

12-2014

# Short-Term Effects of Poultry Litter or Woodchip Biochar Amendment in a Temperate Zone Agronomic System

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Short-Term Effects of Poultry Litter or Woodchip Biochar Amendment in a Temperate Zone  
Agronomic System

Short-Term Effects of Poultry Litter or Woodchip Biochar Amendment in a Temperate Zone  
Agronomic System

A thesis submitted in partial fulfillment  
of the requirements for the degree of  
Master of Science in Crop, Soil, and Environmental Sciences

by

Katy Brantley  
Hendrix College  
Bachelor of Arts in Environmental Studies, 2011

December 2014  
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This thesis is approved for recommendation to the Graduate Council.

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## **Abstract**

Biochar, a charcoal product produced by the anaerobic thermal decomposition of biomass, can provide agronomic benefits when soil applied. However, research is lacking in temperate region soils investigating specific biochar products and their effects on agronomically important crops. A greenhouse study utilizing poultry litter biochar and a field study utilizing pine woodchip biochar were conducted to observe the effects of biochar application to Northwest Arkansas soils on corn growth and nutrient availability. A third experiment investigated poultry litter and pine woodchip biochar influences on soil water retention. In all three experiments, biochar was applied at three rates (0, 5, and 10 Mg ha<sup>-1</sup>). In the greenhouse and field experiments, biochar treatments were applied in combination with three rates of fertilizer based on soil analyses and recommended rates for yield goals [nitrogen (N) for the field, N and phosphorus (P) for the greenhouse]. Greenhouse results indicated that nutrient-rich poultry litter biochar, particularly at the 10 Mg ha<sup>-1</sup> rate, increased corn height and aboveground biomass and root biomass and morphological features, but not percent mycorrhizal infection of roots. The positive effects on improved crop growth were potentially due to direct biochar nutrient addition and greater acquisition of soil and fertilizer phosphorus through the expansion of the root system. Corn yields increased with the application of woodchip biochar in combination with N fertilizer in the field, but corn yields (in the absence of fertilizer) and soil N decreased with biochar. During a laboratory based, soil-rewetting experiment, soil with poultry litter biochar tended to retain more water at very low water potentials. Poultry litter and pine woodchip biochars may both have beneficial impacts on corn production in temperate soil, but the biochar properties and impacts on nutrient availability and soil-water relationships differ. Additional study into potential mechanisms is needed.

## **Acknowledgements**

I would like to thank my advisors, Dr. Mary Savin and Dr. David Longer, for providing the opportunity for me to study at the University of Arkansas and to conduct research with them. I appreciate the extra time and effort spent by my committee member, Dr. Kris Brye, to conduct an additional research experiment with me and to ensure the success of my research. I would also like to thank my committee members, Dr. Mike Evans and Dr. Vaughn Skinner, for their support and guidance with my research. I am very grateful for the support from my fellow graduate students, particularly Jade Ford and Suhartono. I also could not have completed this research successfully without the support from my friends and family. Dr. Trent Roberts, Chester Greub, and Assistant Farm Manager Ron Cox were invaluable for the completion of my field study, and John Guerber was gracious enough to allow me to conduct my research in the U of A Rosen Center greenhouses. Jeff Armstrong and Bradley Jackson with Monsanto provided the corn seed for the greenhouse and field studies. Laboratory support by Kamela Mitchell, Michael Thomas, Missy Fulenwider, and Brina Smith is gratefully acknowledged. Thanks to Dr. Edward Gbur and Jeff Velie for assisting me with statistical analyses and to the USDA Poultry Waste Lab at the University of Arkansas for technical support.

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## 1. Introduction

Biochar is a charcoal product produced by the pyrolysis of biomass and used as a soil amendment. Biochar deposition has occurred for hundreds if not thousands of years in the Amazon River basin (Neves et al., 2003). These Amazonian Terra Preta soils occur on multiple soil types including Oxisols and Ultisols, typically highly leached and weathered and thus nutrient-poor (Lehmann et al., 2003; Smith, 1980; Zech et al., 1990). However, the Terra Preta have greater organic matter and enhanced concentrations of nutrients such as phosphorus (P), potassium (K), calcium (Ca), manganese (Mn), and zinc (Zn), as well as decreased nutrient leaching particularly of nitrogen (N), which are suggested to contribute to the prolonged fertility of these soils (Lehmann et al., 2003; Zech et al., 1990). Additionally, the Terra Preta have greater cation exchange capacities (CEC), base-saturation, and pH than the surrounding non-biochar soils (Zech et al., 1990). Because of the many benefits, aboriginal groups have valued these soils for agricultural production (Smith, 1980).

Research has been conducted in tropical soils, such as those in Brazil, focusing on soil and plant responses to biochar addition. Lehmann et al. (2003) reported increases in cowpea (*Vigna unguiculata* L.) growth and uptake of P, K, Ca, Zn, and copper (Cu) with biochar addition compared to the control. Upland rice (*Oryza sativa* L.) yields increased in a wood biochar-amended soil compared to the control, but yields were much greater when biochar was applied in combination with compost (Nehls, 2002). In an Australian Oxisol, cattle feedlot biochar enhanced the yield response of ryegrass (*Lolium multiflorum* L.) and uptake of N, P, and K when combined with NPK fertilizer compared to NPK fertilizer alone (Sinclair et al., 2008), while a similar fertilizer-biochar interaction effect with wood biochar increased sorghum (*Sorghum bicolor* L.) yields in a Brazilian Oxisol (Steiner et al., 2007). Biological N<sub>2</sub> fixation by

beans (*Phaseolus vulgaris* L.) increased with wood (*Eucalyptus deglupta* L.) biochar in a Typic Haplustox in Colombia, suggested to be due to greater boron (B) and molybdenum (Mo) availability (Rondon et al., 2007). In the same Colombian soil type, maize (*Zea mays* L.) yield did not significantly increase the first year after wood biochar addition, but residual effects increased yields for the next three years (Major et al., 2012).

Although numerous agronomic benefits have been observed with biochar addition to tropical soils, less research has been conducted in temperate region soils. Of the research that does exist, there have been varying results, many due to the type of biochar used. For example, Rajkovich et al. (2012) observed that poultry litter biochar tended to improve corn growth and increase plant N uptake with a wide range of application rates (0 to 91 Mg ha<sup>-1</sup>), but biochars produced from food waste or dairy manure were more likely to decrease biomass and N uptake at greater application rates. In a Kandiudult in Georgia, corn tissue K and Ca concentrations increased with peanut (*Arachis hypogaea* L.) hull biochar application, but no tissue nutrient concentrations differed based on pine (*Pinus* spp.) woodchip biochar addition (Gaskin et al., 2010). Willow (*Salix viminalis* L.) biochar addition decreased soil nitrate more than pine (*Pinus sylvestris* L.) biochar in a sandy loam in Belgium, while both reduced barley (*Hordeum vulgare* L.) and radish (*Raphanus sativus* L.) biomass (Nelissen et al., 2014). Amid the various crop and soil responses to biochar, there are still questions regarding the specific reasons for biochar effects.

Various mechanisms have been proposed for the crop yield, nutrient uptake, and soil fertility alterations with biochar. Biochar was observed to increase total N recovery more than mineral fertilizer or compost in soil, crop residues, and grain, presumably due to reduced N leaching and gaseous losses (Steiner et al., 2008). Decrease leaching leading to increased fertility

has been suggested as a mechanism in multiple additional studies (Laird et al., 2010; Lehmann et al., 2003; Novak et al., 2009; Singh et al., 2010). Increased soil-nutrient retention could result from ammonium adsorption on biochar CEC sites. Biochars can have high porosity and surface area, leading to large sorption potential (Major et al., 2009). Biochar's high porosity and surface area could also contribute to improvements in soil water retention (Laird et al., 2010; Ulyett et al., 2014). Additionally, increased microbial abundance and activity due to biochar could occur, increasing nitrogen mineralization as observed by Domene et al. (2014). Increased mycorrhizal infection has also been reported, a symbiotic relationship known to increase nutrient and water access for plants (Blackwell et al., 2010; Ezawa et al., 2002; Yamato et al., 2006). Mycorrhizae have even been able to directly access phosphorus from the surface of biochar particles (Hammer et al., 2014). There are still many questions about specific biochar effects in temperate region soils and the response of agronomically important crops.

To further investigate biochar effects in temperate soils, three studies were conducted using two types of biochar in laboratory, greenhouse, and field studies. One study investigated a potential mechanism for changes in biochar-applied soil. This laboratory study aimed to determine biochar effects on soil water retention and differences in the relationship between water potential and water content between two biochar products (*Pinus* spp. woodchip and poultry litter biochars). Pine and poultry litter biochars were chosen because these biomass products result from two major industries in Arkansas, where the studies were conducted. Arkansas was the third largest producer of broilers and turkeys in the United States in 2013, and commercial timberland covers over half of the state (NASS-USDA, 2014; Pelkki, 2005). It was hypothesized that poultry litter biochar applied at 10 Mg ha<sup>-1</sup> would alter water-retention

characteristics more than at 5 or 0 Mg ha<sup>-1</sup> or with the woodchip biochar due to the intrinsic differences in the biochar products.

Two studies investigated the potential benefits of growing corn in the presence of poultry litter or woodchip biochar. Roughly 870,000 acres of corn were harvested for grain in Arkansas in 2013, making corn an important commodity crop behind rice and soybeans (NASS, 2014). Nitrogen uptake by irrigated field corn was evaluated in the presence of biochar applied to a silt loam at one of three different rates in combination with nitrogen (N) fertilizer. Soil nutrients, N-use efficiency, and corn ear-leaf N were determined with applications of recommended and reduced rates of fertilizers based on yield goals in soil receiving pine woodchip biochar. Rhizosphere ecology was investigated in addition to soil and corn parameters in the greenhouse study to determine if poultry litter biochar amendments benefitted plants in combination with N and P fertilizers. Soil and root ecological components included microbial biomass, mycorrhizal root infection, soil nutrient availability, and root morphology. For both experiments, it was hypothesized that adequate corn growth and nutrient uptake would occur with biochar addition that could reduce inorganic fertilizer inputs because of the intrinsic biochar properties as well as indirect biochar effects on soil and plant variables.

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## **2. Literature Review**

### **2.1 Terra Preta Soils**

Terra Preta de Indio (Amazonia Dark Earth soils) are Anthrosols located in the Amazon River basin. Terra Preta soils typically encompass areas between 1 to 350 ha, the majority less than 20 ha, but over 50,000 ha exist between the Brazilian Tapajós and Curuá-Una rivers alone (Glaser et al., 2001; Kern et al., 2003; Smith, 1980). The Terra Preta soils contain substantially greater organic matter and cation exchange capacity, higher pH values, greater moisture-holding capacity, and enhanced nutrient levels [e.g. nitrogen (N), phosphorus (P), calcium (Ca), and potassium (K)], as well as lower leaching rates of plant nutrients when compared to texturally and mineralogically similar soils nearby, predominately Oxisols, Ultisols, and Spodosols (Smith, 1980; Zech et al., 1990). Typically, upland Amazonian soils are leached and highly weathered in the humid, warm, and rainy climate (Smith, 1980). However, the anthropogenic A horizon depths of the Terra Preta usually range between 30 and 60 cm (Kern et al., 2003).

The Terra Preta contain fish bones, pottery shards, and other remnants of human life including large amounts of incompletely combusted organic matter or charcoal thought to remain from fires built for cooking and heat (Glaser et al., 2001; Smith, 1980). The productivity and fertility of these soils is attributed to the presence of charcoal, a residual of incomplete combustion from fires, as well as the remains of human habitation such as bones of animals and feces (Glaser et al., 2001). Radiocarbon dating has determined that the charcoal present in these soils originated between 500-2500 years ago (Neves et al., 2003). The supposition of charcoal's importance in these soils had led to research on how to emulate the Terra Preta's fertility and stability in other soils (Lehmann and Joseph, 2009).

## 2.2 Biochar Production

Charcoal applied to soil with the intention of improving soil properties or providing agronomic benefits is called biochar (Lehmann and Joseph, 2009). Biochar is produced using various methods of thermal degradation of biomass, including processes such as hydrothermal conversion, torrefaction, gasification, slow pyrolysis, and fast pyrolysis (Meyer et al., 2011). Two other predominant production outputs besides biochar are syngas and bio-oil. Syngas is a low-energy and low-density mixture of hydrogen (H) gas and carbon monoxide (CO), as well as methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>), water (H<sub>2</sub>O), and several low-molecular weight volatile organic compounds (Laird et al., 2009). Syngas can be used for energy production, but it has a lower heating value (~6 MJ kg<sup>-1</sup>) when compared to natural gas (~54 MJ kg<sup>-1</sup>) heating values (Laird et al., 2009). High-energy and high-density bio-oil is burned as fuel in specially equipped industrial boilers due to complications in burning, acidity, and its unstable nature (Laird et al., 2009). Bio-oil products can differ markedly based on the production conditions and the initial biomass product used. The percentages of the biomass that become biochar, syngas, and bio-oil are dependent on various conditions, including the temperature (typically 350°C to 700°C), the heating rate, gas purge rate, the pressure under which pyrolysis takes place, and the initial biomass composition (Amonette and Joseph, 2009).

Activated charcoal has endured steam or chemical oxidation after pyrolysis (Azargohar and Dalai, 2006; Raave et al., 2014). Activation can increase surface area and adsorption by removing reactive carbon that is blocking pores (Antal and Grønli, 2003). Steam activation can be difficult and not appropriate for all feedstocks and production temperatures, as observed by Gaskin et al. (2008) when peanut hull pellets excessively swelled and clogged the batch reactor. The activation process increases the product cost, with activated carbon costing around \$2,200

Mg<sup>-1</sup>, while non-activated biochar can cost between \$50 and \$390 Mg<sup>-1</sup> (Ghosh et al., 2011; Meyer et al., 2011).

Major differences between biochar production methods include the use or lack of water or steam, amount of atmospheric oxygen (O<sub>2</sub>) available, heating rate, time the biomass resides in the production chamber or retort, and temperature (Amonette and Joseph, 2009). Production machinery can include recovery equipment in order to use the combustible gas produced from one batch to continue powering the next batch (Laird et al., 2009). Torrefaction can be more useful for energy product as opposed to biochar (Spokas et al., 2012). Hydrothermal conversion processes involve biomass in a suspension with water and are capable of using wetter biomass products during the conversion process, meaning less drying is involved (Meyer et al., 2011; Spokas et al., 2012). Gasification more closely resembles traditional charcoal kilns in that it maximizes syngas production and the biomass is partially oxidized (Brown, 2009; Meyer et al., 2011). Fast pyrolysis is a rapid heating, continuous-flow system that maximizes bio-oil production on an industrial scale (Laird et al., 2009). The majority of thermal degradation of biomass that maximizes biochar production utilizes slow pyrolysis (Spokas et al., 2012).

Slow pyrolysis can be conducted in either a batch system, such as a charcoal kiln, or a continuous system. Slow pyrolyzers are less complex than fast pyrolyzers and can be built more easily on a smaller scale (Laird et al., 2009). The temperatures tend to be around or above 400°C and the process is conducted without oxygen (Laird et al., 2009; Meyer et al., 2011; Spokas et al., 2012). Moisture content and particle-size specifications of the feedstock are not crucial for the charcoal kiln method, but they do tend to be more important for continuous systems (Laird et al., 2009). Slow pyrolysis yields roughly 35% biochar, 30% bio-oil, and 35% syngas by mass, and the process is potentially highly energy efficient in that the syngas produced can be capable

of producing enough heat and electricity needed to continue running the pyrolysis system (Laird et al., 2009).

A wide variety of biomass products can be used as the feedstock for the pyrolysis process to produce biochar. Possible feedstocks range from a variety of high C biomass sources including crop residues, woody biomass, sewage sludge, poultry litters, and manures (Spokas et al., 2012). Although biochar can be produced with virtually any biomass as the feedstock, using a waste product can help manage the waste and recycle it back into the soil to benefit crop production. The feedstock choice and various pyrolysis conditions have significant effects on the end biochar product. The resulting highly stable, solid charcoal is typically produced either as pellets, fine powder, or granules.

### **2.3 Biochar Characteristics**

There are various changes that occur to the biomass feedstock during thermal degradation which typically coincide with incremental temperature increases (Amonette and Joseph, 2009). Under slow pyrolysis heating rates, which can include heating rates as slow as  $1\text{-}2^{\circ}\text{C min}^{-1}$ , mostly dehydration occurs until  $250^{\circ}\text{C}$  (Amonette and Joseph, 2009; Brown et al., 2006; Downie et al., 2009). In the temperature range of  $250\text{-}350^{\circ}\text{C}$ , complete cellulose depolymerization results in volatilization of gases such as  $\text{CO}_2$ ,  $\text{CO}$ ,  $\text{H}_2$ , and  $\text{CH}_4$  and mass loss, and an amorphous carbon matrix is formed (Amonette and Joseph, 2009; Antal and Grønli, 2003; Downie et al., 2009). Above  $350^{\circ}\text{C}$ , polyaromatic graphene sheets begin to replace the amorphous-C matrix, and above  $600^{\circ}\text{C}$ , carbonization predominates (Amonette and Joseph, 2009). Hydrogen and oxygen are lost in proportionally greater amounts than carbon, with the proportion of percent C by

weight potentially doubling after pyrolysis compared to the initial biomass concentration (Amonette and Joseph, 2009).

Slow pyrolysis-produced wheat straw biochar had greater C content than that produced by fast pyrolysis as reported by Bruun et al. (2012). The O:C and H:N ratios were 3-4 times greater with fast pyrolysis. Ultimately, it was concluded that the slow pyrolysis biochar had a lower total feedstock C loss when compared to the fast pyrolysis biochar, 43% to 57%, respectively (Bruun et al., 2012). Relatively lower heat treatment temperatures, slower heating rates, slower purge rates, increased pressures, and greater concentrations of lignin in the feedstock tend to produce greater biochar yields and impact the characteristics of the biochar product (Antal and Grønli, 2003; Demirbaş, 2001).

The graphene sheets, which are polycyclic aromatic hydrocarbons, have a large impact on the stability of biochar (Nguyen et al., 2010). The aromatic ring structures are considerably recalcitrant to microbial degradation (Schmidt and Noack, 2000; Zimmerman, 2010). The lingering presence of biochar in soils has prompted investigation to determine the residence time of C in soil. Lehmann et al. (2008) suggested that slow decomposition and high stability of biochar in soils with residence times of 1300-2600 years can be present, specifically in the dry Northern Australian climate in which the study was conducted.

Although biochar has been lauded as a highly stable form of C (Glaser et al., 2001; Lehmann et al., 2008), Cheng and Lehmann (2009) reported that biochar properties change over time. In their 12-month study, there were changes in biochar composition, surface chemistry, and adsorption; the one-year-old Oak (*Quercus* spp.) biochars had greater O<sub>2</sub> and lower C concentrations as well as lower pH and reduced ability to adsorb compounds onto their surfaces compared to fresh biochar. However, the changes seem to occur more on the biochar surface and

in the early stages of biochar incorporation in soils. Biochar incubated at 70°C experienced the greatest change during the 12-month study compared to fresh biochar, while biochar incubated at 30°C and 4°C exhibited fewer changes. Biochar incubated at -22°C showed detectable changes during the aging process, but the rate of change was slower than that of the other biochars incubated at greater temperatures. Since biochar's C backbone has been observed to experience similar stability when it was hundreds of years old compared to thousands of years old, the predominant changes over time occur with regards to the organic matter adsorbed to biochar, as well as the oxidation that occurs on the biochar surface (Cheng and Lehmann, 2009; Cheng et al., 2006, 2008; Liang et al., 2006, 2008).

The remaining inorganic minerals and nutrients, such as P, K, Ca, Zinc (Zn), and other macro- and micronutrients, left after biomass has been oxidized at high temperatures are called ash (Brown, 2009). Mineral matter, or ash, tends to be concentrated in the biochar compared to the original biomass and its percent by weight also increases proportionally. Ash contents can vary from less than 2% to over 70% by weight, with woody biomass feedstock biochars on the low end, grasses and grains proportionally more, and manures and biosolid wastes generally with the largest percent ash contents (Koutcheiko et al., 2007; Skodras et al., 2006; Spokas and Reicosky, 2009; Spokas et al., 2011). Lower temperature pyrolysis biochars, produced at temperatures less than 500°C, tend to store more C and nutrients from the feedstock, react more readily in soils, and better aid soil fertility when compared with biochars produced at temperatures greater than 500°C (Keiluweit et al., 2010; Steinbeiss et al., 2009).

High-ash biochars can have lower surface areas because the ash blocks biochar micropores (Antal and Grønli, 2003; Bruun et al., 2012; Joseph et al., 2009). In addition to ash contents, heating temperature can be responsible for differing biochar surface area and porosity.

Biochar surface area tends to increase with increasing highest treatment temperature (HTT) until a maximum is reached, and then additionally greater temperatures can lead to surface area decreases in some cases (Brown et al., 2006; Downie et al., 2009). Biochar produced with HTTs around 400-450°C has been detected with a small surface area of less than 10 m<sup>2</sup> g<sup>-1</sup> (Brown et al., 2006; Downie et al., 2009; Keiluweit et al., 2010). Large biochar surface areas range from about 400-500 m<sup>2</sup> g<sup>-1</sup> at HTTs of 500°C-900°C in various studies (Antal and Grønli, 2003; Brown et al., 2006; Keiluweit et al., 2010). Biochars with HTTs close to 1000°C had average surface areas in the 200-400 m<sup>2</sup> g<sup>-1</sup> range when faster heating rates (500°C h<sup>-1</sup> to 1000°C h<sup>-1</sup>) were used, but less than 100 m<sup>2</sup> g<sup>-1</sup> when heating rates were slower (350°C h<sup>-1</sup> or less). Temperatures above 550°C result in volatilized residual matter that is responsible for blocked micropores (Antal and Grønli, 2003). Additionally, the internal gases that escape from the feedstock during pyrolysis are suggested to be responsible for biochar's observed high porosity, which contributes to a large surface area (Day et al., 2004).

Biochar has the potential to provide various environmental and agronomic benefits due to its physical and chemical characteristics, such as a large surface area, stable aromatic C components, and high porosity (Schmidt and Noack, 2000). These benefits and various studies are presented in the following section.

## **2.4 Biochar and Agriculture**

Agronomic benefits of biochar include water retention and moisture holding capacity improvements, increases in soil pH, increased nitrification, greater levels of bioavailable nutrients such as P, K, Ca, and Zn, and decreased leaching of nutrients (Ball et al., 2010; Berglund et al., 2004; Chan et al., 2007; Hass et al., 2012; Laird et al., 2010; Lehmann et al.,

2003; Sinclair et al., 2008; Streubel et al., 2011). Increases in cation exchange capacity (CEC), enhanced microbial activity, and a general increase in agricultural productivity including greater plant yields have also been observed in biochar-enriched soils (Amonette and Joseph, 2009; Atkinson et al., 2010; Gundale and DeLuca, 2006; Kolb et al., 2009; Lehmann et al., 2011; Liang et al., 2006; Steinbeiss et al., 2009). Chan et al. (2007) measured reduced soil tensile strength and increased field moisture capacity with biochar additions. Since biochar can have highly porous surfaces and large surface areas, it can be important for providing habitat for soil microorganisms and binding nutrients (Atkinson et al., 2010; Koutcheiko et al., 2007; Ogawa, 1994; Pietikäinen et al., 2000; Steiner et al., 2008b; Warnock et al., 2007). Similar to biochar's properties, the agronomic effects greatly depend on the feedstock and production conditions, the soil properties, the plant requirements, and the longevity of the study (Spokas et al., 2012).

Although biochars chosen for use as soil amendments tends to have alkaline properties, biochar can exhibit a wide range of pH levels, from about 4-12 (Cheng et al., 2006; Lehmann, 2007; Spokas et al., 2012). A change in soil pH depends on the soil buffering capacity and pH of the biochar itself. Sandy soils have lower buffering capacities, thus sandy soils will have rapid and more substantial pH changes (Streubel et al., 2011). Streubel et al. (2011) concluded, from research in Washington, that biochars produced from different feedstocks are capable of affecting soil pH differently. Herbaceous biochar raised soil pH (by 0.4 to 0.8 units) more than woody biochars (by 0.1 to 0.4 units) in all soils observed, including a sandy soil and various silt-loam soils. This pH change is presumably due to the pH of the biochar (9.4 for herbaceous and 7.4 for woody) and greater ash concentrations with the herbaceous feedstocks (Streubel et al., 2011).

The increase in soil pH from biochar can aid in decreasing liming requirements and can be influenced by the amount of biochar applied. The soil pH change tends to become more significant at greater application rates, such as 40 g biochar kg<sup>-1</sup> soil compared to 5 g kg<sup>-1</sup> or 39 Mg ha<sup>-1</sup> compared to 9.8 Mg ha<sup>-1</sup> (Hass et al., 2012; Streubel et al., 2011). Using greenwaste biochar in an Alfisol, soil pH increased with each increasing biochar application (10, 50, 100 Mg ha<sup>-1</sup>) in the treatment with biochar and no fertilizer, but increases were only observed in the biochar and N fertilizer treatment with the 100 Mg ha<sup>-1</sup> biochar rate (Chan et al., 2007). Without N fertilizer, the pH increased from 4.8 with no biochar to 6.0 with 100 Mg ha<sup>-1</sup>. With N fertilizer, the corresponding pH increase was from 4.6 to 5.2 (Chan et al., 2007).

A pH increase by 1.0 to 1.5 units was observed in three different Japanese soils using woody feedstock biochar applied at a 37 Mg ha<sup>-1</sup> rate (Yamato et al., 2006). In a three-year study in a temperate-climate sandy clay loam, significant increases in soil pH were detected from applications of woody biochar at rates of 25 and 50 Mg ha<sup>-1</sup> compared to no biochar in years two (7.2 with biochar, 6.9 without) and three (6.6 with biochar, 6.4 without) of the study (Jones et al., 2012). However, decreases in soil pH have been observed in other studies (Gaskin et al., 2010; Jones et al., 2012). In a two-year experiment in Georgia, Gaskin et al. (2010) investigated the effects of peanut (*Arachis hypogaea* L.) hull and pine (*Pinus* spp.) chip biochars on corn (*Zea mays* L.) production in loamy sand. When the alkaline biochars were applied at rates of 11 and 22 Mg ha<sup>-1</sup>, the soil pH decreased from year one to year two at soil depths of 0-15 cm and 15-30 cm. Gaining better understanding of soil changes over time is important for knowing the long-term implications of biochar addition to soils.

Biochar has been observed to readily adsorb nutrients onto its surface (Mizuta et al., 2004; Lima and Marshall, 2005). This adsorption can be enhanced by biochar's potentially large

surface area and its subsequent affinity for cation exchange. Oxidation of the biochar occurs over time in soils, which, in addition to adsorbed organic matter onto biochar's surface, enhances soil CEC (Cheng et al., 2006). Cheng et al. (2006) concluded that the formation of carboxylic functional groups caused significant enhancement in CEC during biochar oxidation.

Increases in soil CEC have been observed with biochar addition (Chan et al., 2007; Van Zwieten et al., 2008, 2010). In soils with biochar, CEC has been nearly twice as great as in nearby soils of the same mineralogy but lacking biochar (Liang et al., 2006). A study was conducted in an Alfisol in Australia investigating soil quality after application of two rates of N fertilizer, 0 and 100 kg N ha<sup>-1</sup>, and four rates of greenwaste biochar, 0, 10, 50 and 100 Mg ha<sup>-1</sup>, made from the pyrolysis of grass clippings, plant cuttings, and cotton (*Gossypium* spp.) trash at 450°C (Chan et al., 2007). Results showed that soil CEC increased as the application rate of biochar increased in the treatments with and without N fertilizer. Other feedstock biochars have been responsible for varying CECs. Poultry litter biochar has been reported to have a CEC over 60 cmol kg<sup>-1</sup>, while the CEC of peanut hulls and pine chips can be as low as 5 cmol kg<sup>-1</sup> (Gaskin et al., 2008).

