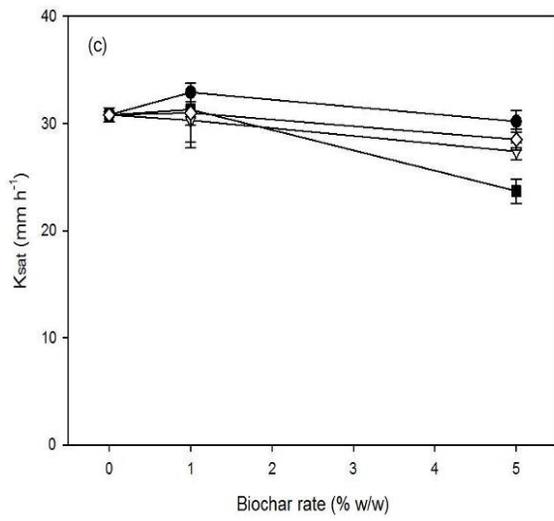


Figure 4. Impact of biochar additions (0, 1, 2, and 5%) of four different biochars on the four different textured soils of (a) Coarse sand, (b) Fine sand, (c) loam-enriched soil, and (d) clay-enriched soil.

Appendix C

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“Biomass or biochar – Which is better at improving soil hydraulic properties?”, by K. Spokas, R. Weis, G. Feyereisen, D. W. Watts, J. M. Novak, T. J. Lim and J.A. Ippolito

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Biomass or biochar – Which is better at improving soil hydraulic properties?

K.A. Spokas, R. Weis, and G. Feyereisen

United States Department of Agriculture
Agriculture

University of Minnesota

Saint Paul, Minnesota USA

D. W. Watts and J. M. Novak

United States Department of

Florence, SC. USA

T. J. Lim

Rural Development Administration
Agriculture

Jeonju-si, Jeollabuk-do 560-500, Republic of Korea

J. A. Ippolito

United States Department of

Kimberly, ID USA

Keywords: Hydraulic conductivity, particle size, pore size, soil amendment

Abstract

When amended to soils, both biochar and biomass impact soil hydraulic properties. However, the exact comparison between these two material forms is not known. The objective of this research was to evaluate and compare the impacts of raw biomass chips with biochar produced from the same feedstock. Both raw biomass (pine chips; *Pinus taeda*) and a corresponding pine chip biochar (slow pyrolysis; 2 hr; 500 °C) were added to a sandy textured Ultisol at three different rates and four incremental particle size fractions (1-2 mm; 0.5 to 1 mm; 0.2 to 0.5 mm; and <0.2 mm).

Results demonstrated that the immediate impact on hydraulic conductivity (K_{sat}) of the amended soil was influenced by application rate and particle size, with remark similarity between the two amendments. All additions significantly reduced both the soil bulk density and K_{sat} ($P<0.05$). These alterations in the hydraulic properties were postulated to be due to the alteration in soil particle packing (i.e., tortuosity). Alterations in pore geometry with blocking of larger macro-pores by the amendment could explain this behavior and was supported by the similar behavior between raw feedstock and biochar of equal particle sizes.

Thus, the immediate alterations in the hydraulic properties of an amended soil were primarily a function of the particle size of the material, regardless whether the raw feedstock had been converted to biochar or not. Both additions with decreasing particle size increased water holding capacity at saturation. This suggests that small particle size additions to a sandy textured soil would reduce infiltration rates and net water gained per precipitation event due to the reduced soil moisture potential gradient and K_{sat} . However, effects are a function of the amendment particle size distribution and the original soil texture.

INTRODUCTION

Soil particle size distribution strongly dictates its capacity to hold soil moisture (Hillel, 1998). Organic wastes have been added to soil since historic times in efforts to improve soil moisture properties (Khaleel *et al.*, 1981). Typically, organic material application results in hydraulic conductivity increases regardless of soil texture. This could be a result of organic materials incorporated bearing a large particle size (> 2 mm), thereby altering the soil particle size distribution (Bose, 2012). An additional mechanism involved in this improvement is increased moisture sorption by the organic material (Gupta *et al.*, 1977). Water sorption on organic surfaces is believed to be controlled by surface functional groups containing oxygen moieties. These O moieties are believed to form hydrophilic domains that allows for H-bonding with water molecules (Novak *et al.*, 2012), which would be aided by amendments with elevated surface areas (Shepherd *et al.*, 2002). In addition, hydraulic improvements can be augmented by soil aggregation processes from the stimulated microbial activity from organic amendments (Shepherd *et al.*, 2002). Together these processes lead to new soil structural packing arrangements, pore geometries, and tortuosity, which alter soil hydraulic properties.

Historically, a chief mechanism for achieving these improvements has been through organic matter amendments. Even though there is relatively rapid mineralization of organic additions (Schneider *et al.*, 2009), this stimulation of microbial activity does result in improved soil properties. For instance, research has confirmed additional benefits in soil physical characteristics with time, such as increasing mean particle size diameter (soil structure), aggregate stability, and increased hydraulic conductivity (Aggelides and Londra, 2000; Schneider *et al.*, 2009). Research on organic materials, such as raw pine chips, have shown that smaller particle sizes increases soil water holding capacity (saturation to wilting point) with a corresponding decrease in total air-filled porosity (Nelson, 2011). In addition to altering soil physical textures, the particle size of soil amendments also influence a variety of processes, such as greenhouse gas production rates (Fangueiro *et al.*, 2012; Sigua *et al.*, 2014; Tejada *et al.*, 2014), bulk density (Zhao *et al.*, 2012), cation exchange capacities (Altland *et al.*, 2014), and pH alterations (Altland *et al.*, 2014).

Biochar has been hypothesized as a material to improve soil moisture characteristics (Novak *et al.*, 2012), while offering longer-term impacts due to the fact that the material is more

resistant to mineralization than the corresponding un-pyrolyzed feedstock (Karhu *et al.*, 2011; Zimmerman *et al.*, 2011). Similar to un-pyrolyzed feedstock, an alteration in soil moisture holding capacity resulting from biochar addition could lead to reduced plant moisture stress (Mulcahy *et al.*, 2013) and have positive implications for plant productivity during periods of water deficit and reduced irrigation water use. Perhaps different from un-pyrolyzed feedstock, the pore water within the biochar is assumed to become available to the soil system during periods of water deficit (Uzoma *et al.*, 2011). Yet, there are no studies that actually confirmed or attempted to simulate this water availability, since this has been based on solely volumetric or gravimetric moisture contents, or differences in limited soil moisture potential assessments (Scott *et al.*, 2014).

Biochar additions have been claimed as an amendment to improve soil water holding and water transport properties (Scott *et al.*, 2014), especially in sandy textured soils. However, raw biomass incorporated into a sandy soil has also been reported to improve moisture capacities (Novak and Watts, 2013). Therefore, to determine which amendment is superior for hydraulic improvements, we evaluated both raw pine chips and pine chip biochar for their impact on soil moisture retention curves and saturated hydraulic conductivity over a range of particle sizes in a sandy loam textured Ultisol.

MATERIALS AND METHODS

Soil

Soil was collected from an agricultural field in the Coastal Plain region of the southeastern US (Florence, SC; Norfolk soil series). This soil is classified as a fine-loamy, kaolinitic, thermic, Typic Kandiodult and has poor water retention characteristics, since it formed in marine sediments (Novak *et al.*, 2012). This soil was air-dried and then sieved (<2 mm) to remove any gravel or plant debris. Overall, the soil had a soil organic carbon content of 0.39%, a pH of 5.9, and a soil texture of 80.7% sand, 16.7% silt, and 2.6% clay (loamy sand; USDA soil textural classification).

Pine Chip Feedstock and Biochar

Pine chips were collected from logging debris located in Cordesville, SC USA. After collection, the pine chips were kept at room temperature and in the air-dried state (10% w/w moisture). To reduce the particle size, collected pine chips were then hammer-milled (PPH1000D; Pellet Pro Davenport, IA, USA) and passed through a Wiley Mill (Thomas Scientific, Sweedesboro, NJ, USA) to achieve <6 mm particle size. A subsample of these flakes was then converted into biochar. Biochar was made using a programmable furnace equipped with a retort (Model 5116HR; Lindberg, Watertown, WI) under an N₂ atmosphere at 500°C for 2 hours.

Pine chip and biochar elemental analysis followed the ultimate and proximal analysis for coal (Hazen Laboratories, Golden, CO USA) following ASTM D-3172 and 3176 standard methods (ASTM, 2006) (Table 1). Finally, the pine chips and biochar were further dry sieved into 4 separate size fractions: 2-1, 1-0.5, 0.5 to 0.25 and < 0.25 mm size classes.

Table 1. Chemical composition of the raw pine chip and pine chip biochar (all values in percentage dry weight basis).

		Proximal				Ultimate				
		Moisture	Ash	Volatil e Matter	Fixed Carbo n	Sulfur	Carbon	Hydroge n	Nitroge n	Oxyge n
Pine chip (loblolly)	Raw feedstock	9.92	4.16	80.88	14.95	0.02	51.88	5.65	0.08	38.21
Pine chip (Loblolly)	Biochar 500 C	4.08	2.61	22.39	75.00	<0.0 2	88.83	3.14	0.45	4.97

Amendment Application

The individual sieved particle size fractions (2-1, 1-0.5, 0.5-0.25, and < 0.25 mm) of both the pine chips and biochar were separately added to the Norfolk soil at the three rates evaluated in this experiment (0, 5 and 10% w/w). The experimental design was a complete randomized experiment, with 3 different factors (2 amendment types x 3 application levels x 4 particle sizes) and conducted with three replications of each combination. The experiments were completed within three months after soil mixing, thus limiting the impacts assessed to purely physical and ignoring longer term microbial aided impacts (Shepherd *et al.*, 2002).

Soil Moisture Potential Curve Determination

The drying portion of the soil moisture potential curve was measured using an automated evaporation ku-pF apparatus (UGT GmbH, Munchenberg, Germany). This instrument allows a maximum of 10 samples to be run concurrently, with the robotic arm switching between the cylinders every 10 minutes. There are two moisture tensiometers that are connect to each cell, and once properly deaired are accurate between 0 to 100 kPa of soil moisture potential. All treatments of the particle size groupings of both biochar and raw pine chip additions were handled in a similar fashion. The soil sample was placed into a cylindrical sample holder and gently tapped to fill the sample ring. After tamping the soil was scraped to be level with the top. Samples were then saturated in a distilled water bath wetting the sample from the bottom (until saturated), tensiometers installed and then placed on the ku-pF instrument. For

several days to weeks of monitoring, the soil water tension readings from the embedded tensiometers and weights of the test cells were recorded at 10 minute intervals for all 8 samples. This monitoring was continued until both tensiometers “popped” ($\psi=-80$ to -100 kPa) in each sample cell. Due to evaporation, each test cell weight change as a function of time along with the measured water tension. Data were processed according to the method outlined in Schindler and Müller (2006). This allowed the calculation of mean water tension values and the corresponding volumetric moisture. Bulk densities were calculated from the weight of the sample materials added to the test cell of known volume (245 cm^3). The data from each individual triplicate was then fitted to calculate the van Genuchten's coefficients (Van Genuchten, 1980), using the interactive *soilwater* function within the **soilphysics** package in R (Silva and Lima, 2015).

Hydraulic Conductivity Measurement

An automated falling head permeameter system was utilized to measure the saturated conductivity on each soil sample (UMS KSAT Benchtop Saturated Hydraulic Conductivity Instrument, Decagon, Pullman, WA). The sample was transferred into the KSAT device again by gently tapping and packing the cylinder until bulk density matched the soil moisture potential curve assessment. The sample was then placed in the apparatus and initially saturated by allowing five pore volumes of water to flow through the sample prior to testing. The K_{sat} was determined through the manufacture's software (KSat; Version 2.1) utilizing a falling head methodology.

Soil Moisture Modeling

A previously validated soil moisture model was used (STM2; Spokas and Forcella, 2009). This model permits a comparison of the annual cycle of soil moisture potential and volumetric moisture utilizing the measured soil hydraulic properties (van Genuchten's coefficients). The model was used in the “advanced mode” and the individual soil moisture properties were entered for the control, pine chip, and biochar at the 10% (w/w) and the <0.25 mm particle size. The average 30-yr climate for Florence, SC USA was used to model the impact of biochar, raw pellet, and the control soil for a typical annual cycle to observe the potential impacts of these additions on the soil moisture profile.

Statistical Treatment of Data

Significance of the biochar treatment was tested by one-way analysis of variance (ANOVA) and Tukeys HSD test (at $P<0.05$) was applied for the differences in mean values.

All the statistical analyses were completed using R (R Core Team, 2014).

RESULTS AND DISCUSSION:

For both amendments, the bulk density decreased when compared to the control (un-amended soil) (Table 2), which suggests alterations in the packing arrangements due to the amendments. For bulk density, the rate of application was the only significant factor; there was no dependency of material type (i.e., pyrolyzed or unpyrolyzed material; $P < 0.05$). Both amendments also increased the volumetric soil moisture capacity at saturation as compared to the control ($P < 0.05$; Table 2). Similar observations have been documented for water retention increasing in the low retention range (< 100 kPa) following organic material additions (Khaleel et al., 1981). Interestingly, the raw pine chips increased saturated soil moisture content to a greater degree than the biochar additions, particularly noticeable at the 10% (w/w) addition. This difference could increase water holding capacity at saturation. This difference could be related to the fact that water molecule sorption is highly dependent on oxygen surface moieties; and oxygen has been lost from the biochar during the pyrolysis (38 to 5% O; Table 1). Thereby, the capacity of biochar to absorb and hold onto water through hydrogen bonding is greatly reduced (Puri *et al.*, 1961). However, biochar still caused an increased in soil moisture content at saturation (Table 2) and thus other phenomena, such as alterations in macro-porosity by particle size distribution and increased tortuosity, are likely responsible for these near saturated water improvements with biochar application. Despite numeric differences, the alteration in the residual moisture was not significant across all treatments (Table 2). The alterations in the α factor was only significant for the 10% pine chip at the 0.25-0.5 and 0.5-1 mm size fractions and 10% biochar at solely the 1-2 mm size fractions. The α factor is related to the air entry value (Tinjum *et al.*, 1997). There was no significant difference in the n parameter (Table 2), which is related to the shape of the soil water characteristic curve.

