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Biochar Amendment for Enhanced Nitrogen Use, Soil Health, and Plant Growth

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I am submitting herewith a thesis written by Xiuwen Li entitled "Biochar Amendment for Enhanced Nitrogen Use, Soil Health, and Plant Growth." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Environmental and Soil Sciences.

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BIOCHAR AMENDMENT FOR ENHANCED NITROGEN USE,

SOIL HEALTH, AND PLANT GROWTH

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Abstract

Biochar is considered as a soil amendment to improve the resilience and productivity of agricultural systems. In particular, co-application of biochar and nitrogen (N) fertilizer has the potential to reduce N losses compared to applying synthetic N alone. However, the positive effects of biochar amendment are tightly linked to several factors. Our overall objective was to determine the effect of biochar types, application methods, and biochar rate on soil health, especially on soil N dynamics, and forage production through bench- and field-scale experiments. A 60-day bench-scale experiment was conducted to study the effect of amending soil with the two types of biochar using two application methods on soil N transformation. This experiment consisted of four treatments (control, 150 mg N kg^{-1} biochar, 150 mg N kg^{-1} urea, 75 mg N kg^{-1} urea + 75 mg N kg^{-1} biochar) with two application methods (surface application and incorporation). When biochar and urea were co-applied, biochar with higher cation exchange capacity inhibited nitrification and biochar with higher ash content reduced nitrous oxide (N_2O) emission compared to urea alone. Biochar applied on soil surface increased 47% mineral N concentration and reduced 20% N_2O emission compared to biochar mixed with soil. A two-year field experiment was conducted in a pasture system in Middle Tennessee to determine the effect of biochar rates (0 to 22.5 Mg ha^{-1}) on forage production and soil properties. Biochar was surface applied in April 2017. Soil samples were collected from 0-15 cm depth biannually beginning June 2017 and plant harvest was done in May 2017 and 2018. Results showed that $\geq$18 Mg ha^{-1} biochar rate significantly affected soil properties and 9 Mg ha^{-1} was the most profitable rate based on the cost-benefit analysis. Also, biochar addition reduced 38-53% soil mineral N within six months while increased 16-22% soil organic carbon and 12-21% extractable phosphorus within two years compared to no biochar addition. Biochar did not increase forage yield but increased plant potassium uptake by 16-26% in 2017. In conclusion, biochar exhibited positive impacts on soil quality, but these effects were influenced by biochar characteristics, application method, and biochar rates.

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CHAPTER I - INTRODUCTION AND LITERATURE REVIEW

Introduction

Biochar is the charred residue produced during thermochemical conversion of plant biomass and carbonaceous organic materials in the absence of oxygen or under substoichiometric oxygen environment required for complete combustion (Lehmann 2007; Joseph et al. 2010). The use of biochar as a soil amendment has received increased attention due to the discovery of *Terra Preta de Índios*, an anthropogenic soil with high fertility and productivity, formed by a combination of activities such as slash-and-burn agriculture, domestic fires, and intentional or unintentional application of biochar on low-fertility soils for thousands of years (Mann 2002; Clement et al. 2015). These soils still retain high fertility and organic carbon (C) content compared to the native soils (Cunha et al. 2009; Rodrigues et al. 2019). Biochar, as a soil amendment, has many advantages. After the thermal conversion of organic feedstocks such as woody materials, manure, and organic waste materials, carbon in the biochar becomes more resistant to microbial decomposition. Thus, biochar amendment enables long-term sequestration of carbon by virtue of its higher stability to microbial decomposition (Wang et al. 2016). Biochar also imparts several positive effects to soil including regulating pH, reducing greenhouse gas emissions, removing pollutants, and providing mineral nutrients (Cayuela et al. 2014; Gul et al. 2015; Brassard et al. 2016; Ding et al. 2016a; Kuppusamy et al. 2016).

The effects of biochar on soil properties and plant growth have been widely studied. When searching "biochar" and "soil" on Web of Science, more than 5,500 results were found since 2006 with topics including soil physical properties (Blanco-Canqui 2017; Zhou et al. 2018; Blanco-Canqui 2019; Fischer et al. 2019), carbon sequestration (Lorenz and Lal 2014; Sarauer et al. 2019; Schmidt et al. 2019), soil fertility (Biederman and Harpole 2013; Rens et al. 2018; El-Naggar et al. 2019; Gao et al. 2019), heavy metal removal (Beesley et al. 2011; Ippolito et al. 2019; Nigam et al. 2019; Rechberger et al. 2019), greenhouse gas emission (Hagemann et al. 2017; Verhoeven et al. 2017; Krause et al.

2018; Borchard et al. 2019), plant productivity (Jeffery et al. 2011b; Aller et al. 2018; Alvarez-Campos et al. 2018; Shahzad et al. 2018; Huang et al. 2019) and microbial activity (Lehmann et al. 2011; Harter et al. 2017; Camenzind et al. 2018; Li et al. 2018c; Novak et al. 2018; Hill et al. 2019). However, studies from the Southeastern United States, a region with hot and humid climatic conditions and less fertile soils, were very limited. This observation and the desire to understand the current state of knowledge regarding biochar amendment in soil motivated the literature review in the next section. We used insights gained from the literature review process to inform our research plan and experimental design.

Literature review

Effect of biochar on soil physical properties

<div align="center">Bulk density and aggregation</div>

Literature shows a general decreasing trend in soil bulk density after biochar addition (Blanco-Canqui 2017). Glab et al. (2016) reported that the effect on soil bulk density was influenced by the biochar particle size and application rate. There was an inverse relationship of particle size and application rate with bulk density (Rogovska et al. 2014). However, Pratiwi and Shinogi (2016) found no change in soil bulk density when 50 Mg ha^{-1} biochar was applied. Rogovska et al. (2016) also reported no change in bulk density with less than 18 Mg ha^{-1} biochar application. One mechanism by which biochar decreases soil bulk density is by enhancing aggregate formation and improving soil porosity. This is observed in several laboratory and greenhouse experiments (Blanco-Canqui 2017).

Biochar addition could promote soil aggregate formation because the hydrophobic characteristics of biochar enhances resistance to slaking and increases interarticular cohesion (Sun and Lu 2014; Zheng et al. 2018; Heikkinen et al. 2019). Though better aggregation was observed after biochar amendment in controlled environment experiments, field experiments from different locations of the United States were not in agreement with this trend. For example, Mukherjee et al. (2014b) found that

water stability of aggregates was not affected by biochar addition in Alfisols. Fungo et al. (2017) found no change in size and distribution of soil aggregates and wet aggregate stability 24 months after biochar addition in Ultisols. Major et al. (2012) reported that particle size distribution in the top 2 m of the Oxisols was not affected by biochar addition after 4.5 years. Burrell et al. (2016) reported that biochar is more useful in forming aggregates in sandy soil than clayey soil.

<p align="center">Soil moisture</p>

Most greenhouse studies proved that biochar can increase soil water content due to high adhesion and cohesion between biochar and water molecules or more water-filled pore spaces (Wang et al. 2019). Several field experiments also showed increased soil moisture content by biochar amendment. Walters and White (2018) studied the impact of biochar on soil hydrological properties in a three-year field study. The results showed that total plant-available water and water retention at permanent wilting point (-1.5 MPa) were increased by 3% and 22 %, respectively, when applying more than 40 Mg ha^{-1} biochar in a fine-loamy Ultisols. Mukherjee et al. (2014c) found that available water capacity was significantly increased by biochar treatment. However, some other studies showed different results. For examples, Hardie et al. (2014) found no significant effect of biochar on the field capacity of a sandy loam soil but the saturated moisture content improved. Jeffery et al. (2015) also reported that biochar application did not improve the hydrologic properties of a sandy soil. These contradictory results suggest that the influence of biochar on soil water content is inconclusive and it may depend on soil type, pyrolysis process, and biochar application rate.

Biochar addition also influences soil water holding capacity, however, the effect varied in different studies. Walters and White (2018) reported that applying more than 40 Mg ha^{-1} biochar increased field capacity by 7% in a fine-loamy Ultisols and Teat et al. (2015) found biochar improved water holding capacity in clay loam but not in sandy clay loam soil. Kinney et al. (2012), however, reported that field capacity increased more than 20% in sandy soil while no effect was found in clayey

soil. Kinney et al. (2012) compared the effect of pyrolysis temperature on hydrologic properties and found that 400-600 °C was the optimal temperature to produce biochar with increased field capacity and minimal hydrophobicity characteristics, regardless of the type of feedstock. Rogovska et al. (2014) found that volumetric water content significantly increased when biochar application rate was increased from 19 to 96 Mg ha^{-1}, while Hardie et al. (2014) reported no significant differences in soil water content with an increase in biochar application rate from 0 to 47 Mg ha^{-1}. These contrasting results inferred that mechanisms by which biochar influence water holding capacity is still unknown.

Effect of biochar on soil chemical properties

<u>Soil pH</u>

Soil pH controls nutrient availability and overall soil fertility. Several studies reported increased soil pH with biochar amendment. Brewer et al. (2011) reported that basic cations released by biochar such as calcium (Ca), magnesium (Mg), potassium (K), and sodium (Na) resulted in an increase in soil pH. Yuan et al. (2011) found that the functional groups on biochar surface such as carboxyl (–COO-) and hydroxyl (–O-) regulate soil pH. Berek and Hue (2016) compared the liming effect of eight biochar types in greenhouse and field experiments. The results from the greenhouse experiment showed that the liming effect of biochar was positively related to the cation exchange capacity (CEC) of biochar, but that effect was not observed from the field experiment. Soil pH increased from biochar application was observed from both field and greenhouse experiments, but the magnitude of increase was different. For example, biochar prepared from mountain gumwood increased soil pH from 4.0 to 4.7 in the field experiment and 4.5 to 4.9 in the greenhouse experiment. Biochar produced from lac tree wood increased soil pH from 4.5 to 5.8 and 4.5 to 6.3 in the greenhouse experiment at 2% and 4% application rate, respectively. However, the pH increase was lower (from 4.0 to 5.1) in the field experiment even after adding 8% of biochar. The differences in the magnitude of soil pH change between greenhouse and field experiments were attributed to many factors including feedstock type,

pyrolysis processing temperature, and how long biochar has been applied to the soil.