It has been proposed that about half of the N in the original feedstock biomass is transferred to the biochar, while the other half is transferred to the bio-oil during pyrolysis (Laird et al., 2009). This suggests that N-rich feedstocks will produce N-rich biochar products. However, the N is predominantly in a highly stable, heterocyclic form, leading to slow release of N into soils from biochar and thus minimal plant availability (Koutcheiko et al., 2007). However, decreased leaching of N fertilizer and increased nutrient availability have been observed (Lehmann et al., 2003; Steiner et al., 2008b). In an experiment with treatments containing both biochar and compost, biochar adsorbed nutrients onto its surface, specifically decreasing N

leaching and increasing N-use efficiency (Steiner et al., 2008b). Another study observed the adsorption of phenolic compounds onto the surface of biochar, which inhibited nitrification and reduced available N for plants (Cheng et al., 2008).

The application of fertilizer and wood biochar at a 37 Mg ha<sup>-1</sup> application rate induced changes in soil chemical properties by increasing total N and available P<sub>2</sub>O<sub>5</sub> concentrations, CEC, amounts of exchangeable cations, and base saturation compared to the fertilizer-only treatment (Yamato et al., 2006). Organic C, Colwell P, and exchangeable K increased as the application rate of greenwaste biochar to an Alfisol increased from 0 to 10, 50 and 100 Mg ha<sup>-1</sup> in treatments with and without 100 kg N ha<sup>-1</sup> fertilizer (Chan et al., 2007). Peanut hull biochar increased soil N, P, K, Ca, and Mg in loamy sand, while pine chip biochar had less impact in terms of nutrient concentrations (Gaskin et al., 2010). When compared to adjacent soils of similar mineralogy but lacking biochar, an increase in K, Ca, and total P was observed in biochar-amended Anthrosols in Brazil (Liang et al., 2006).

In addition to nutrient changes, water-holding capacity can vary depending on soil textures in which biochar is used. Adding biochar to silt loam was observed to increase water-holding capacity, but an effect was not observed in sand (Streubel et al., 2011). Switchgrass (*Panicum virgatum* L.) biochar consistently increased water-holding capacity, whereas woody biochars only produced significant effects with the largest application rate, 39 Mg ha<sup>-1</sup>, and with the silt loams with greater percentages of clay (Streubel et al., 2011). Tensile strength decreased and field moisture capacity increased in an Alfisol, both at increasing rates of biochar production, but only significantly with application rates of 50 and 100 t ha<sup>-1</sup> (Chan et al., 2007). In a 65% clay Oxisol, no change was observed in terms of water percolation (Lehmann et al., 2003). Although no differences were observed in saturated hydraulic conductivity in a Hapludoll,

hardwood biochar-amended soils retained more water at field capacity equilibrium and at less saturated soil matric potentials of -100 and -500 kPa (Laird et al., 2010).

Despite many agronomic benefits, biochar has differing effects on plant growth and yield. For example, various yield increases have occurred when N fertilizer was combined with biochar additions (Asai et al., 2009; Blackwell et al., 2007; Chan et al., 2007, 2008; Rajkovich et al., 2012; Sinclair et al., 2008; Steiner et al., 2007; Van Zwieten et al., 2008). Van Zwieten et al. (2008) attributed improved corn yield to enhanced fertilizer-use efficiency in the presence of poultry litter and paper mill biochars. Poultry manure or litter biochars have been shown to improve yield in mustard (*Brassica juncea* L.), soybean, and corn even without fertilizer when compared to no biochar controls or only fertilizer treatments (Park et al., 2011; Tagoe et al., 2008; Van Zwieten et al., 2008). Wheat (*Triticum aestivum* L.) germination in a sand in Australia was increased with 10 Mg ha<sup>-1</sup> application of certain biochars [oil mallee waste, rice (*Oryza sativa* L.) husk, and wheat straw] but not others (wood waste), while germination was inhibited for wheat and clover (*Trifolium subterraneum* L.) at the 100 mg ha<sup>-1</sup> application rate for all biochars (Solaiman et al., 2012). Mung bean (*Vigna radiate* L.) and clover germination also decreased with 10 Mg ha<sup>-1</sup> for most biochars. Yamato et al. (2006) observed a two-fold increase in corn yield in an Indonesian soil with fertilizer application and biochar from *Acacia mangium* compared to the fertilizer-only treatment, and peanut yield also increased significantly. The fertilizer rate, 75 kg ha<sup>-1</sup> of 15-15-15, was approximately half of the standard rate, and it was suggested that with the use of the 37 Mg ha<sup>-1</sup> woody biochar, the necessary fertilizer amount could be reduced below the standard rate (Yamato et al., 2006). In another study, only at the greater application rates of 50 and 100 Mg greenwaste biochar ha<sup>-1</sup> and N fertilizer were significant increases in radish (*Raphanus sativus* L.) yield observed (Chan et al., 2007). Biochar

addition alone did not significantly affect yield but perhaps aided in improving fertilizer-use efficiency when paired with the addition of N fertilizer.

Other studies have shown little or no improvement in plant growth or yield compared to non-biochar-amended soils. Some studies even detected decreases in yield (summarized in Spokas et al., 2012). Corn grain yields decreased at the largest application rate, 22 Mg ha<sup>-1</sup>, of peanut hull biochar with fertilizer treatment in loamy sand (Gaskin et al., 2010). With pine chip biochar, corn yields decreased with increasing application rates (0, 11, and 22 Mg ha<sup>-1</sup>), but only in the first year of the two-year study. Van Zwieten et al. (2010) observed decreases in wheat biomass with paper mill waste biochar addition to soils with and without fertilizer and no affect on soybean (*Glycine max* L.) with biochar and no fertilizer treatment. There were no differences with woody biochar addition at rates of 25 and 50 Mg ha<sup>-1</sup> during the first year cropping of corn in sandy clay loam with regards to grain N, foliar N, total dry biomass, and crop height, and only a significant difference in foliar N of grass (*Dactylis glomerata* L.) was observed in year two (Jones et al., 2012). However, biochar increased microbial growth rate by year three of the trial, and significant increases in aboveground grass biomass were observed in the third year as well (Jones et al. 2012).

## **2.5 Biochar and Soil Microorganisms**

Soil microorganisms play an integral part in soil ecosystems. Microorganisms decompose plant tissues and integrate animal manures, various soil amendments, and organic materials into the soil. Microorganisms synthesize compounds that stabilize soil aggregates and aide in humus formation, thus improving soil tilth and structure (Brady and Weil, 2008). Microorganisms digest and excrete nutrients such as N, P, K, and sulfur (S) into soil solution, which turns organically

bound compounds into minerals accessible for plants. Microorganisms produce enzymes that break down toxic compounds and therefore detoxify the soil. Microorganisms are instrumental in oxidation and reduction reactions involving compounds with S, N, iron (Fe), and Mn, altering forms and thus availability of these nutrients for other soil inhabitants.

Understanding the important attributes and functions of soil organisms is approached in various ways. Abundance is measured for soil individuals per unit area, biomass per unit volume or area of soil can be measured, and metabolic activity, such as respiration, can be determined. Multiple approaches are frequently undertaken in microbial ecology because of the challenges of acquiring understanding of microscopic organisms in a physically and chemically complex and dynamic medium. Examining diversity is also useful. High species diversity arises when there are many species with a roughly even distribution. A high degree of functional diversity means that the microbial community has the ability to utilize a wide variety of substrates and carry out an assortment of processes (Brady and Weil, 2008). In soils with healthy microbial activity, different species simultaneously carry out the thousands of enzymatic and physical processes. This functional redundancy contributes to stable and resilient soil ecosystems (Brady and Weil, 2008).

Microorganisms are the most numerous of the different size groups of soil organisms. For example, the prokaryotes Bacteria and Archaea, taken as a group, can have numbers ranging from  $10^{14}$  to  $10^{15}$   $m^{-2}$  and 40 to 500  $g\ m^{-2}$  biomass (Brady and Weil, 2008). It is difficult to count fungi in soil because of the extensive filamentous morphology, which is why scientists use biomass or hyphal length  $m^{-2}$ . Fungal biomass may range between 100 and 1500  $g\ m^{-2}$ . This is significant compared to the biomass of soil macroorganisms like earthworms. Earthworms can number between 10 and  $10^3$   $m^{-2}$  with biomass between 10 and 400  $g\ m^{-2}$  (Brady and Weil, 2008).

Microbes provide many positive contributions to the soil ecosystem, but occasionally they can be a detriment to higher plants. The competition between microbes and plants includes the struggle over soluble nutrients and oxygen in soils with poor aeration. Bacteria and fungi are responsible for the majority of plant diseases originating in soils, including root rots, leaf blights, wilts, and crown gall disease. Some rhizobacteria are responsible for stunted plant growth, wilting, nutrient deficiency, and even eventual plant death.

Rhizobacteria are the bacteria that coat the surface of roots and the surrounding soil volume and thus are involved in many of the plant-soil interactions, including nutrient uptake, disease inhibition, and hormone stimulation. The plant root zone houses significant populations of microorganisms, averaging two to ten times more microbes in the rhizosphere than in bulk soil (Brady and Weil, 2008). Mycorrhizal fungi form symbiotic relationships with plant roots, allowing the fungi to receive C from plants while the plants can access much greater amounts of water and nutrients through the increase in root surface area by fungal hyphae.

Although it is evident that microorganisms are an integral part of soil health and fertility, less is known about the effects of biochar on microorganisms. There have been some studies investigating these effects, including community structure and diversity, abundance, biomass, and metabolic activity, and the effects on specific soil organisms such as mycorrhizal fungi.

Researchers have investigated the effects of the Anthrosols of the Amazon River basin on microbial abundance, diversity, and activity (Grossman et al., 2010; Kim et al., 2007; O'Neill et al., 2009). In addition to the numerous benefits related to soil quality and tilth attributed to these soils, large bacterial populations have been observed to lower soil depths and greater species richness when compared to nearby soils lacking biochar (O'Neill et al., 2009). Bacterial species richness was 25% greater in one study using oligonucleotide fingerprint grouping of 16S

ribosomal ribonucleic acid (rRNA) gene (Kim et al., 2007). Grossman et al. (2010) observed that the Anthrosols in Brazil also have distinctly different microbial communities than the adjacent soils lacking biochar. The bacterial community differed based on denaturing gradient gel electrophoresis (DGGE) banding patterns by about 80% and Archaea differed by over 90%. Community deoxyribonucleic acid (DNA) was amplified using nested polymerase chain reaction (PCR) of the 16S rRNA V3 region for Bacteria and Archaea.

Lehmann et al. (2011) presented a summary of possible mechanisms that could affect microbial abundance. These mechanisms include protection from grazers, improved hydration, greater nutrient availability, pH alteration, sorption of signaling or inhibitory compounds, as well as sorption of microorganisms or dissolved organic matter used as microorganism energy sources. Because of biochar's highly porous nature, high internal surface area, and capacity for adsorption of inorganic nutrients and organic matter, biochar can serve as a habitat for microorganisms. Bacteria, actinomycetes, and arbuscular mycorrhizal fungi could potentially colonize, grow, and multiply within or on the surface of this biochar habitat, which could serve for some as a refuge from larger predators (Thies and Rillig, 2009). For example, Ogawa (1994) noted that charcoal's smaller pores could house bacteria and the larger pores and cracks could aid other microorganisms like molds.

Research with forest soils containing recent charcoal deposition (i.e., a forest fire within a 94-year period) showed increased ammonia-oxidizing bacteria (AOB) abundance compared to soils without recent charcoal addition (Ball et al., 2010). The O<sub>a</sub> horizon showed the greatest difference, with a 12.3-fold greater AOB abundance in the charcoal soil versus the soil without recent charcoal addition, while the A horizon comprised the greatest AOB abundance with  $4.90 \times 10^3$  cells g<sup>-1</sup> soil in recently burned soils compared to  $1.03 \times 10^3$  cells g<sup>-1</sup> in the control. The

researchers suggested that the increase in abundance was due to either pH alteration resulting in a more conducive habitat or charcoal adsorption of compounds that inhibit microbial activity.

Pietikäinen et al. (2000) used biochar from Black Crowberry (*Empetrum nigrum* L.) twigs and from forest humus to simulate a wildfire-produced charcoal layer atop a forest humus layer in a microcosm experiment. A newly forming litter layer was also applied on top of all of the treatments. Both of the biochar layers contained greater numbers of bacterial cells than the pumice control,  $6.3$  and  $7.5 \times 10^8$  cells  $g^{-1}$  dry matter (dm) compared to  $2.2 \times 10^8$  cells  $g^{-1}$  dm in the control. The bacterial growth rate in the twig biochar and the humus biochar was around  $24$  and  $35 \text{ mol } g^{-1} \text{ h}^{-1} \times 10^{-12}$ , respectively (measured as thymidine incorporation), compared to only around  $7 \text{ mol } g^{-1} \text{ h}^{-1} \times 10^{-12}$  in the pumice control (Pietikäinen et al., 2000). Biochar is known to adsorb organic compounds that microbes decompose, so the biochar perhaps provided a new habitat for these organisms. Basal respiration activity of the humus beneath the twig biochar was significantly greater than that of the control and the humus biochar (Pietikäinen et al., 2000). The amount of microbial biomass in the humus layer was not affected by the presence of a biochar layer. However, the microbial community in the humus layer under twig biochar differed from the community in the control treatment, presumably due to an increase in humus pH by twig biochar. The increases in basal respiration activity and the microbial communities' substrate utilization patterns in the underlying humus were, as Pietikäinen et al. (2000) suggested, a result of the increase of humus pH from 4.3 to greater than 5 affected by the twig biochar.

The pH of biochar also affected the soil microbial communities in other studies. Fungi generally can tolerate lower pH, while bacteria prefer more near neutral or slightly alkaline pH (Thies and Rillig, 2009). Ogawa and Okimori (2010) summarized various charcoal experiments conducted in Japan. They noted that charcoal fertilizers with a pH greater than 8 altered

microbial populations. Contrary to Thies and Rillig (2009), soil fungi were inhibited in one study, but bacterial and actinomycetes propagation was enhanced. Two months after application, the communities began to return to the pre-application state.

When observing the effects of yeast-derived biochar on microbial communities, fungal biomass increased by 16% when compared to initial soil samples from arable and forest soils, and Gram-positive and Gram-negative bacteria decreased by 7-14% (Steinbeiss et al., 2009). Yoo and Kang (2012) also observed positive effects on fungi in an eight-week laboratory experiment. With soils containing barley (*Hordeum vulgare* L.) stover biochar, there was a smaller total fungal biomass in pasture soil when compared to the control soil, while a larger total fungal biomass in rice paddy soils. The suggested mechanism for these results was that materials beneficial for fungal growth in the biochar were effective in the rice paddy soil, while materials toxic to fungi were more effective in pasture soil (Yoo and Kang, 2012). To better observe whether the change in fungal abundance would play a role in nutrient retention or plant growth, Yoo and Kang (2012) suggested additional longer-term experiments and field trials with plants.

In the same study by Yoo and Kang (2012), the addition of swine manure biochar decreased the soil C:N ratio from about 11 to 3 and enhanced microbial biomass N from about 14 to 38  $\mu\text{g N g}^{-1}$  soil compared to the control, suggesting microbial immobilization of N and a possible shift in community structure. There was, however, an increase in net mineralization of N by 119% in rice-paddy soil and by 61% in pasture soil when compared to the control soil, which suggested that manure biochar could supplement N-fertilizers when added to soil. There was no change in microbial biomass C in swine manure biochar-added soils, which was consistent with the poultry litter biochar results from Van Zwieten et al. (2009). Van Zwieten et

al. (2009) observed a decrease in microbial biomass C by 40% with greenwaste biochar, but microbial biomass was not affected by poultry litter biochar amendment during a 72-hour incubation study. With the application of wood biochar to a highly weathered, nutrient-poor, Amazonian upland soil, an increase in microbial biomass was observed from about  $252 \mu\text{g C g}^{-1}$  soil with no biochar added to about  $290 \mu\text{g C g}^{-1}$  with  $150 \text{ g of biochar kg}^{-1}$  soil (Steiner et al., 2008a). Basal respiration increased with the same rate of applied charcoal from a little over 2 to almost  $4.5 \mu\text{L CO}_2 \text{ h}^{-1} \text{ g}^{-1}$ .

In a three-year study in sandy clay loam, woody biochar at application rates of 25 and 50  $\text{Mg ha}^{-1}$  significantly increased soil respiration and fungal and bacterial growth rate in year two (Jones et al., 2012). Bacteria growth increased by about 80% from year one to year two, while fungal growth rate increased by about 21%, with an overall decrease in the fungal-to-bacterial growth ratio by over 30% (Jones et al., 2012). Significant differences between the control and biochar soils were no longer observable in year three.

Manure plus pine biochar was added to four temperate region soils (i.e., clay loam, sandy loam, loamy sand, and silt loam) in a microcosm experiment (Kolb et al., 2009). Microbial biomass and activity increased significantly in all soils with increasing charcoal application, from 0-0.1  $\text{kg kg}^{-1}$ , and basal respiration increased over a three month period. Bray P increased over time with increasing charcoal rates in all soils. Except for the sandy loam, extractable N tended to decrease with increasing charcoal application rates at one and a half months and at three months (Kolb et al., 2009). As was also observed by Berglund et al. (2004), increases in microbial activity caused immobilization of N because of the high N demand and nutrient limitations (Kolb et al., 2009).

Thies and Rillig (2009) speculated that the soil microorganisms that colonize freshly

applied biochar still containing post-pyrolysis substances on its surface will differ from those that colonize after these condensates, such as water-soluble compounds like alcohols, acids, and sugars, and ash have been metabolized. The later colonizers use the adsorbed C substrates and inorganic nutrients on biochar surfaces. Berglund et al. (2004) also conjectured that soluble organic substances adsorb onto charcoal and serve as a food supply for microorganisms.

An increase in colony number of bacteria (from almost  $10 \times 10^6$  to over  $30 \times 10^6$ ) was observed in tropical soil sprayed with charcoal powder and in N-fixing bacteria (from around  $5 \times 10^6$  to  $10 \times 10^6$ ) when compared to the control soil lacking charcoal (Ogawa, 1994). After the inoculation of common beans (*Phaseolus vulgaris* L.) with *Rhizobium* bacteria in a Haplustox, the proportion of N from biological N-fixation increased from 50% with no biochar amendment to 72% with  $90 \text{ g kg}^{-1}$  biochar amendment (Rondon et al., 2007). Rondon et al. (2007) suggested that the reasons for the increase in biological N-fixation are an increase in boron (B) and molybdenum (Mo), as well as other nutrients to a lesser extent, and low levels of N availability due to the high C:N ratio of the evergreen wood biochar.

Kolton et al. (2011) used DGGE of 16S rRNA gene fragments to determine that, although there were no significant differences in genus richness levels between the sandy soil with  $60 \text{ Mg ha}^{-1}$  wood biochar and the control (198 and 172 genera, respectively), select genera were significantly different from one treatment compared to the control. *Flavobacterium* abundance increased by an average of 15.4% in  $60 \text{ Mg ha}^{-1}$  biochar-amended samples. *Flavobacterium* are important in mineralizing organic matter, such as carbohydrates, amino acids, proteins, and polysaccharides (Kolton et al., 2011). Other genera that were positively affected by biochar-amended soil were chitin and cellulose degraders. *Chitinophaga* relative abundance increased from 0.05% of total root-associated operational taxonomic units in the control to 0.5% in the

biochar-amended soil. *Cellvibrio* relative abundance increased from 0.06% of total root-associated operational taxonomic units in the control to 1.6% in the biochar-amended soil. Aromatic compound degraders, such as *Hydrogenophaga* and *Dechloromonas*, experienced increased abundance from 0.2 to 0.7% and 0.06 to 1.6%, respectively. From the biochar analysis, it was evident that various aromatic compounds were present in the biochar, providing a possible explanation for the increase in abundance (Kolton et al., 2011). Negative effects were served with the genus *Pseudoxanthomonas*, in which multiple species are known plant pathogens. The abundance of operational taxonomic units of this genus was reduced 5.7%. Proteobacteria phylum relative abundance decreased from 72% in the control to 48% in the biochar-amended samples (Kolton et al., 2011).

Warnock et al. (2007) proposed four mechanisms explaining how biochar alters the total abundance and activity of mycorrhizal fungi in soil as well as in plant roots. First, the addition of biochar into soils alters nutrient availability or other physical or chemical factors that in turn affect plants and mycorrhizal fungi. Additionally, there can be alterations in soils with beneficial or detrimental effects on other soil microorganisms that can then have an indirect effect on the mycorrhizae. Biochar can also alter root colonization by the fungi by disturbing plant-mycorrhizal fungi signaling. Lastly, biochar can act as a refuge for fungi and bacteria as a way to escape predators. Warnock et al. (2007) mentioned that not enough research has been done to fully understand these relationships and to make definite conclusions regarding any of the mechanisms.

Mycorrhizae can suffer from biochar additions. In fact, application of pine wood biochar at rates of 40 and 80 Mg ha<sup>-1</sup> contributed to declines in arbuscular mycorrhizal fungal (AMF) abundance in narrowleaf plantain (*Plantago lanceolata* L.) roots by 58 and 73%, respectively

(Warnock et al., 2010). Along with the decline in AMF infection of the roots was a decrease in soil P availability, 28% with 40 mg ha<sup>-1</sup> and 34% with 80 Mg ha<sup>-1</sup> biochar, which was suspected to be due to adsorption of P from the soil onto the low P-containing pine wood biochar. Warnock et al. (2010) also studied three peanut shell biochars and mango wood (*Mangifera indica* L.) biochar and observed increases in P, but decreases in AMF root abundance. At a 200 Mg ha<sup>-1</sup> addition rate of peanut shell biochar produced at 360°C, there was a 101% increase in P, 74% decrease of AMF root colonization, and 95% decrease in extraradical hyphal lengths. Field application rates of 23.2 and 116.1 Mg ha<sup>-1</sup> of mango wood biochar resulted in an increase in P by 163 and 208% and a decrease in AMF abundance in soil by 43 and 77%, respectively. Phosphate desorption from the greater P-rich peanut shell biochars and mango wood biochar could have been responsible for the increases in P availability, but the mechanisms behind the declines in AMF abundance were unclear in both experiments (Warnock et al., 2010).

Others have supported various mechanisms proposed by Warnock et al. (2007), including the suggestion that the biochar pores are a habitat for colonizing microbes, protecting them from grazers and allowing them to propagate (Ogawa, 1994; Saito and Marumoto, 2002). The inorganic, porous nature of biochar enhanced the effectiveness of AMF in aiding plant growth by increasing the amount of larger pores in a Japanese subsoil (Ezawa et al., 2002). Ezawa et al. (2002) observed that AMF colonization doubled from 20% to 40% in the presence of rice-hull biochar when compared to the control soil with no biochar.

From the available literature, it is evident that biochar alters soil ecosystems at scales relevant to microbes, but it is still unclear how this is achieved. There is evidence of positive and negative effects on microorganism activity, abundance, diversity, and community structure, but more research is necessary to better understand how changes occur in the soil ecosystem. With

an emphasis on soil ecology, many feedstock types and production procedures of biochar need to be investigated to observe the effects on soil biota from many different biochars (Lehmann et al., 2011). These studies should also include, according to Lehmann et al. (2011), documentation about microbially available C, biochar surface area, pore-size distribution, pH, ash content, and an analysis of the elements and nutrients present and available.

## **2.6 Biochar's Potential Impact in Arkansas**

Poultry manure and litter biochar could have an enormous impact in the state of Arkansas. In 2013, Arkansas ranked third in the nation for the number of turkeys and broiler chickens produced; of the 8.52 billion broilers produced in the country, Arkansas grew nearly 1 billion (NASS, 2014). Each broiler, weighing 2.2 to 2.7 kg at the harvest age of 7 weeks, produced an average of 1.18 kg of litter (i.e., manure and bedding) during its lifetime, so the total litter production in 2011 in Arkansas was almost 1 billion kg, just with broilers (Moore et al., 2011).

Poultry manure contains many essential plant nutrients and is an excellent fertilizer (Chan et al., 2008; Harmel et al., 2009; Williams et al., 1999). On an “as-is” basis, opposed to a dry weight basis that would be corrected for moisture content, Sharpley et al. (2009) categorized various poultry manures as having total N contents ranging from 1.0 to 4.4%, total P varying from 0.62 to 2.6%, and K between 1.1 and 3.4%. Poultry litter is known to increase organic matter content in soils while improving the water-holding capacity, increasing the oxygen diffusion into the soil, and improving soil aggregation (Adeli et al., 2009).

Unfortunately, the application of poultry manure or litter onto agricultural lands can lead to environmental problems. Poultry litter typically includes not just the manure, but also bedding

material, feathers, and feed, as well as plant nutrients, pesticide residues, pharmaceuticals, endocrine disruptors, and microorganisms (Bolan et al., 2010). The poultry litter can contain potentially toxic levels of trace elements such as arsenic (As), copper (Cu), and zinc (Zn) (Jackson et al., 2003; Subramanian and Gupta, 2006; Toor and Haggard, 2009). Sharpley and Moyer (2000) determined that dissolved inorganic P comprised 74% or more of the P in poultry manure and litters used in their study, which was consistent with other reported ranges (e.g., Cooke et al., 2011). The P leaching potential was closely related to the water extractable inorganic P concentration (Sharpley and Moyer, 2000), also confirmed by Sauer et al. (2000). Leaching losses of N and P can contaminate surface water and groundwater as well as increase the metal content in soils (Harmel et al., 2009; Pote et al., 2003; Sharpley et al., 2007; Vories et al., 2001; Williams et al., 1999). Much of the poultry litter has been applied historically to meet crop N requirements, which results in a large buildup of P that can be lost in runoff (Vories et al., 2001). Surface runoff and groundwater containing increased levels of N and P from the poultry manure can cause eutrophication of lakes, ponds, and other bodies of water. This, in turn, causes algal blooms, low dissolved O<sub>2</sub> levels, greater turbidity, increased sedimentation, and even fish death (Cooke et al., 2011; Sharpley et al., 1998, 2001).

Charring poultry litter could provide a solution to the P contamination of water sources. Since biochar is recalcitrant in nature as described earlier, it is possible that the N and P nutrients would not as readily leach from the biochar as they do from the poultry litter or manure form when applied to soils. Unfortunately, research is lacking in this area. Research that has been conducted has actually discovered that N is not available in the biochar at rates that are sufficient for healthy plant growth (Chan et al., 2008). Examples of the research with poultry litter biochar are discussed in the following paragraphs.

Poultry litter biochar applied to an Alfisol at rates of 10, 25, and 50 Mg ha<sup>-1</sup> was observed to increase total dry matter of radishes, even without N fertilizer, as well as increase the N, P, S, Ca, sodium (Na), and magnesium (Mg) concentrations in the plants (Chan et al., 2008). Radish yield increased by 42% with 10 t ha<sup>-1</sup> and by as much as 96% with 50 t ha<sup>-1</sup> biochar application when compared to the control. However, there was still N deficiency without the addition of N fertilizer (Chan et al., 2008). The combination of N fertilizer and biochar produced yield increases even greater than those observed with N fertilizer alone, suggesting the ability of biochar to increase N availability and uptake efficiency (Chan et al., 2008). The alkaline poultry litter biochars used by Chan et al. (2008) significantly increased soil pH. It is important to note, however, that the pot trial lasted only six weeks and thus data for soil pH is relevant to short-term change.