Hydraulic Conductivity

Despite the reduction in soil bulk density (Table 2), there was also an observed reduction in the hydraulic conductivity for both the pine chip and biochar particles at the 10% (w/w) level (Figure 1A). There was no statistical dependency on the material type (biochar or raw pine chips), therefore the data was pooled to examine the relationships between particle size and addition rate. Both the < 0.25 and $0.25 - 0.50$ mm particle size additions resulted in a reduced hydraulic conductivity across both rates and the larger particle size additions did not alter ($0.5 - 1$ mm) or increased ($1-2$ mm) the K_{sat} (Figure 1B). Similar results were observed with other biochar additions to sandy textured soils (e.g., Brockhoff *et al.*, 2010).

Table 2. Summary of bulk density and the results of the fits of the van Genuchten's parameters.

Addition	Size	Rate	BD (g cm ⁻³)	Θ_R	Θ_S	α kPa ⁻¹	n
Control	--	0	1.57 ^a	0.14 ^a	0.33 ^f	0.15 ^d	2.16 ^{abcd}
Raw Pine Chips	<0.25mm	5	1.33 ^{cde}	0.17 ^a	0.44 ^{abcd}	0.17 ^{cd}	2.65 ^{abc}
	0.25-0.5mm	5	1.36 ^{bc}	0.12 ^a	0.42 ^{bcde}	0.24 ^{bcd}	1.96 ^{abcd}
	0.5-1.0mm	5	1.36 ^{bc}	0.12 ^a	0.38 ^{cdef}	0.18 ^{cd}	1.94 ^{abcd}
	1-2mm	5	1.38 ^{bc}	0.14 ^a	0.35 ^{def}	0.17 ^{cd}	2.05 ^{abcd}
	<0.25mm	10	1.15 ^{def}	0.17 ^a	0.53 ^a	0.21 ^{cd}	2.80 ^a
	0.25-0.5mm	10	1.07 ^f	0.13 ^a	0.50 ^{ab}	0.37 ^a	1.96 ^{abcd}
	0.5-1.0mm	10	1.11 ^f	0.07 ^a	0.44 ^{abcd}	0.33 ^{ab}	1.68 ^d
	1-2mm	10	1.13 ^{ef}	0.08 ^a	0.41 ^{bcdef}	0.25 ^{bcd}	1.68 ^d
Biochar	<0.25mm	5	1.36 ^{cd}	0.11 ^a	0.37 ^{def}	0.16 ^{cd}	1.93 ^{bcd}
	0.25-0.5mm	5	1.35 ^{cd}	0.05 ^a	0.37 ^{cdef}	0.22 ^{bcd}	1.50 ^d
	0.5-1.0mm	5	1.39 ^{abc}	0.15 ^a	0.38 ^{cdef}	0.18 ^{cd}	1.93 ^{bcd}
	1-2mm	5	1.38 ^{bc}	0.16 ^a	0.37 ^{def}	0.16 ^{cd}	1.90 ^{cd}
	<0.25mm	10	1.1 ^f	0.17 ^a	0.47 ^{abc}	0.20 ^{cd}	2.79 ^{ab}
	0.25-0.5mm	10	1.13 ^{ef}	0.13 ^a	0.36 ^{def}	0.24 ^{bcd}	2.02 ^{abcd}
	0.5-1.0mm	10	1.07 ^f	0.12 ^a	0.37 ^{def}	0.24 ^{bcd}	1.70 ^d
	1-2mm	10	1.09 ^f	0.09 ^a	0.41 ^{bcdef}	0.27 ^{abc}	1.77 ^d

Notes: BD – bulk density, the Θ_R , Θ_S , α , and n are the van Genuchten's parameters for residual moisture content, saturated moisture content, inverse of the air entry potential, and parameter related to the pore-size distribution, respectively. Means with the same letters are not significantly different as evaluated through the Tukey's HSD test in the **agricolae** package library in R (Mendiburu, 2014).

Figure 1. Comparing (A) the impact of particle size and (B) amendment application rate on the soil hydraulic conductivity. Due to the similarity in the response of the biochar and raw biomass particles, the results were pooled across material type.

Simulation Modeling:

The impact of raw pine chip and pine chip biochar on an annual cycle (assuming no alteration in the soil moisture potential curve with time; Table 2) was completed for the 30-year average climate for Florence, SC (34.2° N; 79.7° W; annual air temp average = 17.4 °C; 1180 mm total precipitation). The 10% (w/w) of <0.25 mm amendments of biochar and pine chip, along with the un-amended soil (control) were modeled for this typical climate data. Figure 2 illustrates the average rainfall (Fig. 2A) and air temperatures (Fig. 2B) and the corresponding results for the volumetric soil moisture and soil moisture retention curves at 1, 5, 10 and 20 cm depths for the control and amended soils (Figs. 2C & 2D).

Figure 2. Illustration of the (A) predicted precipitation and (B) air temperature for Florence, SC USA which was used as inputs to the STM2 model to simulate a 10% (w/w) <0.25 mm addition of a biochar (BC) and pine chip (PC) amendments to a sandy loam soil (control) for four different depths (1, 5, 10, and 20 cm; labeled in the gray margins of the graphs) illustrating (C) volumetric soil moisture and (D) soil moisture potential (kPa).

Both the biochar and pine chip additions contain a greater amount of soil moisture (Figure 2C). However, this was also accompanied by a lower soil moisture potential (Figure 2D), indicating that this higher moisture is actually less available. This is due to the similar structured pores in both materials: the biochar and pine chip materials (data not shown). Due to the reduced saturated conductivity, when there is a precipitation event, the model predicts a reduced rate of infiltration into the profile, thus reducing the recharge volume of moisture from each precipitation event in the biochar and pine chip amended soil. On the other hand, this reduced hydraulic conductivity translates into more time for the infiltration front being in contact with plant roots. This will be a larger advantage in sandy textured soils, and could explain biochar's improved yields in sandy textured soils (Jeffery *et al.*, 2011). Whether this is of agronomic importance will be a function of the soil hydrodynamics and climate at each individual site.

The major differences between biochar amended and control soils will likely be manifested during periods of drought stress. In other words, the biochar or pine chip amended plot might contain more absolute soil moisture, but it would be held stronger by the amended soil. This is analogous to the soil moisture relationship of different soil texture classes (Hillel, 1998).

CONCLUSIONS

This study investigated the impact of raw versus biochar amendments by particle size on soil hydraulic properties. The data from this study supports the conclusion that the immediate impacts are similar on both materials; however, if the biochar survives for a longer time in the soil system, then a one-time application could lead to longer-term improvements than typically obtained from a one-time organic amendment application (due to mineralization losses). On the other hand, organic amendment applications will likely be required to equal infrequent biochar applications. Regular organic addition will result in a larger microbial stimulation, due to continual increases in degradable organic matter added to the soil.

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Appendix D

Hall, K. E., M. J. Calderon, K. A. Spokas, L. Cox, W. C. Koskinen, J. Novak and K. Cantrell (2014). "Phenolic Acid Sorption to Biochars from Mixtures of Feedstock Materials." Water, Air, & Soil Pollution **225**(7): 1-9.

Phenolic acid sorption to biochars from mixtures of feedstock materials

K. E. Hall¹, M. J. Calderon², K. A. Spokas^{3*}, L. Cox², W. C. Koskinen³, J. Novak⁴, K. Cantrell⁴

¹ Department of Soil Water & Climate, University of Minnesota, 1991 Upper Buford Circle; St. Paul, MN USA 55108

² CSIC-IRNAS, Avda. María Luisa s/n. Pabellón de Perú; 41013 Sevilla, Spain

³ USDA-Agricultural Research Service, 1991 Upper Buford Circle; St. Paul, MN USA 55108

⁴ USDA-Agricultural Research Service, 2611 West Lucas Street; Florence, SC USA 29501

*K. A. Spokas

USDA-ARS, Soil and Water Management Unit

1991 Upper Buford Circle, 439 Borlaug Hall, St. Paul, MN 55108

Email: kurt.spokas@ars.usda.gov ; phone 612-626-2834

Abstract

In an effort to customize biochars for soil amendments, multiple feedstocks have been combined in various ratios prior to pyrolysis at 350°C. The resulting variation in the chemistry and structure can affect the adsorption capacity of biochar and thus influence the bioavailability of many chemical compounds in the soil system including phenolic acids. This study examines the sorption of ¹⁴C-labeled ferulic acid, syringic acid, and chlorocatechol to four biochars prepared from individual feedstocks and four biochars produced from mixed feedstocks using batch equilibration. Pure feedstock biochar sorption followed switchgrass < swine solids < poultry litter < pine chip for both ferulic ($K_d = 1.4\text{-}75 \text{ L kg}^{-1}$) and syringic acid ($K_d = 0.07\text{-}6.03 \text{ L kg}^{-1}$).

Sorption appeared to be influenced by the properties of the biochars as well as the structure of the chemicals. All biochar K_d values, except pine chip, were consistently lower than that of the reference silt loam soil. The sorptive properties of biochars produced from combined feedstocks could not be predicted from their pure feedstock components and sorption coefficients were both higher and lower than the individual parent materials' biochars. Further research is necessary to understand the characteristics of these combination biochars, particularly their sorption, which this study has shown is not merely an average of its components.

Keywords syringic acid, ferulic acid, sorption, allelopathy, feedstock, soil organic matter

1 Introduction

Biochar, a carbon-rich product of biomass pyrolysis, is defined primarily by its intended use in carbon sequestration and as a soil amendment (Lehmann and Joseph 2009). Incorporation of biochar as a soil additive has been associated with numerous benefits including increased crop yield, plant growth, nutrient retention, water holding capacity, and enhanced biological activity (Chan et al. 2007; Graber et al. 2010). On the other hand, neutral and negative effects (e.g. plant growth suppression, decreased arbuscular mycorrhizal fungi) have also been observed (Deenik et al. 2010; Gundale and DeLuca 2007; Rajkovich 2010; Warnock et al. 2010).

Remarkable diversity exists among the chemical and physical properties of different biochars, mainly as a result of variation in feedstock materials and pyrolysis conditions (Kookana et al. 2011), as well as post-production factors (Azargohar and Dalai 2008). Because quality issues vary from soil to soil, Novak et al. (2013b) proposed the use of what has been termed "designer biochars"- biochars tailored to meet the needs of specific soils. Altering the original

feedstock produces numerous unique biochars (Mukome et al. 2013; Novak et al. 2013a), and mixing these carbonaceous materials in various ratios (prior to pyrolysis) further enhances the diversity and potential customization of properties (Novak et al. 2013b). For example, mixing nutrient-rich poultry litter with carbon-enriched pine chips may produce a biochar which improves soil fertility and sequesters carbon without disturbing the phosphorus balance or pH (Novak et al. 2013b). The properties of these combination biochars cannot be predicted based on the characteristics of the individual parent material biochars (Zhao et al. 2013a; Zhao et al. 2013b). They have been observed to have unique chemistries different from their original components, with organic chemical sorption capacities controlled by the resulting surface chemistry differences (Mukherjee et al. 2011; Shafeeyan et al. 2011; Uchimiya et al. 2011) and/or associated mineral oxide forms (Long et al. 2011; Yao et al. 2011).

When studying the impacts of biochar as a soil amendment, it is critical to recognize that the term “biochar” encompasses a range of heterogeneous materials with non-uniform effects and behaviors (Antal and Gronli 2003; Lehmann and Joseph 2009; Ronsse et al. 2013). Variability in characteristics such as specific surface area (SSA), aromaticity, and microporosity of biochars, for example, affect their sorption capacity, which in part governs the bioavailability of many chemical compounds in soil (Kookana et al. 2011). To date, research on the sorptive properties of biochar has focused heavily on the sorption of pesticides (Spokas et al. 2009; Yu et al. 2009; Sun et al. 2011a) and environmental contaminants (Cao et al. 2011; Chen and Chen 2009; Chen and Yuan 2011; Sun et al. 2011b). However, naturally occurring compounds such as aromatic acids (i.e. phenolic acids) from root exudates and vegetative material in the rhizosphere would also be subject to potential immobilization by biochar sorption (Jones et al. 2012). Phenolic acids released into the soil system influence a number of processes including nutrient uptake, protein synthesis, humus formation, plant signaling, development of mutualistic relationships, and allelopathy (Dalton et al. 1989; Mersie and Singh 1993). The allelopathic effects of phenolic acids make them compounds of interest for their potential use in weed management in agroecosystems (Bhadoria 2011; Pandino et al. 2011; Weston 1996; Won et al. 2013). Simple phenolic acids such as *p*-hydroxybenzoic, vanillic, *p*-coumaric, syringic, and ferulic acids in wheat (*Triticum vulgare* L.) and its residues, for example, are known to contribute to its allelopathic action (Lodhi et al. 1987).

The phytotoxicity of phenolic acids is affected by their bioavailability, persistence, and

fate in the soil (Tharayil et al. 2006). Because phenolic acids are effective as allelopathic agents only when they are in their free form (unbound) (Blum et al. 1999), studies on the sorption of these compounds in soil are required to determine potential biological availability and, in turn, efficacy (e.g. Dalton et al. 1989). The sorption-desorption of five phenolic acids on soils of varying physicochemical properties has previously been characterized (Cecchi et al. 2004) as well as the preferential sorption of phenolic phytotoxins on soil (Tharayil et al. 2006). However, information concerning the sorption of these allelopathic compounds by biochar is lacking.

Some of the initial reported effects of biochar on allelochemicals are from plant growth studies and those investigating the influence of biochar on mycorrhizal associations. Assorted biochars have been found to greatly differ in their ability to disrupt the function of allelopathic chemicals leached from corn residues (*Zea mays* L.) (assumed via adsorption) and thus reduce their inhibitory effect on corn seedling growth (Rogovska et al. 2012). Asparagus (*Asparagus officinalis* L.) similarly releases phenolic acid allelochemicals that suppress seedling growth, which is thought to be partly due to their negative effects on arbuscular mycorrhizal (AM) root colonization (Yeasmin et al. 2013). Warnock et al. (2007) proposed four mechanisms by which biochar impacts mycorrhizal abundance and/or functioning, one being through the detoxification of allelochemicals or the alteration of plant-fungus signaling, although no specific data was presented on their sorption potential. Elmer and Pignatello (2011) found that allelochemicals added to soil without biochar significantly suppressed AM root colonization in asparagus; however, an increase in AM colonization with the addition of biochar was observed in both the presence and absence of allelochemicals.