Dai et al. (2017) reviewed how feedstock types and pyrolysis temperature influenced the liming effect of biochar. They found that liming effect was not influenced by factors other than biochar alkalinity, which is positively related to total base cation concentration and the oxygenated functional groups (Gezahegn et al. 2019). Some studies reported that biochar provides only a short-term liming effect (Yuan et al. 2011; Hardy et al. 2017). Base cation concentration was mainly determined by feedstock type while oxygenated functional groups were determined by the pyrolysis processing conditions and time since biochar addition in soil (Verhoeven and Six 2014; Suliman et al. 2016). Brewer et al. (2011) found that slow pyrolysis increased soil pH more than fast pyrolysis, but fast pyrolysis produced more oxygen-containing functional groups. Mukherjee et al. (2014a) observed that the oxidation of functional groups due to weathering decreased biochar pH. According to Verhoeven and Six (2014), soil pH increased significantly soon after biochar application, but had no influence one year later, suggesting that biochar liming effect may decrease with time and repeated application is necessary to avoid soil acidity problems. Blending different feedstocks performed better in regulating soil pH than individual feedstocks (Novak et al. 2014b).

Although feedstock type and pyrolysis processing conditions influenced soil pH, it is hard to quantify the magnitude and the duration of the liming effect. Currently, available information is mostly obtained from laboratory-scale incubation experiments, which are useful to understand the short-term relationship between biochar characteristics and soil pH but it is important to conduct more field experiments to determine the longer-term soil pH changes from biochar addition.

<u>Soil organic carbon</u>

Soil organic carbon (SOC) serves as an indicator of soil fertility and overall soil quality by influencing water holding capacity, nutrient availability, and aggregate stability (Franzluebbers 2002; Kay 2018). Results from both lab and field experiments consistently showed increased SOC content

with biochar amendment (Novak et al. 2009; Stanton et al. 2018). Though biochar amendment is generally regarded as a strategy for C sequestration due to the high C content and stability, it can also decrease or increase native SOC decomposition depending on the amount of labile C compounds present in biochar (Luo et al. 2011; Wang et al. 2016). Therefore, SOC increased by biochar amendment could be higher or lower than the amount of C added through biochar. Results from Suddick and Six (2013) showed an increase in SOC one year after biochar application. In this experiment, total SOC concentration should have been 15 g kg^{-1} after adding 5 Mg ha^{-1} of biochar while the actual measured SOC concentration was 17 g kg^{-1}. Gao et al. (2016) applied 20 Mg ha^{-1} biochar which approximately added 19.2 g C kg^{-1} soil. After three months, SOC concentration was 12 g kg^{-1} higher in biochar amended soil than control soils but was lower than the amount of C added as biochar. Ballantine et al. (2012) showed that SOC difference between biochar amended and control treatments increased one year after application but returned to the original state after three years. Woolf et al. (2010) estimated that biochar addition would reduce 1.8 Pg CO_2-C emissions annually. These studies revealed that the extent of C sequestration potential by biochar application is highly variable.

Zhu et al. (2017) summarized that biochar can affect soil C storage through multiple mechanisms including the addition of recalcitrant C, the formation of aggregates to protect native SOC from decomposition, and favorable effect on soil microbial community structure and soil enzyme activities that control SOC decomposition. These findings were supported by Kelly et al. (2017), who found enhanced aggregate formation and increased C content eight weeks after biochar addition. In another study, Guo and Chen (2014) found that increasing pyrolysis temperature above 500 °C led to biochar with more stable and crystalline silica protected aromatic C. More stable C in biochar attributes to longer-term C sequestration in soil.

Soil nutrients

Availability of soil nutrients is very important for increasing crop productivity (Biederman and Harpole 2013). Generally, biochar increases soil nutrient concentration through two mechanisms, i) by releasing nutrients to the soil, and ii) by reducing nutrient leaching loss from the soils (Laird et al. 2010). The former is tightly related to biochar feedstock properties and the latter is tightly related to biochar surface area, porous structure and surface charge.

Nitrogen (N) is a critical nutrient that plants need for growth and productivity. Plants take up N in inorganic forms such as ammonium (NH_4^+) and nitrate (NO_3^-). Nitrification is the process which converts NH_4^+ to NO_3^- while NO_3^- is converted to nitrous oxide (N_2O) and ultimately to elemental N (N_2) through denitrification process. If N is not properly managed, significant N loss can happen through volatilization, denitrification and leaching processes. Several studies reported increased soil NH_4^+ concentration after biochar addition. Gao et al. (2016) reported that the acidic functional groups on the surface of biochar adsorbed NH_4^+, which resulted in a significant increase in extractable NH_4^+ in sandy soil. However, this response was short-term because no significant difference in soil NH_4^+ was observed at the end of the growing season. Deng et al. (2015) found that biochar increased soil NH_4^+ concentration and decreased N_2O emission in a three-year field experiment in Middle Tennessee. They suggested that biochar responded similarly to the nitrification inhibitors in increasing NH_4^+ concentration. Increase in soil extractable NH_4^+ was also found when biochar was co-applied with urea and manure (Laird et al. 2010; Güereña et al. 2013). Decreased NO_3^- loss through leaching was also observed after biochar application in some studies mainly due to the sorption of NO_3^- on biochar surface (Güereña et al. 2013; Bradley et al. 2015; Sanford et al. 2019). However, some studies reported that biochar had no or negative effect on soil mineral N content, probably due to the enhanced N immobilization by the labile organic C compounds present in biochar (Deenik et al. 2010; Foster et al. 2016; Rhoades et al. 2017).

Based on the review articles by Clough et al. (2013) and Nguyen et al. (2017b), the capacity of biochar to interact with NO_3^- and NH_4^+ is influenced by the feedstock type and pyrolysis temperature. Feedstock type affects the control of biochar on soil N. The effect of feedstock type on soil mineral N is attributed by its influence on C: N ratio of biochar (Mukome et al. 2013b). The addition of biochar with C: N ratio of ≥ 20 led to N immobilization in soil (Chan and Xu 2009). However, this effect varies with the content of easily mineralizable C. For example, biochar produced from wood chips generally has higher C: N ratio than biochar produced from plant litter, but the former resulted in less N immobilization due to less degradable C content (Nguyen et al. 2017). In addition, feedstock type and pyrolysis temperature influence the sorption of NH_4^+ and NO_3^- due to the direct effect on specific surface area and surficial functional groups (Zhang et al. 2015). For example, biochar produced from pepperwood and peanut hull above 600°C reduced the amount of NO_3^- and NH_4^+ in the leachates, while biochar produced from bagasse and bamboo or below 600°C did not adsorb NO_3^- and NH_4^+ significantly (Yao et al. 2012). Since the exchange capacity and the range of pore size changes with respect to time (Mukherjee et al. 2014c), the ability of biochar to improve soil inorganic N content may also vary with time.

Phosphorus (P) is another important major plant nutrient. In acidic soils, the amount of bioavailable P is very limited partly due to the fixation of plant-available P with iron ion Fe^{3+} or aluminum ion Al^{3+} (Bünemann 2015). Biochar application can increase available P concentration in soil in multiple ways. Ducey et al. (2015) reported that biochar produced from P-rich feedstocks can provide sufficient plant-available P. Laird et al. (2010) found that biochar can reduce P leaching via chemical sorption of P to metals or metal oxides present in biochar. Biochar can also reduce Al-P mineral form via increasing soil pH, thus increase soil P availability (DeLuca et al. 2015).

Similar to N, the effect of biochar on P also varied with feedstock and pyrolysis temperature (Gao et al. 2019). Novak et al. (2014a) compared the effect of biochar produced from different feedstocks

on soil extractable P in a pot experiment. Results showed that biochar produced from only one feedstock (swine solids) increased extractable P concentration due to the excessive P content in the feedstock. Ngatia et al. (2017) reported that biochar produced at high temperature increased P sorption to biochar surface due to more aromatic structure and higher alkalinity, thus, reduced P availability.

Studies also reported that concentration of soil potassium (K) and other cations such as Ca, Mg, and Na, increased with biochar addition (Gaskin et al. 2010; Suddick and Six 2013; Ducey et al. 2015). Gaskin et al. (2010) reported that K, Ca and Mg concentrations increased linearly at 0-15 cm depth with biochar rates. Suddick and Six (2013) found that soil retained more K and Ca with biochar addition. Ducey et al. (2015) also reported that Ca, K, Mg and Na increased with biochar addition and the feedstock type showed a significant influence on the concentrations of these nutrients.

<u>Heavy metals</u>

Biochar is regarded as an inexpensive means to clean up pollutants from soil because it has a relatively larger surface area for binding toxic elements. The adsorption capacity depends on biochar properties such as organic functional groups, mineral content, and cation exchange capacity. Several potentially toxic elements such as cadmium (Cd), lead (Pb), and arsenic (As) are adsorbed onto biochar and the extent of sorption is controlled by biochar feedstock and pyrolysis temperature. Past studies reported five mechanisms of sorption of heavy metals by biochar, including complexation with organic functional groups, precipitation with anions released by the minerals, cation exchange, chemical reduction and coordination with delocalized electrons (Li et al. 2017). Mineral content is positively related to pyrolysis temperature while the content of acidic organic functional groups is negatively related to pyrolysis temperature (Zama et al. 2017).

Sorption of Cd (II) was mostly influenced by precipitation with minerals and complexation with oxygen-containing functional groups in the biochar (Chen et al. 2015; Cui et al. 2016). Precipitation occurred between Cd (II) and some anions such as carbonate (CO_3^{2-}), phosphate (PO_4^{3-}), and sulfate

(SO_4^{2-}) released from biochar. This effect increased with increase in pyrolysis temperature up to 500°C because more anions were released at high temperature due to the cleavage of carboxyl bonds. However, when the temperature was higher than 500°C, the sorption capacity decreased because anions were reduced due to the conversion to volatile compounds such as carbon dioxide (CO_2) and sulfur dioxide (SO_2) (Cui et al. 2016). Cation exchange capacity is also an important factor influencing the Cd (II) sorption because Cd (II) can form complexation with ionized oxygen-containing functional groups in the biochar (e.g., $-COO^-$, $-O^-$) through ion exchange (Goswami et al. 2016). It is controlled mostly by the feedstock type than the pyrolysis temperature (Yasmin Khan et al. 2017; Zama et al. 2017). The mechanisms of Pb (II) sorption to biochar are similar to Cd (II), and the sorption capacity was also reduced with increase in pyrolysis temperature (Wang et al. 2015b; Ding et al. 2016b; Clemente et al. 2017).