In a comparison study of multiple different biochars produced from plant residues [i.e., hazelnut (*Corylus* spp.) shells, pine, oak, and corn stover] and wastes products (i.e., paper waste, food waste, dairy manure, and poultry litter), poultry litter biochars produced at slow-pyrolysis temperatures of 300, 400, 500, and 600°C were investigated (Rajkovich et al., 2012). The lowest pyrolysis temperature biochar retained the most N from the feedstock compared to those produced from the greater temperatures (21.5 compared to 9.4 mg N g<sup>-1</sup> biochar), and it also had the lowest pH (8.1 compared to the 10.7 of the 600°C biochar). Significantly greater amounts of P were contained in all of the poultry litter biochars compared to the other types of biochars observed. The 400°C biochar had the least P of the poultry litter biochars with almost 18 g P kg<sup>-1</sup> biochar while the 500°C poultry litter biochar had 30.5 g P kg<sup>-1</sup> biochar (Rajkovich et al., 2012). The biochar with the next greatest P concentration was 600°C dairy manure with 8.3 g P kg<sup>-1</sup>. When these biochars were used in a short-term corn trial, the tissue-N concentrations increased

only when grown in soil with 300°C poultry litter biochar. The largest tissue-N concentrations were 16.0 mg N g<sup>-1</sup> with 2.6 Mg biochar ha<sup>-1</sup> application rate and 15.2 mg N g<sup>-1</sup> with 91 Mg biochar ha<sup>-1</sup> (Rajkovich et al., 2012). With the majority of the biochars produced from poultry litter and other feedstocks, there was a negligible or negative effect on tissue-N concentrations. Other negative effects were measured in this trial with certain biochars. Food waste biochar produced at all four temperatures generally decreased plant biomass compared to no biochar addition. At the greatest application rate of 91 Mg biochar ha<sup>-1</sup>, corn total biomass decreased in the presence of dairy manure, paper mill waste, and food waste biochars (Rajkovich et al., 2012). This research was conducted in pots in a greenhouse, so the potential effects of these biochar products in a field trial are not known from this study.

The elemental concentrations for the poultry litter biochar used in Gaskin et al. (2008) were slightly different. The initial poultry litter feedstock had a N concentration of 45.1 g kg<sup>-1</sup> and a P concentration of 19.1 g kg<sup>-1</sup> and was pyrolyzed at 400 and 500°C. The 400°C biochars had an average N concentration of 34.7 g kg<sup>-1</sup> and P concentration of 30.1 g kg<sup>-1</sup>. The 500°C biochar had an average N concentration of 30.9 g kg<sup>-1</sup> and P concentration of 35.9 g kg<sup>-1</sup>. These N and P concentrations were significantly greater than those in the other two biochar types studied (i.e., peanut hull and pine chip), as were the concentrations of K, Ca, Mg, B, Cu, Fe, Mn, Na, and Zn, demonstrating the nutrient richness of the poultry litter even when charred (Gaskin et al., 2008).

Hass et al. (2012) investigated the use of poultry litter biochar as a soil amendment in a Hapludult. Soil pH, nutrient availability, and soil leachate composition were evaluated. Slow pyrolysis at 350 and 700°C was used to produce the biochars, and samples of each were steam-activated. The non-activated poultry litter biochar produced at 700°C had the greatest pH of 12.4.

Both of the non-activated biochars had larger N concentrations when compared to the activated chars, 38 g N kg<sup>-1</sup> with 350°C and 23 g N kg<sup>-1</sup> with 700°C. The activated biochars possessed greater P levels, 44.5 g P kg<sup>-1</sup> with 350°C and 49.1 g P kg<sup>-1</sup> with 700°C. With an increase in pyrolysis temperature, there was an increase in feedstock mass loss. Of the original dry mass, 57% was recovered in the 350°C non-activated biochar and 38% in the 700°C non-activated biochar (Hass et al., 2012). Carbon and N were lost more than some of the other less volatile elements, with an increase in N loss with increased production temperature (C:N ratios of 11 at 350°C and 17 at 700°C). Biochar treatments were overall more effective in maintaining the availability of soil nutrients, as well as reducing toxic element mobility and solubility, compared to AgLime (Hass et al., 2012). However, it would necessitate nearly 12-fold more poultry litter biochar to increase the soil pH by the same amount as AgLime, so increasing soil pH with biochar may not be the most economical alternative (Hass et al., 2012). This study was conducted in climate-controlled chambers for only eight weeks, so the long-term effects under field conditions were not investigated in this study.

Although the study by Hass et al. (2012) was conducted under controlled conditions, it is of particular relevance to Arkansas because the Udult used in the study is a common soil type in Arkansas and in the southeast region of the United States (NRCS, 2012). Poultry litter biochar has the potential to be a valuable addition to Udults as a soil amendment because of many of the characteristics discussed, particularly the increase in pH, ability to increase nutrient availability, and improved water infiltration and moisture-holding capacity that are useful for the hot summers in the southeast region. There is a lack of knowledge about the effects of poultry litter biochar on soil organisms and soils typical of agricultural production in the southern U.S., so research in this area is necessary. Investigation into the effects of poultry-litter biochar on

Arkansas soils will be critical to determine if this is an advisable use of the excess of poultry litter produced in geographically concentrated areas of the state.

The poultry industry is not the only major agricultural sector in Arkansas. The forestry sector, which primarily includes commercial logging, forest products, and processing of furniture, wood, and paper, contributed \$2.0 billion in labor income and \$2.9 billion in value added in the state in 2011 (English et al., 2013). Almost 490,000 cubic feet of softwood and hardwood industrial products were produced in 2009 (Brandeis et al., 2011). Nearly 60% of Arkansas, or 18.6 million acres, is commercial timberland (Pelkki, 2005). Although wood waste can be used to fulfill part of the energy needs of the forestry industry (Pelkki, 2005), the production of biochar could also be an option for recycling wood waste.

Wood biochar has been used in previous research. Pine woodchip biochar incorporated into a Kandiudult in Georgia resulted in decreased corn yields and minimal effects on plant nutrients (Gaskin et al., 2010). In a Ferrosol and loamy Calcarosol in Australia, two biochars were added made from paper mill waste which resulted in varying soil fertility, agronomic, and microbial activity responses (Van Zwieten et al., 2010). Rajkovich et al. (2012) investigated the effects of various biochars, including oak wood, pine wood, and paper mill waste, on corn in silt loam and loam soils in New York. Corn biomass was either not affected by the wood and paper mill waste biochars or negatively affected, usually when the biochar was applied at rates near 90 Mg ha<sup>-1</sup>. Pine wood biochar applied to a sandy soil in South Africa reduced ammonium and nitrate leaching but also reduced the amounts of exchangeable ammonium and nitrate, suggesting that the nutrients were potentially sorbed into the biochar pores (Sika and Hardie, 2014). In Montana, bark and wood from ponderosa pine (*Pinus ponderosa* L.) and Douglas-fir (*Pseudotsuga menziesii* L.) were pyrolyzed to observe differences in wood source and pyrolysis

temperature (Gundale and DeLuca, 2006). Most of the biochars increased net nitrification in the Dystrustept soil, and many of the biochar characteristics were much more variable based on temperature differences opposed to source material. Brown et al. (2006) conducted research in an attempt to produce pine wood biochar with similar properties as natural char produced from forest fires. More research will be important with different wood varieties, sources, and in different soils to gain a better understanding of wood biochar's impacts, especially in the southeast.

## 2.7 References

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### **3. Soil Water Retention as Affected by Biochar Source and Application Rate**

#### **3.1. Abstract**

Biochar is a stable organic carbon substance produced by the pyrolysis of biomass and used as a soil amendment. Biochar application to soil has been reported to result in many potential agronomic benefits, including improved water-holding capacity. However, limited studies exist quantifying different biochars' roles in water retention in soils, especially when the soil is drier than field moisture capacity. Various soil application rates and production techniques, including pyrolysis temperature and biochar feedstock source, can impact the effectiveness of biochar as a beneficial soil amendment. The objective of this laboratory study was to investigate the effects of poultry litter and woodchip biochars, applied at various rates (i.e., 0, 5, and 10 Mg ha<sup>-1</sup>) to a loam soil, on the relationship between soil water potential and water content across a wide range of moisture conditions. Soil water potentials were measured directly with a Dewpoint PotentialMeter. Based on analysis of variance, the relationship between water potential and water content differed ( $P < 0.05$ ) between biochar sources based on differing ( $P < 0.02$ ) power-function coefficients, with poultry litter biochar having better water retention characteristics, but was unaffected by application rate. Based on regression analyses, application rate affected the relationship between water potential and water content more for poultry litter than for woodchip biochar. Results indicate biochar may not generally improve water retention at all water contents and with one-time application rates. Additional experiments will be necessary to understand the impact on water retention of biochars produced from different feedstocks under varying pyrolysis conditions.

### 3.2. Introduction

Biochar is a charcoal product produced in high-heat, low-oxygen conditions that can be used as a soil amendment with the potential for enhancing soil properties and plant growth. Biochar can have large surface area and high porosity, tending to increase with increasing pyrolysis temperature until around 850°C (Brown et al., 2006; Lua et al., 2004). Brown et al. (2006) reported pitch pine (*Pinus rigida* L.) biochar surface area peaking near 400 m<sup>2</sup> g<sup>-1</sup>, while Lua et al. (2004) reported a surface area over 700 m<sup>2</sup> g<sup>-1</sup> and pore volume exceeding 0.45 cm<sup>3</sup> g<sup>-1</sup> with pistachio (*Pistacia vera* L.) nut biochar. Like ashes, many biochar products have alkaline pHs (Gaskin et al., 2008; Spokas et al., 2012), which can decrease soil acidity and create a more favorable habitat for many plants and microbes. Soils with biochar addition have been shown to increase nutrient contents and improve nutrient-holding capacity, potentially through direct nutrient addition (Gaskin et al., 2008; Glaser et al., 2001; Lehmann et al., 2002; Lehmann et al., 2003), as well as increase soil water-holding capacity (Brown et al., 2006; Laird et al., 2010).

Research is ongoing to confirm these reports since the assumption that benefits will occur may not hold true with all biochar products (i.e., feedstocks and processing characteristics), field and soil conditions, management practices, and crops grown. In a greenhouse experiment, Rajkovich et al. (2012) investigated the effects of over 30 different biochars produced from corn (*Zea mays* L.) stover, nut shells, woodchips, manures, and sawdust bedding at four different pyrolysis temperatures on corn growth and reported differences between feedstock source, pyrolysis temperature, and application rate on corn biomass, plant growth, nitrogen concentration, and total nitrogen uptake. Novak et al. (2009) determined that greater biochar production temperatures could produce more alkaline pHs, greater ash contents, and greater surface areas in the biochar and could result in increased soil pH.

Biochar has also been observed to increase soil water retention (Gaskin et al., 2007; Karhu et al., 2011; Laird et al., 2010; Major et al., 2009). Laird et al. (2010) reported greater water retention at gravity-drained equilibrium and -100 and -500 kPa water potentials in a Hapludoll from Iowa with mixed hardwood biochar. Utilizing an incubation and leaching experiment to determine the percentage of water retained in pots, Novak et al. (2009) reported varied results depending on the biochar feedstock used; switchgrass (*Panicum virgatum* L.) biochar maximized soil water retention when applied at a 40 Mg ha<sup>-1</sup> application rate to loamy sand compared to poultry litter, pecan (*Carya illinoensis* L.) shell, and peanut (*Arachis hypogaea* L.) hull biochars. A similar experimental design was used to test soil-water retention at -5 and -60 kPa with switchgrass biochar produced at 500°C resulting in the greatest soil water retention (Novak et al., 2012). Birch (*Betula* spp.) biochar added to a silt loam in Finland increased soil water-holding capacity by 11% compared to the unamended control (Karhu et al., 2011). Based on moisture release curve data from a laboratory experiment with loamy sand, only the addition of peanut hull biochar applied at 88 Mg ha<sup>-1</sup> increased soil water-holding capacity compared to lower biochar rates and various woodchip biochar types (Gaskin et al., 2007). In a Brazilian sandy clay loam, sugarcane (*Saccharum* spp.) filtercake biochar addition led to greater water retention within the plant-available water range, even though drier soils were also evaluated (Eykelbosh et al., 2014).

Many of the preexisting studies related to the effects of biochar on water-holding capacity involved the use of techniques that evaluate maximum water-holding capacity and plant-available water (i.e., field capacity at ~-0.03 MPa to permanent wilting point at ~-1.5 MPa) in soil (Fellet et al., 2011; Jones et al., 2010; Laird et al., 2010; Mukherjee and Lal, 2013). The relationship between soil water potential and soil water content can be determined from data

generated by drying saturated soil cores with the application of pressure using a pressure-plate apparatus (Basso, 2012; Fellet et al., 2011; Jones et al., 2010; Laird et al., 2010; Piccolo et al., 1997). Another method quantified the volume of water in samples centrifuged at speeds corresponding to matric potentials between saturation and the permanent wilting point (de Melo Carvalho et al., 2014). Pot-water-holding capacities were used by Novak et al. (2009; 2012) to represent gravimetric soil moisture contents in incubation leaching experiments, while Major et al. (2012) used tensiometers in the field. Investigating a wider range of moisture conditions, Sun et al. (2013) investigated water retention to matric potentials close to -7 MPa, and sugarcane water retention effects were examined in Brazil using a Dewpoint Potentiometer (Eykelbosh et al., 2014).

The use of a DewPoint Potentiometer (Decagon Devices, Inc., Pullman, WA) enables measurements at much drier moisture contents because the soil is wet from an air-dry state. Soil wetting curves can be constructed using this technique, displaying the relationship between water content and water potential in soils. Brye (2003) utilized this technique to determine the effect of years of cultivation and land use on soil water-retention characteristics. The use of a DewPoint Potentiometer and soil wetting curves could acquire data to better understand biochar's effect on water relations under less-than-optimum soil moisture conditions.

Though most previous studies have concentrated on moist-soil conditions, it is not always possible to keep managed fields at field capacity. Dry-land agriculture in particular is prone to drought conditions and may become more common as changes in climate continue.

Consequently, additional research is necessary to shed light on potential water-holding capacity improvements from biochar amendment over a wider range of soil moisture conditions.

Therefore, the objective of this study was to investigate the effect of poultry litter and woodchip

biochars, applied at various rates to a loam soil, on the relationship between a wide range of soil water potentials and water contents using soil wetting curves. It was predicted that the type (i.e., feedstock source) of biochar added to soil at varying rates would differentially affect water-retention characteristics determined using soil wetting curves. Specifically, it was hypothesized that poultry litter biochar applied at the greatest rate would alter water-retention characteristics more than at lower rates or with the woodchip biochar due to the intrinsic differences in the biochar products.

### **3.3. Materials and Methods**

#### **3.3.1. Soil Sample Collection and Initial Characterization**

Soil was collected from approximately the top 10 cm of a floodplain at the University of Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas in an area that was classified as a Razort loam (fine-loamy, mixed, active, mesic Mollic Hapludalf; USDA-NRCS, 2014). The soil was homogenized, air-dried for 72 hours, ground, and sieved to pass a 2-mm mesh screen. Four sub-samples were dried for 24 hours at 105°C to quantify the initial moisture content of the air-dried soil. Soil particle-size analysis was conducted on oven-dry sub-samples of the soil sieved through a 2-mm mesh screen using an adaptation of the 12-hr hydrometer method (Gee and Bauder, 1986). Mehlich-3 extractable nutrients [i.e., phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), and copper (Cu); Tucker, 1992] were determined by inductively coupled plasma (ICP) spectrometry (SPECTRO ARCOS, SPECTRO Analytical Instruments GmbH, Kleve, Germany). Electrical conductivity (EC) and pH were determined potentiometrically on a 1:2 soil:water paste, and organic matter concentration was determined by loss-on-ignition in a muffle furnace.

### 3.3.2. Biochar Characteristics

Granular pine (*Pinus* spp.) woodchip biochar (Waste To Energy Solutions Inc., Destin, FL), produced through pyrolysis at 500°C, was obtained for use in this study. Pelletized poultry litter biochar (Whitfield Biochar, Burlington, WA), produced through pyrolysis at 500-520°C, was also obtained for use in this study. Total recoverable minerals [i.e., P, K, Ca, Mg, S, sodium (Na), Fe, Mn, zinc (Zn), Cu, and boron (B)] were determined from acid digests (US-EPA, 1996) by ICP spectrometry. Both biochars were dried for at least 48 hours at 70°C then ground to pass a 40-mesh screen. Biochar pH and EC were determined potentiometrically on a 1:2 sample:water paste. Sub-samples of each biochar were subsequently dried for 24 hours at 105°C to obtain moisture contents of both biochar materials. The persistence of biochar water repellency was measured using a modification of the water droplet penetration test (WDPT) described by Herath et al. (2013). Three drops of water from a Pasteur pipet were added to 2 g of each biochar and the time of penetration was recorded (Bisdorn et al., 1993; Dekker et al., 1990).

### 3.3.3. Wetting Curve Determinations

The relationship between water potential and water content was determined for each of five replicate treatment combinations by re-wetting air-dry soil from randomly selected biochar source-rate treatment combinations following the procedure in Brye (2003). Eight sub-samples of air-dried sieved soil from each treatment combination were prepared by mixing  $5 \pm 0.01$  g of soil with 0, 28, or 57 mg of biochar (i.e., equivalent to 0, 5, and 10 Mg ha<sup>-1</sup> rate at the field scale). The soil and biochar mixtures were added to small plastic cups, 3.8 cm in diameter and 1 cm tall, and lightly tamped to fit into the bottom 0.5 cm of the cup. Then, 1, 2, 4, 6, 10, 12, 15,

and 20 drops of distilled water were added to each sample cup, respectively, using a Pasteur pipet and covered. A WP4 Dewpoint PotentialMeter was used to measure water potential on each sample after equilibrating overnight at room temperature. The potentiometer was calibrated using 0.5 molal KCl standard solution. After the water potential was measured for each sample, the gravimetric water content was determined by weighing, oven-drying at 105°C for at least 12 hours, and reweighing.

#### 3.3.4. Data Analyses

Initial biochar properties were compared between biochar sources by analysis of variance (ANOVA) using SAS (version 9.2, SAS Institute, Inc., Cary, NC). When appropriate, means were separated by least significant difference at  $\alpha = 0.05$ .

The relationship between soil water potential ( $\psi$ ) and gravimetric water content ( $\theta_g$ ) was determined using nonlinear regression as described in Brye (2003) using the power function:

$$\Psi = a(\theta_g)^{-b} \quad [1]$$

The power function was fit to the soil wetting curve data for each replicate treatment combination, with  $\psi$  representing soil water potential (in MPa),  $\theta_g$  representing the gravimetric water content of a given sample (in  $\text{g g}^{-1}$ ), and  $a$  and  $b$  were empirically derived coefficients. Based on a completely random design, a two-factor ANOVA was conducted using SAS to determine the effects of biochar source, rate, and their interaction on modeled water-retention characteristics (i.e.,  $a$  and  $b$  coefficients). When appropriate, means were separated by least significant difference at  $\alpha = 0.05$ . A multiple regression analysis was also conducted on the modeled water-retention characteristics using Minitab (version 13.31, Minitab, Inc., State College, PA) to further investigate the effect of biochar rate.

### 3.4. Results and Discussion

#### 3.4.1. Initial Soil and Biochar Properties

Initial soil and biochar properties were similar to expectations. Particle-size analyses confirmed the texture of the soil to be loam and the soil pH was determined to be 6.7 (Table 1). The poultry litter and woodchip biochars possessed alkaline pHs of 8.0 and 8.8, respectively (Table 2). The majority of the nutrient concentrations and EC differed ( $P < 0.05$ ) between the two biochars. Except for iron, the poultry litter biochar possessed greater ( $P < 0.05$ ) concentrations of all other total recoverable elements measured (i.e., P, K, Ca, Mg, S, Na, Mn, Zn, Cu; Table 2). The water repellency differed between the two biochars ( $P < 0.05$ ), with greater water repellency in the poultry litter ( $> 60$  seconds for water to penetrate into biochar) compared to the woodchip biochar (2.5 seconds).

Biochars can have large surface areas (200 to  $> 500 \text{ m}^2 \text{ g}^{-1}$ ) and are highly porous when produced at intermediate temperatures around 450 to 750°C (Brown et al., 2006; Downie et al., 2009; Lua et al., 2004), a range which includes the temperatures that produced the biochars used in this study. These physical biochar attributes have affected water relationships in soil by increasing soil porosity and changing the pore-size distribution (Jones et al., 2010; Ouyang et al., 2013). In this study, the short duration of the experiment and the pre-treatment of the biochars, including air-drying and sieving, eliminated some possible mechanisms of biochar effect on soil water retention (e.g., improved soil structure, aggregate stability, and the increase of macropores). Grinding the biochar created a more uniform comparison between biochars so that their intrinsic property differences rather than their structural differences (i.e., pellet vs. granular)

were likely responsible for subsequent measured differences in the relationship between water potential and water content.

### 3.4.2. Soil Wetting Curve Differences

The power function [Eq. 1] fit the soil wetting curve data well, with  $R^2$  values ranging from 0.94 to 0.96 for all treatment combinations (Figure 1). Water contents measured in this study included those in the plant-available water range as well as those drier than permanent wilting point (i.e., water potentials ranging from  $\sim -0.2$  to  $\sim -80$  MPa; Figure 1). The  $a$  coefficient of the wetting-curve model, an experimentally derived coefficient, differed ( $P = 0.018$ ) between biochar sources (Table 3). Averaged across biochar rate, the  $a$  coefficient from the wetting-curve model averaged 277.1 for the poultry litter and 392.8 for the woodchip biochars (Figure 2). However, despite the differences in the initial rate of biochar addition, biochar rate did not affect ( $P > 0.05$ ) the  $a$  coefficient of the soil wetting-curve model (Table 3). These results suggest that the samples were uniform across biochar rates but that differences existed based on the biochar source, potentially different pore structures, requiring significant adjustments in the model's  $a$  coefficient (Brye, 2003).

The  $b$  coefficient of Eq. 1 characterizes the rate of water potential decrease as the water content decreased (Brye, 2003). Similar to the  $a$  coefficient, the  $b$  coefficient also differed between biochar sources ( $P = 0.001$ ) but was unaffected ( $P > 0.05$ ) by biochar rate (Table 3). Combined across biochar rates, the  $b$  coefficient from the wetting-curve model averaged -2.36 for the poultry litter and -2.62 for the woodchip biochars (Figure 2). The more negative  $b$  coefficient for the woodchip biochar treatments indicated that the water content decrease was greater for the woodchip than for the poultry litter biochar for the same decrease in water

potential. Consequently, more water was retained at a given water potential with the poultry litter biochar than with the woodchip biochar.

Though ANOVA did not demonstrate a biochar rate effect, regression analyses were conducted to investigate a potential relationship between biochar rate and water retention assuming that biochar rate represented a continuous variable. The  $a$  coefficient for the poultry litter biochar varied significantly ( $P = 0.037$ ) as biochar rate increased, while the  $a$  coefficient for the woodchip biochar and neither  $b$  coefficients were affected by biochar rate (Table 4). The  $a$  coefficient for the poultry litter biochar increased from the 0 to the 5 Mg ha<sup>-1</sup> rate, then decreased from the 5 to the 10 Mg ha<sup>-1</sup> rate (Figure 3, Table 4), suggesting a change in biochar effect at the 5 Mg ha<sup>-1</sup> application rate compared to the control that was no longer observed at the greatest rate.

Considering results for both the  $a$  and  $b$  coefficients, the poultry-litter-biochar-amended soil possessed different overall water-retention characteristics than the woodchip-biochar-amended soil. Since the soil was uniformly treated by air-drying, grinding, and sieving and the biochars were oven-dried and ground, these results suggest that there were factors intrinsic to the biochars that potentially led to water-retention differences between biochar sources. The poultry litter biochar, with a significantly smaller C:N ratio, significantly greater nutrient contents, and almost ten-fold numerically greater EC (Table 2), possessed a strikingly different composition that could have affected water-retention characteristics differently than those of the woodchip biochar. Water could have reacted with the inorganic nutrients and compounds in the poultry litter biochar, creating a physical adhesion effect to retain the water in the soil or biochar pores (Novak et al., 2012).

The greater C:N ratio of the woodchip biochar, which suggests a greater lignin and cellulose concentration than in the poultry litter biochar due to its origin as plant material, could lead to greater hydrophobicity and water repellency. However, the poultry litter biochar possessed greater water repellency than the woodchip biochar. Fresh biochar tends to be water repellent (Lehmann et al., 2009), and Briggs et al. (2012) speculated that the water repellency of their pine biochar samples had to do with the aliphatic compounds that make up biochar as well as the mineral-rich composition clogging outer pores. Tars in biochars can also repel water, and they remain in charcoal produced at temperatures below 600°C (Amonette and Joseph, 2009; Antal and Grønli, 2003; Downie et al., 2009; Kameyama et al., 2010), such as with the biochars used in this study. Water-repelling tar in the nutrient-rich poultry litter biochar could have influenced soil water repellency. The original poultry litter was a hydrophobic pellet, while the woodchip was a coarse, less compacted granular-like form, which could have resulted in differences in the biochars based on the processing and production conditions. Based on visual observation, following grinding to facilitate comparison among intrinsic properties between biochars, the poultry litter biochar was composed of finer particles than the woodchip biochar. Grinding of the biochars for use in this study may have produced finer particles of the poultry litter biochar likely filled soil pores more than the coarser woodchip biochar material to help retain water once soil applied.

Different biochar feedstocks, production temperatures, and environmental conditions (i.e., a greenhouse compared to a field setting) could also produce drastically different results than those observed in this study in terms of their effects on soil water-retention characteristics (Brown et al., 2006). Thirty months after field application of 47 Mg ha<sup>-1</sup> *Acacia* spp. green waste biochar to a silt loam, Hardie et al. (2014) reported no significant effects on soil water retention

or soil moisture content near field capacity, but reported numerically greater water contents near saturation. Soil water-holding capacity was unaffected by 20 Mg ha<sup>-1</sup> of mixed wood species biochar in an Oxisol (Major et al., 2012) or by 11 or 22 Mg ha<sup>-1</sup> of peanut (*Arachis hypogaea* L.) hull, hardwood, or various pine biochars added to loamy sand (Gaskin et al., 2007). In a laboratory experiment, plant-available water was shown to increase in sandy soil, remain unaffected in loamy soil, and decrease in clayey soil with the additions of hardwood and pine biochars from 0 to 15, 30, and 45 % (w/w) application rates (Tryon, 1948). After investigating 60 combinations of soils and biochars, Strebel et al. (2011) reported 25 combinations as resulting in significantly greater soil water-holding capacity.

Though only a one-time biochar application was used in this study, there is potential that differing results could be observed if greater rates were used or if multiple biochar additions were made over time in a longer study. However, greater rates could prove uneconomical for field application (Piccolo et al., 1997). The Na content in poultry litter biochar was roughly 50 times greater than that in the woodchip biochar; thus, repeated biochar additions, especially with the poultry litter biochar, could negatively impact soil water-holding capacity by increasing soil dispersion and off-setting the potential for improved soil structure. Future studies should also investigate the effects of other biochar products produced from different feedstocks and under various production and environmental conditions on soil water-retention characteristics.

### **3.5. Conclusions**

Water-retention characteristics of a loam soil differed as a result of a single addition of poultry litter and woodchip biochar. Poultry litter biochar had different chemical characteristics and affected water-retention characteristics differently than woodchip biochar, although both

biochars were produced from pyrolysis at approximately 500°C. More water was retained at a given water potential with the poultry litter biochar than with the woodchip biochar, but rate effects were minimal. The results of this study demonstrate that all biochars may not improve soil water retention over a wide range of soil moisture conditions at all application rates.

Additional research with various biochar products applied at a range of application rates and with multiple applications would enhance knowledge of the effects of biochar amendment on soil water-retention characteristics.