Although the research on the effects of biochar on allelopathy frequently discusses the potential sorption of phytotoxins by biochar, studies actually characterizing the sorption of allelochemicals are scant, and ones accounting for the physicochemical variability of biochars are fewer still. Of the limited available studies, one by Ni et al. (2011) describes the mechanism of allelopathic aromatic acid adsorption to biochar and reports isotherms for cinnamic and coumaric acids, which could not be fit to Freundlich or Langmuir models.

The objectives of this study were to examine the sorption of two phenolic acids, ferulic acid (3-(4-hydroxy-3-methoxy-phenyl)prop-2-enoic acid) and syringic acid (4-hydroxy-3,5-dimethoxybenzoic acid), and chlorocatechol (4-chlorobenzene-1,2-diol) on a variety of biochars and to determine how the sorption by biochar prepared from mixtures of feedstock materials

differs from those prepared from the pure feedstocks. Ferulic and syringic acid were selected based on their ubiquity and known allelopathic properties. Chlorocatechol was included to compare the reactivity of other phenolic groups.

2 Materials and methods

2.1 Biochar/ soil

Eight low temperature (350°C, 2 h residence time) biochars prepared from different feedstock/ feedstock mixtures were selected for this experiment. The feedstock materials used in the pure feedstock biochars include switchgrass (SG), swine solids (SS), poultry litter (PL), and pine chips (PC). These same feedstocks were mixed in measured ratios (w/w%) to create 4 additional biochars. The SG:SS (80:20) biochar, for example, was prepared from the same switchgrass and swine solids that were used to create SG and SS biochars. The parent materials were mixed prior to pyrolysis and prepared under conditions identical to the singular feedstock biochars. Properties of these biochars are listed in Table 1. The lignocellulosic-based and manure-based feedstock mixtures in this study were designed to rebalance soil phosphorus concentrations and improve moisture retention (Novak et al. 2013b). As these are common soil quality issues, use of similar mixtures may be widely adopted and are therefore important to study. A Waukegan silt loam soil (fine-silty, mixed, mesic Typic Hapludoll; Rosemount, MN) (6.0 pH/H₂O, 2.5 % organic carbon (OC), 15% clay, and 33% sand) was included for comparison.

2.2 Chemicals

¹⁴C-ring labeled ferulic acid, syringic acid, 4-chlorocatechol acid were synthesized and provided by Dr. Konrad Haider, Deisenhofen, Germany. The chemicals were purified by thin-layer chromatography using Si250-F-PA plates (J.T. Baker Chemical Co., Phillipsburg, NJ) with a toluene, ethyl acetate, formic acid (30:25:5) mixture acting as the liquid phase. Chemical structures for the polyphenols used in this study appear in Fig. 1.

2.3 Sorption

Sorption studies were performed in duplicate using the batch equilibration method. Biochar or soil (0.5 g) was weighed into 35 mL glass centrifuge tubes with Teflon lined caps and 5 mL of a 1 $\mu\text{g mL}^{-1}$ phenolic acid ($>17 \text{ Bq mL}^{-1} \text{ }^{14}\text{C}$) in 0.005 M CaCl_2 solution was added. A blank tube containing solution with no soil or biochar was included as a method blank. The tubes were oriented horizontally on a shaker and subsequently shaken approximately 18 h, which is considered to be sufficient time for equilibration on soil (Cecchi et al. 2004). Samples were centrifuged for 30 min at 1280xg and the supernatants collected using disposable glass pipettes. Duplicate 1-mL aliquots were combined with 5 mL scintillation cocktail (EcoLite(+)TM, MP Biomedicals, LLC, Solon, OH) in 7 mL vials and thoroughly mixed. After sitting overnight, the solutions were analyzed for ^{14}C by liquid scintillation counting with a Packard 1500 counter (Packard Tri-Carb, Downers Grover, IL). No chemiluminescence was observed.

The amount of chemical sorbed was calculated as the difference between the amount of chemical in the initial solution and amount of chemical in the final supernatant solution after equilibration. The sorption distribution coefficient, K_d (L kg^{-1}), was determined from the concentration sorbed (C_s) and the concentration remaining in solution after equilibration (C_w) according to the following relationship: $K_d = C_s/C_w$. K_d values were calculated to compare sorption of the phenolic acids to biochars at the single concentration used. Sorption normalized to OC was calculated: $K_{OC} = (K_d/\%OC) \cdot 100$, (L kg^{-1}). Measured total carbon (C) values of the biochars (Table 1) were used for this calculation since the biochars in this study are known to contain <1% inorganic carbon. Reported K_d and K_{OC} values are the averages of the duplicate samples.

3 Results and discussion

3.1 Phenolic acid/ pure feedstock biochar sorption

Biochars prepared from a single feedstock had sorption distribution coefficients, K_d , ranging from 1.4 to 75 for ferulic acid and from 0.07 to 6.03 for syringic acid (Table 2). Chlorocatechol was completely sorbed on all biochars except the poultry litter ($K_d = 25$). Sorption of the three compounds consistently increased in the following order: syringic acid < ferulic acid

< chlorocatechol, a trend which could be related to the chemistries of the compounds (Fig. 1).

According to previous studies, sorption is influenced by the phenolic acid structure. One reported observation is that cinnamic acid derivatives sorb more to soil as compared to benzoic acid derivatives (Dalton et al. 1989). The results from this study appear to be in accordance with this finding; ferulic acid (a cinnamic acid derivative) showed greater sorption to both the soil and biochars than syringic acid (a benzoic acid derivative). A study by Cecchi et al. (2004) found that chemicals having free phenolic groups displayed greater sorption, consistent with our data. Chlorocatechol, which has two highly reactive phenolic groups, had the highest sorption of the three chemicals followed by ferulic acid and lastly, syringic acid. Although ferulic and syringic acid each have a single phenolic group, steric hindrance from the two methoxy groups on syringic acid may lessen its reactivity as compared to ferulic acid.

Whether phenolic acids exist as ions or neutral compounds can also affect sorptive behavior; neutral compounds can more readily sorb to organic matter (Weed and Weber 1974) and anions to oxides (Green 1974). The negative surface charge of the biochars would sorb chemicals in their molecular form more so than their anionic species (Moreno-Castilla 2004). At the pHs of the biochars in this study (pH=6.4-9.4), ferulic ($pK_a=4.58$) and syringic acid ($pK_a=4.34$) exist predominantly as anions (although ferulic acid would have a slightly higher proportion of its molecular form than syringic), while chlorocatechol, assuming a pK_a similar to that of catechol (9.48), remains neutral.

The pure feedstock biochars had K_d and K_{oc} values increasing in the following order: SG < SS < PL < PC for both ferulic and syringic acid. Because this order is the same for both chemicals, certain characteristics of the biochars must also be affecting sorption. Correlations have been found between phenolic acid sorption and OC, pH, and clay content in a soil matrix, although no single variable could be used singularly to predict sorption (Cecchi et al. 2004). In the present study, no such correlations between measured biochar properties and sorption were observed. The order of sorption magnitude did not correlate to any of the chemical and physical properties listed in Table 1 including pH, total C, and ash content. A distinct increase in sorption with an increase in OC was not observed and correcting for OC content did not reduce the variability among biochars.

The PC biochar had a notably higher sorption coefficient than the other three biochars

with both ferulic and syringic acid. Efforts have been made to identify trends in biochar characteristics associated with feedstock materials and these are typically discussed in terms of general groups, mainly wood and non-wood, or subgroups of hard-wood, soft-wood, grass, and manure (Mukome et al. 2013). Based on this broad division, wood biochars are found to have a lower ash content, lower pH, higher C/N, and higher SSA than non-wood chars (Mukome et al. 2013; Singh et al. 2010). The pine chip biochar did in fact have a lower % ash and pH than the switchgrass, swine solids, and poultry litter in this study; however the lack of correlation between these characteristics and sorption suggests others are exerting a greater influence.

Sorption is typically reported to increase with SSA of biochars. However, PC biochar possessed the lowest SSA ($<0.1 \text{ m}^2\text{g}^{-1}$) of all biochars studied, as measured by BET nitrogen adsorption (Brunauer et al. 1938). The low observed SSA may be due to resins, tars, or oils blocking sorption in pore spaces, since pine chips are a particularly oil rich biomass (Conner and Rowe, 1975). Additionally, the resins in the wood may alter the surface properties of the pores and in turn the adsorption capacity (Keech et al. 2005). Biochars are known to maintain relic structures of the parent material; therefore pore distribution can vary among them (Keech et al. 2005). Warnock et al. (2007) states that feedstock materials with large diameter cells can result in biochars with more macropores, which can adsorb large molecules such as phenolic compounds (Keech et al. 2005). Measurements of SSA, however, do not account for differences in the size and shapes of pores, which may be influential. SSA and feedstock material do not have a transparent relationship beyond the general wood/ non-wood distinction and SSA is found to be largely temperature dependent (Brown et al. 2006; Chun et al. 2004; Ronsse et al. 2013).

Pyrolysis temperature is known to be a principal factor influencing biochar characteristics. Increasing production temperatures has been shown to increase SSA, microporosity, and decrease the H/C ratio (i.e. aromaticity), cation exchange capacity, and % volatile matter (Kookana et al. 2011; Mukherjee et al. 2011; Mukome et al. 2013). The biochars in this experiment were prepared at a single temperature (350°C) to strictly compare sorption differences from feedstock variability, however the capacity of high temperature biochars to sorb phenolic acids merits further study. A high temperature (550°) olive mill waste biochar, for example, had a greater sorption coefficient than the soil for syringic acid ($K_d = 14.58$ vs. 12.04) and well above the soil and PC biochar for ferulic acid ($K_d = 236$ vs. 29 and 75, respectively)

(unpublished data). This may be related to the higher SSA of this biochar ($9.82 \text{ m}^2\text{g}^{-1}$).

Our assumption that sorption is the sole mechanism responsible for removing the chemicals from solution in this study cannot be made with absolute certainty. The removal of metal oxides from soils largely decreased sorption (Cecchi et al. 2004), although the influence of metal oxides may go beyond sorption alone. The two phenolic acids examined here have been observed to react with iron and manganese oxides very rapidly, with 70% of ferulic and 90% of the syringic acid disappearing in 4 hours (Lehmann et al. 1987). Biochars and the soil evaluated here do contain both of these metals, and therefore could provide pathways for abiotic interactions with the metal oxides. The extent of this influence was not analyzed here.

3.2 Phenolic acid/ combination biochar sorption

The K_d values of the four mixed biochars [SG:SS(80:20), PC:PL(90:10), PC:PL(80:20), and PC:PL(50:50)] differed from their components for both phenolic acids. An 80:20 blend of switchgrass and swine solids feedstocks, had a *higher* K_d value than either of its constituent biochars (SG, SS) (Fig. 2). A similar counterintuitive effect was observed with the 90:10 mixture of pine chip and poultry litter. Unlike the SG:SS(80:20) biochar, this feedstock combination led to a biochar with a K_d value *lower* than either of its individual components (PC, PL). The weighted averages of the amount sorbed to the pure feedstock components failed to predict the amount sorbed of the blended biochar. This clearly shows that extreme caution needs to be used when predicting the sorptive behavior of mixed feedstock biochars.

The sorption differences between biochars prepared from a known mixture of feedstock materials and those from the individual components could arise from physicochemical alterations to the biochar during pyrolysis. Variation in trace metal constituents, which may act as catalysts during pyrolysis (Agblevor and Besler 1996; Okuna et al. 2005), can lead to differences in observed surface chemistries of the biochar. The surface properties are the primary factor determining sorption characteristics. While it may be possible to estimate the chemical properties of biochars based on trends relating to chemical and physical characteristics of a single feedstock (Mukome et al. 2013), sorption properties of combination biochars have proven more challenging to predict. Further research on the sorptive behaviors of these combination biochars is necessary before customization can be achieved based on feedstocks.

3.3 Soil

The silt loam soil in this study sorbed syringic acid to a greater extent than the biochars (both pure and mixed feedstock). Ferulic acid also sorbed more strongly to the soil than the biochars with the exception of the PC biochar. The normalization of the K_d values to OC provided K_{oc} values that remained relatively low for the majority of the biochars (Table 2). However, when the soil K_d was adjusted for its OC content, which was much lower than that of the biochars, the resulting K_{oc} drastically increased, with the highest biochar K_{oc} value (PC) being over an order of magnitude less for ferulic acid and syringic acid. The generally higher sorption of the phenolic acids to soil may also result from mineral interactions (Cecchi et al. 2004; Tharayil et al. 2006).

3.4 Impacts

Because the majority of the biochars have sorption coefficient values below that of the soil, if they were incorporated into this silt loam soil, their impact on the sorption of the phenolic acids would be inconsequential. However, the addition of these biochars to soils with lower sorptive capacities or in the presence of other phenolic acids with different chemistries (eg. more free phenolic groups) may have greater impacts on the immobilization of these compounds and interfere with their allelopathic effects. Biochars prepared with different feedstocks, feedstock mixtures, higher pyrolysis temperatures, or activation may sorb phenolic acids to a larger degree and must also be studied.

4 Conclusion

All of the biochars examined in this study, with the exception of the pure pine chip biochar, sorbed ferulic and syringic acid less than the reference soil and therefore would not likely alter the bioavailability of these chemicals in the soil environment to a large degree. It was observed that the structure of the phenolic acid, particularly the hydroxyl group, may impact its sorption to biochar. Chlorocatechol showed the greatest sorption with two available hydroxyl groups followed by ferulic acid with one and lastly syringic acid, whose single hydroxyl group is

less accessible due to steric hindrance. Sorption did not appear to be correlated with biochar pH, OC, % ash, or SSA and the sorption of the phenolic acids to the mixed feedstock biochars could not be predicted from the behavior of their pure feedstock components.