Biochar adsorbs As (III) through electrostatic attraction, which is a very weak bonding, so the sorption of As (III) is not as strong as Pb or Cd (Liu et al. 2016b; Zama et al. 2017). The sorption of As (V) to biochar also depends on electrostatic interactions between the negative surface charge of biochar and As (V) as well as metal precipitation, which increased with large surface area and more functional groups at the biochar surface (Jin et al. 2014). Besides, the addition of biochar could reduce the bioavailability of As by enhancing the transformation from As (III) to As (V) (Vithanage et al. 2017).

Effect of biochar on soil biological properties

Microbial biomass and soil enzyme activity are influenced by biochar application. Zhou et al. (2017) reviewed the effect of biochar on soil microbial activity. Based on 97 published articles they found that biochar application increased microbial biomass carbon (MBC) in short-term (< six months) lab experiments, but unaffected in the longer-term field or greenhouse experiments (<12 months). On the contrary, no effect of biochar amendment on MBC was found in lab experiments

which are longer than six months and in field or greenhouse experiments which are longer than 12 months. Soil type also controlled the effect of biochar on MBC with mean values increased in Aridisols and unaffected in Inceptisols after 112 days of incubation experiment (Jiang et al. 2016). Biochar from pinewood had no influence on MBC in a field study conducted in Mollic Haplustalfs of Arkansas (Brantley et al. 2015), while slightly decreased MBC in a field study conducted in Aridic Haplustalfs of Colorado (Foster et al. 2016). Microbial biomass can also be estimated from phospholipid fatty acid (PLFA) analysis. Jiang et al. (2016) showed that biochar application increased PLFA in Mollisols after 30 months of incubation experiment, while Gomez et al. (2014) reported that biochar decreased PLFA in four temperate soils after 12 months of incubation experiment. These contrasting results suggest that soil type plays an important role in determining the effect of biochar on microbial biomass.

Due to the abundance of microporosity on the surface, biochar exhibits excellent sorption of microbes, enzymes, and nutrients. As a result, enzyme activity may be inhibited which has direct implications in the cycling of C and N in soil. Chintala et al. (2014) found that biochar application had negative effects on the activities of enzymes such as fluorescein diacetate (FDA) hydrolase, dehydrogenase (DHA), β-glucosidase and protease after 120 days of incubation. However, field studies showed different patterns. Elzobair et al. (2016) reported that biochar showed no effect on several enzymes including β-D-cellobiosidase, β-glucosidase, and N-acetyl-b-glucosaminidase in short-term (1 year) and long-term (4 years) field experiments. It is quite possible that the negative effect of biochar on enzyme activity becomes significant when the nutrient level is sufficient for microbial growth.

Nutrient transformations driven by the microbial activities are also influenced by biochar addition. Previous researchers used functional marker genes to explore the effects of biochar on microbial activities. For example, one indicator of nitrification activity is the gene encoding ammonia

monooxygenase enzyme, *amo*A. Nitrification is a two-step process which involves the aerobic oxidation of NH_4^+ to nitrite (NO_2^-) and then from NO_2^- to NO_3^-. The first step, also the rate-limiting step, is catalyzed by the ammonia monooxygenase enzyme (Coskun et al. 2017). Thus, measuring the abundance of *amo*A gene can represent the activity of the nitrification process. Teutscherova et al. (2017) observed that biochar addition increased the abundance of *amo*A gene via increasing pH of the acidic soil. Pereira et al. (2015) reported that feedstock type and pyrolysis temperature influenced the effect of biochar on the abundance of *amo*A gene. Results showed that biochar produced from woody materials above 500℃ increased the abundance of *amo*A gene and biochar produced from the walnut shell at 900℃ decreased *amo*A abundance. These inconsistent results suggested that feedstock, pyrolysis temperature, and soil properties influenced microbial response to the biochar addition.

Effect of biochar on plant biomass and yield

Biochar application is regarded as a means to ensure food security due to its potential to favorably affect crop productivity (Spokas et al. 2011). Greenhouse experiments showed increased crop yield from biochar application (Sigua et al. 2016), but the effect was, in general, not very promising from the field experiments. Jeffery et al. (2011b) reviewed the relationship between biochar application and crop yield, and reported that the mean increase in crop productivity from controlled experiments is three times greater than that from field trials. In a pot experiment conducted by Artiola et al. (2012), the biomass of romaine lettuce and bermudagrass were increased with biochar application at 2% rate. Rogovska et al. (2014) found that biochar application at the rate of 58 Mg ha^{-1} increased corn grain yield only in the first year. Brantley et al. (2015) also reported that the application of biochar decreased crop yield after one year. Laird et al. (2017) conducted field experiments at six sites across the United States and found that biochar has the potential to increase yield but only in poor quality soils.

Table 1-1 is a summary of crop yield response to biochar addition based on studies conducted in

Table 1-1 The response of different types of biochar on crop yield in the United States

Study Location	Feedstock Type	Crop	Yield Response	Reference
Ohio	Oak	Corn	Increase	Mukherjee et al. (2014c)
Iowa	Hardwood	Corn	Increase	Rogovska et al. (2014)
Arkansas	Pinewood	Corn	Decrease	Brantley et al. (2015)
Georgia	Pinewood	Corn	Decrease	Gaskin et al. (2010)
	Peanut hulls			
New York	Corn stove	Corn	No effect	Güereña et al. (2013)
Colorado	Pinewood	Corn	No effect	Foster et al. (2016)
Tennessee	Switchgrass	Switchgrass	No effect	Ashworth et al. (2016b)
St. Croix		Guinea grass	Decrease	
New Hampshire	Hardwood	Yellow rattle	No effect	Smith and Cox (2014)
Virginia	Broiler litter	Tall fescue	No effect	Revell et al. (2012)
		Green pepper	No effect	
Arizona	Hardwood	Grassland	No effect	Gebhardt et al. (2017)
Arizona	Pine waste	Romaine lettuce	Increase	Artiola et al. (2012)
		Bermudagrass		
Georgia	Pinewood	Sunflower	No effect	Pfister and Saha (2017)

the United States. It shows that feedstock type, crop species, and geographic region are key factors that influence crop yield. For example, biochar produced from hardwood increased yield (Mukherjee et al. 2014c; Rogovska et al. 2014) while biochar from softwood like pine chips decreased yield (Gaskin et al. 2010; Brantley et al. 2015). Biochar from the walnut shell or herbaceous feedstock materials like corn showed no significant effect on crop productivity (Güereña et al. 2013; Suddick and Six 2013). Feedstock type determines nutrients in biochar; however, no direct relationship between nutrients in feedstocks and yield were established (Spokas et al. 2011). Properties other than nutrient content may control how feedstock affects crop yield, for example, alkalinity (Biederman and Harpole 2013). Addition of biochar increases soil pH, which can increase the availability of some nutrients (e.g., P), and limit the mobility of toxic metals (e.g., Cd), both of which had a positive effect on yield improvement (Spokas et al. 2011).

Problem statement

A thorough literature review revealed that feedstock types, pyrolysis temperature, biochar amount and application time, and soil type play critical roles in determining whether biochar amendment improves soil properties and crop productivity (Joseph et al. 2010; Biederman and Harpole 2013; Gul et al. 2015; Ding et al. 2016a). In addition, application method also plays a key role. For example, through a lab-scale experiment, Page-Dumroese et al. (2015) reported that biochar applied on soil surface reduced water infiltration while that mixed with soil showed the opposite effect. Schnell et al. (2012) found that surface-applied biochar increased nutrient concentration in the surface soil layer (0-5 cm) but enhanced nutrient runoff compared to biochar mixed with soil in a greenhouse experiment. Doydora et al. (2011) reported that a mixture of biochar and litter placed on soil surface reduced phosphorus leaching compared to the mixture which was incorporated into the soil. These different responses of soil properties to biochar application method indicated that the general understanding of biochar's effect on soil properties, which were derived by incorporating biochar with soil, may not

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hold true when biochar is surface-applied. Understanding the effect of biochar's surface application is of particular importance to the agroecosystems of Tennessee as nearly 80% of row crop producers in Tennessee follow no-till management and amendments are mostly applied on the soil surface (USDA 2018a). However, biochar field experiments that studied the effect of surface application method are very limited.

The potential of biochar as a nutrient source or as a tool for nutrient management has not been widely studied. Out of all the plant nutrients, N is particularly important for increasing productivity. Farmers typically rely heavily on synthetic fertilizers to meet the crop demand for N, which often leads to over-fertilization (Galloway et al. 2008). Over-use of synthetic N fertilizers has caused serious environmental problems such as eutrophication in water bodies and deterioration of air quality due to the emission of N_2O, a potent greenhouse gas (Galloway et al. 2003; Liu et al. 2016a). Therefore, it is important to find cost-effective options to minimize the loss of N from the soil system. Our literature review revealed that biochar application is a promising strategy to reduce N losses from soil (Laird et al. 2010; Deng et al. 2015; Sanford et al. 2019), but biochar's ability to act as a sole N source to plants is questionable. Therefore, co-application of biochar and N fertilizer could be an alternative strategy for improving the N use efficiency. However, the potential of biochar to increase soil N retention could vary depending on the characteristics of biochar and soil, and application methods (Blanco-Canqui 2017; Kameyama et al. 2017).

Research objectives

The overall goal of this research is to understand how biochar interacts with synthetic N fertilizer to reduce N loss from the soil, and to determine the overall in-situ response of biochar in a no-till ecosystem. We conducted a laboratory incubation experiment for 60 days and a field experiment for two years to achieve the goal. The specific objectives of the incubation study are to 1) determine the effect of biochar and synthetic N amendment - independently and in combination - on inorganic N

availability and N_2O production in soil, 2) determine the influence of application method on the way biochar and synthetic N fertilizer affect soil inorganic N availability and N_2O production, and 3) understand the change in functional genes related to N cycling in response to biochar and synthetic N amendments as well as the application methods. Our hypotheses are, 1) co-application of urea with biochar that is characterized by higher CEC will decrease nitrification resulting in lower NO_3^--N concentration and N_2O production; 2) co-application of urea and biochar on the soil surface would result in lower nitrification rate and less N_2O emission compared to incorporation of the mixture in the soil; and 3) biochar addition would inhibit *amo*A gene expression.