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### 3.7. Tables

Table 1. Initial mean [ $\pm$  standard error (SE)] particle-size distribution, pH, electrical conductivity (EC), organic matter, and Mehlich-3 extractable nutrients for the Razort soil (n=4)

Soil Property	Mean ( $\pm$ SE)
Particle-size distribution ( $\text{g g}^{-1}$ )	
Sand	0.4 (0.01)
Silt	0.5 (0.01)
Clay	0.1 ( $<0.01$ )
pH	6.7 (0.1)
EC ( $\text{dS m}^{-1}$ )	0.3 (0.2)
Organic matter ( $\text{g kg}^{-1}$ )	34.6 (0.4)
Phosphorus ( $\mu\text{g g}^{-1}$ )	5.2 (0.5)
Potassium ( $\mu\text{g g}^{-1}$ )	84.9 (15)
Calcium ( $\mu\text{g g}^{-1}$ )	1191.0 (26)
Magnesium ( $\mu\text{g g}^{-1}$ )	47.4 (0.2)
Sulfur ( $\mu\text{g g}^{-1}$ )	14.0 (5.9)
Iron ( $\mu\text{g g}^{-1}$ )	48.9 (1.8)
Manganese ( $\mu\text{g g}^{-1}$ )	93.1 (3.7)
Copper ( $\mu\text{g g}^{-1}$ )	1.1 (0.02)

Table 2. Initial mean [ $\pm$  standard error (SE)] pH, electrical conductivity (EC), total carbon (C), total nitrogen (N), C:N ratio, and total recoverable minerals for poultry litter and pine (*Pinus* spp.) woodchip biochar sources, (n = 2)

Biochar Property	Biochar Source	
	Poultry Litter	Woodchip
pH	8.0 (0.04)* a	8.8 (0.03) b
EC (dS m <sup>-1</sup> )	42.0 (0.9) a	4.6 (0.2) b
Total Carbon (mg g <sup>-1</sup> )	337.2 (2.5) a	244.5 (21) b
Total Nitrogen (mg g <sup>-1</sup> )	34.9 (0.01) a	0.7 (0.2) b
C:N ratio	9.7:1 (0.1) a	365.7:1 (64) b
Phosphorus (mg g <sup>-1</sup> )	25.7 (0.2) a	0.8 (0.02) b
Potassium (mg g <sup>-1</sup> )	52.4 (0.2) a	2.1 (0.1) b
Calcium (mg g <sup>-1</sup> )	45.4 (5.8) a	10.1 (0.5) b
Magnesium (mg g <sup>-1</sup> )	12.6 (0.9) a	2.7 (0.2) b
Sulfur (mg g <sup>-1</sup> )	13.6 (0.3) a	0.1 (<0.01) b
Sodium (mg g <sup>-1</sup> )	15.3 (0.6) a	0.3 (0.01) b
Iron (mg g <sup>-1</sup> )	1.4 (0.1) a	0.9 (0.1) a
Manganese (μg g <sup>-1</sup> )	715.0 (18) a	420.5 (30) b
Zinc (μg g <sup>-1</sup> )	829.5 (26) a	0.01** (0) b
Copper (μg g <sup>-1</sup> )	583.0 (40) a	6.5 (0.04) b
Boron (μg g <sup>-1</sup> )	78.0 (4.0) a	10.4 (0.7) b

\*Means in the same row followed by different letters are different ( $P < 0.05$ ).

\*\*Zinc in the woodchip biochar was below the detection limit of the method. Therefore, the detection limit of 0.01 was used for statistical analysis.

Table 3. Analysis of variance summary of the effects of biochar (BC) source, rate, and their interaction on water-retention characteristics from soil wetting curve data fit to the model  $\Psi = a(\theta_g)^{-b}$

Source of Variation	Degrees of Freedom	<i>a</i> Coefficient	<i>b</i> Coefficient
		————— <i>P</i> —————	
BC source	20	0.018	0.001
BC rate	20	0.293	0.085
BC source x BC rate	20	0.365	0.291

Table 4. Summary of multiple regression results for *a* and *b* coefficients from poultry litter and woodchip biochar and the effect of biochar rate and rate<sup>2</sup>

Variable	Biochar Source	Regression Coefficients		Intercept	Overall Model	R <sup>2</sup>
		Rate	Rate <sup>2</sup>			
<i>a</i> coefficient	Poultry litter	78.2*	-7.16*	244*	<i>P</i> 0.037	0.42
	Woodchip	4.05	-0.34	479*	0.998	0.00
<i>b</i> coefficient	Poultry litter	-0.075	0.0086	-2.40*	0.100	0.32
	Woodchip	0.0057	0.00052	-2.70*	0.790	0.04

\*Asterisks indicate values significantly different from 0 ( $P < 0.05$ ).

### 3.8. Figure Captions

Figure 1. Relationship between water potential and gravimetric water content from six different treatment combinations of poultry litter (PL) and woodchip (WC) biochars at three application rates (i.e., 0, 5, and 10 Mg ha<sup>-1</sup>). The resulting fitted power function and associated coefficient of determination ( $R^2$ ) are also reported for each biochar source-rate treatment combination.

Figure 2. Relationship between water potential and gravimetric water content for poultry litter and woodchip biochars averaged over rate. The resulting fitted power function and associated coefficient of determination ( $R^2$ ) are reported for each biochar source. The open circles are data points with 0 Mg ha<sup>-1</sup>, the filled circles with 5 Mg ha<sup>-1</sup>, and the filled squares with 10 Mg ha<sup>-1</sup> biochar application.

Figure 3. Effect of biochar rate on  $a$  and  $b$  coefficients from fitting the power function to soil wetting-curve data for poultry litter (PL) and woodchip (WC) biochars. The plotted line represents a significant regression model, where model parameters are summarized in Table 4.

### 3.9. Figures

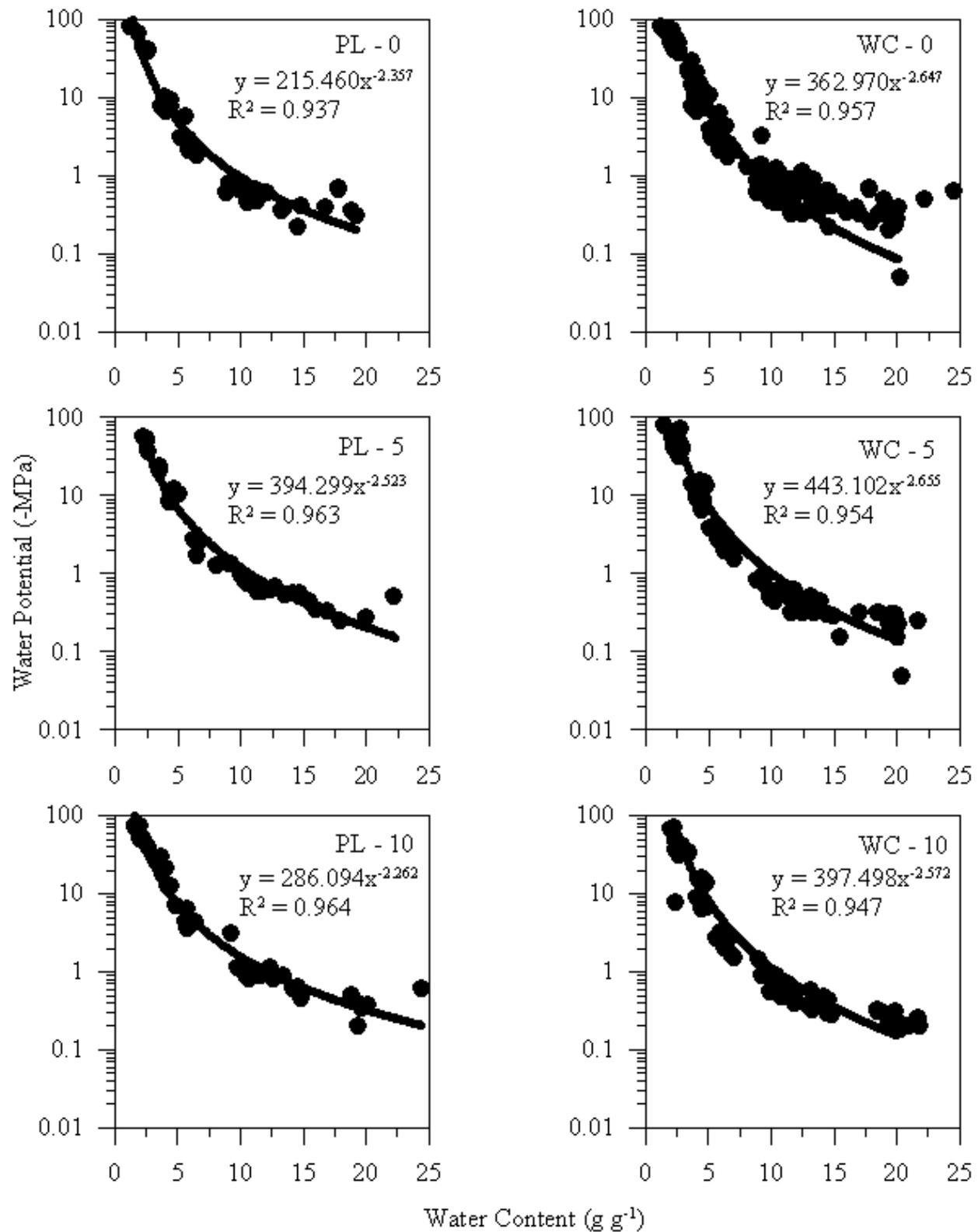


Figure 1

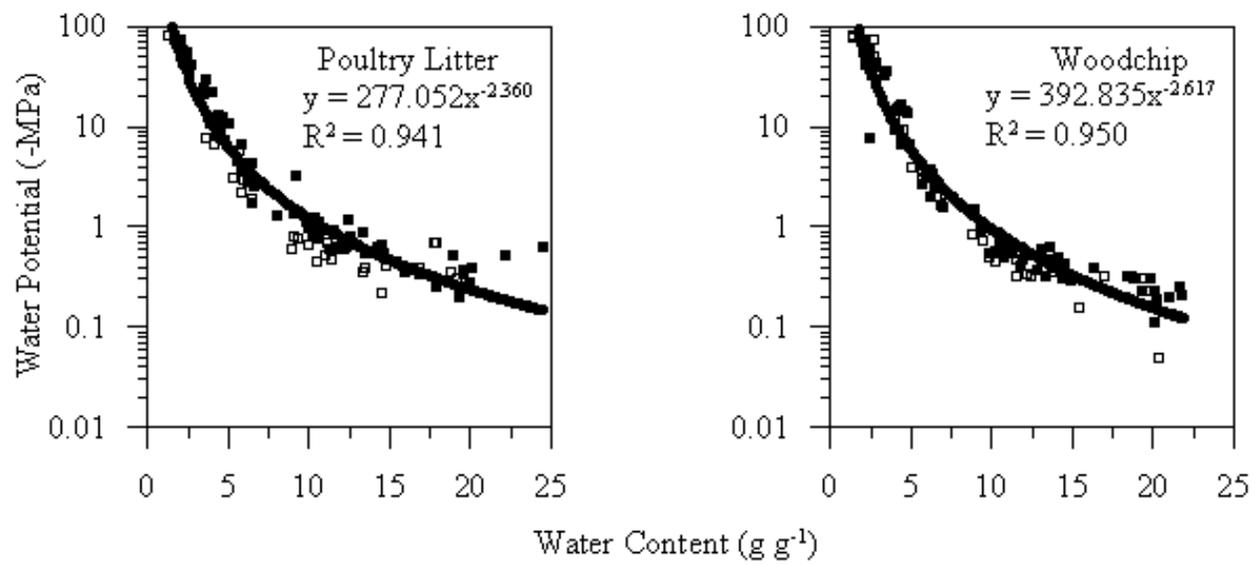


Figure 2

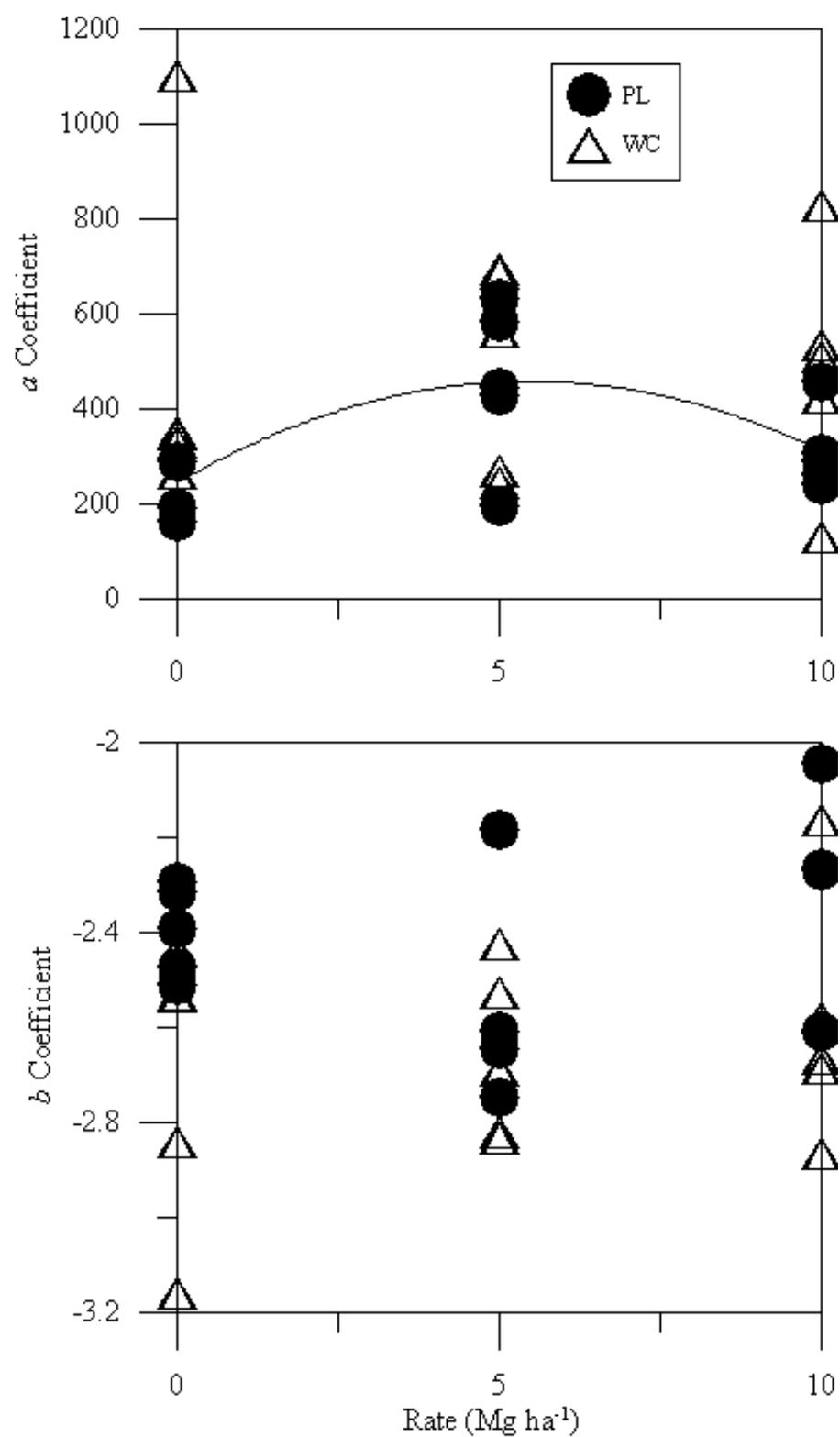


Figure 3

## **4. Poultry Litter Biochar Effects on Corn Biomass, Mycorrhizal Infection, and Soil Nutrient Availability in a Loam**

### **4.1. Abstract**

Soil-applied biochar can provide agronomic benefits. Specifically, biochar may increase plant growth and nutrient uptake indirectly, such as by stimulating mycorrhizal fungal activity and plant infection, or through direct nutrient addition. The effects of biochar in combination with fertilizer on soil nutrient availability and corn (*Zea mays* L.) growth were investigated in a greenhouse study with treatments of poultry litter biochar (0, 5, and 10 Mg ha<sup>-1</sup>) and nitrogen (N) and phosphorus (P) fertilizer (0, half, and full rates). Plant height and aboveground biomass were significantly greater ( $P < 0.05$ ) with biochar addition compared to no addition. Acid phosphatase activities tended to increase ( $P < 0.1$ ) by the end of the experiment in the absence of biochar application. Biochar did not affect mycorrhizal infection or alkaline phosphatase activities, but root biomass and diameter were greater with 10 Mg ha<sup>-1</sup> biochar (9.2 g and 5.5 mm) than with no biochar (6.6 g and 3.2 mm). Root morphological features were greatest in the fully fertilized soil receiving 10 Mg ha<sup>-1</sup> biochar compared to all other treatments. Ear-leaf P and extractable soil P tended to increase with 10 Mg ha<sup>-1</sup> biochar compared to the control. Biochar at the 10 Mg ha<sup>-1</sup> application rate could have increased ear-leaf P directly from the biochar and/or indirectly by increasing root growth and thus improving plant access to soil P. Poultry litter biochar may improve agronomic performance of corn grown in mid-southern U.S. soils, but future research should further investigate other benefits and mechanisms behind biochar effects.

## 4.2. Introduction

Biochar, the charcoal product of anaerobic thermal decomposition of biomass, has been applied to soil in an attempt to provide agronomic benefits (Lehmann and Joseph, 2009; Spokas et al., 2012). Biochar has been observed to positively affect plant growth parameters and soil nutrient availability, especially in tropical regions. For example, coconut (*Cocos nucifera* L.) shell and cattle manure biochars both increased corn (*Zea mays* L.) yield and nitrogen (N), phosphorus (P), and potassium (K) nutrient uptake in a sandy Ustipsamment in Indonesia (Sukartono et al., 2011). *Eucalyptus* spp. wood biochar applied to a Typic Haplustox in Colombia increased bean (*Phaseolus vulgaris* L.) biomass when applied at concentrations up to 60 g kg<sup>-1</sup> while also increasing plant nutrient concentrations [i.e., boron (B), magnesium (Mg), K, P, and calcium (Ca)] in a greenhouse experiment (Rondon et al., 2007). Although 20 Mg ha<sup>-1</sup> of wood biochar applied to the same Colombian soil did not increase corn yield the year after field application, yield increases occurring the following three years were influenced by residual biochar effects (Major et al., 2010).

Biochars can also lead to positive effects when combined with fertilizers. For example, wood biochar applied to a Xanthic Ferralsol in Brazil increased rice (*Oryza sativa* L.) and sorghum (*Sorghum bicolor* L.) yield when applied with NPK fertilizer in a field experiment (Steiner et al., 2007). Poultry litter biochar and N fertilizer applied to an Australian Alfisol increased radish (*Raphanus sativus* L.) yields in a greenhouse compared to the addition of N fertilizer alone (Chan et al., 2008).

In contrast to many of the tropical region studies described above, biochar effects on crop growth and nutrient availability in temperate regions have varied. Mixed hardwood biochar applied at 96 Mg ha<sup>-1</sup> in Iowa increased corn yield in the first year after field application,

although there was no effect on nutrient uptake (Rogovska et al., 2014). Application of biochars produced from dairy manure, paper mill waste, and food waste decreased corn growth when applied at rates of 26 or 91 Mg ha<sup>-1</sup> to a Glossoboric Hapludalf loam soil in a greenhouse experiment in New York, while 91 Mg ha<sup>-1</sup> of wood or crop residue biochar did not typically improve corn growth compared to no biochar addition (Rajkovich et al., 2012). At lower biochar rates (2.6, 6.5, and 26 Mg ha<sup>-1</sup>), however, there were generally improvements in corn growth with these biochar products (Rajkovich et al., 2012). In a sandy loam in Belgium, 10 g kg<sup>-1</sup> concentrations of wood biochar reduced soil nitrate availability and radish yields when grown in a greenhouse (Nelissen et al., 2014), while wood biochar applied to a sandy loam (Typic Hapludalf) field in Denmark generally did not affect oat (*Avena sativa* L.) yields when applied at 20 Mg ha<sup>-1</sup> or corn yields with rates between 10 and 100 Mg ha<sup>-1</sup> (Sun et al., 2014).

Biochar amendment can clearly lead to varying agronomic results because all biochars are not the same and can differ dramatically based on the production characteristics and biomass source used (Gaskin et al., 2008; Rajkovich et al., 2012). Agronomic impacts can also depend on soil texture and biochar application rate (Rajkovich et al., 2012; Streubel et al., 2011). Amid the contradictory results, mechanisms for these changes are not always clear. Low-temperature biochars, produced by pyrolysis at temperatures less than 500°C, have been suggested to retain more carbon and nutrients from the original biomass product, react more readily in soils, and better aid soil fertility when compared with biochars produced at temperatures greater than 500°C (Keiluweit et al., 2010; Steinbeiss et al., 2009). Although biochar consists of highly stable carbon that is fairly recalcitrant to degradation over hundreds of years, biochar does sorb oxygen and participate in surface oxidation and partial decomposition reactions that tend to increase biochar cation exchange capacity (CEC) over time (Cheng et al., 2006; Liang et al., 2006, 2008).

Due to the typically large surface area, CEC, and highly porous biochar surface, soil and fertilizer nutrients may adsorb onto biochar, increasing nutrient availability to plants by decreasing leaching (Atkinson et al., 2010; Cheng et al., 2006, 2008; Liang et al., 2006).

In addition to the potential agronomic benefits, the production of biochar is a potentially viable use of waste products, such as animal manures. Poultry production is a considerable agricultural industry in the United States and is of great economic importance in Arkansas, the third largest producer of broilers and of turkeys in the U.S. (NASS-USDA, 2014a). Because of the narrow N:P ratio relative to plant requirements, excess land application of poultry litter increases soil-test P, which can result in environmental problems, such as eutrophication of nearby surface waters (Sharpley et al., 1998, 2009). Turning poultry litter into biochar could offer a use for the excess poultry litter since pyrolysis yields a more stable product that can still provide agronomic benefits.

When studying biochar and its agronomic utility, it is important to understand biochar effects on crop growth and productivity. One important agricultural commodity in the United States, including Arkansas, is corn. In 2013, over 352,000 ha (870,000 ac) of corn were harvested for grain in Arkansas and almost 35.5 million ha (87.7 million ac) in the United States (NASS-USDA, 2014b). Corn is a high nutrient-demanding crop, especially for N; 12.9 to 19.3 kg N per m<sup>3</sup> of corn (1 to 1.5 lbs N bu<sup>-1</sup>) are recommended (Espinoza and Ross, 2003). Other macronutrients, such as P and K, are also critical for proper plant growth. With the high nutrient content of poultry litter biochar, there is the potential for a fertilizer effect and a resulting decrease in inorganic fertilizer input needs. However, biochar may also positively affect nutrient availability to plants indirectly by altering microorganism, especially mycorrhizal, activity and/or interactions with plants. For example, mycorrhizal fungi have been observed to directly access P

from biochar surfaces and increase P translocation to carrot (*Daucus carota* L.) roots (Hammer et al., 2014). Additional research is necessary to further clarify the effects of biochar on microorganisms like mycorrhizal fungi, plant growth, and nutrient availability.

The objective of this study was to investigate the effects of poultry litter biochar in combination with recommended and reduced rates of N and P fertilizer on temperate soil nutrient availability and corn growth under controlled conditions. It was hypothesized that adequate corn growth and nutrient uptake would occur with biochar addition that could potentially reduce inorganic fertilizer inputs through the intrinsic properties of the biochar and indirect biochar effects on soil and plant variables, particularly increased mycorrhizal infection.

### **4.3. Materials and Methods**

#### **4.3.1. Biochar Characteristics**

Poultry litter biochar (Whitfield Biochar, Burlington, WA) produced through pyrolysis at 500-520°C was used in this study. Biochar was dried for at least 48 hours at 70°C then ground to pass a 40-mesh screen. Total nitrogen & total carbon were determined by combustion with Elementar Variomax (Elementar Americas, Inc., Mt. Laurel, NJ). The C:N ratio was determined from the total N and C concentrations. Total recoverable minerals [i.e., P, K, calcium (Ca), magnesium (Mg), sulfur (S), sodium (Na), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), and boron (B)] were determined from acid digestion (US EPA, 1996) using an ARCOS inductively coupled plasma (ICP) spectrophotometer (SPECTRO Analytical Instruments Inc., Mahwah, NJ). Biochar pH and electrical conductivity (EC) were determined potentiometrically on a 1:2 sample:water paste.

#### 4.3.2. Soil Sample Collection and Initial Characterization

Soil classified as a Razort loam (fine-loamy, mixed, active, mesic Mollic Hapludalf; NRCS, 2014) was collected from approximately the top 10 cm of a floodplain at the University of Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas. Fresh (i.e., moist) soil was homogenized and sieved first through a 6-mm sieve to remove coarse fragments and large debris and then through a 2-mm sieve. Soil particle-size analysis was conducted on oven-dry (i.e., 105°C for 24 hours) sub-samples of the soil using an adaptation of the 12-hr hydrometer method (Gee and Bauder, 1986). Mehlich-3 extractable nutrients (i.e., P, K, Ca, Mg, S, Fe, Mn, and Cu; Tucker, 1992) were determined by ICP spectrophotometry. Electrical conductivity and pH were determined potentiometrically on a 1:2 soil:water paste, and organic matter concentration was determined by loss-on-ignition in a muffle furnace.

Sieved and homogenized soil was added to 36, 11.4-L (3-gal) pots (23.5 cm height). The dry soil mass was determined by calculating the gravimetric soil moisture on oven-dried soil randomly sampled from 10 pots. A total of 3 L of tap water were added to each soil-filled pot in order to wet the soil and encourage microbial activity. The soil was then incubated for 14 days with one addition of 3 L of water on day 6 to maintain moist soil. Then, excess water (5 L) was added to each pot to leach excess nitrate until nitrate test strips (AquaChek, Hach Company, Loveland, CO) indicated that leachate concentrations were approximately 0 ppm nitrate. Soil was incubated an additional 10 days before treatments were imposed and the greenhouse pot experiment was initiated.

#### 4.3.3. Greenhouse Experimental Design and Treatments

The greenhouse maintained a temperature between approximately 21.1 and 29.4°C (70-85°F). Two grow lamps, 277 V and 120 V, supplemented sunlight when sunlight fell below 20 klux to ensure a 14-hour photoperiod.

A randomized complete block design was implemented, blocking for spatial variability in the greenhouse. Treatments consisted of a full factorial design of poultry litter biochar at rates of 0, 5, and 10 Mg ha<sup>-1</sup> and N and P fertilizer at 0, half, and full recommended rates (Espinoza and Ross, 2003). Biochar was added based on soil surface area with 53 g per pot for the 10 Mg ha<sup>-1</sup> treatments and 26.5 g per pot for the 5 Mg ha<sup>-1</sup> treatments. Phosphorus fertilizer rates were calculated based on soil analyses (Table 2), resulting in 78.5 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> for the full rate and 39.2 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> for the half rate. Specifically, 2.3 g P<sub>2</sub>O<sub>5</sub> in the form of super phosphate (0-18-0) were added for the full rate and 1.15 g P<sub>2</sub>O<sub>5</sub> for the half rate. Nitrogen was added at rates of 224 kg ha<sup>-1</sup> N (200 lb ac<sup>-1</sup>) for the full fertilizer rate and 112 kg ha<sup>-1</sup> N (100 lb ac<sup>-1</sup>) for the half rate. These rates were chosen based on recommended nitrogen rates for loam soil to achieve a theoretical corn yield of 12.55 Mg ha<sup>-1</sup> (200 bu ac<sup>-1</sup>). Specifically, 7.5 g N for the full rate and 3.7 g N for the half rate using sodium nitrate (16-0-0) were added to the pots.