Coinciding with previous research, the physicochemical variability among the biochars in this study affected their sorptive behavior and reinforced the importance of acknowledging the diverse effects a biochar amendment may have. While feedstock materials, pyrolysis conditions, and post-production factors are well known to create the observed variability, the interaction of multiple feedstock materials during pyrolysis is not well understood. What is unique to this study is that it demonstrates the counterintuitive effects combining feedstock materials can have on the sorption characteristics of the resulting biochar.

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Table 1 Biochar properties

feedstock	pyrolysis temp (°C)	pH ^a	ash %	VC ^b %	FC ^c %	C %	H %	N %	O %	S %	SSA ^d (m ² g ⁻¹)
Pure feedstock (100%)											
switchgrass (Florence)	350	7.4	3.21	41.40	55.39	75.53	4.55	0.52	16.15	0.04	0.5005
swine solids (Florence)	350	6.5	34.97	37.61	27.42	51.02	3.68	5.94	3.19	1.21	1.0084
poultry litter	350	9.4	32.06	36.15	31.80	51.50	3.56	5.13	6.86	0.89	1.9373
pine chip (loblolly) (Lt 4mm)	350	7.1	1.79	40.41	57.80	78.68	4.87	0.37	14.28	0.02	< 0.100
Feedstock mixtures (w:w ratios)											
<i>Swine solids : switchgrass</i>											
SG:SS(80:20)	350	6.5	7.29	33.74	58.97	75.85	4.55	1.32	10.78	0.22	1.3506
<i>Pine chips : poultry litter</i>											
PC:PL(90:10) (Lt 4mm)	350	6.4	4.36	37.18	58.45	78.13	4.83	0.89	11.70	0.08	1.1148

PC:PL(80:20)	350	7.5	7.3	33.74	58.97	75.8	4.55	1.3	10.78	0.22	1.0888
(<4mm)											
PC:PL(50:50)	350	7.4	18.5	31.55	50.16	63.7	3.80	3.4	10.27	0.38	1.1386
(Lt 4mm)											

^apH was measured in a 1 g biochar/10 mL DI water slurry after 5 min settling time

^bvolatile compounds

^cfixed carbon

^dSpecific surface area as measure by BET nitrogen adsorption

Table 2 Sorption distribution coefficient (K_d) and organic C K_{oc} value

	K_d (L kg ⁻¹)			K_{oc} (L kg ⁻¹)		
	ferulic acid	syringic acid	chloro-catechol	ferulic acid	syringic acid	chloro-catechol
soil	29 ± 0.50	12.04 ± 0.76	56 ± 4.00	1160 ± 20	481.6 ± 30.4	2240 ± 160

Pure feedstock

SG	1.4 ± 0.18	0.07 ± 0.10	*	1.85 ± 0.24	0.09 ± 0.13	*
SS	1.6 ± 0.15	0.41 ± 0.02	*	3.14 ± 0.29	0.80 ± 0.04	*
PL	3.1 ± 0.40	0.43 ± 0.00	25 ± 0.25	6.02 ± 0.78	0.83 ± 0.00	48.54 ± 0.49
PC	75 ± 8.00	6.03 ± 0.06	*	95.32 ± 10.17	7.66 ± 0.08	*
Feedstock mixtures (w:w ratios)						
SG:SS (80:20)	9 ± 0.35	1.03 ± 0.04	*	11.87 ± 0.46	1.36 ± 0.05	*
PC:PL (90:10)	1.8 ± 0.35	0.31 ± 0.14	*	2.30 ± 0.45	0.40 ± 0.18	*
PC:PL (80:20)	3.1 ± 0.13	0.40 ± 0.10	*	4.09 ± 0.17	0.53 ± 0.13	*
PC:PL (50:50)	8.2 ± 0.40	1.02 ± 0.09	*	12.87 ± 0.63	1.60 ± 0.14	*

*Chemical was sorbed completely- K_d could not be calculated

± standard deviation

Fig. 1 Chemical structures of polyphenols used in this study

Fig. 2 Sorption coefficients, K_d ($L\ kg^{-1}$), of combination biochars compared to their pure feedstock component biochars; error bars are standard deviation

Appendix E

Thomazini, A., Spokas, K., Hall, K., Ippolito, J., Lentz, R., & Novak, J. (2015). GHG impacts of biochar: Predictability for the same biochar. *Agriculture, Ecosystems & Environment*, 207, 183-191.

Title:

GHG impacts of biochar: Predictability for the same biochar

Authors:

A. Thomazini ^a, K. Spokas ^{b,c*}, K. Hall ^c, J. Ippolito ^d, R. Lentz ^d, and J. Novak ^e

a - Federal University of Espirito Santo - Agriculture Science Center, Espirito Santo
Brazil

b - USDA-Agricultural Research Service (ARS), Soil and Water Management Unit, St.
Paul, MN USA

c - University of Minnesota, Department of Soil, Water and Climate, 439 Borlaug Hall,
St. Paul, MN USA

d – USDA-ARS, Northwest Irrigation and Soils Research Laboratory, Kimberly, ID USA

e - USDA-ARS, Coastal Plain Soil, Water and Plant Conservation Research, Florence,
South Carolina USA

*** - Corresponding Author**

Kurt A. Spokas; USDA-ARS

1991 Upper Buford Circle – 439 Borlaug Hall

St. Paul, MN 55108

Email: kurt.spokas@ars.usda.gov

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ABSTRACT

One potential strategy to abate increasing atmospheric carbon dioxide (CO₂) levels is to sequester CO₂ as biochar, a structural form of carbon created through the pyrolysis of various biomass materials. Biochar may be applied to soils, but has resulted in variable impacts on net soil greenhouse gas (GHG) emissions, with results spanning from suppression to stimulation. This laboratory incubation study examined the impacts of the same hardwood biochar (fast pyrolysis at 550 °C) to elucidate driving variables affecting previously observed carbon dioxide (CO₂) fluctuations as well as nitrous oxide (N₂O), and methane (CH₄) production impacts across ten different US soils with and without biochar (10% w/w). Biochar application significantly impacted CO₂ (P=0.04) and N₂O (P=0.03) production following amendment across all soils, but there were no differences observed in CH₄ production/oxidation rates (P=0.90). Interestingly, the induced biochar GHG alterations were significantly correlated to the original GHG production activity in the control soil, suggesting a more universal response across various soils to the same biochar than has been previously hypothesized. After correcting for the amount of CO₂ released from the biochar itself [24 μg C g_{BC}⁻¹ d⁻¹], there was no statistically significant alteration in the actual soil CO₂ mineralization rate for any soil. This suggests that the observed increase in CO₂ production was solely attributed to the abiotic CO₂ releases from the biochar. On the other hand, there was an average suppression of 63% in the N₂O production across all soils following biochar addition, which was again correlated to initial N₂O production activity. For this particular biochar, there are predictable impacts on the GHG production potential across various soils despite differences in soil chemistry, texture, and microbial communities.

1.0 INTRODUCTION

The discovery and use of renewable energy sources is critical to the sustainability of the planet. One well known source is biomass, which has been historically used for energy and chemical production (Hawley, 1926). Biomass can originate from numerous sources; for example, in agriculture the generation of biomass waste is particularly high in sectors such as sugarcane production (Ribeiro and Raiher, 2013) and forestry product processing (Thompson *et al.*, 2001). Although biomass is commonly associated with energy production from combustion and its conversion to biofuels (Voivontas *et al.*, 2001), these materials can also be transformed through pyrolysis into biochars (Lehmann, 2007). The addition of the biochar products to soil has been found to improve soil quality and increase carbon sequestration (Atkinson *et al.*, 2010; Ameloot *et al.*, 2013). In the context of global changes in temperature and atmospheric carbon balance, the ability to sequester carbon dioxide (CO₂) via biochar is a mitigation strategy to lower atmospheric CO₂ levels (Goldberg, 1985; Lehmann, 2007).

By definition, biochar is a more stable form of carbon created through pyrolysis for carbon sequestration purposes (Sohi *et al.*, 2010b; Spokas, 2010b; Manyà, 2012). The use of biochar to increase soil carbon and fertility while simultaneously reducing soil greenhouse gas (GHG) emissions has been a growing topic of study in recent years (Asai *et al.*, 2009b; Ameloot *et al.*, 2013; Mašek *et al.*, 2013). The capacity of biochar to sequester carbon is due to the transfer of atmosphere–biosphere cycling carbon to a slower cycling less microbial degradable structural form (Spokas, 2010a; Zimmerman *et al.*, 2011; Harvey *et al.*, 2012). Due to its aromatic chemical nature, biochar is more recalcitrant to microbial decomposition than the original feedstock (Biederman and Harpole, 2013; Gurwick *et al.*, 2013) and thereby postulated to exist for hundreds to thousands of years in the soil (Goldberg, 1985; Zimmerman, 2010; Castaldi *et al.*, 2011; Zimmerman *et al.*, 2011; Harvey *et al.*, 2012; Ameloot *et al.*, 2013; Mašek *et al.*, 2013; Kuzyakov *et al.*, 2014). However, recent studies also highlight its fragile physical nature, thereby physically disintegrating into suspended colloidal fractions (Jaffé *et al.*, 2013; Spokas *et al.*, 2014).

Biochar amendments to soil have the potential to reduce GHG emissions including CO₂, methane (CH₄), and nitrous oxide (N₂O) (Cayuela *et al.*, 2010; Scheer *et al.*, 2011); however the reported effects of biochar on GHG emissions are variable (Taghizadeh-Toosi *et al.*, 2012a, b). Reductions in CO₂ production in biochar amended soils have been observed in some studies (Zimmerman *et al.*, 2011; Harvey *et al.*, 2012; Lentz *et al.*, 2014), while no significant differences or stimulation in CO₂ production between control and biochar amended soils have been reported elsewhere (Spokas and Reicosky, 2009; Zimmerman *et al.*, 2011). Likewise for CH₄, studies show suppressed CH₄ production (Rondon *et al.*, 2007), while another reports observed increased CH₄ production (or reduced methanotrophic activity) (Spokas and Reicosky, 2009; Fungo *et al.*, 2014). In contrast, the incorporation of biochar has more consistently reduced N₂O production in soils, with no clear indication of driving mechanism or responsible properties of the biochar (Cayuela *et al.*, 2013).

Biochar is cited to be capable of sequestering nitrogen within its aromatic structure during the pyrolysis process (Smith *et al.*, 1988; Hilscher and Knicker, 2011) and decreasing

levels of N₂O production following soil applications (Huang *et al.*, 2004; Yanai *et al.*, 2007). Therefore, biochar may act as an agent for mitigating nitrogen losses and potentially serve as a slow-release N-fertilizer in agricultural soils (Laird *et al.*, 2010; Taghizadeh-Toosi *et al.*, 2012a; Clough *et al.*, 2013). There is also the hypothesis that biochar reduces N₂O emissions in soils with high denitrification activity and potentially could increase N₂O emissions in soils dominated by nitrification production (Sánchez-García *et al.*, 2014; Cayuela *et al.*, 2015). However, the exact mechanisms behind these reductions and their duration in soils are not fully known (Cayuela *et al.*, 2013; Clough *et al.*, 2013).

The variability in GHG production following biochar amendments has been linked to a number of factors. The heterogeneity among biochar properties is one such factor contributing to the observed differences. Depending on the biomass material, pyrolysis conditions and post-production processing (Mészáros *et al.*, 2007; Keiluweit *et al.*, 2010; Harris *et al.*, 2013), biochars can vary in both the numbers and type of associated chemical moieties (Singh *et al.*, 2010; Uchimiya *et al.*, 2013) and contain unique mixtures of sorbed volatile organic compounds (Spokas *et al.*, 2011; Quilliam *et al.*, 2012). Furthermore, the inherent variability of soil properties at all scales (e.g., Parkin, 1987) may impact soil-biochar-microbial interactions (Jaiswal *et al.*, 2014) and consequently the GHG production (Cayuela *et al.*, 2013; Cornelissen *et al.*, 2013; Van Zwieten *et al.*, 2014).

At present, the GHG mitigation potential of biochar is difficult to estimate because the mechanisms of the GHG suppression have not yet been fully elucidated (Lehmann *et al.*, 2011; Ameloot *et al.*, 2013; Cayuela *et al.*, 2013). Attempts at deriving unified mechanisms of biochar interactions (Jeffery *et al.*, 2011; Biederman and Harpole, 2013; Liu *et al.*, 2013) have been restricted in part by the variability among biochars studied as well as the variability among soils. It has been known for some time that dissimilar soils have different biochar mineralization potentials (Potter, 1908); however, only a limited number of studies have examined multiple soil types. Therefore, the objective of this study is to evaluate GHG emissions (CO₂, N₂O, and CH₄) in 10 US soils with and without the identical biochar amendment through a laboratory assessment. This will allow an investigation into potential correlations to soil properties across different soils and elucidate potential mechanisms behind biochar GHG suppression or enhancement by reducing the variability due to different site specific climatic conditions (e.g. air and soil temperatures, soil moisture).

2.0 MATERIALS AND METHODS

2.1 Soil sampling

Surface soils (0-5 cm depth) from 10 locations across the US were selected for this study. A random grab soil sample from 0-5 cm was taken from at least 3 locations within a 2 m radius and then homogenized to comprise each of the 10 soil samples. This depth interval typically contains the maximum soil microbial activity for the soil profile (Panettieri *et al.*, 2014). The soil samples were air dried, ground and sieved through a 2-mm sieve. Following this the samples were stored until time of analysis (lab temperature). Agricultural soils were collected from Minnesota, Florida, South Carolina, Idaho, Illinois, Pennsylvania, Idaho, Michigan, and California and forest soils from Minnesota and Wisconsin, which together represent a range of potential soil properties (Table 1). Soils were analyzed for typical micro- and macro- nutrient contents by a commercial soil testing laboratory (A&L Laboratories,

Memphis, TN) (Table 1).

2.2 Biochar

The biochar used in this study was prepared from hardwood sawdust under fast pyrolysis conditions (550 °C; Dynamotive Energy Systems; Vancouver, Canada). The biochar is a very finely grained biochar (<0.3 mm) which facilitates uniform mixing with the soil. This particular biochar was selected since it has been shown to significantly reduce N₂O emissions (Spokas *et al.*, 2009) and nitrate leaching in previous studies (Ippolito *et al.*, 2014). The biochar underwent proximate analysis (ASTM D1762, Hazen Research; Golden, CO), ultimate analysis (ASTM D3176, Hazen Research; Golden, CO) and surface area analysis (BET, N₂, Material Synergy; Oxnard, CA) (Table 2). The biochar was applied at a rate of 10% w/w to all soils in this experiment. The 10% by weight biochar addition has been used in previous laboratory studies (Ippolito *et al.*, 2014); although an unrealistic application rate for agronomic soils, it provides a measurable impact of biochar additions on GHG production (Spokas *et al.*, 2009).