To obtain a broader understanding of the in-situ response of biochar in a no-till ecosystem, we established a field study in a tall fescue dominant no-till pasture system in Middle Tennessee with the specific objectives to 1) determine the optimum rate of biochar needed for desirable soil properties and forage quality in a no-tilled system, and 2) evaluate the temporal effect of biochar amendment on soil properties and crop growth. Our hypotheses are, 1) surface placed biochar can improve soil and forage quality in the no-tilled system, 2) biochar application rate is positively related to soil and plant benefits, and 3) the positive effect from biochar amendment on soil and plant will decrease over time.

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CHAPTER II - CO-APPLICATION OF BIOCHAR AND SYNTHETIC NITROGEN REDUCED NITROGEN LOSSES FROM SOIL

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Abstract

Applying biochar with nitrogen (N) fertilizer has the potential to reduce N losses from soil which can effectively improve producer's revenue and reduces N contamination in water reservoirs. However, the effectiveness of biochar amendment on N management varies with biochar types that differ in physical and chemical properties. This study aimed to determine the effect of two types of biochar (B1 and B2), which varies in moisture content, ash content and cation exchange capacity (CEC), on soil N transformation processes when applied alone and in combination with synthetic N. Soil samples collected from a tall fescue (*Festuca arundinacea*) dominated pasture system in Lebanon, TN, were amended with two types of biochar (designated B1 and B2), urea, and urea plus biochar (B1 or B2). A control sample of the same soil with no amendment was also included and subjected to the same incubation procedure. Soil pH, nitrate N (NO_3^--N), ammonium N (NH_4^+-N) and ammonia-oxidizing bacteria (AOB) *amo*A genes transcripts were determined on day 3, 10, 30, and 60, and nitrous oxide (N_2O) production was determined 16 times during the experiment. The results showed that adding B1 biochar alone decreased NO_3^--N concentration by 21% to 45% compared to control. Soil NH_4^+-N and NO_3^--N concentrations increased immediately after the co-application of biochar and urea, while the effect of B1 + urea was significantly lower than B2 + urea until day 60 of incubation. Compared to the urea alone treatment, cumulative N_2O emission was reduced by 13% and 41% under B1 + urea and B2 + urea, respectively. We also found that urea addition inhibited *amo*A gene transcripts at the beginning of incubation but resulted in an increase after 60 days. Furthermore; B1 addition also reduced *amo*A gene transcripts after 60 days. In conclusion, our study showed that

biochar has the potential to enhance N retention in soil and reduce N_2O emission when it is applied in tandem with urea.

Introduction

Nitrogen (N) is one of the primary nutrients that plants need for growth and productivity. Farmers rely heavily on synthetic N fertilizers to improve crop yield. In 2016, more than 100 million tons of fertilizer N was applied to agricultural lands across the world (IFA 2017). However, only less than 50% of the applied N is taken up by the crops (Lassaletta et al. 2014) and the rest is lost from the system, potentially contributing to eutrophication, lake acidification, biodiversity loss, and global warming (Galloway et al. 2003). Maintaining or improving crop productivity through synthetic fertilization while causing a minimal adverse impact on the environment is one of the global challenges of humankind.

If mineral N (ammonium-N (NH_4^+-N) and nitrate-N (NO_3^--N)), the form of N taken up by the plants, is not managed properly, significant N loss can occur through volatilization, denitrification and leaching processes. Several strategies are proposed for the efficient plant use of mineral N including the selection of appropriate fertilizer types and application methods, and the use of nitrification inhibitors (Cameron et al. 2013). Although the use of NI reduces N leaching and nitrous oxide (N_2O) emission losses by slowing down the nitrification process (Cameron et al. 2013), it can result in other environmental problems such as NH_3 pollution (Romano and Zeng 2007; Adelman et al. 2009).

Biochar amendment in soil is considered an alternative strategy for improving N use efficiency (Mandal et al. 2016; Cao et al. 2019). Biochar, the by-product produced from the thermal conversion of biomass to biofuel under anoxic and high-temperature conditions (Lehmann 2007), is found to be effective in reducing N losses by physical and chemical sorption due to its higher specific surface area and charged surface functional groups (Clough et al. 2013). The oxygenated carboxyl and carbonyl functional groups in biochar can reduce NH_4^+ availability by sorption, resulting in a decreased rate of nitrification. Additionally, hydroxyl and alkyl functional groups can control the availability of NO_3^-, resulting in reduced N leaching and N_2O emission (Mukherjee et al. 2014d; Wang et al. 2015a; Zhao et

al. 2017; Hu et al. 2018). Other studies reported that biochar decreased soil mineral N concentration because it stimulated N immobilization and NH_3 volatilization (Liu et al. 2017; Nguyen et al. 2017a) or facilitated the denitrification process by changing the microbial community structure (Singh et al. 2010; Harter et al. 2014).

The inconsistent response of biochar to soil N transformation processes could be due to the differences in biochar properties, which is attributed, in part, to the differences in feedstock types and conversion temperature. The type of feedstocks used during the production of biochar largely dictates its ash content and C:N ratio (Mukome et al. 2013a). For example, biochar produced from debarked wood chips generally has lower ash content and higher C: N ratio than biochar produced from poultry litter (Nguyen et al. 2017a). Besides the feedstocks, the process conditions employed during biochar production play key roles in determining the biochar physical and chemical properties. For example, conversion temperatures influence biochar pH, specific surface area, and surface functional groups (Zhang et al. 2015). The pH, surface area, and percentage of aryl substituted functional groups of biochar increased when pyrolysis temperature increased from 200 to 700°C (Mukherjee et al. 2014d). Both feedstock and temperature influence cation exchange capacity (CEC) due to the amount of negative charge from oxygen-containing acidic functional groups formed on the biochar surface (Nguyen et al. 2017a). Generally, more oxygen-containing functional groups are expected in biochars produced from grasses because of the higher concentration of cellulose, alkaline salts and alkaline metal oxides in grasses (Harvey et al. 2012). At temperatures higher than 600°C, the conversion of oxygen-containing functional groups to neutral or basic functional groups reduces CEC (Gai et al. 2014). In general, biochar with a higher C: N ratio, higher CEC, and larger surface area reduces soil mineral N concentration (Nguyen et al. 2017a).

The effect of biochar amendment on N cycling also varies with geographic regions and ecosystems. For example, Thomazini et al. (2015) reported that biochar prepared from hardwood

increased NH_4^+ content in agricultural soil from Florida but decreased NH_4^+ in forest soil from Minnesota. Thus, regional-specific studies are necessary to better understand the effect of biochar on soil N dynamics. In this study, we conducted a laboratory experiment using soils collected from a pasture system in Middle Tennessee to determine how biochar influences soil N dynamics in the warm and humid southeastern US region. The specific objectives of this study were to determine the effect of two biochar types - alone and in combination with synthetic N amendment - on (i) soil mineral N content and N_2O production, and (ii) changes in functional genes related to N cycling. We hypothesized that co-application of urea with the biochar containing higher CEC will decrease nitrification resulting in lower NO_3^--N concentration and N_2O production.

Materials and methods

Soil collection

Soil samples were collected from a tall fescue (*Festuca arundinacea*) dominated pasture system in Lebanon city, TN, USA (36°11'45.3"N, 86°15'50.3"W). The mean annual temperature of this location is 14.5°C and the mean annual precipitation is 1342 mm. The soil is classified as Bradyville series (fine, mixed, semiactive, thermic Typic Hapludalfs). In December 2017, 10 to 15 soil samples were randomly collected from 0 to 15 cm depth using a soil auger and composited. Fresh samples were sieved through a 2 mm sieve on the same day of collection and a sub-sample was used for the soil moisture determination using the gravimetric method. After storing another sub-sample at 4°C for the incubation experiment, the rest was air-dried to determine soil physico-chemical properties following standard protocols (Table A-1).

Biochar characterization

Two types of locally available biochar were used in this study, one (B1) was produced from mixed hardwood chips without bark in Lebanon, TN by a gasification process at 700°C and the other (B2) was prepared from mixed hardwood chips with bark by Proton Power Inc. in Lenoir City, TN by

pyrolysis at 1100°C (Table A-2). The properties of both B1 and B2 are listed in Table A-2. Biochar pH

was determined by a pH meter using 1:20 biochar:deionized H_2O (w:v) (Rajkovich et al. 2012).

Biochar moisture content was determined by the ASTM D1762-84 (2007). The surface area was

determined based on CO_2 adsorption using the Brunauer-Emmet-Teller (BET) theory (Brunauer et al.

1938). Cation exchange capacity was determined according to Mukherjee et al. (2011) after slight

modification which included use of 1 μm size filter paper and determination of K concentration by an

Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (Spectro Ciros CCD). Total

carbon (C) and N concentrations were determined by the dry combustion method (Nelson and

Sommers, 1996) using Elementar Vario TOC cube CN analyzer. Both types of biochar were sieved

through a 4 mm sieve before used for the incubation experiment.

Microcosm experimental design

Fresh soils were pre-incubated at room temperature in the dark for seven days before the

experiment. After the pre-incubation, 35 g soil was transferred into a specimen cup which was placed

into a 500 mL mason jar for incubation. On day 0, soil moisture content was 26% and it was

maintained throughout the 60-day incubation by adding Milli Q water with mini pipette every week, if

needed, after weighing the specimen cups. There were four treatments with different biochar and urea

addition ratios: (i) control (soil alone with no biochar and urea), (ii) urea to provide 150 mg N kg^{-1}

soil, (iii) biochar plus urea (biochar to provide 75 mg N kg^{-1} soil + urea to provide 75mg N kg^{-1} soil),

and (iv) biochar to provide 150 mg N kg^{-1} soil. Two sets of experiments were set up with two different

types of biochar, B1 and B2. The amount of biochar and urea were decided based on an extensive

literature review of similar experiments (Kumar et al. 2007; Tong and Xu 2012). After mixing the

biochar and/or urea with the soil using a glass rod, all the jars were tightly closed and incubated at

room temperature (~ 26°C) in the dark. The jars were opened every four to six days and flushed with

room air for 10 min by a small fan to maintain the aerobic environment. There were 72 jars in total

with six treatments, four destructive sampling points, and three replications.