Biochar and fertilizer were uniformly mixed with soil based on treatment rates and returned to each pot to achieve an approximate bulk density of 1.2 g cm<sup>-3</sup>, and water was added to achieve a calculated water content of 0.30 cm<sup>3</sup> cm<sup>-3</sup>. The volumetric moisture content was monitored daily using a SM-150 soil moisture sensor (Delta-T Devices Ltd., Cambridge, England) to ensure that the water content remained between 0.25 and 0.30 cm<sup>3</sup> cm<sup>-3</sup> in the top 6 cm of each pot.

#### 4.3.4. Soil Sampling and Analyses

Soil was sampled at the initiation and conclusion of the study. Moist soil was ground to pass a 2-mm sieve and analyzed for dissolved organic carbon (DOC), dissolved total N (DTN), ammonium ( $\text{NH}_4^+$ ), and nitrate ( $\text{NO}_3^-$ ) using a single extraction approach (Jones and Willett, 2006). Microbial biomass C and N were quantified by calculating the difference between fumigated and unfumigated samples using the chloroform fumigation method (Vance et al., 1987). Fumigated and unfumigated soils were extracted and analyzed for DOC and DTN on a Shimadzu TOC-V PC-controlled total organic carbon with attached total nitrogen analyzer (Shimadzu, Columbia, MD). A Skalar segmented-flow autoanalyzer (Skalar Inc., Norcross, GA) colorimetrically determined  $\text{NH}_4^+$  following the salicylate hypochlorite procedure and  $\text{NO}_3^-$  following a modification of Griess-Ilosvay cadmium-copper reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  procedure (Mulvaney, 1996). Inorganic N ( $\text{N}_i$ ) was calculated by summing  $\text{NO}_3^-$  and  $\text{NH}_4^+$ , and dissolved organic nitrogen (DON) was calculated by subtracting  $\text{N}_i$  from DTN (Jones and Willett, 2006). To obtain water-soluble phosphorus (WSP), 2-g moist soil were extracted using a 1:10 soil:water ratio (Self-Davis et al., 2000) and analyzed by a Skalar Sans-plus segmented-flow autoanalyzer (Skalar Inc., Norcross, GA) using the ascorbic acid method (Kuo, 1996). Acid and alkaline phosphatases were measured using the colorimetric estimation of *p*-nitrophenol (Tabatabai, 1994).

After drying soil at 70°C for at least 48 hours, soil pH and EC were measured using a 1:2 soil:water paste. Organic matter was determined by loss-on-ignition using a muffle furnace. Mehlich-3 extractable nutrients [i.e., P, K, Ca, Mg, S, Fe, Mn, and Cu; Tucker, 1992] were determined by ICP spectrometry.

#### 4.3.5. Corn Planting, Harvesting, and Analyses

DEKALB hybrid DKC64-69 corn with the Genuity VT Triple PRO value-added trait was chosen for the experiment. Five seeds were planted at a 2-cm depth in a radial pattern and thinned to one seedling after 16 days. After 76 days, when the tasseling stage was achieved in the majority of the plants (55%), the experiment was terminated. Ear leaves were harvested for leaf tissue analysis and plant shoots were cut at the soil surface. Ear leaves were dried at 65°C and weighed before digestion for nutrient concentrations (i.e., P, K, Ca, Mg, S, Mn, Cu, Na, Zn, and B) using an organic matter-wet ashing HNO<sub>3</sub> acid digestion and subsequent analysis using ICP spectrometry (Plank, 1992). Ear-leaf N was determined by combustion (Plank, 1992) using a Model Rapid N III (Elementar Americas, Inc., Mt. Laurel, NJ). Aboveground biomass was dried and weighed. Roots were harvested from the pots, rinsed with tap water to remove soil, and stored at -20°C until analyzed.

Mycorrhizal infection, root morphology (e.g., length, surface area, diameter, root volume, and root tips), and root dry weight were measured. Subsamples (0.5 g) of roots roughly 1 mm or less in diameter were cleared and stained in triplicate for each pot using a Trypan blue stain solution (Penn State University, 2014) following a modified procedure of the method by Sylvia (1994). The percentage of infected roots was determined using the gridline intersect method (Giovannetti and Mosse, 1980). Roots were scanned using WinRHIZO root-scanning software (Regent Instruments Inc., Quebec, Canada, 2007) and root morphological characteristics were measured from scanned images. Roots were dried at 65°C for 72 hours to obtain dry weight.

#### 4.3.6. Data Analyses

Data were analyzed by analysis of variance (ANOVA) using SAS (version 9.2, SAS Institute, Inc., Cary, NC). A three-way ANOVA was performed to determine the effects of

biochar, fertilizer, time, and their interactions on WSP, acid and alkaline phosphatase enzyme activities, soil pH, EC, OM, DOC, microbial C, and Mehlich-3 extractable soil nutrient concentrations (i.e., P, K, Ca, Mg, S, Fe, Mn, and Cu). A two-way ANOVA was performed to determine the effects of biochar, fertilizer, and their interaction on ear-leaf weight and nutrient concentrations (i.e., N, P, K, Ca, Mg, S, Mn, Cu, Zn, Na, and B), plant height and aboveground biomass, percent mycorrhizal infection, root weight, root morphology, and end-of-study DTN, nitrate, ammonium, inorganic N, DON, and microbial N. Least significant differences (LSDs) were used to separate treatment means at  $\alpha = 0.05$ . When significant differences were not observed, tendencies for a biochar effect were investigated at  $\alpha = 0.1$ .

#### **4.4. Results**

##### 4.4.1. Initial Biochar and Soil Properties

The biochar, produced from pelletized poultry litter, had an alkaline pH, EC over 40 dS  $m^{-1}$ , C:N ratio near 10:1, and generally large concentrations of recoverable minerals (Table 1). The texture characterization of the soil as loam from Web Soil Survey (NRCS, 2014) was confirmed by particle-size analysis, and the percentages of sand, silt, and clay were 41, 47, and 12%, respectively (Table 2). The soil possessed a near-neutral pH of 6.5 and EC of 0.32 dS  $m^{-1}$ , and Mehlich-3 extractable soil nutrient concentrations (Table 2) that were adequate for corn production (Espinoza and Ross, 2003) except for P, which was added as a treatment.

##### 4.4.2. Corn Growth and Nutrient Concentrations

As a result of the leaching of mineralized and nitrified nitrogen during initial soil preparation, treatments without added fertilizer produced nutrient-deficient plants that were not

capable of producing ear leaves. When ear leaves were present, ear-leaf K differed among fertilizer-biochar treatment combinations ( $P = 0.032$ ; Table 3), with the greatest concentration in the full-rate fertilizer and the 10 Mg ha<sup>-1</sup> biochar treatment combination compared to either biochar treatment with fertilizer applied at the half rate (Figure 1). Ear-leaf K concentration in the treatment with 5 Mg ha<sup>-1</sup> biochar was greater than concentrations in either fertilizer treatment without biochar. Ear-leaf Mg also differed among fertilizer-biochar treatment combinations ( $P = 0.016$ ), with the full fertilizer and the no biochar treatment combination having significantly greater concentrations of Mg (0.21%) than the other treatment combinations (mean = 0.096%). Ear-leaf Cu also differed among fertilizer-biochar treatment combinations ( $P = 0.011$ ), ranging between 1.95 and 4.25 mg kg<sup>-1</sup>, and was generally numerically greater with the full-rate fertilizer (data not shown).

Other ear-leaf nutrients (i.e., Ca, S, Na, and Mn) increased with full- compared to half-rate fertilizer ( $P < 0.019$ ; Table 3). In contrast to other nutrients, biochar addition, irrespective of rate, decreased ear-leaf Ca and Mn ( $P < 0.003$ ; Table 5). Corn height and aboveground biomass differed among biochar rates ( $P < 0.002$ ) and differed among fertilizer rates ( $P < 0.001$ ; Table 3). As expected, fertilizer additions resulted in greater corn height and biomass with increasing application rate (Table 4). Corn height and biomass were also positively influenced by biochar addition compared to the control (Table 5). When ear leaves were present, ear-leaf N concentrations were increased by fertilizer additions ( $P < 0.001$ ; Table 4), while ear-leaf P had a tendency to increase with 10 Mg ha<sup>-1</sup> biochar addition compared to the control ( $P = 0.075$ ; Table 4). There was no biochar effect on ear-leaf N.

#### 4.4.3. Root Morphology and Mycorrhizal Infection

Root length, surface area, volume, and number of root tips differed among biochar rates within fertilizer rates ( $P < 0.039$ ; Table 3). As a general trend for the four root morphological parameters, the addition of fertilizer and biochar resulted in numerically greater values than treatments without fertilizer (Figure 2). Root morphological parameters were statistically greater ( $P < 0.05$ ) with the full-rate fertilizer and the 10 Mg ha<sup>-1</sup> treatment combination compared to all other treatment combinations. Root length, surface area, and volume produced with 10 Mg ha<sup>-1</sup> biochar and the half-rate of fertilizer did not differ from the full-rate of fertilizer alone or in combination with 5 Mg ha<sup>-1</sup> biochar. There was an increase in root tips with the half-rate fertilizer and the 10 Mg ha<sup>-1</sup> treatment combination compared to full-rate fertilizer and no biochar combination, while root tips produced with the 10 Mg ha<sup>-1</sup> biochar and half-rate fertilizer combination were statistically similar to those produced with the full-rate fertilizer and 5 Mg ha<sup>-1</sup> biochar combination.

Root biomass and diameter differed among biochar rates ( $P < 0.049$ ) and differed among fertilizer rates ( $P < 0.001$ ; Table 3). Root weight and average diameter were greatest with the largest biochar treatment (10 Mg ha<sup>-1</sup>) and were also greatest with the full-fertilizer application (Tables 4 and 5). Greater mycorrhizal infection occurred with no fertilizer compared to the full rate ( $P = 0.023$ ; Tables 3 and 4). However, the percent of mycorrhizal infection in corn roots was unaffected by biochar addition ( $P > 0.05$ ).

#### 4.4.4. Additional Soil Microbial Variables and Nutrients

The soil organic matter concentration differed among biochar rates within fertilizer rates over time ( $P = 0.026$ ; Table 6), ranging from 3.4 to 3.7% at the beginning and from 3.8 to 4.9% at the end of the study. Dissolved organic carbon differed among biochar rates over time ( $P =$

0.016; Table 6), with generally greater DOC at the beginning of the study than at the end (Table 7). Mehlich-3 extractable nutrients Mg, Fe, Mn, and Cu increased over time, especially with both the 5 and 10 Mg ha<sup>-1</sup> biochar additions ( $P < 0.041$ ; Tables 8 and 9). Soil pH increased over time with a greater increase with the full-rate fertilizer addition compared to the half-rate or no fertilizer ( $P = 0.001$ ; Tables 6 and 10). The 5 Mg ha<sup>-1</sup> biochar rate and full-rate fertilizer combination resulted in a greater microbial C:N ratio than the 10 Mg ha<sup>-1</sup> with half- or full-rate fertilizer or full fertilizer and no biochar treatment combinations ( $P = 0.021$ ; Table 6 and Figure 3). Alkaline phosphatase enzyme activity had a tendency for greater activity at the beginning with no or the half-rate fertilizer than at the end of the study with no or the full-rate fertilizer ( $P = 0.051$ ; Tables 6 and 10). In contrast, acid phosphatase enzyme activity increased during the experiment and was greater by the end than at the beginning, although acid phosphatase enzyme activity was greater without than with biochar addition by the end of the study ( $P = 0.051$ ; Tables 6 and 7). Despite not having the greatest enzyme activity, the 10 Mg ha<sup>-1</sup> biochar treatments at the end of the study had a tendency to produce the greatest water-soluble P concentrations compared to other biochar rates and time treatment combinations ( $P = 0.05$ ; Tables 6 and 7).

Dissolved total N tended to decrease with 10 Mg ha<sup>-1</sup> biochar addition ( $P = 0.05$ ; Tables 6 and 11) and decreased with the half-rate fertilizer addition ( $P = 0.033$ ; Tables 6 and 12) by the end of the experiment. Ammonium concentrations also tended to decrease with biochar addition by the end of the experiment ( $P = 0.095$ ; Table 6) from 0.7 µg g<sup>-1</sup> without biochar to 0.1 µg g<sup>-1</sup> with biochar, irrespective of rate (Table 11). Nitrate concentrations by the end of the experiment were less ( $P = 0.002$ ; Table 6) in the 10 Mg ha<sup>-1</sup> than in the no biochar treatment (Table 11) and also decreased ( $P = 0.004$ ; Table 6) with fertilizer addition (Table 12). Microbial C increased over time from 31.5 to 43.7 µg g<sup>-1</sup> without a fertilizer or biochar effect ( $P < 0.001$ ; Table 6),

while microbial N by the completion of the study had increased with fertilizer addition ( $P = 0.034$ ; Tables 6 and 12).

Mehlich-3 extractable soil S was greater with biochar addition compared to no addition ( $P = 0.018$ ; Tables 8 and 11). Soil Ca increased over time from 1197 to 1466  $\mu\text{g g}^{-1}$  ( $P < 0.001$ ; Table 8), while soil K decreased over time from 172 to 85.3  $\mu\text{g g}^{-1}$  ( $P = 0.007$ ; Table 8). Soil K tended to increase with biochar addition irrespective of rate compared to no addition, while 10  $\text{Mg ha}^{-1}$  biochar application tended to increase soil P compared to the control ( $P < 0.094$ ; Tables 8 and 11). Soil P had a tendency to increase over time from 8.2 to 11.4  $\mu\text{g g}^{-1}$  ( $P = 0.062$ ; Table 8). Soil EC decreased over time from 493 to 250  $\text{dS m}^{-1}$  ( $P = 0.008$ ; Table 6).

#### 4.5. Discussion

Corn height and biomass increased with biochar addition irrespective of the rate used in this study (5 or 10  $\text{Mg ha}^{-1}$ ). Increases in crop biomass have also been observed with other poultry litter biochar additions. Chan et al. (2008) observed increases in radish yields with increasing rates of poultry litter biochar from 10 to 25 and 50  $\text{Mg ha}^{-1}$  in a greenhouse experiment. Also in a greenhouse study, Rajkovich et al. (2012) reported increases in corn biomass with all poultry litter biochar application rates used (i.e., 2.6, 6.5, 26, and 91  $\text{Mg ha}^{-1}$ ) compared to the unamended soil. However, corn growth with 91  $\text{Mg ha}^{-1}$  of poultry litter biochar was similar to corn growth with 26  $\text{Mg ha}^{-1}$  in the loam soil in New York (Rajkovich et al., 2012). Lettuce (*Lactuca sativa* L.) germination was negatively affected by poultry litter biochar at concentrations around 50  $\text{g kg}^{-1}$  in a silt loam and sandy loam in Virginia (Revell et al. 2012). This suggests that a threshold could have been reached with poultry litter biochar rates somewhere greater than 50  $\text{Mg ha}^{-1}$ , beyond which no additional benefits to crop growth were

observed. In the present experiment, the biochar rates used were apparently not different enough to produce varying biomass or height results. It is possible that biochar rates between 10 and 50 Mg ha<sup>-1</sup>, at least with poultry litter biochar, could be optimal rates for continued study in temperate regions.

Various mechanisms have previously been proposed for changes in P availability with biochar addition. Biochar could serve as a source of soluble P salts and exchangeable P, modify soil pH to make P more available, or enhance microbial activity and indirectly alter mineralization of P (Atkinson et al., 2010; Gundale and DeLuca, 2006). In this case, biochar had no effect on soil pH, and the near-neutral pH was already allowing for optimal P availability.

Considering microbial activity, mycorrhizal fungi can increase P availability for plants (Smith et al., 2011). The colonization of arbuscular mycorrhizal fungi has been stimulated by the addition of rice hull biochar, with a greater percentage increase with ground material compared to intact biochar as observed by Ezawa et al. (2002) in a greenhouse experiment in Japan. Ezawa et al. (2002) concluded that the increase in surface area of the ground material provided a more favorable habitat. Hammer et al. (2014) reported direct biochar P acquisition by fungal hyphae that resulted in increased P in the plant as observed through use of a P isotope tracer. Mycorrhizal colonization of wheat (*Triticum aestivum* L.) roots increased with *Eucalyptus* spp. biochar addition combined with inoculated mineral fertilizer in a sandy clay loam in an Australian field, potentially reducing drought stress and improving the water supply for the plant (Solaiman et al., 2010). Since moisture was kept at an adequate range for plant growth, drought stress was not a major concern in this greenhouse experiment.

Regardless, mycorrhizal infection was unaffected by biochar addition in this study. Similarly, Warnock et al. (2010) observed no influence of pine (*Pinus contorta* L.) biochar at

concentrations of 5 and 10 g kg<sup>-1</sup> on mycorrhizal infection but reported decreases in available soil P with 10 g kg<sup>-1</sup> biochar in a growth chamber experiment. However, in a field experiment, decreases in mycorrhizal infection but increases in nutrient availability with mango (*Mangifera indica* L.) wood biochar addition were reported (Warnock et al., 2010). Increased percent mycorrhizal infection does not explain the positive effect of biochar on plant parameters in this study, particularly increased plant nutrient availability.

Phosphatase enzymes are involved in the hydrolysis of phosphoric acid and are important in organic-P mineralization and P availability to plants (Tabatabai, 1994). With less accumulation of acid phosphatase enzymes, excreted by plants and microorganisms, with biochar addition and the lack of biochar effect on alkaline phosphatase enzyme activity, which is expected to be produced by microorganisms only (Tabatabai, 1994), evidence is lacking to suggest an increase in active soil microbial decomposition of organic phosphate compounds. Additionally, a clear pattern is lacking between fertilizer rate and biochar rate in terms of how treatments affected the microbial C:N ratio, so there was not an obvious shift in microbial community composition. Therefore, some of the potential indirect nutrient addition effects of biochar based on increases in microbial activity and nutrient acquisition for the plant do not appear to be supported in this study.

Even though biochar has been considered to be recalcitrant to degradation in soils, biochar does change once applied to soil (Glaser et al., 2001; Schimmelpfennig and Glaser, 2012; Schmidt and Noack, 2000; Zimmerman, 2010). While the majority is biologically recalcitrant, a small portion of fresh biochar is thought to be easily mineralizable (Chan and Xu, 2009). Biochar is oxidized over time, which produces carboxylic groups on the biochar surface, increases in % O and decreases in % C, and greater cation exchange capacity and biochar

reactivity (Cheng et al., 2008; Glaser et al., 2001; Gundale and DeLuca, 2006). Cheng et al. (2006) observed increases in biochar CEC and increases in oxygen content, potentially due to the formation of carboxylic functional groups during biochar oxidation over only a four-month incubation period at both 30 and 70°C.

As suggested by Warnock et al. (2010), a possible mechanism for changes in soil and plant nutrient concentrations is the direct nutrient addition from biochar occurring due to the biochar nutrient composition, with biochar actually having somewhat of a fertilizer effect, especially with nutrient-rich biochars (Gaskin et al., 2008). Animal wastes are biomass products that yield nutrient-rich biochars relative to other biomass sources, such as woodchips or crop residues (Chan and Xu, 2009; Rajkovich et al., 2012; Singh et al., 2010). For example, a pine wood biochar and a poultry manure biochar, both produced using slow pyrolysis at 500°C, possessed drastically different nutrient concentrations; the wood biochar had 0.001 g kg<sup>-1</sup> total P and 0.7 g kg<sup>-1</sup> total K, while the poultry manure biochar contained 30 g kg<sup>-1</sup> total P and 28 g kg<sup>-1</sup> total K (Rajkovich et al., 2012).

With a tendency for more extractable P in the soil with the application of 10 Mg ha<sup>-1</sup> biochar, there was less reliance on the associations with microorganisms and more P available for the plant to access. There were more roots to access available nutrients in more of the soil volume, which in turn led to increases in aboveground growth and nutrient demand, and potentially what contributed to the general tendency for decreases in soil N concentrations with biochar addition. Additionally, the statistically similar results in root morphology for the combination of 10 Mg ha<sup>-1</sup> biochar and the half-rate of fertilizer compared to full fertilizer alone or in combination with the lesser biochar rate (5 Mg ha<sup>-1</sup>) suggest that the biochar could potentially replace some of the inorganic fertilizer inputs and still produce similar results in

terms of root ability to access nutrients. With a more extensive root system produced with the 10 Mg ha<sup>-1</sup> biochar treatment, there was more potential for root contact with biochar particles. Roots could have also grown towards the biochar to better and more directly access any available P on the biochar surface or in the surrounding soil (Prendergast-Miller et al., 2013).

#### **4.6. Summary and Conclusions**

Poultry litter biochar addition at 5 and 10 Mg ha<sup>-1</sup> to a loam soil in Northwest Arkansas increased corn height and aboveground biomass. Additions of 10 Mg ha<sup>-1</sup> biochar increased root weight and diameter and tended to increase WSP and Mehlich-3 extractable P, while tending to decrease acid phosphatase enzyme activity at the end of the experiment. Regarding root morphological parameters, the 10 Mg ha<sup>-1</sup> biochar and the half-rate of fertilizer treatment combination produced roots with similar root length, surface area, volume, and number of root tips as with the application of the full-rate of fertilizer alone. A direct biochar nutrient addition may have contributed to increases in soil nutrients, such as water-soluble and Mehlich-3 exchangeable P. Conversely, or perhaps in conjunction with increased nutrient availability, the increase in roots resulted in greater plant nutrient accessibility and thus the tendency for increases in ear-leaf P with the 10 Mg ha<sup>-1</sup> poultry litter biochar treatment. Therefore, there is the potential for decreases in inorganic fertilizer with biochar addition. However, additional research will be necessary to better understand the effects of biochar on soil variables not investigated here, such as improvements in water-holding capacity and decreased nutrient leaching, that could provide additional benefits beyond those capable of inorganic fertilizer inputs alone.

Additionally, there is the need to further investigate the biochar effects on soil and plant nutrients, especially N. In this study, ear-leaf N was unaffected by biochar addition, while soil N

tended to decrease with biochar addition, presumably due to the increases in N demand by the larger plants. Additional studies utilizing techniques such as isotope tracers as in Hammer et al. (2014) will be critical to support conclusions of direct nutrient addition from biochar products, the potential for a fertilizer effect of biochar (and thus a potential reduction in inorganic fertilizer inputs), and to better understand the fate of any exchangeable nutrients.

#### 4.7. References

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#### 4.8. Tables

Table 1. Initial mean [ $\pm$  standard error (SE)] pH, electrical conductivity (EC), total carbon (C), total nitrogen (N), C:N ratio, and total recoverable minerals for poultry litter biochar, (n = 2)

Biochar Property	Mean ( $\pm$ SE)
pH <sup>1</sup>	9.3 (0.1)
EC (dS m <sup>-1</sup> ) <sup>1</sup>	32.5 (0.2)
Total Carbon (mg g <sup>-1</sup> )	337.2 (0.3)
Total Nitrogen (mg g <sup>-1</sup> )	34.9 (0.01)
C:N ratio	9.7:1 (0.1)
Phosphorus (mg g <sup>-1</sup> )	25.7 (0.2)
Potassium (mg g <sup>-1</sup> )	52.4 (0.2)
Calcium (mg g <sup>-1</sup> )	45.4 (5.8)
Magnesium (mg g <sup>-1</sup> )	12.6 (0.9)
Sulfur (mg g <sup>-1</sup> )	13.6 (0.3)
Sodium (mg g <sup>-1</sup> )	15.3 (0.6)
Iron (mg g <sup>-1</sup> )	1.4 (0.1)
Manganese ( $\mu$ g g <sup>-1</sup> )	715.0 (18.0)
Zinc ( $\mu$ g g <sup>-1</sup> )	829.5 (26.5)
Copper ( $\mu$ g g <sup>-1</sup> )	583.0 (40.0)
Boron ( $\mu$ g g <sup>-1</sup> )	78.0 (4.0)

<sup>1</sup>1:2 saturated paste

Table 2. Initial mean [ $\pm$  standard error (SE)] particle-size distribution, pH, electrical conductivity (EC), organic matter, and Mehlich-3 extractable nutrients for the Razort soil (n = 3)

Soil Property	Mean ( $\pm$ SE)
Particle-size distribution ( $\text{g g}^{-1}$ )	
Sand	0.4 (0.2)
Silt	0.5 (0.2)
Clay	0.1 (0.2)
pH <sup>1</sup>	6.5 (0.1)
EC ( $\text{dS m}^{-1}$ ) <sup>1</sup>	0.3 (0.01)
Organic matter ( $\text{mg g}^{-1}$ )	28.4 (4.4)
Total N ( $\text{mg g}^{-1}$ )	0.9 (0.01)
Total C ( $\text{mg g}^{-1}$ )	9.8 (0.2)
Phosphorus ( $\mu\text{g g}^{-1}$ )	13.0 (0.8)
Potassium ( $\mu\text{g g}^{-1}$ )	76.1 (1.4)
Calcium ( $\mu\text{g g}^{-1}$ )	1590.0 (44.4)
Magnesium ( $\mu\text{g g}^{-1}$ )	73.8 (2.0)
Sulfur ( $\mu\text{g g}^{-1}$ )	7.6 (0.04)
Sodium ( $\mu\text{g g}^{-1}$ )	5.7 (0.2)
Iron ( $\mu\text{g g}^{-1}$ )	97.2 (2.0)
Zinc ( $\mu\text{g g}^{-1}$ )	1.8 (0.1)
Manganese ( $\mu\text{g g}^{-1}$ )	215.7 (5.8)
Copper ( $\mu\text{g g}^{-1}$ )	1.8 (0.1)
Boron ( $\mu\text{g g}^{-1}$ )	0.3 (0.01)

<sup>1</sup>1:2 saturated paste

Table 3. Analysis of variance summary of the effects of fertilizer, biochar, and their interaction (Fert x BC) on corn plant height, aboveground biomass, ear-leaf weight, ear-leaf nutrients nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), sodium (Na), manganese (Mn), zinc (Zn), copper (Cu), and boron (B), mycorrhizal infection, and root biomass, diameter, length, surface area, volume, and tips

Plant Variable	<i>P</i> - Value		
	Fertilizer	Biochar	Fert x BC
Plant height	<0.001**	0.001**	0.839
Aboveground biomass	<0.001**	0.002**	0.227
Ear-leaf weight	0.110	0.581	0.485
N	<0.001**	0.223	0.262
P	0.300	0.075*	0.850
K	0.751	<0.001**	0.032**
Ca	<0.001**	0.003**	0.214
Mg	0.001**	<0.001**	0.016**
S	<0.001**	0.124	0.607
Na	0.016**	0.244	0.308
Mn	0.019**	0.001**	0.386
Zn	0.225	0.472	0.849
Cu	0.001**	0.686	0.011**
B	0.867	0.440	0.690
Mycorrhizal infection	0.023**	0.448	0.257
Root biomass	<0.001**	0.049**	0.896
Root diameter	<0.001**	0.002**	0.499
Root length	<0.001**	<0.001**	0.022**
Root surface area	<0.001**	<0.001**	0.011**
Root volume	<0.001**	<0.001**	0.039**
Root tips	<0.001**	0.001**	0.011**

\*  $P < 0.1$

\*\*  $P < 0.05$

Table 4. Means of plant height, plant biomass, percent mycorrhizal infection, root weight, average root diameter, and corn ear-leaf nitrogen (N), calcium (Ca), sulfur (S), iron (Fe), manganese (Mn), and sodium (Na) concentrations as affected by fertilizer rate. The N and phosphorus (P) fertilizer rates are no, half (112 kg ha<sup>-1</sup> N, 39.25 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>), and full (224 kg ha<sup>-1</sup> N, 78.5 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) rates

Corn Variable	Fertilizer		
	No <sup>1</sup>	Half	Full
Plant height (cm)	94.7 c	157.3 b	177.3 a
Plant biomass (g)	8.5 c	55.0 b	84.2 a
Infection (%)	23.0 a	19.9 ab	16.7 b
Root weight (g)	3.3 c	7.8 b	12.0 a
Root diameter (mm)	1.4 c	4.5 b	6.8 a
N (mg g <sup>-1</sup> )	-	9.1 b	13.7 a
Ca (mg g <sup>-1</sup> )	-	2.2 b	3.2 a
S (mg g <sup>-1</sup> )	-	0.8 b	1.2 a
Mn (μg g <sup>-1</sup> )	-	27.7 b	39.9 a
Na (μg g <sup>-1</sup> )	-	9.9 b	48.3 a

Means followed by different letters in the same row are statistically different ( $P < 0.05$ ).