2.3 GHG Incubations

Quadruplicate incubations were conducted for each soil (S) with and without biochar (B). For each soil type, the treatments were:

- 1- (S+B) = Soil (5g) + Biochar (0.5g) + DI Water
- 2- (S) = Soil (5g) + DI Water
- 3- (BC) = Biochar Control : 1 g BC + 0.3 mL DI water

The amount of DI water that was added was sufficient to bring each soil up to field capacity (-33 kPa). There were no differences in the amount of water added to the biochar incubations for each soil type, since there were no significant differences observed in the water holding capacity of any soil (drained from saturated state; data not shown). Soils and biochars were manually mixed in 125 mL serum bottles prior to water addition. Then, soils were pre-incubated for 7 days prior to the start of the incubation to ensure re-establishment of stabilized microbial dynamics, and avoid the initial spike in GHG production following rewetting (Franzluebbers *et al.*, 1996; Lamparter *et al.*, 2009). Biochar control incubations were conducted to assess the production or consumption of CO₂, N₂O and CH₄ from the biochar itself in an aerobic environment. DI water was added to these incubations, since previous data has shown that the presence of moisture increases the abiotic release of CO₂ from biochar (McBain *et al.*, 1933; Zimmerman, 2010; Jones *et al.*, 2011).

All incubations were conducted in pre-sterilized serum vials (Wheaton Glass, Millville, NJ) and sealed with red butyl rubber septa (Grace, Deerfield, IL). Gas samples were periodically withdrawn from the incubations for analysis on a gas chromatographic system to quantify gas production over a 45-d incubation period. Gas samples were taken at different time intervals throughout the incubation, with biweekly reading for the first two weeks, then weekly for the remainder of the experiment. O₂ headspace levels remained >15% throughout the incubation, ensuring aerobic conditions. The gas chromatographic system consisted of a headspace sampler (Agilent, Foster City, CA, model 7694) that was modified with the addition of a 10-port diaphragm sample valve (Valco, Houston, TX, model DV22-2116). Initially, 5 mL of lab air (known composition) was injected into the sealed vials to allow the withdrawal of a 5 mL headspace sample without altering headspace gas pressure. The syringe was flushed 3

times to allow for adequate mixing of the serum bottle headspace. Five mL of gas was then pulled back into the syringe and subsequently injected into an autosampler vial that was previously helium-flushed for analysis. Concentrations from the GC were mathematically corrected for dilution from the 5 mL of air and converted to a mass basis by the ideal gas law. The GC system used was previously described in Spokas and Bogner (2011). The rate of production of each gas was calculated as the linear increase in the gas concentration as a function of time ($R^2 > 0.90$) for the 45 day incubation period.

2.4 Ammonia, Nitrate and Nitrite

At the conclusion of the experiment (day 45), two of the replicate GHG incubations were extracted with 2 M KCl for 1 hr at a soil to liquid ratio of 1:5. After settling for 0.5 h, extracts were centrifuged and filtered (no. 42; Whatman, Maidstone, UK). Filtrates were then stored (-20°C) until analysis. Filtrate samples were analyzed for ammonium-N [NH_4^+ -N] and the sum of nitrite-N and nitrate-N [NO_2^- -N + NO_3^- -N] using a flow-through injection analyzer (Lachat, Milwaukee, WI). Filtrates were then analyzed solely for nitrite-N [NO_2^- -N] and the amount of nitrate-N was calculated by difference.

2.5 Statistical analysis

Results for the CO_2 , N_2O , and CH_4 production rates were reported as the arithmetic means of the four replicates, while ammonia-N, nitrate-N and nitrite-N results were averages of duplicate samples. The average GHG production rates and extractable nitrate concentration between control and biochar treatments were analyzed across the different soil types using one-way ANOVAs with post-hoc Tukey's test to analyze for significant interactions among the soil types. The assumption of normality was verified with the Kolmogorov–Smirnov test and homogeneity of variance was confirmed with the Bartlett test. Linear regression analyses were used to further explore relationships among soil variables. Significance was defined as $p \leq 0.05$, unless otherwise indicated. R statistical software was used for all analyses (R Core Team, 2014).

3.0 RESULTS

3.1 GHG Production Impacts

Figure 1 presents the observed average cumulative rates of CO_2 , N_2O and CH_4 production in the various soils with and without biochar. In order to account for production of CO_2 from the biochar itself, the subtraction of a biochar control (with no soil) was used (Spokas et al., 2009). The biochar in this study produced $24 \mu\text{g C g}_{\text{BC}}^{-1} \text{d}^{-1}$. This rate suggests a loss of $\sim 4.4 \text{ mg C g}_{\text{BC}}^{-1} \text{yr}^{-1}$ from abiotic oxidation (or $0.4\% \text{ C yr}^{-1}$). Contrary to the observed CO_2 production in the biochar control, no significant N_2O or CH_4 production/consumption was observed. Therefore, no biochar correction was applied to the CH_4 and N_2O production data. After applying this correction for the BC production, no significant differences between the control and biochar amended soils were observed (Fig. 1a), despite the fact that there were significant differences in the uncorrected CO_2 production rates ($P=0.038$).

Statistically significant differences between the biochar and non-amended soils for N_2O production were observed across all soils ($P=0.027$). N_2O production from soils without biochar were observed to be highest in FL, MN-A and SC soils (3.43 ; 3.8 and $3.1 \text{ ng N g}^{-1} \text{d}^{-1}$,

respectively; Fig. 1b) and lowest in the MN-F soil ($0.1 \text{ ng N g}^{-1} \text{ d}^{-1}$; Fig. 1B).

No significant differences were observed in the CH_4 production rates ($P=0.897$) across all soils due to the high standard deviations. Total methane flux was the lowest in the ID, WI-F, and MI soils (-1.2 and $-0.93 \text{ ng C g}^{-1} \text{ d}^{-1}$, respectively) both with and without biochar. A negative methane flux indicates net soil methane oxidation activity.

3.2 Ammonia, Nitrate and Nitrite

The available inorganic N at the conclusion of the 45 day laboratory incubations did vary among soils. The FL soil was the only soil with a statistically significant higher ammonia level following the addition of biochar (186%), with the MN forest soil was the only soil with a 46% lower availability. The remaining 8 soils had no significant difference in ammonia availability following biochar addition.

On the other hand, there was 43-96% lower nitrate availability in the biochar treatments compared to the controls across the 7 statistically significant reductions (Table 3). The MN-F, CA, and PA soils possessed no difference in nitrate availability as a function of biochar addition. In addition, there were no statistically significant differences observed in nitrite concentration in any soil following the 45 d incubations between the control and biochar treatments (Table 3).

3.3 Correlation Analysis

Correlation analysis of the observed GHG production rates (control and the uncorrected biochar rates), final inorganic-N availability, and the corresponding soil properties were then conducted (Table 4). Examining solely the GHG_{production} rates (last 6 rows), soil OM content was correlated with control rate CO_2 (CO_2) ($R=0.69$). In addition, there were two significant correlations observed between K with control rate CO_2 and the biochar CO_2 (B_CO_2) production. However, the more significant results were the significant correlations between the mean GHG production rates of the control (unamended soils; CO_2 , N_2O , and CH_4) vs. the production rates of the biochar amended soils (BC_CO_2 , $\text{BC_N}_2\text{O}$, and BC_CH_4) with R ranging from 0.94 to 0.97 ($P<0.001$; Table 4). This resulted in significant linear relationships for all three GHG gases with biochar related to their associated control rate: CO_2 [$\text{CO}_{2\text{Biochar}} = 0.944 (\text{CO}_{2\text{control}}) + 11.14 \mu\text{g C g}_{\text{soil}}^{-1} \text{ d}^{-1}$; $R^2=0.97$; $P<0.001$], N_2O [$\text{N}_{2\text{OBiochar}} = 0.3709 (\text{N}_{2\text{Ocontrol}}) - 0.05 \text{ ng N g}^{-1} \text{ d}^{-1}$; $R^2=0.63$; $P<0.0058$], and CH_4 [$\text{CH}_{4\text{Biochar}} = 0.714 (\text{CH}_{4\text{control}}) - 1.02 \text{ ng C g}^{-1} \text{ d}^{-1}$; $R^2=0.90$; $P<0.0001$] (Figure 2). Incidentally, the rate of CO_2 production of the biochar blank (0.5 g biochar in control) is very close to the value of the intercept from the linear regression between the control and biochar CO_2 production rates ($11.14 \mu\text{g C g}_{\text{soil}}^{-1} \text{ d}^{-1}$; Figure 2a). Similarly, the negligible N_2O ($0.05 \text{ ng N g}^{-1} \text{ d}^{-1}$; Fig. 2b) and CH_4 intercept values ($-1.02 \text{ ng C g}^{-1} \text{ d}^{-1}$; Fig 2c) are consistent with the insignificant N_2O or CH_4 flux observed in the biochar only controls.

From the linear regression between control and biochar-amended rates (Figure 2b), N_2O production following biochar addition was suppressed by an average of 63%. This suppression was statistically significant in 60% of the soils (WI-F, CA, FL, MN-A, SC, and PA). The soils that did not show a statistically significant suppression were also those with the

lowest basal rate of N₂O production in the soil control (<1.5 ng N g⁻¹ d⁻¹).

4.0 DISCUSSION

The chemical, physical and biological properties of soil, which can be altered by biochar application, directly influence soil-plant-atmosphere processes. For example, biochar has been reported to increase soil pH for acidic soils and in turn impact the availability soil nutrients (Lentz and Ippolito, 2012). Additionally, several studies have shown higher productivity rates in biochar amended soils related to improved soil conditions (Chan *et al.*, 2007; Glaser *et al.*, 2009). Asai *et al.* (2009a) observed an increase in yield due to an increase in plant-available P content, and Laird *et al.* (2010) noted an increase in soil extractable P, K, Mg, and Ca in soil treated with biochar. Biological alterations in soil following the addition of biochar can include changes in the composition and abundance of the biological community, as well as enzyme activities (Lehmann *et al.*, 2011). It is commonly reported in literature that GHG production is highly associated with such soil attributes (Sohi *et al.*, 2010a); thus, it may be important to consider potential alterations in soil characteristics (Yao *et al.*, 2010; Jones *et al.*, 2012; Spokas, 2013; Zheng *et al.*, 2013). However, studies with the same biochar added to multiple soils are lacking.

Our results demonstrate that with the identical biochar addition to soil, GHG dynamics may be less tightly linked to soil properties (i.e. CEC, microbial biomass & community structure), but may be driven by the biochar-nitrate interaction (Cayuela *et al.*, 2013). This is supported by the linear relationships observed between the control and biochar amended soil GHG production rates (Figure 2). Since biochar's impact on GHG production is correlated across different soil types suggests that these impacts would be driven by the biochar properties and not influenced as greatly by soil chemical and microbial differences. These linear relationships observed for CO₂, N₂O, and CH₄ production show that a single biochar reacts more uniformly across different soil chemistries and microbial activities than currently hypothesized (e.g., Sohi *et al.*, 2010a). Therefore, the impact of biochar on a soil's GHG production could potentially be predicted based on its original, un-amended GHG production activity. The mechanisms responsible for these observed correlations with a soil's initial GHG production, however, remain ambiguous.

Increases in CO₂ production in soils following biochar application could be due to the abiotic production from chemisorptions of oxygen to the surface of biochar (Puri *et al.*, 1958) or microbial biomass already present on the biochar. Either of these would explain the positive intercept (Figure 2a) of the biochar control incubation. Given the linear response observed for all the soils evaluated here, we hypothesize that the majority of this effect is due to abiotic processes stimulated by the biochar addition, particularly supported by the fact that the biochar control resulted in a similar production rate for a 0.5 g of biochar. Such processes would be influenced by biochar characteristics, which vary with feedstock conditions, pyrolysis temperature and post-production handling conditions. For example, Ameloot *et al.* (2013) observed greater net C mineralization from low temperature biochars compared to the control and the treatments with high temperature biochars and Sigua *et al.* (2014) observed a significant impact with particle size. Additionally, greater CO₂ and N₂O production were found in low temperature (350°C) compared to high temperature (700 °C) biochar (Ameloot *et al.*, 2013), which has been correlated to the degree of incomplete carbonization (Fabbri *et al.*,

2012). Thus, mineralization rates and production of CO₂ present different behaviors according to individual biochar properties and it is unlikely that an average biochar factor for CO₂ production will be determined. The time elapsed since the production of a biochar also influences its ability to impact the GHG production in soils as a result of decomposition, weathering, or microbial activity (Spokas, 2013; Borchard *et al.*, 2014). This hinders our ability to extract information from meta-analyses across all biochars, since different processes are likely active with different biochars. We need to understand the mechanism of interaction allowing us to normalize the observed responses.

Compared to the control soils, observed CH₄ concentrations were not significantly different with biochar additions to soils in this study. Other studies have reported a reduction in CH₄ production or increasing CH₄ oxidation with the addition of biochar (Laird, 2008). However, this inconsistency could be related to differences in biochar chemical and physical factors leading to changes in soil redox state or the differences in sorbed organic compounds and inorganic constituents are known stimulants or inhibitors of methane oxidation (Hubley *et al.*, 1975; Hazeu and Bruyn, 1980). Furthermore, the aerobic conditions in this experiment would not favor CH₄ production (methanogens).