Measurement of nitrous oxide production

Gas samples were collected 16 times from three replicate jars of each treatment on day 0, 1, 2, 4, 6, 8, 10, 13, 16, 21, 26, 31, 37, 43, 51, and 60. Samples were taken from the headspace through the sampling port on the center of the jar lids by injecting a needle attached to a 20 mL polypropylene syringe and stored in 12 mL pre-evacuated glass vials sealed with butyl rubber septa after flushing the vials with 10 mL samples. Air samples were also collected from the room atmosphere and stored in the vials. The concentration of N_2O in the samples was determined within a week of collection by a gas chromatograph (Model GC-2014, Shimadzu, Japan) with an electron capture detector. The amount of N_2O production on day i was calculated as below:

$$N_2O_{dayi} = [(N_2O_{sample.-dayi} - N_2O_{air-dayi}) \times V]/ m \tag{1}$$

where $N_2O_{sample-dayi}$ is the N_2O concentration (mg N L^{-1}) in the sample on day i, $N_2O_{air-dayi}$ is the N_2O concentration (mg N L^{-1}) in the atmosphere on day i, V is the headspace volume of the jars (L), and m is the dry mass of soils used for incubation (kg). Cumulative N_2O emission was calculated by adding the N_2O production from individual measurements.

Destructive sampling and soil analysis

On days 0, 3, 10, 30, and 60, soils from three replicated jars were destructively sampled to measure soil pH, mineral N and N cycling genes. Each sample was divided into two subsamples, one was air-dried for soil pH and mineral N analysis, and the other was frozen at -80°C for RNA extraction. Soil pH was measured in 1: 2 soil: water ratio using an electron pH meter (Thomas 1996). To measure mineral N, 5 g air-dried soils were shaken for 30 min with 25 ml of 2 M KCl solution, and the NH_4^+-N and NO_3^--N concentrations in the filtered extracts were determined using a Continuous Flow Analyzer (Skalar Analytical B.V., The Netherlands) (Maynard et al. 1993).

RNA extraction and ammonia-oxidizing bacteria amoA gene quantification

The abundance of ammonia-oxidizing bacteria (AOB) *amo*A gene transcripts was determined on day 10 and day 60. Only two time points were selected because this measurement was expensive, and we did not have the resources to consider all the samples. The RNA from soil was extracted from 2 g frozen soil stored at -80°C by an RNeasy PowerSoil Total RNA Kit (Qiagen, Hilden, German) following the manufacturer's protocol. Polymerase chain reaction (PCR) amplification of the RNA templates was performed in 20 μL reaction mixture consisted of 3 μL RNA template with primer pairs *amo*A-1F/*amo*A-2R to check for remaining DNA (Rotthauwe et al. 1997; Haddad et al. 2007). The quality and quantity of RNA were determined using Nanodrop One[C] (Thermo Fisher, DE) to ensure high-quality RNA yield (nuclear acid concentration > 30 ng μL^{-1}, A260/A230 > 1.7 and A260/280 > 1.8). SuperScript IV First-Strand Synthesis System (Thermo Fisher, MA) was used to synthesize cDNA with random hexamer primers (Invitrogen) according to the manufacturer's protocol. After synthesis, cDNA was stored at -20°C.

Quantitative real-time PCR (qPCR) was carried out for quantifying the abundance of AOB *amo*A gene transcripts on CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories Inc., Hercules, CA) using PowerUp SYBR Green Master Mix (Thermo Fisher, MA). A standard curve was generated from serial 10× dilutions of plasmid DNA from one representative clone containing the *amoA* gene. Triplicate analyses per sample were conducted in 20 μL reaction mixtures containing 10 μL of SYBR Green Master Mix, 0.5 μL of each primer, and 3 μL of cDNA template containing approximately 20-25 ng of cDNA. The *amo*A genes were quantified using the primer pairs *amo*A-1F/*amo*A-2R (Rotthauwe et al. 1997). A negative control was included in each run with sterilized distilled water as the template instead of a cDNA sample. The qPCR condition was controlled in the following order: 95°C for 3 min, 40 cycles of 60 s at 94°C, 45 s at 56°C, 60 s at 72°C, and 72°C for 10 min. The R^2 values were 0.991-0.997 and the primer efficiencies were 71-72 %.

Calculations and statistical analysis

Net ammonification rate was calculated by Equation (2):

$$NAR = (N_{ai} - N_{a0})/\triangle t \tag{2}$$

where NAR (mg kg^{-1} day^{-1}) is the net ammonification rate of the treatment compared to the control, N_{ai} (mg kg^{-1}) is the NH$_4^+$-N concentration observed during incubation, N_{a0} (mg kg^{-1}) is the initial NH$_4^+$-N concentration on day 0, and $\triangle t$ is the time (day) when NH$_4^+$-N concentration was observed (Ste-Marie and ParC 1999).

Net nitrification rate was calculated by Equation (3):

$$NNR = (N_{ni} - N_{n0})/\triangle t \tag{3}$$

where NNR (mg kg^{-1} day^{-1}) were the net nitrification rate of the treatment, N_{ni} was the concentration of NO$_3^-$-N observed on day i, N_{n0} (mg kg^{-1}) was the initial concentration of NO$_3^-$-N on day 0, $\triangle t$ was the interval of incubation (Wang et al. 2006).

Treatment effects on NH$_4^+$-N, NO$_3^-$-N, pH, cumulative N$_2$O emission, NAR, NNR and the gene copies were analyzed by the one-way analysis of variance (ANOVA) using GLIMMIX procedure of SAS (version 9.4 Cary, NC) with treatment as fixed effects and replication as random effects for each sampling points separately. Normality was tested by Kolmogorov-Smirnov test and the homogeneity of variance was tested by Levene test before ANOVA. Statistical differences among treatments were determined by Fisher's protected least significant difference (LSD) at 5% confidence level ($p \leq 0.05$). Bivariate correlations between NH$_4^+$-N, NO$_3^-$-N, pH, and AOB *amo*A gene transcripts were determined by Pearson's correlation analysis. Before analysis, the abundance of *amoA* gene transcripts was logarithm transformed.

Results

Soil ammonium nitrogen content

Urea application alone significantly increased NH$_4^+$-N content in the soil during the first month of

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incubation ($p < 0.05$) with the highest amount of 49.5 mg N kg^{-1} soil observed on day 10 and

decreased to 15 mg N kg^{-1} soil on day 60 (Figure A-1). Co-application of biochar and urea resulted in

a general reduction in NH$_4^+$-N content compared to the application of urea alone, except in the case of

B2 + urea on day 3. Compared to the co-application of B2 and urea, B1 and urea decreased NH$_4^+$-N

concentration by 19.7 %, 71.3% and 36.9% on day 3, 10 and 30, respectively. Although the total N

contained in biochar alone treatments was similar to that in urea alone and biochar + urea treatments,

soils amended with biochar alone had the least amount of NH$_4^+$-N, which was statistically similar to

the soil only control.

Net ammonification rate was calculated by Equation (2). Biochar alone treatments resulted in

negligible NAR from -0.21 to 0.35 mg N kg^{-1} day^{-1} in B1 treatment and -0.28 to 0.27 mg N kg^{-1} day^{-1} in B2

treatment, respectively which was significantly lower than that in the urea added treatments (0.18-11.2 mg

N kg^{-1} day^{-1}) (Table A-3). Co-application of B2 and urea caused higher NAR than B1 + urea treatment until

day 60. The highest NAR was observed in B2 + urea treatment on day 3 (12.6 mg N kg^{-1} day^{-1}), which was

almost six times higher than the highest NAR from the B1+urea treatment (1.86 mg N kg^{-1} day^{-1}).

Soil nitrate nitrogen content

The NO$_3^-$-N concentration was progressively increased in all the treatments with the length of

incubation increased (Figure A-2). Urea application significantly increased soil NO$_3^-$-N concentration

from 38.2 mg N kg^{-1} on day 3 to 371 mg N kg^{-1} on day 60. Co-application of biochar and urea

significantly reduced NO$_3^-$-N concentration compared to urea alone. When the two biochar treatments

were compared, B1 + urea produced lower amount of NO$_3^-$-N than B2 + urea in the first 30 days.

However, on day 60, B1 + urea had 316 mg N kg^{-1} soil NO$_3^-$-N concentration, which was 53% higher

than that from B2 + urea. Unlike the NH$_4^+$-N results, NO$_3^-$-N from B1 alone was lower than control

during the first month while that from B2 alone was higher than control during the first 10 days. On

day 60, B1, B2, and control had similar soil NO$_3^-$-N concentrations.

The net nitrification rate was calculated by Equation (3) (Table A-3). Similar to NAR, soil amended with biochar alone had lower NNR than control. When two types of biochar were compared, B1 had lower NNR than B2. Co-application of biochar and urea significantly reduced NNR when compared to urea alone treatment. B1 + urea had significantly lower NNR than B2 + urea in the first 30 days, while the NNR of B1 + urea was increased to the highest value (4.83 mg N kg^{-1} day^{-1}) after 60 days, which is 33% higher than that of B2 + urea.

Cumulative nitrous oxide emission

Cumulative N_2O emission after 60 days of incubation varied significantly across the treatments (Figure A-3). Urea application alone resulted in the greatest amount of N_2O emission (0.15 mg N kg^{-1} soil), which was 90% more than the control. Co-application of B1 and urea also resulted in higher N_2O emission (0.13 mg N kg^{-1} soil), which was 70% higher than control, however, B2 + urea, biochar alone and control treatments produced the lowest amount of N_2O.

The abundance of amoA gene transcripts

The abundance of the AOB *amo*A genes transcripts in control ranged between 3.3×10^4 and 8.3×10^4 copies g^{-1} dry soil during incubation (Figure A-4). Urea amendment, alone and in combination with biochar, significantly reduced the abundance of AOB *amo*A genes transcripts on day 10 compared to biochar alone and control treatments, but then increased at day 60, with values ranging from 6.3×10^3 to 1.5×10^5 copies g^{-1} dry soil. We also observed decreased content of transcripts in B1 alone treatment compared to control at both time points, which was not the case for the B2 treatment. After the 60-day incubation, the abundance of *amo*A transcripts in B1 treatment was 71% lower than control. A correlation analysis between the logarithm transformed *amo*A gene transcripts and mineral N was shown in Figure A-5. The *amo*A gene transcripts were positively correlated to NO_3^--N concentration and negatively correlated to NH_4^+-N concentration and soil pH in urea amended treatments (Figure A-5 b, d, f). No correlation was observed in control and biochar alone treatments

(Figure A-5 a, c, e).