<sup>1</sup> No ear leaves were produced in the no fertilizer treatment.

Table 5. Means of plant height, biomass, root weight, average root diameter, and ear-leaf phosphorus (P), calcium (Ca), and manganese (Mn) as affected by biochar rate (0, 5 and 10 Mg ha<sup>-1</sup>)

Corn Variable	Biochar rate (Mg ha <sup>-1</sup> )		
	0	5	10
Plant height (cm)	128.4 b	144.6 a	156.3 a
Plant biomass (g)	37.9 b	50.2 a	59.6 a
Root weight (g)	6.6 b	7.3 b	9.2 a
Root diameter (mm)	3.2 b	3.9 b	5.5 a
P (mg g <sup>-1</sup> )	1.0 b	1.1 b	1.3 a
Ca (mg g <sup>-1</sup> )	3.3 a	2.5 b	2.3 b
Mn (μg g <sup>-1</sup> )	50.5 a	27.4 b	23.4 b

Means followed by different letters in the same row are statistically different ( $P < 0.05$  for all variables except ear leaf P, for which  $P < 0.1$ ).

Table 6. Analysis of variance summary of the effects of fertilizer (Fert), biochar (BC), time, and their interactions on soil water (H<sub>2</sub>O), pH, electrical conductivity (EC), organic matter (OM), dissolved organic carbon (DOC), dissolved total nitrogen (DTN), microbial carbon (Mic C) and N (Mic N), microbial C:N ratio (Mic C:N), nitrate (NO<sub>3</sub><sup>-</sup>), ammonium (NH<sub>4</sub><sup>+</sup>), inorganic N (Inorg N), dissolved organic N (DON), acid and alkaline (alk) phosphatase (Pase), and water soluble phosphorus (WSP). The N data presented are from the end of the experiment since N variables were not analyzed over time

Soil Variable	<i>P</i> - Value						
	Fert	BC	Time	Fert x BC	Fert x Time	BC x Time	Fert x BC x Time
H <sub>2</sub> O	0.049**	0.118	<0.001**	0.926	0.029**	0.607	0.122
pH	0.042**	0.874	<0.001**	0.960	0.001**	0.893	0.968
EC	0.092*	0.490	0.008**	0.856	0.227	0.434	0.644
OM	0.322	0.075*	<0.001**	0.128	0.618	0.112	0.026**
DOC	0.349	0.276	<0.001**	0.258	0.156	0.016**	0.452
Mic C	0.208	0.463	<0.001**	0.582	0.447	0.209	0.252
Mic N	0.034**	0.238	-	0.688	-	-	-
Mic C:N	0.979	0.342	-	0.021**	-	-	-
DTN	0.033**	0.050*	-	0.868	-	-	-
NO <sub>3</sub> <sup>-</sup>	0.004**	0.002**	-	0.817	-	-	-
NO <sub>4</sub> <sup>+</sup>	0.388	0.095*	-	0.433	-	-	-
Inorg N	0.042**	0.006**	-	0.558	-	-	-
DON	0.422	0.001**	-	0.048**	-	-	-
Acid Pase	0.142	0.096*	<0.001**	0.148	0.166	0.051*	0.654
Alk Pase	0.013**	0.450	0.001**	0.481	0.051*	0.375	0.380
WSP	0.107	0.073*	0.006**	0.320	0.236	0.050*	0.432

\* *P* < 0.1

\*\* *P* < 0.05

Table 7. Dissolved organic C (DOC), water-soluble phosphorus (WSP) and acid phosphatase (Acid Pase) as affected by biochar rate (0, 5, and 10 Mg ha<sup>-1</sup>) over time

Time	Biochar (Mg ha <sup>-1</sup> )	DOC ( $\mu\text{g g}^{-1}$ )	WSP ( $\mu\text{g g}^{-1}$ )	Acid Pase ( $\mu\text{g g}^{-1} \text{ hr}^{-1}$ )
Start	0	33.1 a	0.7 b	140.0 c
	5	27.2 b	0.5 b	164.1 c
	10	32.2 a	0.5 b	140.9 c
End	0	22.3 c	0.8 b	346.3 a
	5	24.3 bc	0.9 b	292.5 b
	10	23.3 c	2.0 a	261.9 b

Means followed by different letters in the same column are statistically different ( $P < 0.05$  for DOC and  $P < 0.1$  for WSP and Acid Pase).

Table 8. Analysis of variance summary of the effects of fertilizer (Fert), biochar (BC), time, and their interactions on Mehlich-3 extractable phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), iron (Fe), manganese (Mn), and copper (Cu) concentrations

Mehlich-3 Soil Variable	<i>P</i> - Value						
	Fert	BC	Time	Fert x BC	Fert x Time	BC x Time	Fert x BC x Time
P	0.204	0.094*	0.062*	0.629	0.539	0.126	0.530
K	0.878	0.053*	0.007**	0.218	0.348	0.507	0.274
Ca	0.172	0.329	<0.001**	0.651	0.346	0.457	0.959
Mg	0.001**	<0.001**	<0.001**	0.313	0.321	<0.001**	0.987
S	0.445	0.018**	0.672	0.200	0.637	0.312	0.331
Fe	0.773	0.003**	<0.001**	0.735	0.174	0.002**	0.109
Mn	0.566	0.003**	<0.001**	0.964	0.149	0.002**	0.403
Cu	0.170	<0.001**	<0.001**	0.691	0.638	0.041**	0.322

\*  $P < 0.1$

\*\*  $P < 0.05$

Table 9. Mehlich-3 extractable soil nutrients [i.e., magnesium (Mg), iron (Fe), manganese (Mn), and copper (Cu)] as affected by biochar rate (0, 5, and 10 Mg ha<sup>-1</sup>) over time

Time	Biochar (Mg ha <sup>-1</sup> )	Mg ( $\mu\text{g g}^{-1}$ )	Fe ( $\mu\text{g g}^{-1}$ )	Mn ( $\mu\text{g g}^{-1}$ )	Cu ( $\mu\text{g g}^{-1}$ )
Start	0	47.2 d	49.9 c	95.7 e	1.1 d
	5	49.8 cd	52.2 c	101.1 de	1.2 c
	10	53.4 c	55.7 bc	108.5 d	1.2 c
End	0	54.3 c	52.6 c	149.0 c	1.3 b
	5	74.8 b	64.9 a	175.9 a	1.5 a
	10	90.0 a	59.4 b	160.5 b	1.5 a

Means followed by different letters in the same column are statistically different ( $P < 0.05$ ).

Table 10. Means of alkaline phosphatase (Alk Pase) and pH as affected by fertilizer rate over time. The N and phosphorus (P) fertilizer rates are no, half (112 kg ha<sup>-1</sup> N, 39.25 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>), and full (224 kg ha<sup>-1</sup> N, 78.5 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) rates

Time	Fertilizer	Alk Pase ( $\mu\text{g g}^{-1} \text{hr}^{-1}$ )	pH
Start	No	165.7 a	6.6 c
	Half	176.9 a	6.6 c
	Full	153.2 ab	6.7 c
End	No	94.7 c	7.2 b
	Half	156.9 ab	7.3 b
	Full	136.8 b	7.6 a

Means followed by different letters in the same column are statistically different ( $P < 0.1$  for Alk Pase and  $P < 0.05$  for pH).

Table 11. Means of soil dissolved total N (DTN), nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), inorganic N, and Mehlich-3 soil phosphorus (P), potassium (K), and sulfur (S) as affected by biochar rate (0, 5, and 10  $\text{Mg ha}^{-1}$ ). The N data presented are from the end of the experiment since N variables were not analyzed over time

Soil Variable	Biochar rate ( $\text{Mg ha}^{-1}$ )		
	0	5	10
P ( $\mu\text{g g}^{-1}$ )	7.7 b	9.7 ab	12.0 a
K ( $\mu\text{g g}^{-1}$ )	73.6 b	153.1 a	159.1 a
S ( $\mu\text{g g}^{-1}$ )	18.9 b	50.7 a	59.7 a
DTN ( $\mu\text{g g}^{-1}$ )	4.4 a	4.0 ab	3.6 b
$\text{NO}_3^-$ ( $\mu\text{g g}^{-1}$ )	0.9 a	0.5 ab	0.2 b
$\text{NH}_4^+$ ( $\mu\text{g g}^{-1}$ )	0.7 a	0.1 b	0.1 b
Inorganic N ( $\mu\text{g g}^{-1}$ )	1.6 a	0.6 b	0.2 b

Means followed by different letters in the same row are statistically different ( $P < 0.05$  for all variables except P, K, DTN and  $\text{NH}_4$ , for which  $P < 0.1$ ).

Table 12. Means of Mehlich-3 extractable soil magnesium (Mg) and end of experiment dissolved total nitrogen (DTN), microbial nitrogen (Mic N), nitrate (NO<sub>3</sub><sup>-</sup>), and inorganic N as affected by fertilizer rate. The N and phosphorus (P) fertilizer rates are no, half (112 kg ha<sup>-1</sup> N, 39.25 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>), and full (224 kg ha<sup>-1</sup> N, 78.5 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub>) rates

Soil Variable	Fertilizer		
	No	Half	Full
Mg (µg g <sup>-1</sup> )	63.4 a	62.9 a	58.5 b
DTN (µg g <sup>-1</sup> )	4.5 a	3.6 b	3.9 ab
Mic N (µg g <sup>-1</sup> )	6.6 b	7.5 a	7.5 a
NO <sub>3</sub> <sup>-</sup> (µg g <sup>-1</sup> )	0.9 a	0.2 b	0.5 b
Inorganic N (µg g <sup>-1</sup> )	1.4 a	0.4 b	0.6 ab

Means followed by different letters in the same row are statistically different ( $P < 0.05$ ).

#### 4.9. Figure Captions

Figure 1. Concentration of ear leaf potassium (K) as influenced by poultry litter biochar and fertilizer rate. The fertilizer rates are no (0 kg N ha<sup>-1</sup>; 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>), half (112 kg N ha<sup>-1</sup>; 39 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>), and full (224 kg N ha<sup>-1</sup>; 78 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) nitrogen and phosphorus rates. The biochar treatments are displayed in the shaded boxes at rates of 0, 5, and 10 Mg ha<sup>-1</sup>. No ears were produced with the 0 fertilizer treatments, so no ear leaves were harvested. Bars with different letters are statistically different from each other ( $P < 0.05$ ).

Figure 2. Corn root morphology (i.e., root length, surface area, volume, and number of root tips) as influenced by poultry litter biochar and fertilizer rate. The fertilizer rates are no (0 kg N ha<sup>-1</sup>; 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>), half (112 kg N ha<sup>-1</sup>; 39 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>), and full (224 kg N ha<sup>-1</sup>; 78 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) nitrogen and phosphorus rates. The biochar treatments are displayed in the shaded boxes at rates of 0, 5, and 10 Mg ha<sup>-1</sup>. Bars with different letters are statistically different from each other ( $P < 0.05$ ).

Figure 3. Microbial C:N ratio as influenced by poultry litter biochar and fertilizer rates. The fertilizer rates are no (0 kg N ha<sup>-1</sup>; 0 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>), half (112 kg N ha<sup>-1</sup>; 39 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>), and full (224 kg N ha<sup>-1</sup>; 78 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) nitrogen and phosphorus rates. The biochar treatments are displayed in the shaded boxes at rates of 0, 5, and 10 Mg ha<sup>-1</sup>. Bars with different letters are statistically different from each other ( $P < 0.05$ ).

#### 4.10. Figures

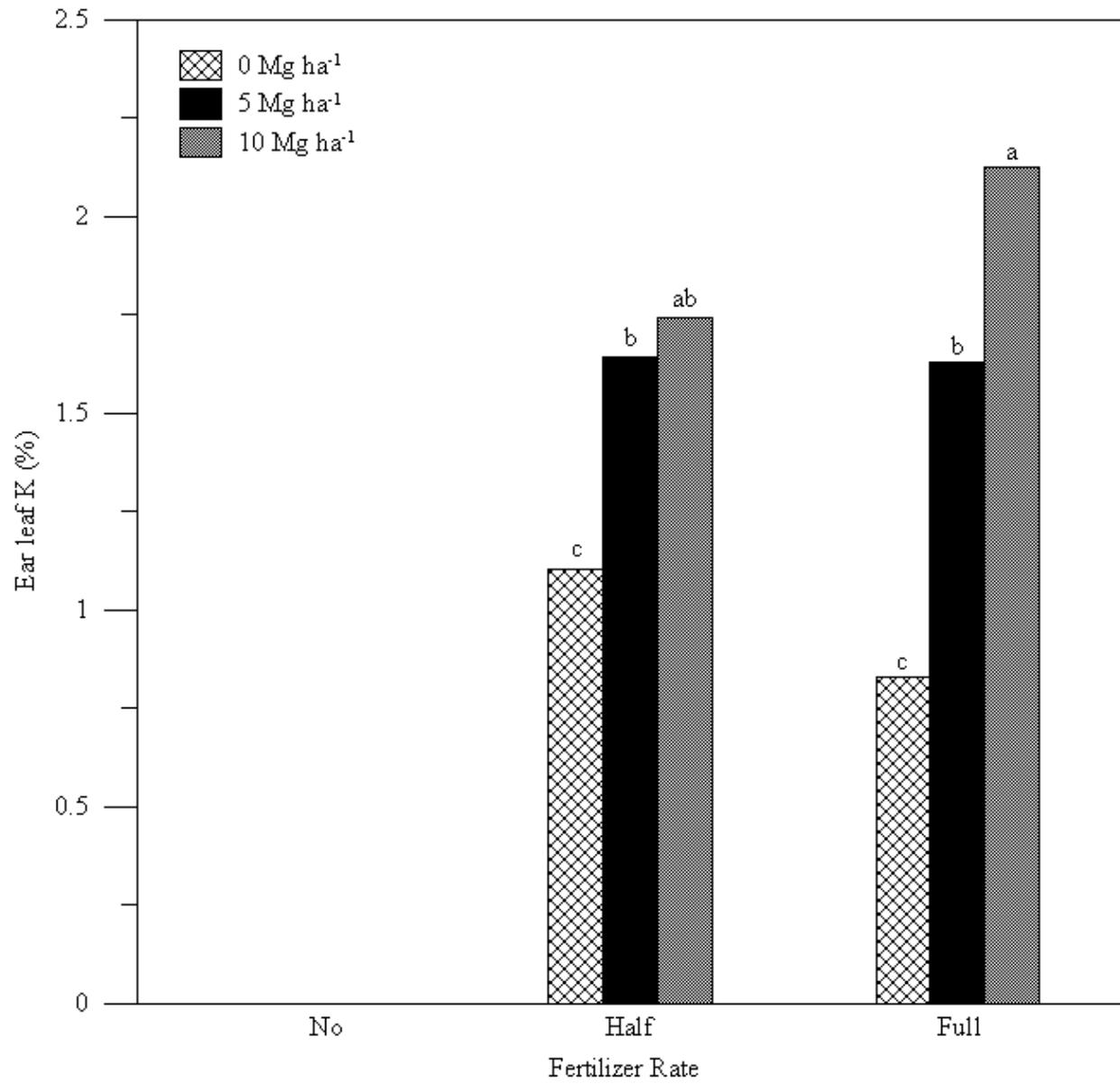


Figure 1

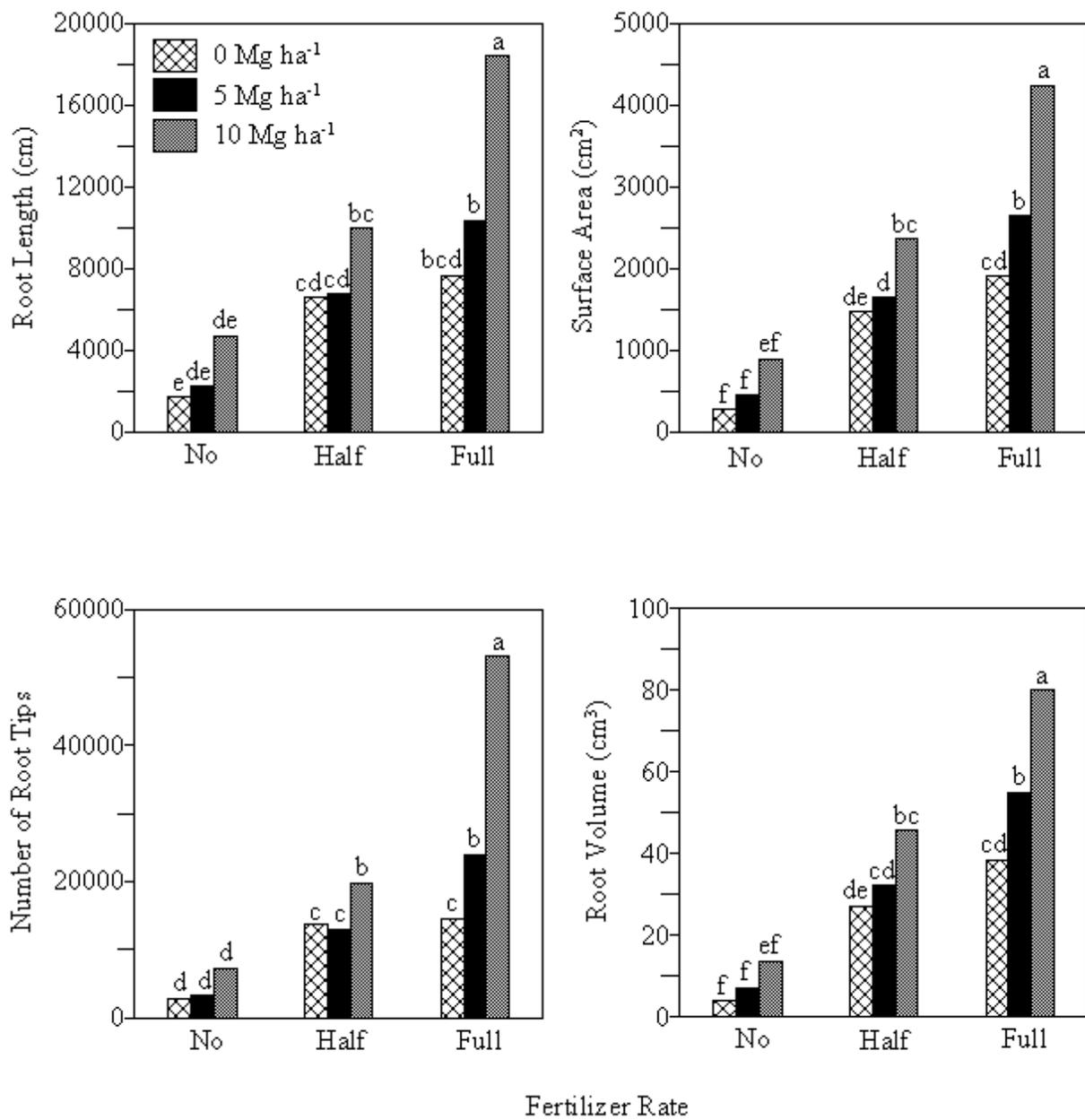


Figure 2

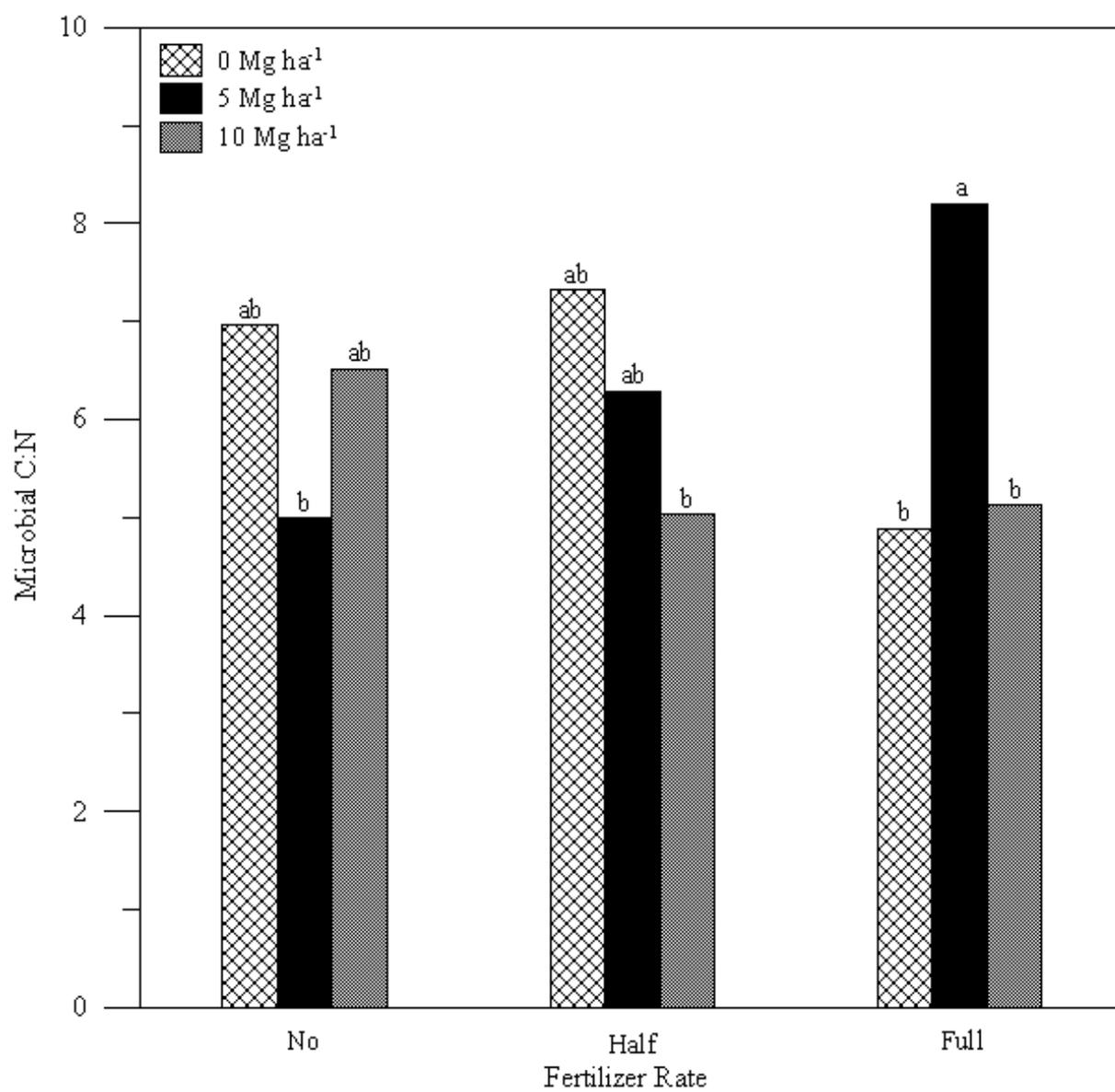


Figure 3

## **5. Pine Woodchip Biochar Impact on Soil Nutrient Concentrations and Corn Yield in a Silt Loam in the Mid-Southern U.S.**

### **5.1. Abstract**

Biochar has altered plant yields and soil nutrient concentrations and availability in tropical soils, but less research exists involving biochar additions to temperate cropping systems. The objective of this study was to determine the effects of pine (*Pinus* spp.) woodchip biochar applied at 0, 5, and 10 Mg ha<sup>-1</sup> rates in combination with varying rates of inorganic nitrogen (N) fertilizer (0, 112, and 224 kg N ha<sup>-1</sup>) on soil chemical property changes and corn (*Zea mays* L.) yield under field conditions in the first growing season after biochar addition in a silt-loam alluvial soil in Arkansas. Biochar combined with fertilizer numerically increased corn yields, while biochar alone numerically decreased corn yields, compared to a non-amended control. Corn nitrogen use efficiency (NUE) was greater with the 10 Mg ha<sup>-1</sup> biochar treatment (44.4%) compared to the no biochar treatment (12.2%), while the 224 kg N ha<sup>-1</sup> fertilizer rate increased grain N and ear-leaf N concentrations compared to the non-fertilized treatment ( $P < 0.05$ ). The 224 kg N ha<sup>-1</sup> fertilizer rate also increased soil nitrate concentrations but decreased dissolved organic N concentrations in the top 10 cm compared to the non-fertilized treatment. There were limited biochar effects on soil nutrients, but biochar decreased nitrate, total dissolved N, and Mehlich-3 extractable sulfur and manganese concentrations in the top 10 cm. Pine woodchip biochar combined with N fertilizer has the potential to improve corn production when grown in silt-loam soil in the mid-southern U.S. by improving NUE and increasing yield.

## 5.2. Introduction

Fertile and carbon-rich soils have been discovered throughout the Amazon River basin, an area where soils are typically nutrient leached and weathered (Kern et al., 2003; Lehmann, 2006). Terra Preta soils, or Amazonian dark earth soils, occur in locations classified as Oxisols and Ultisols with similar mineralogical properties as surrounding soils. However, the Terra Preta soils are differentiated by their darker color due to large amounts of organic matter (reportedly nearly  $90 \text{ g kg}^{-1}$  in the surface horizon or  $250 \text{ Mg ha}^{-1} \text{ m}^{-1}$  compared to around  $30 \text{ g kg}^{-1}$  or  $100 \text{ Mg ha}^{-1} \text{ m}^{-1}$  in surrounding Oxisols), charcoal, and A horizons ranging from 30 to 60 cm opposed to the typical 10- to 15-cm depths in adjacent soils (Glaser et al., 2001; Kern et al., 2003; Smith, 1980; Zech et al., 1990). Dark earth soils contain greater concentrations of phosphorus (P), calcium (Ca), magnesium (Mg), manganese (Mn), zinc (Zn), potassium (K), and copper (Cu) and lower iron (Fe) concentrations than adjacent soils (Glaser et al., 2001; Lehmann et al., 2003). With the presence of pottery shards and charcoal thought to be deposited from decades of low-heat domestic fires, these soils are often referred to as Anthrosols (Glaser et al., 2001; Neves et al., 2003; Smith, 1980).

In light of the observed fertility of Amazonian Anthrosols, presumably due in part to the presence of charcoal, research is ongoing to determine the effects of charcoal addition in different soils and charcoal's agronomic impact as a soil amendment. Biochar, or charcoal added to soils for the purpose of improving agronomic soil properties, can be produced by pyrolysis. Pyrolysis is the thermal conversion of biomass under no or minimal oxygen conditions with temperatures generally between 300 and  $700^\circ\text{C}$  (Demirbaş, 2001; Gaskin et al., 2008; Kloss et al., 2012; Skodras et al., 2006). The wide range of biochar products produced through various

combinations of biomass types and production conditions can lead to different results when applied to various soils and at different application rates.