For all of the soils in this study, there was a reduction in N₂O production rate and typically a reduction in extractable nitrate with the addition of this hardwood fast pyrolysis biochar. Sorption of ammonia and nitrate to biochar has been cited as a possible mechanism for the suppression of soil N₂O production and nitrate leaching (Laird, 2008). Other studies have observed increases in gene abundance with N-fixation and denitrification (Ducey *et al.*, 2013). Given the fact that the suppression observed here was correlated across different soils with different N₂O production potentials, we hypothesize that direct effects on microbial populations are not a likely explanation for this biochar. Similar to the result of this study, others have observed the ability of biochar to decrease total N₂O productions to be independent of soil texture and mineralogy, but highly correlated with initial soil nitrate concentrations and dissolved organic C (Cayuela *et al.* 2013), which would be assessed in this study through the initial GHG production activity. In addition, Lin *et al.* (2014) also could not link biochar's N₂O suppression to any microbial group through the use of selective microbial inhibitors, supporting an abiotic mechanism for the interaction of a macadamia nut shell biochar. These findings along with the results observed here, suggest that biochar participates in abiotic reduction of nitrate/nitrite to N₂(g). This mechanism is typically dismissed as a trivial contributor in soils (i.e., Nelson and Bremner, 1970). Nevertheless, these chemical interactions could be more important in biochar amended soils, analogous to observations of N₂O production in Antarctica soils (Samarkin *et al.*, 2010) and the critical role of iron in moderating nitrogen transformations (Zhu *et al.*, 2013a).–

The abiotic transformations of nitrite/nitrate by the charcoal-cation metal systems have been known for some time (Moraghan and Buresh, 1977; Hansen *et al.*, 1996; Huang and Zhang, 2004; Huang *et al.*, 2009). These reactions include the chemical conversion of nitrate/nitrite directly to N₂ gas, which could be an important process when evaluating alterations in biochar N₂O mitigation and reduction in nitrate leaching. In other words, biochar additions might increase the importance of direct chemical reaction pathways terminating in N₂ formation (Zhu *et al.*, 2013b; Dhakal *et al.*, 2014), thus reducing the reliability of the N₂O:N₂

ratio that has been used as evidence of increased microbial denitrification. Furthermore, a potential negative consequence of this chemical interaction is that instead of nitrate being sorbed to the biochar and available through desorption, it might be removed from the soil system entirely. This could explain the reduced nitrate in final biochar extractions, and the reduction of N in existing leaching experiments (e.g. Laird, 2008). This reduction in available nitrate in biochar amended soil also could lead to decreases in plant growth and explain the historical suggestions to co-apply biochar with a synthetic or organic fertilizer (Priestley, 1770; Davy, 1856; Blake, 1893). In the long-term, biochar is hypothesized to promote improved fertilizer availability (Raynbird, 1847; Davy, 1856; Terne, 1882; Khan *et al.*, 2008); although complete understanding of these mechanisms and long-term effects are lacking.

Biochars are complex heterogeneous materials on many levels; it has different surface chemistries, diverse microbial populations and its responses to nitrate and ammonium sorption could differ as a result of these and other chemical variations (Asada *et al.*, 2006; Seredych *et al.*, 2010; Long *et al.*, 2011; Seredych *et al.*, 2011). The sorption and reaction potential of biochar with nitrogen depends on the surface oxygen groups (Fujitsu *et al.*, 1993; Seredych and Bandosz, 2007; Huang *et al.*, 2008; Shafeeyan *et al.*, 2011) as well as the retention/trapping of dissolved nitrogen species in biochar micropores (Kameyama *et al.*, 2012). Thereby, greater concentrations of surface oxygen groups on biochar with aging (Qian and Chen, 2014) could result in a biochar with decreased carbon sequestration potential (Spokas, 2013; Naisse *et al.*, 2014; Qian and Chen, 2014) and increased reactivity with inorganic N forms.

5.0 Conclusion

This study examined the universality in potential GHG mitigation due to the same biochar application. The addition of this hardwood biochar reduced both the production of N₂O and extractable nitrate concentrations across a variety of soils studied. These corresponding reductions are hypothesized to be the result of biochar-nitrate interactions (chemical reaction and not sorption), since this would explain both the observed suppression of N₂O and nitrate following biochar addition. Our results show that in the short term, the alteration in GHG production is more uniform than hypothesized from compiling existing studies using different soils and different biochars, depending solely on original GHG production rates. This study provides insight that the inconsistent effects across existing biochar studies partly result from variability in biochar properties, and care should be utilized when comparing biochar effects across different studies. Comparing dissimilar biochars confounds our ability to synthesize results from different studies, due to the variability in the functionality and mechanistic differences between biochars. While the results from this study show that applying an identical biochar to different soils can result in predictable impacts on GHG production, these relationships are likely different for various biochars.

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Table 1. Soil properties

Soil Location	Soil Taxonomy	OM ^a	CEC ^b	pH	P	K	Ca	Mg	S	Na	Zn	Mn	Fe	Cu	B
		%			-----ppm-----										
Forest Soils															
Minnesota – F (MN-F)	Mixed, frigid Spodic Udipsamment	2.10	3.10	4.90	145	52	318	38	23	17	2	25	447	1.3	0.20
Wisconsin-F (WI-F)	Sandy, mixed, frigid, Entic Haplorthod	0.50	1.10	6.89	55	22	128	27	18	16	0	16	76	1.3	0.10
Agricultural Soils															
California (CA)	Coarse-loamy, mixed, superactive, nonacid, thermic Typic Xerorthents	1.30	9.60	7.50	98	112	1816	262	19	25	14	78	75	22	0.70
Florida (FL)	Siliceous, hyperthermic Mollic Psammaquents	0.90	3.90	7.30	73	21	894	29	13	17	4	7	118	4	0.20
Minnesota – A (MN-A)	fine-silty over skeletal, mixed, superactive, mesic Typic Hapludoll	4.80	16.90	6.10	60	156	2568	507	12	25	4	119	190	3	1.00
South Carolina (SC)	Fine, kaolinitic, thermic Typic Paleaquults	1.30	2.40	6.20	69	106	300	73	11	16	6	14	99	2	0.10
Idaho (ID)	Coarse-silty, mixed, superactive, mesic Durinodic Xeric Haplocalcid	1.50	19.0	7.60	18	176	345	152	1	62	2	15	6	2	0.10

Illinois (IL)	Fine-silty, mixed, superactive, mesic Typic Endoaquolls	4.30	15.80	6.30	21	152	2552	472	18	22	2	60	144	3	0.60
Michigan (MI)	Coarse-loamy, mixed, mesic Typic Hapludalfs	1.80	8.10	5.80	31	104	1172	208	12	25	2	61	196	2	0.20
Pennsylvania (PA)	Fine-loamy, mixed, active, mesic Ultic Hapludalfs	2.80	9.10	6.50	39	103	1810	126	9	20	1	202	118	2	0.40

^aOM = organic matter

^bCEC = cation exchange capacity (cmol_c/dm³)

Table 2. Biochar properties

Feedstock	hardwood sawdust
Pyrolysis Temperature (°C)	550
pH	7.1
% C _{total}	63.9
% C _{inorganic}	2.1
% C _{organic}	61.8
% N	0.44
% O	11.8
% H	3
% Ash	21.1
% VM ^a	26.1
% FC ^b	52.8
SSA ^c (m ² g ⁻¹)	0.8

Notes:

All composition percentages are based on oven-dried (105 °C) weight basis.

^a VM = volatile matter

^b FC = fixed carbon

^c SSA = specific surface area by BET N₂ adsorption method (Brunauer *et al.*, 1938).

Table 3. Values for ammonia, nitrate, and nitrite (mg N kg_{soil}⁻¹) in soils with (S+BC) and without (S) biochar after the 45 day incubation

Soil Location	Ammonia		Nitrate		Nitrite	
	S	S+BC	S	S+BC	S	S+BC
Forest Soils						
Minnesota - F	628.33 a	337.50 b	18.83 a	16.90 a	15.00 a	16.38 a
Wisconsin - F	0.54 a	1.3 a	12.4 a	0.5 b	<10	<10
Agricultural Soils						
California	18.08 a	20.42 a	1615.83 a	1249.17 a	14.27	<10
Florida	17.14 a	31.92 b	419.17 a	35.25 b	13.93 a	14.10 a
Minnesota - A	35.42 a	22.25 a	1537.50 a	834.17 b	<10	<10
South Carolina	25.17 a	29.58 a	700.83 a	227.50 b	<10	<10
Idaho	6.54 a	5.21 a	32.42 a	6.42 b	<10	<10
Illinois	22.58 a	21.08 a	1061.67 a	600.00 b	16.08 a	15.53 a
Michigan	28.75 a	22.17 a	763.33 a	411.67 b	17.25 a	17.67 a
Pennsylvania	17.58 a	17.21 a	678.33 a	386.67 a	<10	<10

Note: Within each variable for the soil and the (soil + biochar) followed by the same letter are not significantly different (p > 0.05) by Student's t-test.

Table 4. Pearson correlations between GHG production potential with and without biochar compared to soil attributes

	OM	CEC	pH	P	K	Ca	Mg	S	Na	Zn	Mn	Fe	Cu	B	CO ₂	N ₂ O	CH ₄	BC_CO ₂	BC_N ₂ O	
OM																				
CEC	0.89**																			
pH	-0.35	-0.01																		
P	-0.31	-0.43	-0.24																	
K	0.80**	0.86**	-0.16	-0.4																
Ca	0.82**	0.98***	0.15	-0.44	0.81**															
Mg	0.86**	0.96***	-0.03	-0.4	0.87**	0.90***														
S	-0.13	-0.16	-0.18	0.61	-0.26	-0.2	-0.06													
Na	0.53	0.80*	0.06	-0.3	0.72*	0.78*	0.78*	-0.12												
Zn	-0.17	0.13	0.48	0.36	0.25	0.2	0.17	0.13	0.4											
Mn	0.54	0.58	0.02	-0.34	0.53	0.65	0.39	-0.46	0.5	0										
Fe	0.22	-0.07	-0.85**	0.6	-0.09	-0.18	-0.08	0.48	-0.08	-0.29	-0.12									
Cu	-0.18	0.19	0.58	0.27	0.18	0.29	0.19	0.28	0.48	0.92***	0.09	-0.32								
B	0.76*	0.89**	0.14	-0.11	0.74*	0.89**	0.88**	-0.03	0.75*	0.4	0.54	-0.08	0.42							
CO ₂	0.69*	0.55	-0.54	-0.29	0.76*	0.42	0.58	-0.44	0.39	-0.1	0.25	0.21	-0.34	0.37						
N ₂ O	0.44	0.45	0.13	-0.25	0.45	0.43	0.45	-0.64	0.26	0.16	0.17	-0.24	-0.1	0.48	0.63					

CH ₄	-0.31	-0.35	-0.22	-0.12	-0.38	-0.48	-0.21	0.22	-0.15	-0.48	-0.42	0.09	-0.37	-0.4	-0.17	-0.36			
BC_CO ₂	0.6	0.47	-0.49	-0.16	0.69*	0.36	0.49	-0.46	0.33	0.02	0.18	0.23	-0.27	0.32	0.97***	0.70*	-0.32		
BC_N ₂ O	0.55	0.48	-0.04	-0.34	0.59	0.42	0.52	-0.65	0.25	0.05	0.22	-0.21	-0.23	0.47	0.78*	0.95***	-0.26	0.79*	
BC_CH ₄	-0.4	-0.55	-0.35	-0.1	-0.48	-0.66	-0.43	0.09	-0.38	-0.61	-0.44	0.16	-0.58	-0.65	-0.12	-0.38	0.94***	-0.23	-0.25

P<0.05 indicates a significant difference

MB= microbial biomass; OM = organic matter; CEC = cation exchange capacity

Figure 1. Observed cumulative production rates of (a) CO₂, (b) N₂O and (c) CH₄ from soils with and without biochar over the 45 day incubation. Error bars represent one standard deviation of the quadruplicate samples. The symbol is the location abbreviation (Table 1), MN-F: Minnesota Forest; WI-F: Wisconsin Forest; CA: California; FL: Florida; MN-A: Minnesota Agriculture; SC: South Carolina; IL: Illinois; ID: Idaho; MI: Michigan; and PA: Pennsylvania.

Figure 2. Observed relationships between the biochar amended and control incubations for (a) CO₂, (b) N₂O, and (c) CH₄ production between all soil types. Error bars represent corresponding one standard deviation of the associated rates. The symbol is the location abbreviation (Table 1), MN-F: Minnesota Forest; WI-F: Wisconsin Forest; CA: California; FL: Florida; MN-A: Minnesota Agriculture; SC: South Carolina; IL: Illinois; ID: Idaho; MI: Michigan; and PA: Pennsylvania

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Appendix F

Vozhdayev, G. V., Spokas, K. A., Molde, J. S., Heilmann, S. M., Wood, B. M., & Valentas, K. J. (2015). Response of maize germination and growth to hydrothermal carbonization filtrate type and amount. *Plant and Soil*, 1-10.

Title:

Response of maize germination and growth to hydrothermal carbonization filtrate type and amount

Authors:

Georgiy V. Vozhdayev¹, Kurt A. Spokas^{2,3}, Joseph S. Molde¹, Steven M. Heilmann¹, Brandon M. Wood¹, and Kenneth J. Valentas¹

Affiliations:

1 –University of Minnesota, BioTechnology Institute, 140 Gortner Laboratory, 1479 Gortner Ave., St. Paul, MN 55108-1041, USA

2 – United States Department of Agriculture – Agricultural Research Service, Soil and Water Management Research Unit, 1991 Upper Buford Circle, 439 Borlaug Hall, St. Paul, MN. 55108 USA

3 – University of Minnesota, Department of Soil, Water, and Climate, 1991 Upper Buford Circle, 439 Borlaug Hall, St. Paul, MN. 55108 USA

4 – University of California Berkeley, College of Engineering - Applied Science and Technology, 210 Hearst Memorial Mining Building, Berkeley, CA 94720

Corresponding Author:

Kurt A. Spokas

1991 Upper Buford Circle – 439 Borlaug Hall

St. Paul, MN 55108

Phone: (612) 626-2834

Email: kurt.spokas@ars.usda.gov

Fax (651) 649-5175

Abstract:

Aims: The option of using hydrothermal carbonization (HTC) filtrate as a liquid based fertilizer for agricultural crop production was evaluated through germination and plant growth studies using corn (*Zea Mays* L.).

Methods: Corn growth trials were conducted in a growth chamber with artificial lighting and controlled temperature programming in washed silica sand amended with condensed distillers soluble (CDS), swine manure, or poultry litter HTC filtrates. Seedling growth trials were conducted over a period of three weeks and evaluated for overall plant height, above ground biomass, below ground biomass, and total biomass in response to various filtrate applications. Impacts on germination were studied by quantifying germination time and of corn seeds in response to various amounts of condensed distillers solubles (CDS) and swine HTC filtrates.