Discussion

Effect of urea and biochar on nitrogen mineralization

Soil NH_4^+-N concentration was increased from day 0 to day 10 of incubation and then sharply decreased for all the urea added treatments (Figure A-1), which is consistent with the results from other studies (Nielsen et al. 1998; Baiga and Rao 2017; Tambone and Adani 2017). Also, NAR was closer to zero when only biochar was added and increased several-fold with urea addition suggesting that soil NH_4^+-N concentration was increased mainly due to urea hydrolysis. Urea-added treatments resulted in higher substrate (NH_4^+-N) enhanced nitrification, evidenced by increased soil NO_3^--N concentration (Figure A-2), NNR (Table A-3), and N_2O emission (Figure A-3). Urea addition can also influence nitrifier activity as ureolysis produces CO_2, which can be a C source for nitrifiers to stimulate nitrification (Mobley and Hausinger 1989; Denecke and Liebig 2003). However, our data showed that *amoA* gene transcripts, which encode the enzyme that catalyzes the NH_4^+-N oxidation step in the nitrification process, were reduced by urea in the early phase of incubation (day 10) and then increased after 60 days (Figure A-4). The initial inhibition effect was probably caused by the excessive amount of NH_3, which can be toxic to nitrifiers (Anthonisen et al. 1976; Geisseler and Scow 2014). This finding is consistent with the study by Staley et al. (2018), which observed lower nitrifier diversity in the soil with a higher concentration of urea.

In contrast to the effect of urea, the addition of biochar alone had a negative effect on nitrification compared to control, which may be because of the reduced NH_4^+-N availability as a result of NH_4^+ absorption on biochar (Yang et al. 2015). Biochar has previously been shown to stimulate the activity of N-immobilizing heterotrophs, leading to the consumption of available NH_4^+ by both nitrifiers and N-immobilizing heterotrophic microorganisms and overall inhibition of nitrification (Martin et al. 2015). Wang et al. (2017) found that biochar amendment slowed the nitrification process by reducing

the abundance of AOB. In this experiment, the abundance of *amo*A gene transcripts was lower in biochar-treated treatments, especially in the case of B1, as compared to the control treatment (Figure A-4), which also indicated that biochar addition inhibited nitrifier's activity.

Biochar type significantly influenced N transformation when biochar was co-applied with urea. For example, B1 resulted in lower NAR than B2, which could be attributed to the higher CEC of B1 than B2 (Table A-1), leading to more urea absorption by B1. We also found a delay of NO_3^--N production when B1 was co-applied with urea as it took a longer time to reach the maximum NNR (Table A-3). This reiterates the ability of B1 to retain urea within their exchange sites longer than B2. A previous study reported that urea loading onto biochar surface resulted in slow and incomplete (70-80 %) release of NH_4^+-N from urea (Manikandan and Subramanian 2013). Saha et al. (2017) also reported that charcoal with higher CEC reduced urea mineralization. In addition, the slow release of urea can reduce NH_3 toxicity, thus enhances long-term microbial activity (Timilsena et al. 2015). This is supported by our finding that AOB *amoA* gene expression in B1 + urea was significantly higher than B2 + urea after 60 days (Figure A-4).

Our results showed that urea stimulated N mineralization by enhancing both ammonification and nitrification processes while biochar inhibited N mineralization by slowing nitrification. The mineral N (NH_4^+-N and NO_3^--N) concentration in urea alone treatment after 60 days of incubation was 386 mg N kg^{-1}, which was much higher than the sum of added amount of N and the mineral N derived from soil alone (150 mg N kg^{-1} as urea + 81 mg N kg^{-1} from soil, which is called expected mineral N concentration below). This indicated that more organic N from soil was transformed to mineral N, exhibiting a positive priming effect. The enhanced organic N transformation was also observed when urea was co-applied with biochar. Compared to the expected mineral N concentration (231 mg N kg^{-1}), the amount of mineral N on day 60 in B1 + urea and B2 + urea treatments were 44% and 10% higher, respectively. Similar to our findings, Baiga and Rao (2017) found that co-application of biochar and

urea enhanced microbial activity resulting in higher N mineralization rate compared to urea alone treatment and control. Fiorentino et al. (2019) also reported higher soil mineral N concentration in biochar treatment compared to control, with or without urea. However, they attributed this increase to the displacement of N from the bound N pool or the sorption of NH_3 rather than enhanced microbial activity. Other studies showed evidence for no or negative effect of biochar on N mineralization when applied with N fertilizer (Dempster et al. 2012).

Effect of urea and biochar on nitrous oxides emission

As expected, urea treatment had the highest cumulative N_2O emission after 60 days of incubation (Figure A-3). However, co-application of biochar and urea reduced N_2O emission significantly. Grutzmacher et al. (2018) also reported that the application of biochar and N fertilizer together decreased N_2O emission by 67-95% compared to N fertilizer alone treatment when the same amount of ammonium nitrate was added. The favorable effect of biochar in reducing N_2O emission could be attributed to biochar's ability to inhibit nitrification (as described in the previous section) as well as denitrification processes. Some studies attributed lower N_2O production from biochar amendment to enhanced redox reaction that converts N_2O to N_2, which can be influenced by metal ions or organic radicals in the biochar (Obia et al. 2015; Quin et al. 2015; Grutzmacher et al. 2018). This could be the potential dominant mechanism for the substantial reduction in N_2O from the B2 + urea treatment in our study, since B2 contains 2 times more ash content than B1 (Table A-1) and thus possibly has greater metal ion content and the ability to function as an "electron shuttle" (Grutzmacher et al. 2018). Some other studies reported that biochar-reduces N_2O production due to the entrapment of N_2O on the biochar surface area which slows down the gaseous diffusion (Harter et al. 2016). Although the total surface area of both B1 and B2 were similar, the moisture content of B1 was > five times higher than B2 (Table A-1). So, B2 should have more effective surface area for absorbing N_2O, resulting in lower N_2O emission.

Abundance of amoA genes transcripts and its relationship with nitrogen fertilizer

The abundance of *amoA* gene transcripts is an important indicator of the AOB activity in the nitrification process. In urea added treatments, NO_3^--N concentration was positively related to *amo*A gene transcript (Figure A-5b), however, the nitrification rate showed no increase during the 60 days of incubation (Table A-3). This indicates that more *amoA* genes were expressed as incubation progressed to maintain the same rate of nitrification as in the beginning, and it also suggests a decrease in specific cell activity with the length of incubation. The decrease of cell activity was also reported by Prosser and Nicol (2012) and they linked this effect to soil pH changes. The nitrifying bacteria *Nitrosomonas* and *Nitrobacter* are sensitive to pH changes (Norton and Stark 2011) and a pH range of 6.6-8.0 is considered as optimal for nitrification (Calderon et al. 2005). In our experiment, soil pH for the urea alone treatment was 5.71 on day 60, which was considerably lower than the initial pH (6.5) (Figure A-6)).

Although a significant relationship of *amoA* gene transcripts with mineral N and soil pH was observed in urea added treatment (Figure A-5 b, d, f), no relationship was observed in no urea added treatments (Figure A-5 a, c, e), which indicated that AOB has a control on the N mineralization processes occurring in urea added treatments only, due to the higher mineral N content from urea addition. This result indicated that other microbes, like ammonia-oxidizing archaea (AOA), played a greater role than AOB in nitrification, which is influenced by the depth of soil and management practices (e.g. agricultural liming, animal grazing, and nutrient fertilization) (Leininger et al. 2006; Prosser and Nicol 2008; Daebeler et al. 2014; Egan et al. 2018). Although the relative importance of AOA and AOB in N mineralization process is still unclear, some other studies revealed that AOB outcompete AOA under high N input while AOA is functionally dominant in soils with low-N input (Di et al. 2009; Lu et al. 2012; Ouyang et al. 2017; Egan et al. 2018).

Conclusions

This 60-day incubation experiment compared the effect of applying two types of biochar with and without urea on N dynamics in soils from fescue dominated system in Middle Tennessee. Despite adding the same amount of N in all the treatments except control, the N mineralization, N_2O emission, and AOB *amoA* gene expression were different among treatments. Urea addition increased ammonification and nitrification as well as AOB activity as incubation progressed. However, co-application of biochar and urea reduced both NH_4^+-N and NO_3^--N concentrations and N_2O production as compared to urea alone treatment, indicating that providing N as two sources (organic form by biochar and inorganic form by urea) is better in retaining N longer in soil. When applied with urea, biochar with higher CEC effectively reduced N losses by decreasing ammonification and nitrification rates, while biochar with higher ash content and more effective surface area reduced N losses by decreasing N_2O production. Overall, applying synthetic N fertilizer and biochar together showed promise in reducing N losses from the system. Future studies are needed to determine the effect of co-application of biochar and urea on N use efficiency in the field considering the effect on plant growth, plant nutrient uptake, and nutrient losses.

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Appendix A: Tables and Figures

Table A-1 Properties of the soil used for the incubation experiment

Soil property	Unit	Mean value
pH (H$_2$O)		6.3
Moisture content	%	26
Total organic C	g C kg^{-1}	18.9
Total N	g N kg^{-1}	1.7
C: N ratio		11:1
NH$_4^+$-N	mg N kg^{-1}	4.3
NO$_3^-$-N	mg N kg^{-1}	26.4
P	mg kg^{-1}	1.91
K	mg kg^{-1}	176
Ca	mg kg^{-1}	1195
Na	mg kg^{-1}	1.34
Mg	mg kg^{-1}	177
Al	mg kg^{-1}	154
Cu	mg kg^{-1}	0.58
Fe	mg kg^{-1}	3.27

Table A-2 Physico-chemical properties of biochar

	Biochar 1 (B1)	Biochar 2 (B2)
pH (H_2O)	10.4	8.96
Total C (g C kg^{-1})	830	855
Total N (g N kg^{-1})	10.5	8.1
C:N ratio	79:1	105:1
Moisture content (%)	56.1	9.29
Surface area (m^2 g^{-1})	279	295
Ash content (%)	3.17	8.13
Cation exchange capacity (cmol$_c$ kg^{-1})	202	71.6

Table A-3 Net ammonification rate (NAR) and net nitrification rate (NNR) (mg N kg^{-1} day^{-1}) for all treatments at different time point of the incubation.