With a focus beyond tropical soils, research has been conducted to investigate the effects of soil application of various biochar products in temperate regions. For example, birch (*Betula* spp.) wood biochar applied at 20 Mg ha<sup>-1</sup> to a sandy loam (Typic Hapludalf) in Denmark did not increase oat (*Avena sativa* L.) biomass or yield, but barley (*Hordeum vulgare* L.) grown the following year experienced significant biomass increases with biochar addition (Sun et al., 2014). In a sandy clay loam (Eutric Cambisol) in Wales, woodchip biochar applied at 25 and 50 Mg ha<sup>-1</sup> had no effect on corn (*Zea mays* L.) growth or nutrient concentration, although hay grass (*Dactylis glomerata* L.) grown the year after corn experienced increased foliar N with 50 Mg ha<sup>-1</sup> biochar compared to the control and increased biomass in the third year of the study (Jones et al., 2012). Poultry litter biochar addition to an Alfisol in Australia increased radish (*Raphanus sativus* L.) yield with increasing biochar addition from rates of 10, 25, to 50 Mg ha<sup>-1</sup> in a pot trial (Chan et al., 2008). In a sandy loam in Belgium, there was a general reduction in soil nitrate availability and nitrogen-use efficiency as well as reduced biomass in radish and spring barley with the addition of 10 g kg<sup>-1</sup> willow (*Salix* spp.) or pine (*Pinus* spp.) biochar, with greater reduction in soil nitrate with the willow biochar compared to the pine biochar in the pot study (Nelissen et al., 2014). Considering these and other observations, the results regarding biochar addition have been mixed based on the biochar products used, soil textures, and the specific crops grown in these temperate region studies.

Although research is accumulating in the United States regarding biochar addition to soils and the agronomic implications, research results have been inconsistent concerning the effects of biochar application in the field environment, specifically in terms of corn production. Corn is an

important commodity crop in the United States. In 2013, nearly 35.5 million ha (87.7 million ac) were harvested for grain, with grain production at a record high of almost 0.49 billion m<sup>3</sup> (13.9 billion bu) across the country (NASS, 2014). Corn also requires substantial N inputs, with recommendations of 12.9 to 19.3 kg N per m<sup>3</sup> of corn (1 to 1.5 lbs N bu<sup>-1</sup>) to meet yield goals (Espinoza and Ross, 2003). If biochar can enhance soil fertility of corn production systems in temperate agroecosystems, there is potential to improve soil quality characteristics, increase yields, and reduce commercial fertilizer-N inputs, thereby improving the sustainability of production systems.

Previous temperate region field research has included reports in Iowa of increases in corn yield but no effects on nutrient uptake after the addition of mixed hardwood biochar (96 Mg ha<sup>-1</sup>) to a Typic Hapludoll (Rogovska et al., 2014). Peanut (*Arachis hypogaea* L.) hull biochar increased soil N concentrations but did not affect corn tissue N when applied at 11.2 and 22.4 Mg ha<sup>-1</sup> to loamy sand in Georgia, while pine woodchip biochar did not increase soil N or tissue N (Gaskin et al., 2010). Corn yield was reduced with the addition of 11.2 Mg ha<sup>-1</sup> of the peanut hull biochar, but the application of 22.4 Mg ha<sup>-1</sup> produced yields similar to those of the unamended control. The pine woodchip biochar decreased corn yield with increasing application rate in the first year after biochar addition, but the second corn crop experienced increased grain yields with biochar application compared to the control (Gaskin et al., 2010). Zheng et al. (2013) observed increases in corn biomass with giant reed (*Arundo donax* L.) biochar irrespective of biochar concentration (0.1, 0.2, and 0.5 g kg<sup>-1</sup>) added to a silt loam in a pot experiment in China.

Corn was planted in a Glossoboric Hapludalf in a greenhouse study in New York with biochars produced from food waste, paper mill waste, manures, and plant residues at 300, 400, 500, or 600 °C (Rajkovich et al., 2012). Feedstock type and rates were ultimately the most

influential variables impacting corn growth in this study. Plant residue biochars tended to increase biomass when applied at rates of 2.6 and 6.5 Mg ha<sup>-1</sup>, with minimal differences at greater applications (i.e., 26 and 91 Mg ha<sup>-1</sup>). Poultry manure biochar application produced a similar pattern, while food waste, paper mill waste, and dairy manure applied at 26 and 91 Mg ha<sup>-1</sup> tended to decrease corn biomass. This study emphasizes the importance of investigating individual biochars and their agronomic effects at various rates, since clearly not all biochars are the same or produce similar effects. In Arkansas, nearly 60% of the state, or 7.5 million ha (18.6 million ac), is commercial timberland (Pelkki, 2005). Thus, pine woodchip biochar could be a potential use of wood waste from the forestry industry. Additionally, to the authors' knowledge, there are no published studies conducted in Arkansas regarding biochar application to soils.

The objective of this study was to determine the effects of pine woodchip biochar in combination with varying amounts of inorganic N fertilizer on soil chemical property changes and corn yield under field conditions in the first growing season after biochar addition. Specifically, it was hypothesized that adequate corn yields and nutrient uptake would occur with biochar addition that could reduce inorganic fertilizer inputs due to the intrinsic properties of the biochar and indirect biochar effects on soil and plant variables.

### **5.3. Materials and Methods**

#### **5.3.1. Biochar Characteristics**

Pine woodchip biochar (Waste to Energy Solutions Inc., Destin, FL), which was produced through pyrolysis at 500°C, was selected for this field study. Biochar was dried for 48 hours at 70°C then ground to pass a 40-mesh screen before pH and EC were determined potentiometrically on a 1:2 sample:water paste. Total nitrogen & total carbon concentrations

were determined by combustion with Elementar Variomax (Elementar Americas, Inc., Mt. Laurel, NJ). The C:N ratio was calculated from the total N and C concentrations. Total recoverable minerals [i.e., P, K, Ca, Mg, sulfur (S), sodium (Na), Fe, Mn, Zn, Cu, and boron (B)] were determined from acid digestion (US-EPA, 1996) using an ARCOS inductively coupled plasma (ICP) spectrophotometer (SPECTRO Analytical Instruments Inc., Mahwah, NJ).

### 5.3.2. Site Description and Experimental Design

The field experiment was conducted at the University of Arkansas Agricultural Research and Extension Center in Fayetteville, Arkansas in summer 2013. The Global Positioning System data for the four corners of the field were collected using World Geodetic System 1984 in latitude, longitude format (36.09780719°N, 94.16717458°W; 36.09780275°N, 94.16708997°W; 36.09846935°N, 94.16705646°W; 36.09846342°N, 94.16713982°W). Annual precipitation from 30-year normal data (NCDC, 2011) was 126.5 cm (49.8 in), average annual maximum air temperature was 20.2°C (68.4°F), and average annual minimum air temperature was 8.7°C (47.7°F). The soil within the field was classified as a Razort silt loam, occasionally flooded (fine-loamy, mixed, active, mesic Mollic Hapludalf; NRCS, 2014). There was a slight positive slope from north to south. The 0.26-ha field was planted the previous two years in cotton (*Gossypium hirsutum* L.). The old cotton stalks had been mowed in fall 2012.

The experimental design was a full factorial randomized complete block. There were 36 plots, each 6 m (20 ft) long and 3.6 m (12 ft) wide and consisting of 4 rows. The row spacing was 0.9 m (36 in). The total field was 73 m by 11 m (240 ft by 36 ft). A random number generator was used to place the treatments in each block. Pine woodchip biochar was added at rates of 0, 5, and 10 Mg ha<sup>-1</sup>. For the 5 Mg ha<sup>-1</sup> biochar application rate, 11.2 kg of biochar were

used per plot, and 22.5 kg were used per plot for the 10 Mg ha<sup>-1</sup> biochar application rate. Biochar was manually applied on May 28 and was incorporated with mechanical tillage into approximately the top 5 cm before rows were bedded and knocked down for planting.

Corn, DEKALB hybrid DKC64-69 with the Genuity VT Triple PRO value-added trait, was planted 74,100 seeds ha<sup>-1</sup> (30,000 seeds ac<sup>-1</sup>), which equated to 7 to 10 seeds m<sup>-1</sup> (2 to 3 seeds ft<sup>-1</sup>), on May 29 with a four-row planter. Full emergence occurred after one week, and no thinning of seedlings was required. The rows were watered by furrow irrigation as needed with the use of the Arkansas online irrigation scheduler (University of Arkansas, 2013).

Nitrogen fertilizer was applied at 0, half, and full recommended rates in a split application. The full rate was chosen to achieve a theoretical corn yield of 12.5 Mg ha<sup>-1</sup> (200 bu ac<sup>-1</sup>; Espinoza and Ross, 2003). Therefore, 224 kg N ha<sup>-1</sup> (200 lb N ac<sup>-1</sup>) were added as the full rate and 112 kg N ha<sup>-1</sup> (100 lb N ac<sup>-1</sup>) were added as the half rate of fertilizer, both in the form of urea (46-0-0). Based on initial soil analyses, no other fertilizer amendments were required. The first urea application was manually applied June 20, and the split application was applied July 9. Herbicide application consisted of 1.3 L ha<sup>-1</sup> (1.5 pt ac<sup>-1</sup>) of broadcasted Cornerstone herbicide on June 20 and a directed spray of 1.7 L ha<sup>-1</sup> (2 pt ac<sup>-1</sup>) Atrazine plus 1.7 L ha<sup>-1</sup> (2 pts ac<sup>-1</sup>) Cornerstone on July 10.

### 5.3.3. Soil Analyses

Soil was sampled at the 0- to 10-cm depth prior to treatment application to document initial properties and assess initial plot variability. Soil sampling was also conducted at the end of the growing season to assess potential treatment effects. After moist soil was sieved through a 2-mm mesh screen, soil was analyzed for dissolved organic C (DOC), dissolved total N (DTN),

ammonium ( $\text{NH}_4^+$ ), and nitrate ( $\text{NO}_3^-$ ) using a single extraction approach (Jones and Willett, 2006). A Skalar segmented-flow autoanalyzer (Skalar Inc., Norcross, GA) colorimetrically determined  $\text{NH}_4^+$  following the salicylate hypochlorite procedure and  $\text{NO}_3^-$  following a modification of Griess-Ilosvay cadmium-copper reduction of  $\text{NO}_3^-$  to  $\text{NO}_2^-$  procedure (Mulvaney, 1996). Using the chloroform-fumigation method, microbial biomass C and N were quantified by calculating the difference between fumigated and unfumigated samples for both C and N (Vance et al., 1987). Fumigated and unfumigated soils were extracted and analyzed for DOC and DTN on a Shimadzu TOC-V PC-controlled total organic carbon with attached total nitrogen analyzer (Shimadzu, Columbia, MD). Inorganic N ( $\text{N}_i$ ) was calculated by summing concentrations of  $\text{NO}_3^-$  and  $\text{NH}_4^+$ . Dissolved organic nitrogen (DON) concentration was calculated by subtracting  $\text{N}_i$  from DTN (Jones and Willett, 2006).

Water-soluble phosphorus (WSP) was obtained from extractions from 2-g moist soil samples using a 1:10 soil-to-water ratio (Self-Davis et al., 2000) and analyzed by a Skalar Sans-plus segmented-flow autoanalyzer (Skalar Inc., Norcross, GA) using the ascorbic acid method (Kuo, 1996). Acid and alkaline phosphatase activities were measured using the colorimetric estimation of *p*-nitrophenol produced by phosphatase enzyme activity after soil incubation in buffered sodium *p*-nitrophenyl phosphate solution (Tabatabai, 1994).

After drying soil at 70°C for at least 48 hours, soil pH and EC were measured using a 1:2 soil:water paste. Organic matter was determined by loss-on-ignition using a muffle furnace. Mehlich-3 extractable soil nutrients [i.e., P, K, Ca, Mg, S, Fe, Mn, Cu; Tucker, 1992] were determined by ICP spectrometry.

One soil core per plot, 4.8 cm in diameter, was collected at the 0- to 10-cm depth on July 29. After drying at 70°C for at least 48 hours, soil cores were weighed for bulk density

determinations. Oven-dried soil was sieved through a 2-mm mesh screen and particle-size analysis was conducted using an adaptation of the 12-hr hydrometer method (Gee and Bauder, 1986).

#### 5.3.4. Corn Analyses

Ear leaves were harvested at tasseling from the outer two rows of each plot for leaf tissue-N analysis. Ear leaves were dried at 65°C, ground to pass a 40-mesh screen, and weighed before ear-leaf total N was determined by combustion (Plank, 1992) using a Model Rapid N III (Elementar Americas, Inc., Mt. Laurel, NJ). The harvested yield area was the center 1.5 m (5 ft) of the center two rows in each plot. Grain was harvested on September 28 once physiological maturity had been reached, and yield was calculated based on grain dry weight. Grain samples were ground prior to analysis for total N by combustion. Nitrogen use efficiency (NUE) was calculated using the difference method, where NUE in grain was equal to the difference between N removed in grain and the N removed in the unamended control grain divided by the fertilizer-N applied (Pomares-Garcia and Pratt, 1978). The N removed in grain was calculated by multiplying the N concentration in the grain by the mass of grain (yield), assuming 720 kg grain per m<sup>3</sup> (56 lb bu<sup>-1</sup>).

#### 5.3.5. Data Analyses

A two-way analysis of variance (ANOVA) was performed using SAS (version 9.2, SAS Institute, Inc., Cary, NC) to determine any initial soil differences in the plots on the soil characteristics soil pH, EC, OM, DOC, DTN, microbial C, N, and C:N ratio, NO<sub>3</sub><sup>-</sup>, NH<sub>4</sub><sup>+</sup>, N<sub>i</sub>, DON, WSP, acid and alkaline phosphatase enzyme activities, and Mehlich-3 extractable soil

nutrient concentrations (i.e., P, K, Ca, Mg, S, Fe, Mn, and Cu). A two-way ANOVA was also performed using the data from the soil harvested at the end of the growing season to determine the effects of biochar, fertilizer, and their interaction on similar soil characteristics as were analyzed for the initial soil. An additional ANOVA was performed to determine the effect of biochar, fertilizer, and their interaction on soil bulk density and particle-size fractions sand, silt, and clay. A two-way ANOVA was performed to determine the effects of biochar, fertilizer, and their interaction on ear-leaf weight and N, corn yield, grain total N, and NUE. Least significant differences were used to separate treatment means at  $\alpha = 0.05$ .

## **5.4. Results**

### **5.4.1. Initial Biochar and Soil Properties**

The pine woodchip biochar had an alkaline pH, EC over  $5 \text{ dS m}^{-1}$ , and a C:N ratio of 365:1 (Table 1). The surface texture was confirmed to be silt loam with percentages of sand, silt, and clay of 26, 65, and 9%, respectively (Table 2). The soil possessed a near-neutral pH of 6.4 and EC of  $0.16 \text{ dS m}^{-1}$ . Ideal soil pH for corn growth ranges from 5.8-7 (Espinoza and Ross, 2003). Since the initial soil pH fell within this range, no additional liming was necessary. All initial soil property variables except for Mehlich-3 extractable soil P were statistically similar ( $P < 0.05$ ) among all plots. Mehlich-3 soil P ranged from 29.3 to  $35.2 \mu\text{g g}^{-1}$ . Mehlich-3 extractable soil nutrient concentrations (Table 2) were adequate for corn production (Espinoza and Ross, 2003). Soil bulk density differed among fertilizer-biochar treatment combinations when analyzed during the middle of the experiment. However, a clear pattern was lacking between fertilizer rate and biochar rate in terms of how treatments affected soil bulk density, which ranged from  $1.18$  to  $1.33 \text{ g cm}^{-3}$ .

#### 5.4.2. Post-Harvest Soil Characteristics

In terms of treatment effects on various post-harvest soil characteristics, biochar had minimal influence. However, alkaline phosphatase enzyme activity differed among fertilizer-biochar treatment combinations ( $P = 0.046$ ; Table 3). Alkaline phosphatase enzyme activity was greater in the no fertilizer with 5 Mg ha<sup>-1</sup> biochar treatment combination and the 112 kg ha<sup>-1</sup> fertilizer with 0 Mg ha<sup>-1</sup> biochar treatment combination than that in the 10 Mg ha<sup>-1</sup> biochar with 224 kg ha<sup>-1</sup> fertilizer combination and the no fertilizer with 0 or 10 Mg ha<sup>-1</sup> biochar treatment combinations (Figure 1). Water-soluble P also differed among fertilizer-biochar treatment combinations ( $P = 0.045$ ; Table 3), with similar concentrations across fertilizer rates with 10 Mg ha<sup>-1</sup> biochar, but with greater WSP in the 224 kg ha<sup>-1</sup> fertilizer and no biochar treatment combination compared to other treatments without biochar (Figure 2). Among the 5 Mg ha<sup>-1</sup> biochar treatments, WSP concentrations were lowest with the 112 kg ha<sup>-1</sup> fertilizer rate.

Nitrate and DON concentrations differed among fertilizer rates ( $P < 0.031$ ; Table 3). Nitrate increased with the 224 kg ha<sup>-1</sup> fertilizer treatment (3.9 µg g<sup>-1</sup>) compared to the 0 kg ha<sup>-1</sup> fertilizer treatment (2.4 µg g<sup>-1</sup>), while DON was lower with the 224 kg ha<sup>-1</sup> fertilizer treatment compared to the 0 and 112 kg ha<sup>-1</sup> fertilizer treatments (Table 4). Nitrate and DTN concentrations differed among biochar rates ( $P < 0.044$ ; Table 3). The addition of biochar, irrespective of rate, decreased DTN concentrations compared to no biochar addition, while nitrate concentrations decreased with the 10 Mg ha<sup>-1</sup> biochar treatment (2.5 µg g<sup>-1</sup>) compared to no biochar addition (3.8 µg g<sup>-1</sup>; Table 5).

Dissolved organic C concentrations increased ( $P = 0.041$ ; Table 3) with the 112 kg ha<sup>-1</sup> (12.4 µg g<sup>-1</sup>) compared to the 0 kg ha<sup>-1</sup> (8.6 µg g<sup>-1</sup>) fertilizer treatment (Table 4). Mehlich-3

extractable soil S and Mn differed among biochar rates ( $P < 0.045$ ; Table 6). Soil S decreased with the 10 Mg ha<sup>-1</sup> biochar treatment compared to the 0 and 5 Mg ha<sup>-1</sup> treatments (Table 5). Manganese decreased with the 10 Mg ha<sup>-1</sup> biochar treatment (72.3 μg g<sup>-1</sup>) compared to the no biochar treatment (80.6 μg g<sup>-1</sup>). Conversely, Fe increased ( $P = 0.035$ ; Table 6) with the 224 kg ha<sup>-1</sup> fertilizer treatment (30.6 μg g<sup>-1</sup>) compared to the no fertilizer treatment (25.3 μg g<sup>-1</sup>; Table 4).

#### 5.4.3. Corn Characteristics

Corn yield differed among fertilizer-biochar treatment combinations ( $P = 0.011$ ; Table 7), with the 224 kg N ha<sup>-1</sup> fertilizer and 10 Mg ha<sup>-1</sup> biochar treatment combination resulting in a greater yield [15.7 Mg ha<sup>-1</sup> (250 bu ac<sup>-1</sup>)] than those produced by treatments with no fertilizer or no biochar (Figure 3). Yields were similar between the 10 Mg ha<sup>-1</sup> biochar treatment combined with either the 112 or 224 kg ha<sup>-1</sup> fertilizer N. Yields produced with the 5 Mg ha<sup>-1</sup> biochar treatment combined with the 112 or 224 kg ha<sup>-1</sup> fertilizer treatment were similar to yields produced with all treatment combinations involving fertilizer, except the 112 kg ha<sup>-1</sup> fertilizer and 5 Mg ha<sup>-1</sup> biochar treatment combination produced lower yields than the 224 kg ha<sup>-1</sup> fertilizer and 10 Mg ha<sup>-1</sup> biochar combination. When no fertilizer was applied, the 10 Mg ha<sup>-1</sup> biochar treatment reduced yield [11.4 Mg ha<sup>-1</sup> (182 bu ac<sup>-1</sup>)] compared to the no fertilizer and no biochar treatment combination [13.2 Mg ha<sup>-1</sup> (210 bu ac<sup>-1</sup>)].

In contrast to the differences in corn yield among fertilizer-biochar treatment combinations, grain N concentrations differed among fertilizer rates ( $P = 0.014$ ; Table 7). The 224 kg ha<sup>-1</sup> fertilizer treatment produced greater grain N (12.4 mg N g<sup>-1</sup>) than the 112 kg ha<sup>-1</sup> fertilizer treatment (11.4 mg N g<sup>-1</sup>) and the no fertilizer treatment (11.1 mg N g<sup>-1</sup>; Table 4). The

addition of fertilizer at either rate increased ear-leaf weight and ear-leaf N concentrations compared to the no fertilizer treatment (Table 4). While biochar did not significantly affect ( $P > 0.05$ ; Table 7) grain N concentrations, ear-leaf weight, or ear-leaf N, NUE was greatest ( $P = 0.003$ ; Table 7) with the 10 Mg ha<sup>-1</sup> biochar treatment compared to the 5 or 0 Mg ha<sup>-1</sup> biochar treatments (Table 5).

## 5.5. Discussion

Average corn yields in Arkansas for 2012 and 2013 were close to 11.3 Mg ha<sup>-1</sup> (180 bu ac<sup>-1</sup>) and consistent with the lowest yields in this study (NASS, 2014). Nutrients were therefore not limiting and were adequate before treatment applications to produce average state yields. Variety testing in 2013 of irrigated corn using the specific corn variety used in this study, DEKALB DKC64-69, yielded 15.8 Mg ha<sup>-1</sup> (252 bu ac<sup>-1</sup>) when grown in silt loam at the University of Arkansas Experiment stations in Stuttgart, Rohwer, and Bell Farm (Bond et al., 2013). The greatest yields from this study were consistent with those reported from the variety testing in the same growing year. The above-state-average yields even with the 112 kg ha<sup>-1</sup> fertilizer treatment were potentially due to residual N in the soil. In this case, even though yields produced with the no fertilizer and no biochar treatment combination were equivalent to the state average for corn, the addition of biochar combined with fertilizer improved crop production to reach the yield potential achieved in the variety testing trials.

In another wood biochar field study, increases in soybean (*Glycine max* L.) yields and doubling of forage biomass with 3.9 Mg ha<sup>-1</sup> hardwood biochar were observed in a high-P clay loam in Quebec (Husk and Major, 2011). However, hardwood biochar applied at rates of 0, 25, and 50 Mg ha<sup>-1</sup> did not affect growth performance (corn height or biomass) and did not affect

grain N concentrations in a sandy clay loam (Eutric Cambisol) in a field trial in Wales (Jones et al., 2012). The lack of a wood biochar effect on plant N concentrations in temperate soil was also observed in this study, with only fertilizer influencing grain and ear-leaf N concentrations.

Although changes in N concentrations in the grain and ear leaf were not influenced by biochar treatments, there was an increase in NUE with the 10 Mg ha<sup>-1</sup> biochar application compared to the 0 or 5 Mg ha<sup>-1</sup> biochar treatments. In a silt loam in an Italian field study, durum wheat (*Triticum durum* L.) grain production increased with biochar addition (30 and 60 Mg ha<sup>-1</sup>) compared to no biochar addition, while there was no biochar effect on grain N concentration (Vaccari et al., 2011). Since grain N concentrations were unaffected by biochar addition, there was no N-dilution effect corresponding with the increase in yield, suggesting greater fertilizer NUE with biochar treatments than without (Vaccari et al., 2011). The lack of a N-dilution effect also resulted in a field study conducted in a Typic Hapludoll in Iowa with hardwood biochar applied at five rates between 0 and 95.8 Mg ha<sup>-1</sup>, in which corn yield increased with biochar addition, but biochar did not affect plant tissue N concentrations (Rogovska et al., 2014). Zheng et al. (2013) reported lower concentrations of N absorbed by corn seedlings in a three-month pot experiment but also greater biomass production with the amount of N that was absorbed compared to the no biochar treatment. Therefore, it was suggested that based on the lower N accumulation efficiency but greater NUE with biochar compared to the no biochar treatment, biochar could have enhanced N availability to the corn plants and therefore decreased N fertilizer demand (Zheng et al., 2013).

In this study, corn yields actually decreased with the addition of 10 Mg ha<sup>-1</sup> biochar in the no fertilizer treatment compared to the no fertilizer and no biochar treatment combination. There was the possibility of biochar increasing microbial immobilization or sorption of soil N in the

treatments lacking fertilizer. Once fertilizer was added, corn yield increased numerically with biochar additions, so any reduction of N release by microbial immobilization or N sorption by biochar was presumably not negatively impacting yield when inorganic N fertilizer was applied.

Three Wisconsin cropped soils, a Typic Hapludalf (clay loam), Typic Udipamment (loamy sand), and Typic Argiudoll (silt loam), all experienced increased microbial biomass and activity with mixed manure and pine biochar application in a soil microcosm experiment; microbial biomass and activity increased with increasing biochar concentration (0, 10, 25, 50, and 100 g kg<sup>-1</sup>) and extractable-N decreased (Kolb et al., 2009). In contrast, Streubel et al. (2011) reported decreased N mineralization in a silt loam with wood biochar in Washington. Immobilization of soil or biochar N from microbial decomposition of the pine woodchip biochar may have occurred in this study based on the wide C:N ratio of the biochar, but there was no significant biochar effect on microbial biomass C, N, or C:N ratio, thus failing to provide support that changes in the microbial community explain differences in soil N concentrations or NUE of corn with biochar treatments.

Lower soil N concentrations with biochar applications may have resulted in part from N loss mechanisms such as leaching, erosion, and denitrification, which were not directly investigated in this study. Since the fertilizer used was urea, there was the potential for N loss by ammonia volatilization. However, since biochar did not affect soil water content or pH, and ammonia volatilization was not quantified, there was no evidence to suggest that ammonia volatilization differed among biochar treatments and thus does not explain differences between soil N concentrations based on biochar treatments. After ammonia was converted to ammonium, the ammonium could have been adsorbed onto the cation exchange capacity (CEC) sites of the biochar. This could have reduced N losses by leaching if nitrification was delayed or reduced,

which could consequently reduce the pool of nitrate subject to gaseous losses during denitrification following irrigation events.

Zheng et al. (2013) reported reduced nitrate leaching after nitrate or ammonium fertilizer addition as well as increased microbial activity in giant reed biochar-amended soil. Increased N immobilization in the presence of biochar could have temporarily retained the N in the organic form and reduced the potential for inorganic N leaching (Zheng et al., 2013). In a study by Güereña et al. (2013), there was less N leaching with biochar addition combined with the recommended N fertilizer rate than with the fertilizer addition alone. The increases in soil N retention were thought to be due to increases in microbial biomass and thus N immobilization, lower gaseous and erosion losses, and increased retention of organic N on biochar surfaces. Additional research has suggested physical, in addition to biological, biochar effects to explain decreased N leaching. A hardwood biochar decreased N leached from a manure-amended Typic Hapludoll (Laird et al., 2010b), while increasing total soil N (Laird et al., 2010a). Laird et al. (2010b) suggested that biochar adsorbed ammonium onto CEC sites and reduced nitrification and in turn the potential for nitrate leaching.

Prendergast-Miller et al. (2011, 2013) observed nitrate retained by biochar as well, presumably in solution in biochar pores rather than on CEC sites, and proposed that nitrate could potentially be released from biochar pores by diffusion gradients (Prendergast-Miller et al., 2011). Prendergast-Miller et al. (2011, 2013) also suggested that biochar localized nitrate in the rhizosphere soil and that roots grew preferentially towards biochar particles. Deciduous wood biochar applied at 20 and 60 Mg ha<sup>-1</sup> produced longer wheat roots that increased the root-soil contact compared to non-biochar amended soil and could have increased root N uptake in biochar treatments (Prendergast-Miller et al., 2011). A more extensive root system could have

increased plant ability to access nutrients in the soil (Zheng et al., 2013), thus providing an explanation for the decrease in dissolved total N, nitrate, and Mehlich-3 extractable soil S and Mn concentrations with the 10 Mg ha<sup>-1</sup> biochar treatment compared to the no biochar treatment in this experiment. Additional belowground sampling would be necessary in future studies to better understand the effects of pine woodchip biochar on rhizosphere soil and corn roots.