Results: Inhibitory effects on corn seed germination and seedling growth were dependent on HTC filtrate type and application amount, where at dilutions greater than 1:2 (filtrate : total volume) corn germination was not inhibited and swine based filtrate extending the seed germination delay (lag phase). Low filtrate applications were statistically equal to control responses.

Conclusions: These results suggest a potential opportunity for utilization of HTC filtrates as an agricultural liquid fertilizer, thereby recycling critical plant nutrients, once inhibitory compounds are treated.

Keywords: Filtrate; hydrochar; plant growth; seed germination

Abbreviations

HTC – hydrothermal carbonization; P - phosphorous; MGT - mean germination time;

Introduction

Hydrothermal carbonization (HTC) is process that provides an option for nutrient reclamation, and more specifically, the recovery of phosphorous (P). Because HTC filtrates are enriched with abundant levels of solubilized ammonium, phosphate, and potassium, they have the potential to provide a renewable source of nutrients necessary for agricultural crop production (Heilmann et al. 2010). Since the aqueous phase of the HTC process makes up a major fraction of final products (Poerschmann et al. 2014), it is essential that a useful application of this by-product be developed in order for this treatment to become an

economically viable option.

Several studies to date have analyzed the effect of hydrochars on plant growth and have had mixed results with respect to growth stimulation and phytotoxicity (Bargmann et al. 2013; Bargmann et al. 2014; Busch et al. 2012; Busch et al. 2013; George et al. 2012; Rillig et al. 2010). However, only limited studies have been identified that examined the HTC filtrate. Not all of the studies isolated the impact of the filtrate, with HTC filtrates being mixed with the respective hydrochar and other organic raw materials, and then subjecting the mixture to composting prior to soil application (Busch et al. 2013). Other studies identified solely the effect of HTC filtrate application on plant germination and growth (Bargmann et al. 2013; XiaoHan et al. 2014). There have been a variety of different responses observed following HTC filtrate application, ranging from inhibition to stimulation. These impacts have been linked to dissolved salts (Nakhshinieiev et al. 2014) and the presence of various organic compounds in the filtrate (Biller et al. 2012; Libra et al. 2011). However, these studies are limited in scope, since a majority examined solely a single feedstock (e.g., Poerschmann et al. 2014; XiaoHan et al. 2014). In addition to fertilizer applications, HTC process waters have been examined for the potential use as a substrate for algal growth as well as bio-energy resources (Hognon et al. 2015; Libra et al. 2011).

Of interest to US agriculture in the corn-belt region is the impact of HTC filtrate on corn growth (*Zea Mays* L.). To better understand how filtrate type and concentration impact germination and corn growth, studies were conducted that utilized HTC filtrates collected from three diverse waste streams that had undergone the HTC treatment: swine manure, poultry litter, and condensed distiller's solubles (CDS) from the dry-grind ethanol industry. This study also examined the impact of filtrate aging on these observed impacts.

MATERIALS AND METHODS

HTC Filtrate Preparation

Swine manure, poultry manure, and condensed distillers solubles (CDS) were used as feedstock materials. All of these underwent hydrothermal carbonization (HTC) for 2 hr at 225 °C. All HTC reactions were conducted in a laboratory-scale stirred stainless steel reactor fitted with a heating mantel system (1 L; Parr Instruments, Inc.; Moline, IL, USA). The feedstock was poured into the reactor, stirred at 88 rpm, and heated to the specified temperature for 2 hr. Pressure during HTC reaction was autogenous. After the 2 hr reaction time was reached, the system was allowed to cool to 40 °C. At this time, the reactor was disassembled and the contents filtered (VWR Filter Paper, #415) (Wood et al., 2013). The end result was solid hydrochar and the aqueous filtrate products. Only the filtrate phase (liquid) was utilized in these experiments.

HTC Filtrate Aging

After producing the HTC filtrate, an aliquot of the original filtrate was used to establish aging trials in order to simulate filtrate being stored in a tank prior to use. A 500 mL wide mouth glass bottle was used to simulate a storage tank (ThermoScientific, part #2100-0016). For each

treatment, 250 mL of filtrate was initially placed in each bottle. The lid was left open to allow evaporation and volatilization to occur. To compensate for evaporative losses, weekly ultrapure HPLC water (Aqua Solutions; Deer Park, TX, USA) was added to replace the water lost by mass difference to keep dissolved salt concentrations (osmotic potential) equal between the two treatments.

This aging does not adequately account for field storage scenarios, but was used to assess if volatilization loss of chemicals would result translate to differences in seed germination and seedling growth. Nitrate, ammonium and phosphate nutrient analysis was performed on all of the initial filtrates using a Lachat auto-analyzer (Lachat Instruments, Loveland CO, USA).

Germination Studies

Germination effects were studied by observing germination over the course of a week in response to various dilutions of filtrate, while keeping the moisture addition constant. The experiment was conducted by evenly spreading 10 corn seeds across the 8.6 cm circular, blue blotter paper (Anchor Paper Co.; St. Paul, MN, USA) inside a standard petri dish (9 cm diameter). Corn seeds were selected for uniform size (20 g per 100 seeds) prior to placement within each petri dish. Blotter paper of each treatment was saturated with 5 mL of the particular dilution being evaluated (6 levels - Undiluted, 1:2, 1:5, 1:20, 1:50, and a deionized water control). This was established as a full factorial design with each filtrate treatment level being run in triplicate and two separate germination experiments were performed for the aged and fresh filtrates (2 filtrates x 6 treatment levels x 2 aging treatments x 3 replicates).

These germination studies were conducted in a growth chamber 1.8 x 1.4 x 2.4 m (Controlled Environments, Winnipeg, Manitoba, Canada). The light cycle in the growth chamber was set for 16-hour days and 8-hour nights and temperature was held constant at 20 ± 2 °C. Light intensity in the growth chamber was recorded with a light sensor which was placed at the same level at the petri dishes in the chamber (average intensity was $200 \mu\text{mol m}^{-2} \text{s}^{-1}$). Daily visual assessments were made to monitor germination progress. Seed germination was taken as the time when the radicle could be seen emerging from the seed coat and cumulative germination percentage data was then used to assess mean germination time (MGT) (Chang and Sung 1998).

Corn Seedling Growth Trials: Silica Based Sand Media

Growth trials were conducted in a climate controlled chamber that was set to run on a 16-hour light period, with daytime temperature of 31 °C with relative humidity of 60%, and a nighttime temperature of 26 °C and relative humidity of 50%. Temperature and humidity were recorded over the course of the 21 days at 5-minute intervals with a humidity and temperature data-logger (Extech Instruments, RHT10, Melrose, MA, USA) to verify operational set points.

Washed silica sand was used as the growth media, which was rinsed initially for 30 minutes with tap water (5 L min^{-1}) to eliminate any existing contaminants and then was air dried prior to use. This silica sand was used to eliminate the complication of soil organic matter

adsorbing compounds within the filtrate (Pignatello 2013), which allows the isolation of the filtrate impact. The sand was lightly packed into a 10.2 x 10.2 x 10.2 cm pot containing drainage holes. There was 1.3 kg of sand added per container (bulk density $\sim 1.12 \text{ g cm}^{-3}$; total pore volume $\sim 670 \text{ mL}$). Due to the lack of nutrient availability in the silica sand, a fertilized control was used versus distilled water. The control treatments received an aqueous trace element solution to supplement initial micronutrient contents (12.3 mg MgSO_4 ; 0.5 mg H_3BO_3 ; and 7.2 ng of $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ per pot). These levels were below the upper range of nutrient concentrations that has been observed to restrict corn germination (Cummins and Parks 1961). Therefore, the fertilizer addition was assumed to have no impact on seed germination. Each individual pot was watered with 100 mL (0.15 pore volumes) of autoclaved distilled water every other day for the length of the 3 week period. No observable seedling water stress or drainage was noted during the experiment. Furthermore, no leachate was collected or sampled.

The growth trials consisted of three types of filtrates: condensed distillers solubles (CDS), swine manure, and poultry litter filtrates. A full factorial design was planned with each filtrate treatment level being run in triplicate and two separate growth experiments were performed for the aged and fresh filtrates (3 filtrates x 2 treatment levels x 2 aging treatments x 3 replicates). However, due to limited filtrate available from the same batch, only poultry manure filtrates were run fresh and aged, the remainder of the filtrates were all run as 90 day aged filtrates. Therefore, the analysis was focused on the aged treatments (3 aged filtrates x 2 treatment levels x 4 replicates). Based on the germination testing conducted prior to the plant growth trials, filtrates were diluted to 1:2 and 1:10 (Table 1) and then applied to the triplicate soil pots with a one-time application at the initial watering event. A fertilizer control (Peat-Lite™ 20-10-20 fertilizer stock solution; 1.32 g L^{-1} in distilled water) was used as the control in this seedling growth experiment. These control nutrient levels have not been observed to restrict corn germination (Cummins and Parks 1961).

After the treatments were applied, each pot was seeded with three corn seeds of uniform size (20 g per 100 seeds). Upon emergence, the sprouts were thinned to one per pot, selectively pulling the smaller sprouts. All replicates were randomly placed within the growth chamber, and under an average photo-synthetically active radiation (PAR) of $470 \pm 9 \mu\text{mol m}^{-2} \text{ s}^{-1}$ (FieldScout Light Sensor, Spectrum Technologies, Aurora IL, USA). The seedlings were harvested on day 22 and the roots were manually washed. This was the length of time prior to roots emerging from the bottom of the pots. The root system was separated from the seedling at the pot soil surface. Biomass samples (above and below ground) were placed into separate, pre-weighed paper bags for drying in an 85°C oven. The bags used for drying of the plant mass, had also been dried in the 85°C oven prior to being weighed.

Chemical Analysis of HTC Filtrates

A comprehensive 2-dimensional gas chromatograph–time of flight-mass spectrometer (Pegasus-4D; GCxGC-TOF-MS; LECO, St. Joseph, MO, USA) was used, equipped with a cryogenic inlet system (CIS) injector and a thermal desorption unit (TDU) (Gerstel Inc., Baltimore, MD, USA). The analytical column set consisted of a non-polar primary column [30 m x 0.25 mm x

0.25 μm DB-5; 95% polydimethylsiloxane, Agilent, Santa Clara, CA, USA] and a mid-polarity secondary column [2 m \times 0.10 mm \times 0.1 μm BPx50 50% phenyl polysilphenylene-siloxane, SGE Analytical Science, Austin TX, USA]. All analytical hardware was computer controlled (LECO ChromaTOF software; version 4.50). Chemical species were analyzed by evaporating 1 μL in the TDU [40°C, 60° C/ min, 300°C (5min)]. These eluted compounds were then cyro-focused and injected into the analytical column flow (split mode; 1:20). GC separation method was performed as published previously (Strong et al. 2014).

Statistics

For the germination assessments, data presented represents the means of the triplicate samples for all treatments. One-way analysis of variance (ANOVA) was performed using the R software environment (R Development Core Team 2010) to determine statistical significance of the filtrate type and dilution rate. If statistical significance was present, the Tukey-Kramer Multiple Comparisons Test was then used to test between means of seed germination by treatment [HSD.test; (Mendiburu 2014)]. Aging effects were compared by using a Student's t-test to evaluate differences between germination rate and percentage for the fresh and aged filtrates. A value of $P < 0.05$ was used to assess statistical significance. The time series data of the cumulative germination for each treatment was fit to an empirical germination curve and analyzed for mean germination time (MGT) according to methods used in Chang and Sung (1998).

For plant growth dynamics, plant growth data (height) was transformed into necessary matrices in order to analyze with the “grofit” R package library (Kahm et al. 2010). The 3-parameter sigmoidal curve (Gompertz function) for plant growth is:

$$y(t) = A \left[e^{-e^{-\mu e^{\left[\frac{1}{A(\gamma-t)+1} \right]}}} \right],$$

where $y(t)$ is the plant height at the time (t), μ represents the maximum growth rate, γ is the lag phase, and A is the maximum growth achieved (Richards 1959). The resulting model parameters were then assessed with a two-way ANOVA to determine if statistical differences existed between treatments and dilution rates in the fitted variables ($P < 0.05$). If a significant difference is present, then the means were further evaluated by the Tukey-Kramer Multiple Comparisons Test [HSD.test; (Mendiburu 2014)] to assess if any particular treatment mean significantly differs from any other treatment. Again, statistical significance was taken at $P < 0.05$.

For peak resolution and quantification of the GCxGC-TOF-MS analyses, the software integrated preprocessing tools corrected for instrumental fluctuations and noise, followed by mathematical resolution of overlapping peaks (LECO Stat. Compare software; version 1.6). Automated mass spectral matching with the National Institute of Standards data library (NIST-2011) identified compounds. Due to the complexity and number of compounds identified

(<248 separate individual compounds), peak quantification was not possible and peak area was utilized for comparing relative differences between samples.

RESULTS

HTC Filtrate Aging

The mass of each open container undergoing an aging period was recorded before and after each H₂O addition. Observed evaporation rates for the filtrates ranged from 20 to 50 mm week⁻¹ as a function of the climatic fluctuations in the laboratory fume hood (e.g., air temperature, barometric pressure, etc.; actual data not shown). Overall, there were no differences in the evaporation rate as a function of filtrate type. Table 1 presents the inorganic analyses and Table S1 for the top 10 organic chemical species identified in the aged and fresh filtrates. There were no significant differences observed in the inorganic N and P contents of the fresh and aged filtrates. Despite this similarity in inorganic contents, there were different organic compounds and varying abundances and types of organic compounds as a consequence of the aging treatment (Table S1; Figure S1).

Corn Germination Study

Germination studies confirmed that the HTC filtrate concentration inhibited germination. These results are presented in Table 2. These results will be discussed by filtrate treatment type.