Time	Control	B1	B2	B1+urea	B2+urea	Urea
			Net ammonification rate (mg N kg^{-1} day^{-1})			
Day 3	-0.32[c]	-0.21[c]	-0.28[c]	1.27[b]	12.3[a]	11.2[a]
Day 10	0.36[d]	0.35[d]	0.27[d]	2.22[c]	2.87[b]	4.52[a]
Day 30	0.01[d]	0.05[c]	0.07[c]	0.05[c]	0.37[b]	1.20[a]
Day 60	0.07[c]	0.10[bc]	0.13[ab]	0.20[a]	0.12[ab]	0.18[a]
			Net nitrification rate (mg N kg^{-1} day^{-1})			
Day 3	-1.22[c]	-2.84[d]	1.07[b]	0.28[b]	3.33[a]	3.94[a]
Day 10	0.56[e]	-0.26[f]	1.56[d]	3.16[c]	5.13[b]	5.63[a]
Day 30	1.50[cd]	0.43[e]	1.24[d]	1.71[c]	4.68[b]	5.94[a]
Day 60	0.77[d]	0.41[d]	0.83[d]	4.83[b]	3.61[c]	5.75[a]

Treatments followed by different letters within each measurement time were significantly different ($p \leq$ 0.05).
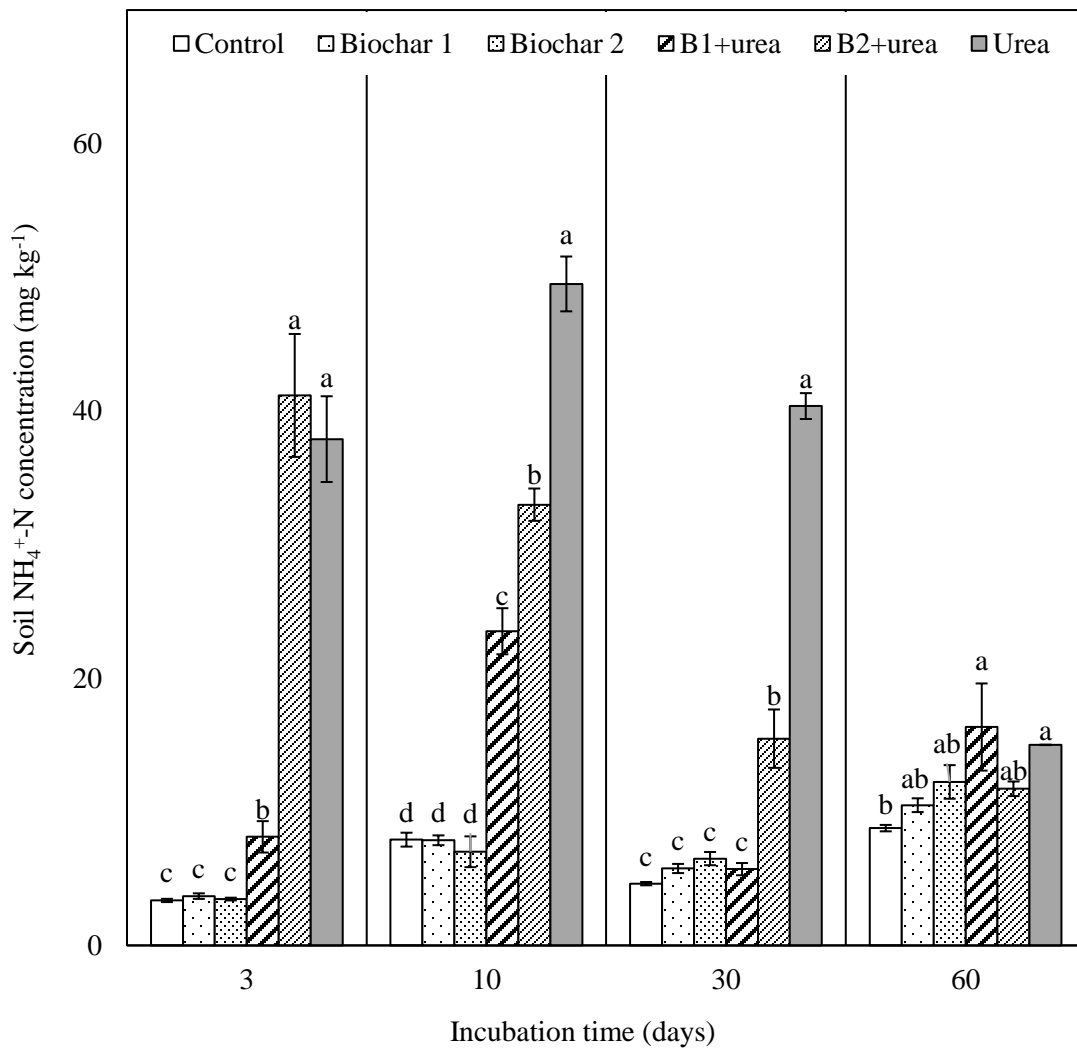
Figure A-1 Changes in soil NH_4^+-N concentration from all the treatments on day 3, day 10, day 30 and day 60 of incubation. Error bars represent standard error (n = 3). Different letters above the bars within each panel means a significant difference between treatments ($p \leq 0.05$).
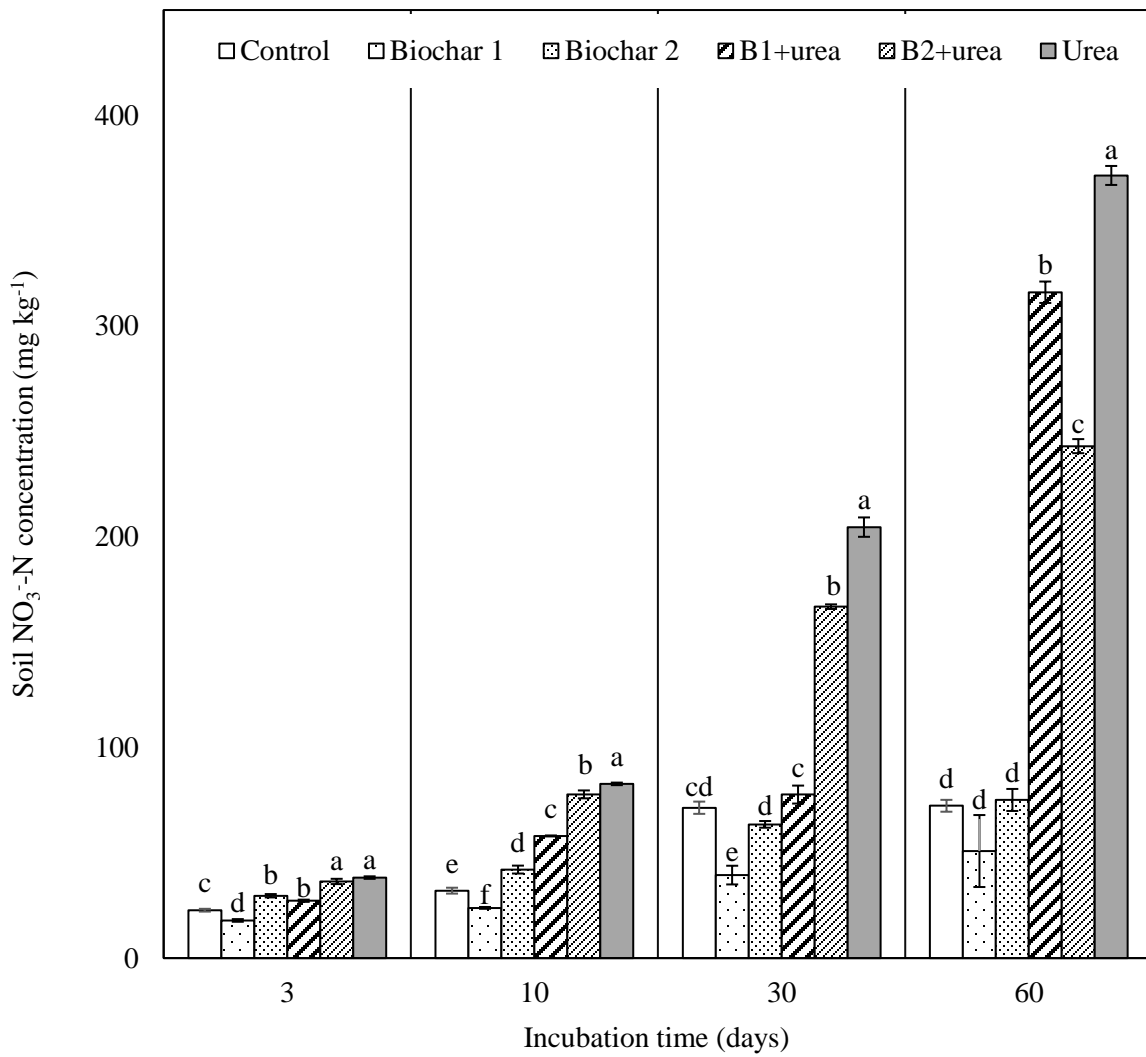