Increased radish NUE with greenwaste biochar occurred in an acidic, Australian Alfisol, attributed in part to increases in pH, extractable soil P and K, and field capacity water content in a pot trial (Chan et al., 2007). However, increases in pH and extractable soil P and K do not seem to be reasons for improvement in this study, or in the case of field capacity water content, were not analyzed. Biochar did not alter soil pH despite the biochar's alkaline pH. In this experiment, the soil was initially similar, except for soil P concentrations, despite attempts at homogenizing the soil and uniform field preparation. However, by the end of the experiment, there were no differences based on treatment effects with Mehlich-3 soil P. The changes in WSP based on fertilizer and biochar addition did not seem to have a clear pattern, perhaps impacted by the differences in initial Mehlich-3 soil P and increases in alkaline phosphatase enzyme activity by the end of the experiment. The alkaline phosphatase enzyme activity was greatest with 5 Mg ha<sup>-1</sup> biochar application without fertilizer, possibly related to the greatest residual P in the initial soil later amended with the 5 Mg ha<sup>-1</sup> biochar treatment. With fertilizer addition, the activity seemed to level out across biochar treatments without significant differences within fertilizer treatments.

## **5.6. Summary and Conclusions**

Pine woodchip biochar applied at rates of 5 and 10 Mg ha<sup>-1</sup> in combination with N fertilizer to a fertile silt loam in northwest Arkansas numerically increased corn yields compared

to fertilizer application without biochar. However, biochar addition numerically decreased yields when applied without fertilizer, leading to statistically lower yields with the 10 Mg ha<sup>-1</sup> biochar treatment combined with no fertilizer compared to the no fertilizer treatment alone. Nitrogen use efficiency was greatest with 10 Mg ha<sup>-1</sup> biochar application, while the same rate of biochar application reduced soil nitrate concentrations. Biochar potentially altered N availability in soil, although the exact mechanisms regarding biochar-soil-plant nutrient relations need further study to elucidate. Pine woodchip biochar can improve corn NUE even in a fertile, temperate, alluvial soil and can increase corn yields in combination with N fertilizer.

## 5.7. References

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## 5.8. Tables

Table 1. Initial mean [ $\pm$  standard error (SE)] pH, electrical conductivity (EC), total carbon (C), total nitrogen (N), C:N ratio, and total recoverable mineral concentrations determined using a nitric acid digest for pine (*Pinus* spp.) woodchip biochar source, (n = 2)

Biochar Property	Mean ( $\pm$ SE)
pH <sup>1</sup>	8.7 (0.03)
EC (dS m <sup>-1</sup> ) <sup>1</sup>	5.3 (0.2)
Total Carbon (mg g <sup>-1</sup> )	244.5 (21)
Total Nitrogen (mg g <sup>-1</sup> )	0.7 (0.2)
C:N ratio	365.7:1 (64)
Potassium (mg g <sup>-1</sup> )	2.1 (0.1)
Calcium (mg g <sup>-1</sup> )	10.1 (0.5)
Magnesium (mg g <sup>-1</sup> )	2.7 (0.2)
Phosphorus ( $\mu$ g g <sup>-1</sup> )	770.5 (17)
Sulfur ( $\mu$ g g <sup>-1</sup> )	128.5 (1.5)
Sodium ( $\mu$ g g <sup>-1</sup> )	321.5 (13.5)
Iron ( $\mu$ g g <sup>-1</sup> )	868.0 (57)
Manganese ( $\mu$ g g <sup>-1</sup> )	420.5 (30)
Copper ( $\mu$ g g <sup>-1</sup> )	6.5 (0.04)
Boron ( $\mu$ g g <sup>-1</sup> )	10.4 (0.7)
Zinc ( $\mu$ g g <sup>-1</sup> ) <sup>2</sup>	0.01 (0)

<sup>1</sup> pH and EC were determined using a 1:2 soil:water saturated paste.

<sup>2</sup> Zinc in the woodchip biochar was below the detection limit of the method. Therefore, the detection limit of 0.01 was used for statistical analysis.

Table 2. Initial mean [ $\pm$  standard error (SE)] of particle-size distribution, pH, electrical conductivity (EC), organic matter, dissolved organic carbon (DOC), microbial C:N ratio, dissolved total nitrogen (DTN), nitrate ( $\text{NO}_3^-$ ), ammonium ( $\text{NH}_4^+$ ), inorganic N, dissolved organic N (DON), acid and alkaline phosphatase enzyme activities (acid Pase and alk Pase, respectively), water soluble phosphorus (WSP), and Mehlich-3 extractable nutrient concentrations for the Razort silt loam prior to treatment applications (n = 36)

Soil Property	Mean ( $\pm$ SE)
Particle-size distribution ( $\text{g g}^{-1}$ )	
Sand	0.3 (0.3)
Silt	0.6 (0.3)
Clay	0.1 (0.2)
pH <sup>1</sup>	6.4 (0.03)
EC ( $\text{dS m}^{-1}$ ) <sup>1</sup>	0.2 (0.1)
Organic matter ( $\text{mg g}^{-1}$ )	27.1 (0.4)
DOC ( $\mu\text{g C g}^{-1}$ )	33.8 (1.2)
Mic C:N ratio	5.3:1 (0.2)
DTN ( $\mu\text{g N g}^{-1}$ )	9.2 (0.2)
$\text{NO}_3^-$ ( $\mu\text{g N g}^{-1}$ )	7.6 (0.2)
$\text{NH}_4^+$ ( $\mu\text{g N g}^{-1}$ )	0.02 (0.02)
Inorganic N ( $\mu\text{g N g}^{-1}$ )	7.6 (0.2)
DON ( $\mu\text{g N g}^{-1}$ )	1.6 (0.1)
Acid Pase ( $\mu\text{g g}^{-1} \text{hr}^{-1}$ )	271.9 (14)
Alk Pase ( $\mu\text{g g}^{-1} \text{hr}^{-1}$ )	102.8 (7.2)
WSP ( $\mu\text{g g}^{-1}$ )	5.3 (0.3)
Phosphorus ( $\mu\text{g g}^{-1}$ )	31.7 (1.1)
Potassium ( $\mu\text{g g}^{-1}$ )	104.4 (4.7)
Calcium ( $\mu\text{g g}^{-1}$ )	923.4 (13)
Magnesium ( $\mu\text{g g}^{-1}$ )	46.4 (0.8)
Sulfur ( $\mu\text{g g}^{-1}$ )	5.5 (0.2)
Iron ( $\mu\text{g g}^{-1}$ )	51.1 (1.7)
Manganese ( $\mu\text{g g}^{-1}$ )	161.7 (3.7)
Copper ( $\mu\text{g g}^{-1}$ )	1.9 (0.1)

<sup>1</sup> pH and EC were determined using a 1:2 soil:water saturated paste.

Table 3. Analysis of variance summary of the effects of fertilizer, biochar, and their interaction (Fert x BC) on soil variables soil moisture (H<sub>2</sub>O), pH, electrical conductivity (EC), organic matter (OM), dissolved organic carbon (DOC), dissolved total nitrogen (DTN), microbial carbon (Mic C), microbial nitrogen (Mic N), and their ratio (Mic C:N), nitrate (NO<sub>3</sub><sup>-</sup>), ammonium (NH<sub>4</sub><sup>+</sup>), inorganic N (Inorg N), dissolved organic nitrogen (DON), acid and alkaline phosphatase enzyme activities (acid Pase and alk Pase, respectively), and water soluble phosphorus (WSP) concentrations

Soil Variable	<i>P</i> - Value		
	Fertilizer	Biochar	Fert x BC
H <sub>2</sub> O	0.754	0.065	0.335
pH	0.248	0.105	0.417
EC	0.639	0.308	0.865
OM	0.587	0.104	0.671
DOC	0.041*	0.356	0.241
DTN	0.093	0.022*	0.727
Mic C	0.257	0.778	0.435
Mic N	0.255	0.768	0.477
Mic C:N	0.385	0.577	0.419
NO <sub>3</sub> <sup>-</sup>	0.031*	0.044*	0.570
NH <sub>4</sub> <sup>+</sup>	1.000	1.000	1.000
Inorg N	0.031*	0.044*	0.570
DON	0.002*	0.644	0.203
Acid Pase	0.513	0.603	0.882
Alk Pase	0.143	0.235	0.046*
WSP	0.015*	0.767	0.045*

\* *P* < 0.05

Table 4. Mean corn grain total nitrogen (N), ear-leaf weight, and ear-leaf N concentration, and soil dissolved organic N (DON), nitrate (NO<sub>3</sub>), inorganic N, dissolved organic C (DOC), and Mehlich-3 extractable soil iron concentration, as affected by fertilizer rate

Plant/Soil	Variable	Fertilizer Rate (kg N ha <sup>-1</sup> )		
		0	112	224
Plant	Grain total N (mg g <sup>-1</sup> )	11.1 b	11.4 b	12.4 a
	Ear-leaf weight (g)	14.0 b	15.7 a	16.7 a
	Ear-leaf N (mg g <sup>-1</sup> )	23.1 b	26.7 a	28.0 a
Soil	DON (µg g <sup>-1</sup> )	2.0 a	2.1 a	1.6 b
	NO <sub>3</sub> (µg g <sup>-1</sup> )	2.4 b	2.8 ab	3.9 a
	Inorganic N (µg g <sup>-1</sup> )	2.4 b	2.8 ab	3.9 a
	DOC (µg g <sup>-1</sup> )	8.6 b	12.4 a	11.0 ab
	Iron (µg g <sup>-1</sup> )	25.3 b	28.5 ab	30.6 a

Means followed by different letters in the same row are statistically different ( $P < 0.05$ ).

Table 5. Mean nitrogen use efficiency (NUE) in corn and soil dissolved total N (DTN), nitrate ( $\text{NO}_3^-$ ), inorganic N, and Mehlich-3 extractable soil sulfur and manganese concentrations as affected by biochar rate

Plant/Soil	Variable	Biochar rate ( $\text{Mg ha}^{-1}$ )		
		0	5	10
Plant	NUE (%)	12.2 b	21.8 b	44.4 a
Soil	DTN ( $\mu\text{g g}^{-1}$ )	5.7 a	4.8 b	4.4 b
	$\text{NO}_3$ ( $\mu\text{g g}^{-1}$ )	3.8 a	2.8 ab	2.5 b
	Inorganic N ( $\mu\text{g g}^{-1}$ )	3.8 a	2.8 ab	2.5 b
	Sulfur ( $\mu\text{g g}^{-1}$ )	12.3 a	11.4 a	9.4 b
	Manganese ( $\mu\text{g g}^{-1}$ )	80.6 a	78.2 ab	72.3 b

Means followed by different letters in the same row are statistically different ( $P < 0.05$ ).

Table 6. Analysis of variance summary of the effects of fertilizer, biochar, and their interaction (Fert x BC) on Mehlich-3 extractable phosphorus, potassium, calcium, magnesium, sulfur, iron, manganese, copper, sodium, and zinc concentrations

Mehlich-3 Soil Variable	<i>P</i> - Value		
	Fertilizer	Biochar	Fert x BC
Phosphorus	0.103	0.403	0.661
Potassium	0.708	0.697	0.785
Calcium	0.094	0.146	0.064
Magnesium	0.641	0.580	0.903
Sulfur	0.582	0.008*	0.519
Iron	0.035*	0.453	0.520
Manganese	0.150	0.045*	0.105
Copper	0.952	0.226	0.942

\*  $P < 0.05$

Table 7. Analysis of variance summary of the effects of fertilizer, biochar, and their interaction (Fert x BC) on corn plant variables yield, grain total nitrogen (N), nitrogen use efficiency (NUE), corn ear-leaf weight, and corn ear-leaf N concentrations

Plant Variable	<i>P</i> - Value		
	Fertilizer	Biochar	Fert x BC
Yield	<0.001*	0.439	0.011*
Grain total N	0.014*	0.491	0.477
NUE	0.170	0.003*	0.300
Ear-leaf weight	0.005*	0.548	0.333
Ear-leaf N	<0.001*	0.555	0.710

\*  $P < 0.05$

## 5.9. Figure Captions

Figure 1. Alkaline phosphatase (Alk Pase) enzyme activity as influenced by pine woodchip biochar and fertilizer rates. The fertilizer rates are 0, 112, and 224 kg nitrogen (N) ha<sup>-1</sup> rates. The biochar treatments are displayed in the shaded boxes at rates of 0, 5, and 10 Mg ha<sup>-1</sup>. Bars with different letters are statistically different from each other ( $P < 0.05$ ).

Figure 2. Water soluble phosphorus (WSP) concentrations as influenced by pine woodchip biochar and fertilizer rates. The fertilizer rates are 0, 112, and 224 kg nitrogen (N) ha<sup>-1</sup> rates. The biochar treatments are displayed in the shaded boxes at rates of 0, 5, and 10 Mg ha<sup>-1</sup>. Bars with different letters are statistically different from each other ( $P < 0.05$ ).

Figure 3. Corn yield as influenced by pine woodchip biochar and fertilizer rates. The fertilizer rates are 0, 112, and 224 kg nitrogen (N) ha<sup>-1</sup> rates. The biochar treatments are displayed in the shaded boxes at rates of 0, 5, and 10 Mg ha<sup>-1</sup>. Bars with different letters are statistically different from each other ( $P < 0.05$ ).

## 5.10. Figures

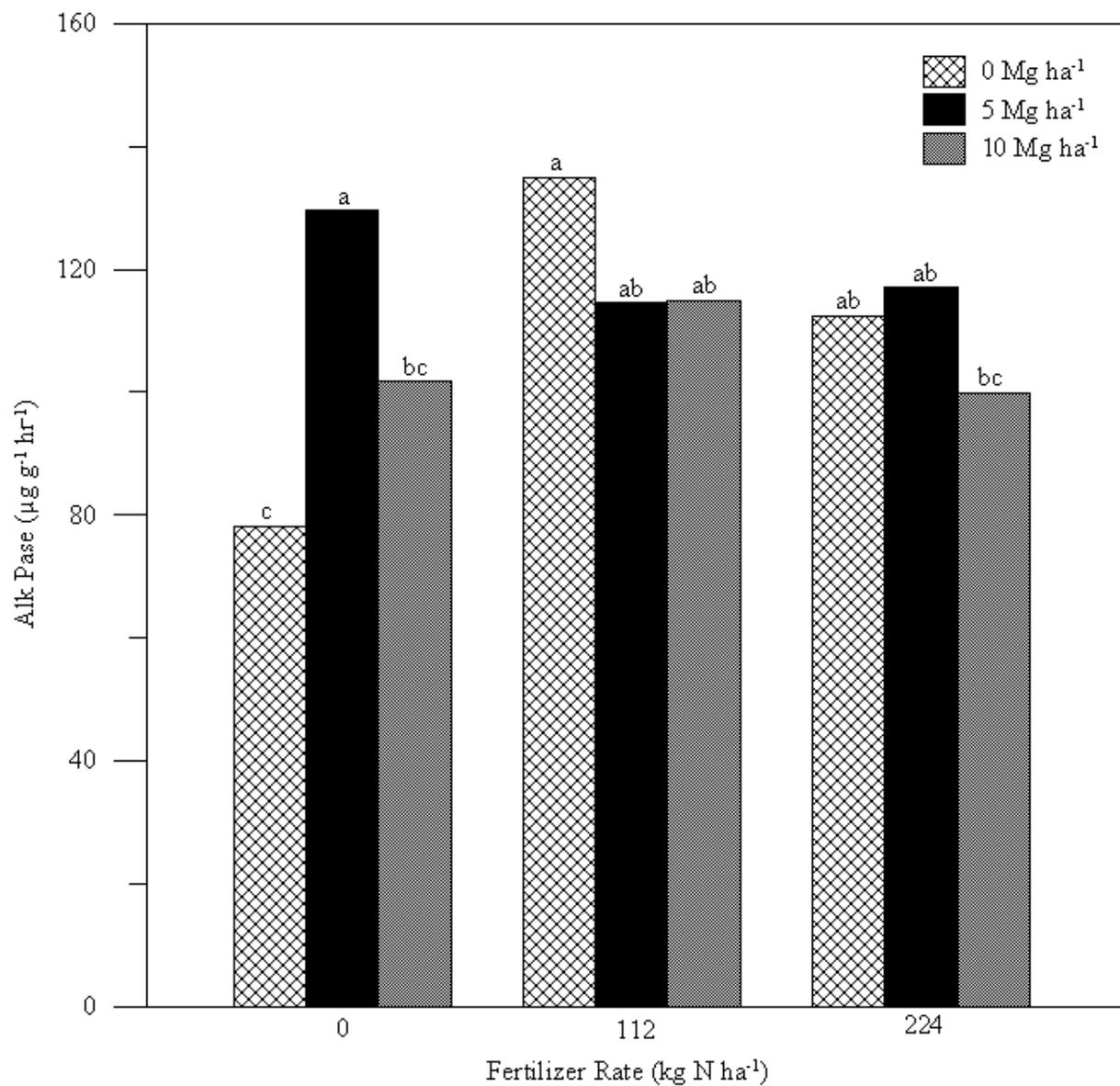


Figure 1

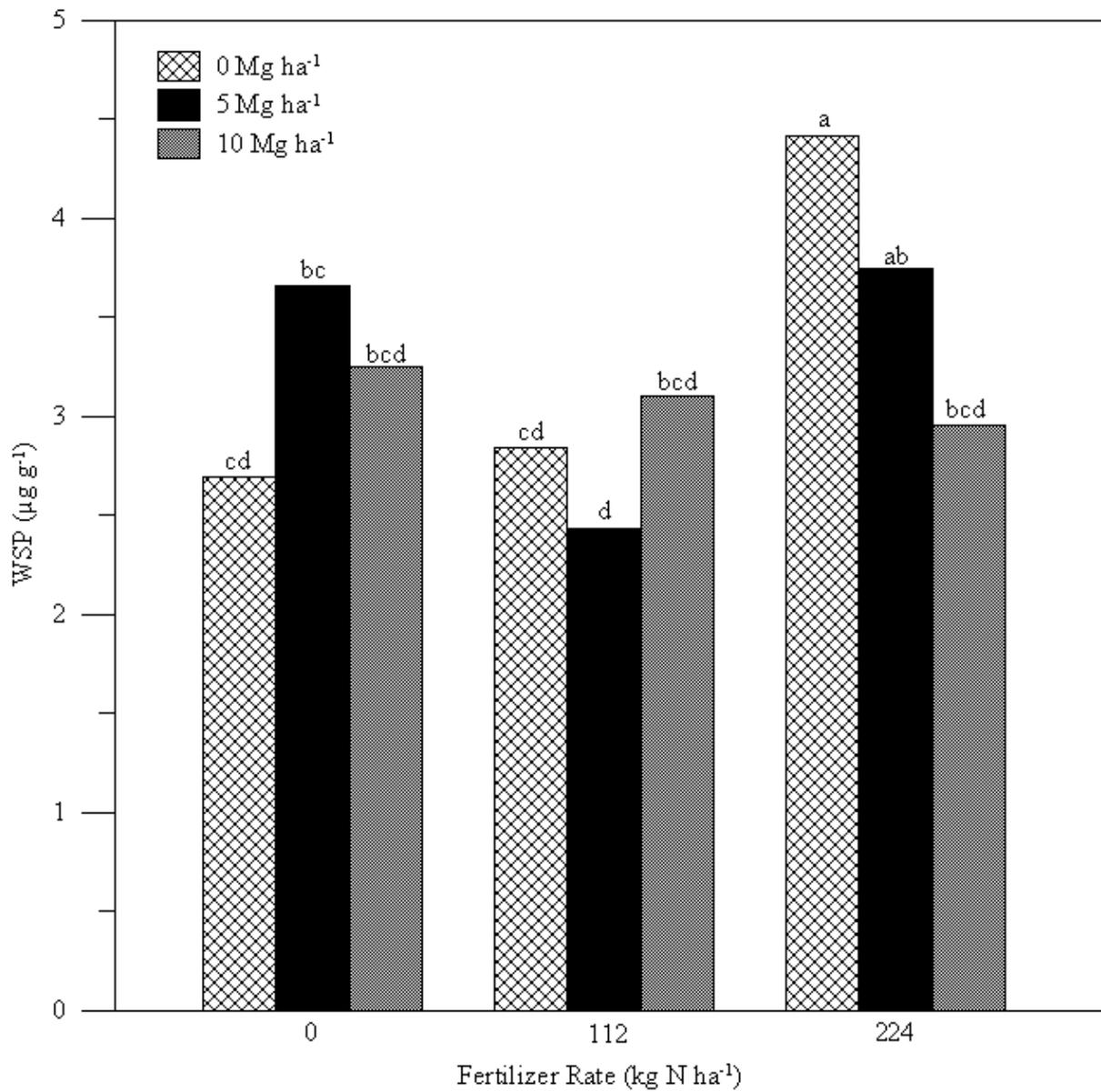


Figure 2

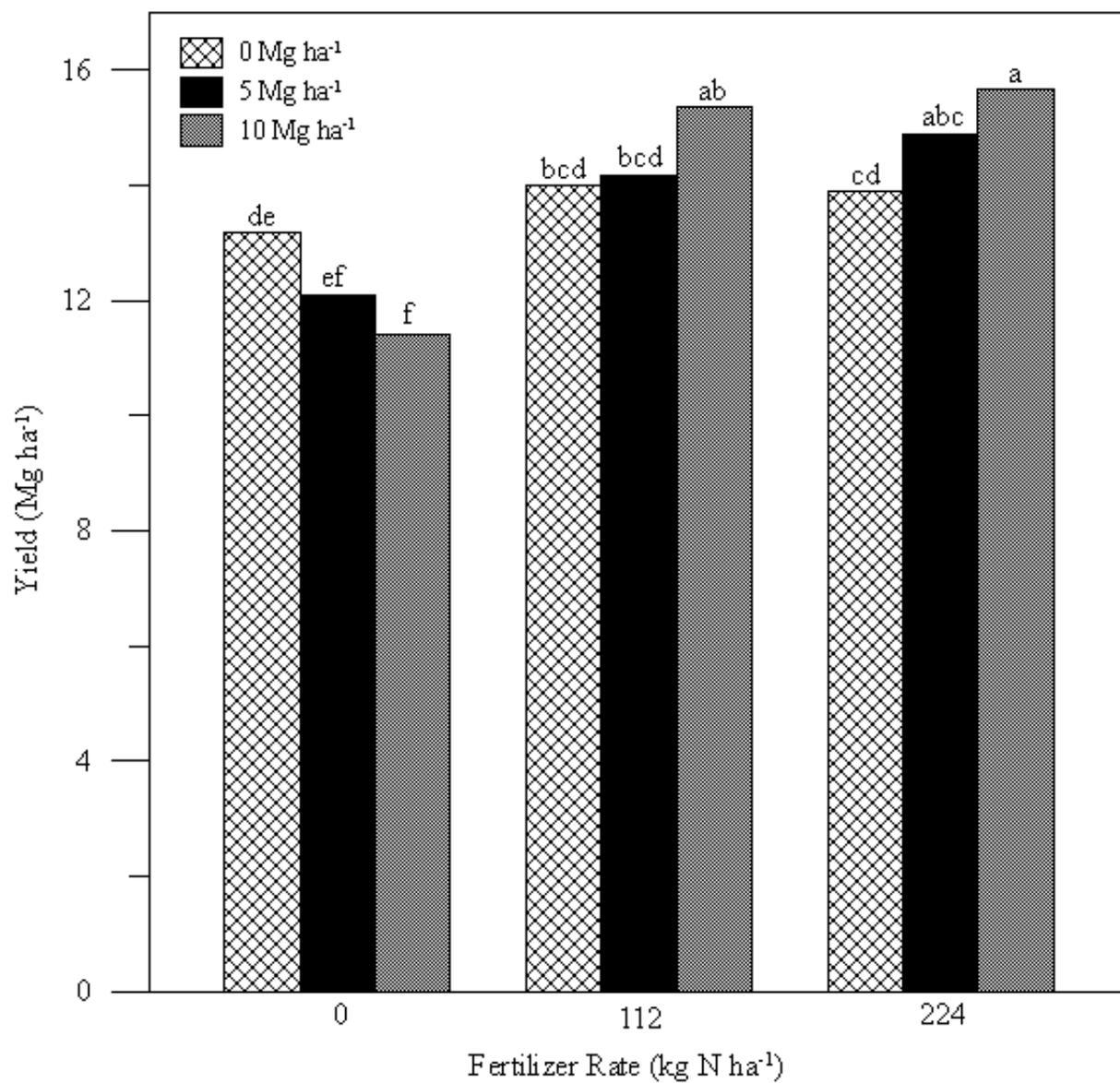


Figure 3

## 6. Conclusion

These studies investigated the effects of poultry litter and woodchip biochars on soil and corn parameters and the potential for reduced inorganic fertilizer application in Northwest Arkansas. Results from these studies suggest that biochar addition can improve corn growth. With poultry litter biochar, there is the potential for direct nutrient addition and for more extensive root growth, thus improving the ability of plant roots to access nutrients. Therefore, there is the potential for reduced inorganic fertilizer application while still obtaining adequate corn growth.

However, there is a need to further explore mechanisms behind crop improvements with biochar addition. Additionally, it is evident that biochar type and soil application rate can impact soil and plant parameters differently. Poultry litter biochar applied at  $10 \text{ Mg ha}^{-1}$  produced greater belowground growth when combined with nitrogen (N) and phosphorus (P) fertilizers than when applied at  $5 \text{ Mg ha}^{-1}$  and combined with fertilizers. Poultry litter biochar applied at rates greater than  $10 \text{ Mg ha}^{-1}$  could provide additional nutrients to plants, but Revell et al. (2012a; b) reported increases in soil Mehlich-1 P above recommended levels with poultry litter biochar addition at rates below  $10 \text{ Mg ha}^{-1}$  to high soil-P silt loam in Virginia. Therefore, initial soil nutrient concentrations need to be considered and additional variables analyzed, such as nutrient leaching and retention in soils, to better understand the environmental implications of these particular biochar amendments.

Nutrient leaching and volatilization have not been prevalent with plant and wood-based biochars. In fact, improved nitrogen retention is commonly reported (Ding et al., 2010; Güereña et al., 2013; Singh et al., 2010; Steiner et al., 2008, 2010; Zheng et al., 2013). Therefore, the use of a woodchip biochar at rates greater than  $10 \text{ Mg ha}^{-1}$  could potentially provide benefits to soils

and crops without the risk of nutrient loss as with the nutrient-rich manure biochars. Based on the results from the field study, the combination of the full-rate of fertilizer and 10 Mg ha<sup>-1</sup> woodchip biochar improved corn yield opposed to fertilizer alone. Considering that woodchip biochar improved nitrogen use efficiency (NUE), further investigation into the mechanisms behind this improved performance, such as improved fertilizer nutrient retention and NUE determined using <sup>15</sup>N isotope tracers, is necessary.

Based on results from the wetting-curve model in the water-retention experiment, more water was retained at a given water potential with the poultry litter biochar than with the woodchip biochar. Although general soil water-retention improvements were not observed at all water contents based on biochar rates (5 and 10 Mg ha<sup>-1</sup>) after one application in loam soil, additional experiments could further investigate the effects on soil water retention of poultry litter and woodchip biochars applied at greater rates and in other soils. The Mississippi River Alluvial Plain is an important crop-producing region in Arkansas, so additional research needs to be conducted in this region to observe not only the biochar effects on water retention but also additional crop responses to biochar addition such as soybean (*Glycine max* L.) and rice (*Oryza sativa* L.). Furthermore, the reported studies were short-term, thus only evaluating the effects of biochar addition to soils immediately or a few months after application. Future research should investigate longer-term biochar effects on soils and plants.

## 6.1. References

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