Fresh Filtrates

Universally, the undiluted fresh filtrate of both fresh swine and CDS filtrates inhibited corn seed germination completely during the observation period (Table 2). There was no significant impact of the 1:50, 1:20, and 1:5 fresh dilutions of the swine and the 1:50 and 1:20 dilutions of the CDS filtrates on germination percentage or estimated mean germination time (MGT) (Table 2). There was an observable difference in the rate of germination at the 1:2 dilution of the fresh CDS (an increase of 5.2 d for the MGT) and a doubling of the MGT at the 1:5 dilution of the fresh CDS filtrate (Table 2). On the other hand, the 1:2 dilution of the fresh swine filtrate increased MGT by only 0.8 d, with no decreases observed with the other dilutions (Table 2). These data suggest that the undiluted and 1:2 diluted fresh filtrates inhibited corn seed germination.

Aged Filtrates

Upon aging, seed germination increased compared to the fresh filtrates (Table 2). It is also noteworthy that the aged CDS filtrate at the 1:2 did have a greater germination percentage and a shorter MGT compared to the fresh filtrate (Table 2), despite the similarity in the inorganic N and P levels (Table 1).

Overall, application of filtrates at low amounts does not inhibit corn germination and

early radicle formation. A difference in inhibition between swine and CDS filtrates becomes apparent when comparing the 1:2 dilution of the two filtrates, with CDS having a larger inhibitory effect. Inhibition of germination was universally observed when undiluted filtrate was used, regardless of the feedstock type.

Corn Growth Trials: Plant Growth Dynamics

There were significant differences observed in the characteristics of corn seedling growth as a function of filtrate, dilution rate, and the interaction of filtrate x dilution rate (Table S2). Measurements of the light concentrations confirmed a statistically uniform light field across all treatments, with the average light intensity of $460 \pm 10 \mu\text{mol m}^{-2} \text{s}^{-1}$, with no statistically significant difference between treatments in light intensity ($P > 0.05$). The results from each filtrate will be presented separately.

Swine

For the swine filtrate, there was a 56% decrease in total plant growth observed for the 1:2 dilution level, which was statistically significant when compared to the control ($P < 0.05$). The total plant growth at the 1:10 dilution level was numerically equivalent to the control (Figure 1A). As seen in the fitted plant growth parameters (Table 3), the rate of maximum growth (μ) was suppressed by 60% in the 1:2 dilution level with a corresponding increase in the lag phase of 2.1 days and 30 cm lower achieved plant height (Table 3). The 1:10 dilution level did possess a lower plant height than the control (5.6 cm lower), but there were no significant differences observed in the lag or growth rate for the 1:10 swine dilution.

CDS

For the 1:2 CDS filtrate, there was a 49% decrease in maximum plant growth rate and a 65% reduction in achieved plant height, but a similar lag phase as the control (Table 3). The 1:10 dilution level had similar growth characteristics (lag and maximum growth rate) as the control, with an increase of 12% in plant height (Table 3). This was the only treatment with an observed increase in plant growth compared to the control. Interestingly, there was no lag phase alterations observed in the seedling growth for the CDS filtrates.

Poultry Litter

Unlike the previous two HTC filtrates, the poultry litter material did not possess a decrease in predicted plant productivity properties for the 1:2 dilution level. The total plant productivity was still lower than the fertilizer control for the diluted filtrate (1:10) (Figure 1C), despite greater N and P concentrations in the poultry filtrate (Table 1).

Total Plant Productivity (above and below ground biomass).

In addition to the dynamics of seedling growth, there were interactions observed for the partitioning of plant growth (Table 4; Table S2). The above ground and total (above + below) biomass were strongly related to the filtrate application rate and type, as well as the interaction

between these factors ($P < 0.001$; Table S3). However, there were no differences observed in the ratio of the above to below ground biomass for any of the treatments (Table 4), despite the observed plant height difference with the 1:10 CDS filtrate (Table 3).

DISCUSSION

Germination studies confirmed that the HTC filtrate concentration inhibited corn seed germination. The data presented on the corn seed germination with varying concentration of filtrates clearly demonstrate that there are different impacts in regards to filtrate concentrations and their effect on germination (Table 2). There was an association between filtrate type and applied rates with regards to corn germination and seedling phytotoxicity. The germination studies suggested that swine HTC filtrate could initially be applied at higher amounts than CDS filtrate, without negatively impacting plant growth. Osmotic (salt) stress (Chan et al. 2008), fluoride (Chang 1967), nano-scale ZnO (Lin and Xing 2007), and high level of N-nutrients (Libra et al. 2011) have been linked to reduced corn seed germination and seedling growth. Historically, it is known that high concentrations of nutrients can inhibit microbial and plant processes (e.g., Eno and Blue 1957). Analogous to the results observed here, high concentrations of filtrate from fresh swine manure and poultry litter have inhibited seed germination, which has traditionally been attributed to osmotic interactions (Miller 1962).

However, the aged and fresh filtrate treatments in this experiment possessed statistically equal concentrations of N and P (Table 1). Therefore, suppression observed in the germination rates could be linked to the presence of allelopathic compounds. The chemical and thermal carbonization could produce inhibitory compounds similar to those observed during microbial crop residue decomposition (e.g., Martin et al. 1990; Patrick and Koch 1958; Vaughn and Boydston 1997).

The qualitative analysis of the filtrates (Table S1) demonstrates that the different filtrates have unique chemical fingerprints. Even though the analysis requires further improvement, the acquired data provides a starting point for further characterizations, and gives a general glimpse into the chemistry. Note that the major alterations observed from aging are in the abundances of compound classes, transforming from diols (double alcohol groups on the compound) in the fresh filtrate to single alcohols, ketones and cyclic-N compounds in the aged filtrate (Table S1). It is important to remember that a diol can be converted to a cyclic compound through diol cyclization, which is aided by an acid catalyst (March, 1985). The carbon-carbon bond in a vicinal (adjacent) diol (also called a glycol) can be cleaved and replaced with two carbon-oxygen double bonds resulting in either a ketone or aldehyde (March, 1985). These reactions along with volatilization losses and other microbial transformations could lead to these observed differences.

There have been some previous attempts at characterizing HTC filtrates using a wide-range of analytical tools. Because HTC filtrate is compositionally very complex, a wide array of analytical tools has been utilized for characterization. Various organic constituents have been

determined using pyrolysis gas chromatography-mass spectrometry (py-GC/MS, Anastasakis, 2011) and high-pressure liquid chromatography (HPLC) in attempts to characterize the aqueous phase generated in the hydrothermal liquefaction of brown macro-alga (*Laminaria saccharina*). Jena and Kastner (2011) used HPLC to analyze the liquid phase produced during liquefaction of *Spirulina* algae. Eibisch (2013) utilized inductively coupled plasma (ICP) and HPLC coupled with ultraviolet (UV) and refractive index (RI) detection to separate and analyze several classes of organic compounds within the HTC filtrate of grass, straw and woodchips. Organic compounds present in the filtrate were also examined by Poerschmann (2013), who performed solvent extraction analysis, followed by saponification and derivitization of olive mill waste (OMW) hydrochar by gas chromatography- mass spectrometry (GC-MS). Further advancements were performed by Stemann (2013), who used a combination of UV absorbance, size exclusion chromatography, organic carbon detection (LC-OCD, DOC) and combustion GC-MS to evaluate the chemical composition of poplar woodchips HTC filtrate. Levine et al. (2013) also demonstrated the chemical complexity of the *N. oculata* microalgae filtrate through their analysis by HPLC, GC-MS, and FT-ICR-MS. Poerschmann et al. (2014) determined that the most abundant products in the hydrothermal liquid phase of spent brewer's yeast distillers grain were phenols and volatile fatty acids. However, even trace level (sub-ppm) organic chemicals can be an important signaling agents for both germination and seedling growth (Nelson et al. 2012).

Compounds known to produce negative germination effects have been attributed to certain aldehydes [e.g., (E)-2-hexenal, nonanal, 3-methylbutanal, 5-hydroxymethyl-furfural-1-aldehyde (HMF), and furfural] (Bradow and Connick 1990), phenols (Williams and Hoagland 1982), polycyclic aromatic hydrocarbons (PAH's) (Rogovska et al. 2012), dioxins, and volatile organic acids such as acetic acid (Titirici et al. 2008). Some of these compounds are present in the filtrates (Table S1).

The data for the two aged filtrates questioned the dominant role of osmotic drivers, since the differences in germination of the aged and fresh filtrates could not be linked to nutrient concentrations (Table 1). The extremely high levels of NH_4 in all filtrate types is a likely inhibitory agent, as it has been previously shown that root toxicity can occur at concentrations around 35 ppm NH_4^+ (Eno and Blue 1957). Furthermore, soil microbe stress can occur at levels greater than 200 ppm NH_4^+ (Eno and Blue 1957). Despite an inhibitory effect seen in plant growth with high filtrate applications, higher dilutions of CDS filtrate exhibited an increase in plant mass after 3 weeks compared to the fertilized control (Table 1). Similar increases have also been observed for Japanese mustard spinach (*Brassica rapa var. periviridis*) following hydrothermal sewage sludge filtrate applications (XiaoHan et al. 2014). On the other hand, poultry and swine HTC filtrates resulted in lower total corn plant mass than the fertilizer control. This suggests some chemical differences in the organic compounds present in each filtrate (Bagnoud-Velásquez et al. 2014; Becker et al. 2014; Biller et al. 2012) or even plant species specific responses. Due to the fact that there were statistical differences due to aging for germination percentages and timing (Table 2), suggests volatile organic compounds are one of the responsible mechanisms (Table S1).

Both the germination and the plant growth studies, provided data on the positive effects in regards to low applications rates of filtrate. Therefore, to achieve a greater improvement in overall plant growth, filtrates should be applied at low-doses to agricultural soil, with the correct delay offset for mineralization or chemical imbalances. However, site specific data (soil and plant evaluation) would be needed to formulate this guidance. It is envisioned that these recommendations will be similar in form to those used for the incorporation of green manures and corresponding planting delays (Känkänen et al. 2008; Lahti and Kuikman 2003; Vaughn and Boydston 1997). Future work should examine growth trials that are more representative of real-life farm practices (field plots & tank aging) with additional attention to the soil microbial populations. Soil microbes are important agents in controlling N availability and overall plant growth (Bargmann et al. 2014; Busch et al. 2012; Busch et al. 2013).

COMPLIANCE WITH ETHICAL STANDARDS

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Figure 1. Estimated total plant growth from the integration of the fitted plant model curve from “grofit” R package (Kahm et al. 2010) for the A) Swine, B) CDS, and C) Poultry HTC filtrate compared to the Control and 2 filtrate dilution levels (1:2 and 1:10).

Note: Error bars illustrate one standard deviation of the measurements.

Appendix G:

EVAPORATION OF H₂O FROM SOILS GROWN WITH CORN AND BIOCHAR

MATERIALS AND METHODS

Soil Sampling and Analysis

The experiment was conducted in a greenhouse located at the University of Minnesota in 2015. The experiment was established in a completely randomized design (CRD) with a factorial 3 x 5 x 3, referring to three soil types (RM: Rosemount, MN; PS: MVP; and a: UM:University of MN soil) incubated with five different biochar (ICM: Pine chip biochar; RO: Royal Oak hardwood lump charcoal, AAC: Accurel activated charcoal; B: Bamboo and MC: Macadamia nut) and a treatment control.

Soil samples were collected in the 0 - 20 cm layer and then air-dried, sieved (<2 mm) A portion of the soil was sent for chemical and physical attributes characterization. For determining the soil texture we used the pipette method, for potassium (K⁺), calcium (Ca⁺²), magnesium (Mg⁺²), potential acidity (H + Al), pH (1:10 H₂O) and available phosphorous (P), determination was according to Embrapa (1997). The total soil nitrogen (TN) was determined according to the Kjeldahl (Embrapa, 1997) and TOC by Yeomans and Bremner (1988). These results are shown in Table 1.

Pots with a height of 15 cm and width 10.5 cm (1298.8 cm³) total volume were used for the growth experiments. A coffee filter was used to prevent loss of soil by the addition of water (Figure 1A and B). Added with soil 1% of biochar in the total volume of soil. After conditioning the soil in the pots added five corn seeds (*Zea mays*) and the initial volume of 80 ml of the water on first day of incubation reaching %, % and % field capacity for soil RM, MN and PS, respectively. On the fifth day after planting the corn seed was made desbate the plants remaining only one maize plant per pot (Figure 1B and C).

Figure 1. Conditions utilized for incubation of soil in pots (Figure 1A and B), experimental design (Figure 1C) and wet sieving of the roots (Figure 1D). Shown is only one soil in the greenhouse experiment. The location of the trays was randomized to avoid any systematic bias in the greenhouse conditions on the bench.

Monitoring of the Soil evaporation

Evaluation of evaporation from the soil were conducted at 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 15 and 16 days after incubation (DAI). At the moment of incubation performed the initial soil dry weight condition obtaining an average of 450; 400 and 100 g of soil in each pot, respectively, for Solso A, RM and PS.

The evaporation was calculated according to formula 1, which calculated the moisture of the difference in the 24 hours evaluation: M with weights Soil dry (before the add the 80ml of H₂O) and soil wet (after the add the 80ml of H₂O) . The difference was divided by the initial amount of dry soil.

$$EVP = \frac{\text{Soil Dry}_{\text{before}} - \text{Soil wet}_{\text{after}}}{\text{Soil dry}_{\text{Initial}}}$$

Monitoring of the corn plants

After 17 days of incubation, the corn plants were evaluated for plant height using a ruler according to the measurement of ground base to the largest leaf. After this the media was held cutting and subsequent measurement of humid weight of vegetative parts (leaf and stem) of maize plants.

To determine the weight of roots used the sieve for washing the roots and remove the soil removal (Figure 1C). Subsequently, the roots and the vegetative part of corn plants were directed to the greenhouse at 72 ° C for 72 hours to obtain the dry weight.

Statistical analysis

Variable variability (Evaporação, altura de plantas, peso vegetativo) was calculated by first determining descriptive statistics such as mean, standard deviation, minimum, maximum and median. The results were submitted to normality tests (Shapiro-Wilk test, SPSS Inc., USA) and homogeneity of variances (Bartlett test, SPSS Inc., USA) and the significant H0 obtained.

The evaporation (EVP) for effect soil and biochar were analyzed by cumulate evaporation for all days. Evaporation, plant height, root part weight and vegetative was tested by the F-test. When the H0 hypothesis was rejected and H1 accepted, the means were compared by the Tukey test at 5% probability (Sisvar Inc., Brasil).

(A)