Figure A-2 Changes in soil NO_3^--N concentration from all treatments on day 3, day 10, day 30 and day 60 of incubation. Error bars represent standard error (n = 3). Different letters above the bars within each panel means a significant difference between treatments ($p \leq 0.05$).
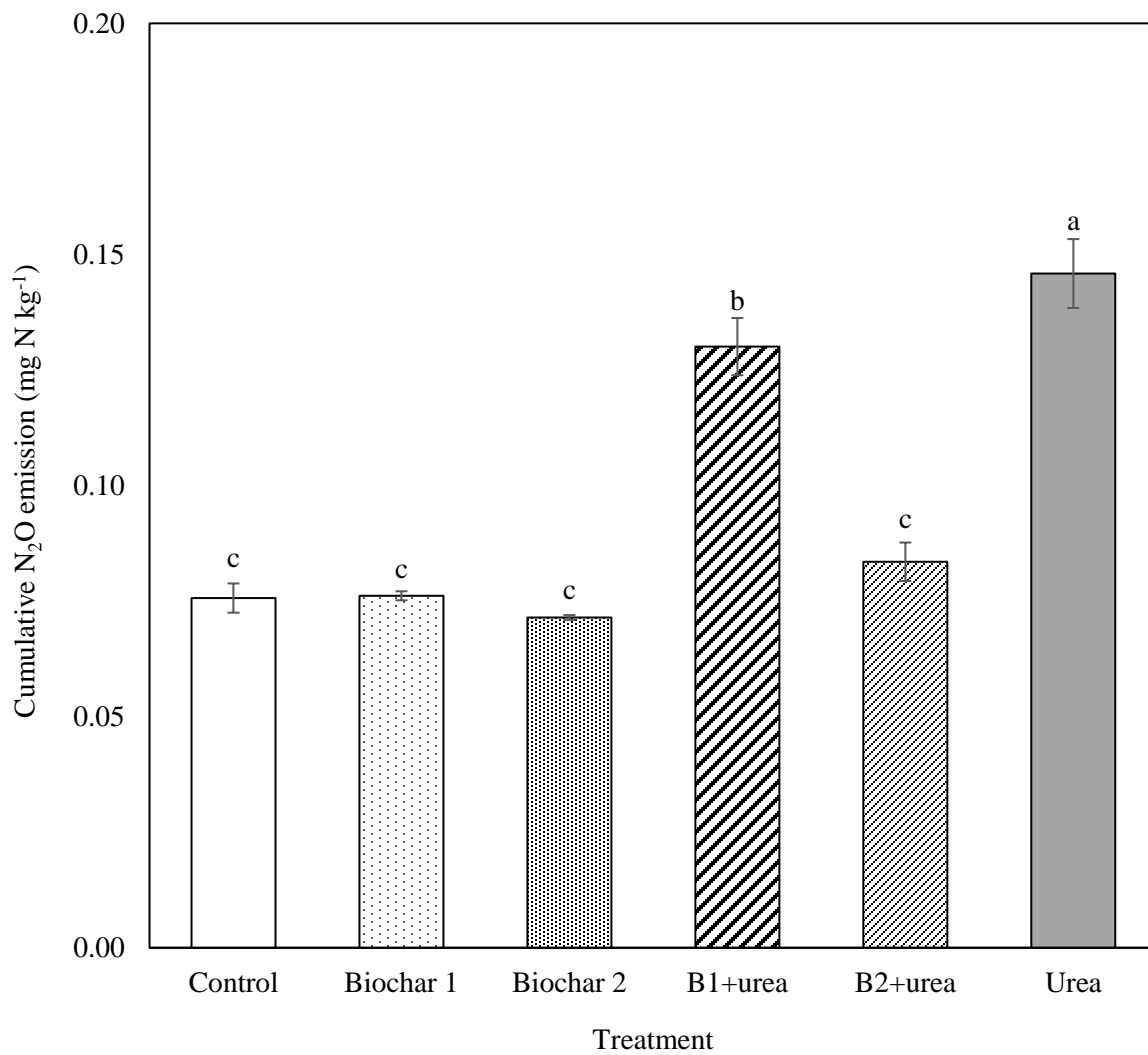
Figure A-3 Cumulative N$_2$O emission from all treatments at the end of incubation (day 60). Error bars represent standard error (n = 3). Different letters above the bars within each panel means a significant difference between treatments ($p \le 0.05$).
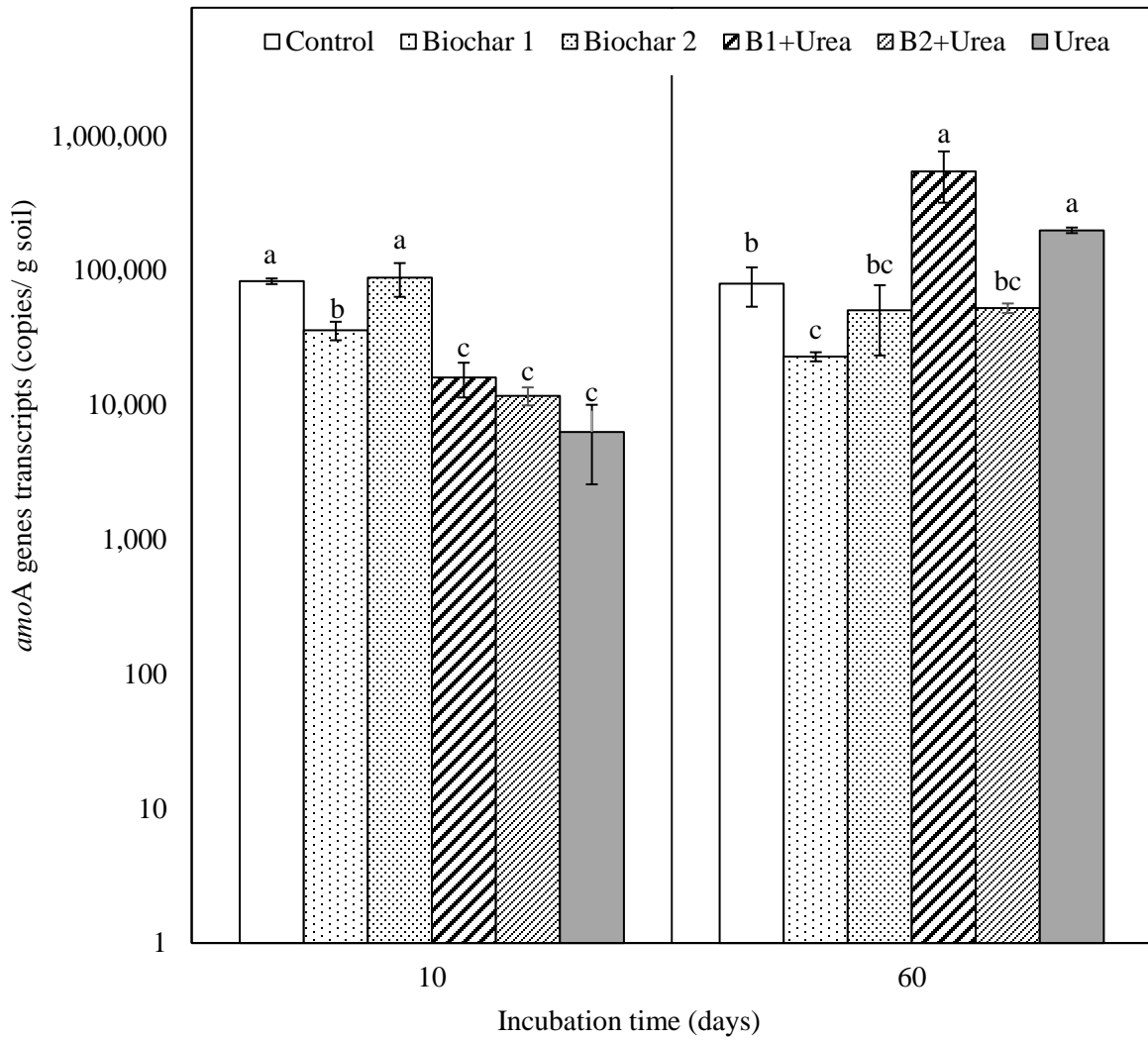
Figure A-4 The abundance of AOB *amo*A gene transcripts from all treatments on day 10 and day 60 of incubation. Error bars represent standard error (n = 3). Different letters above the bars within each panel means a significant difference between treatments (p ≤ 0.05).
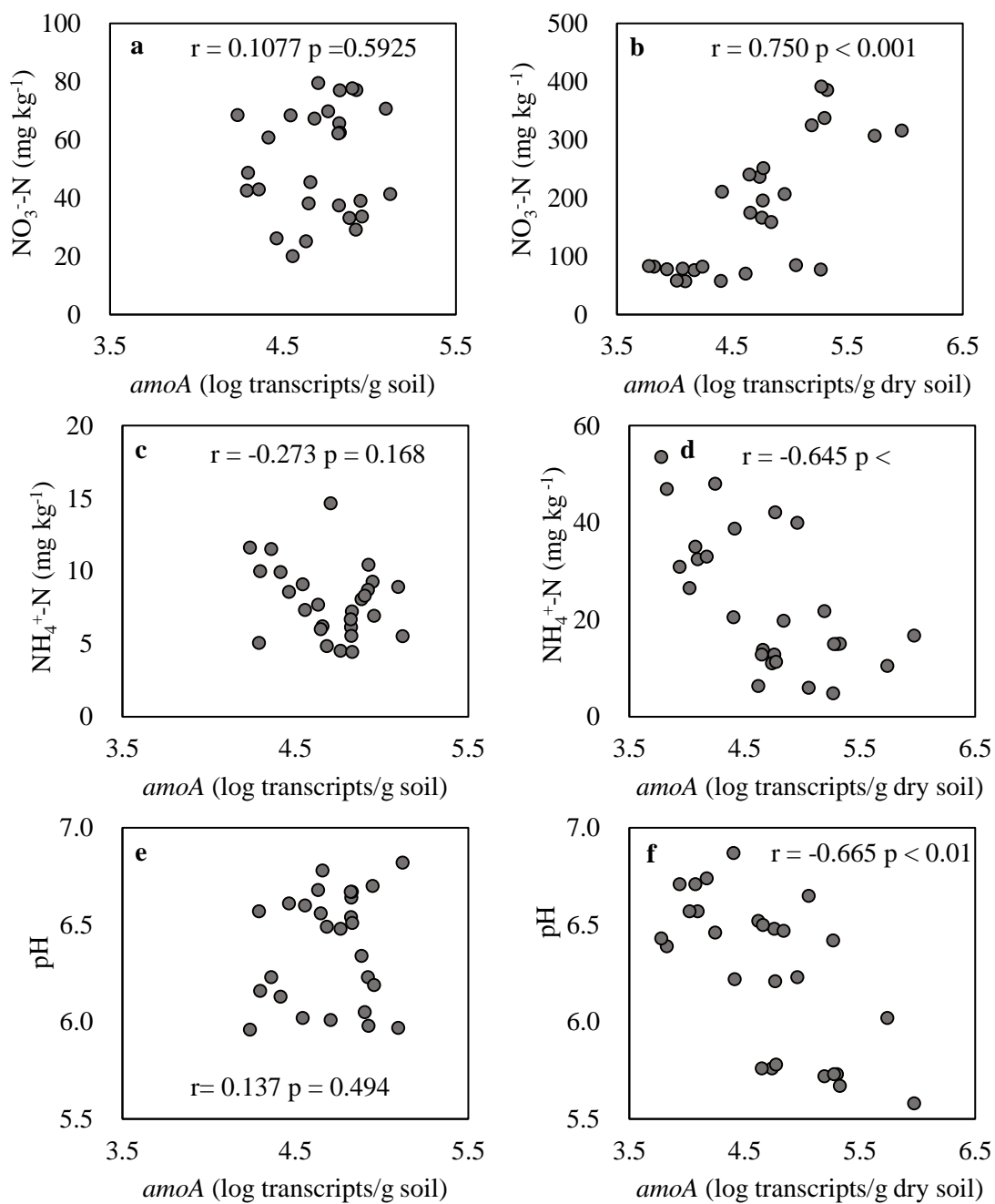
Figure A-5. The correlation of *amo*A gene transcripts with NO_3^--N, NH_4^+-N, and pH in treatments with no urea (a,c,e) and with urea application (b, d, f).
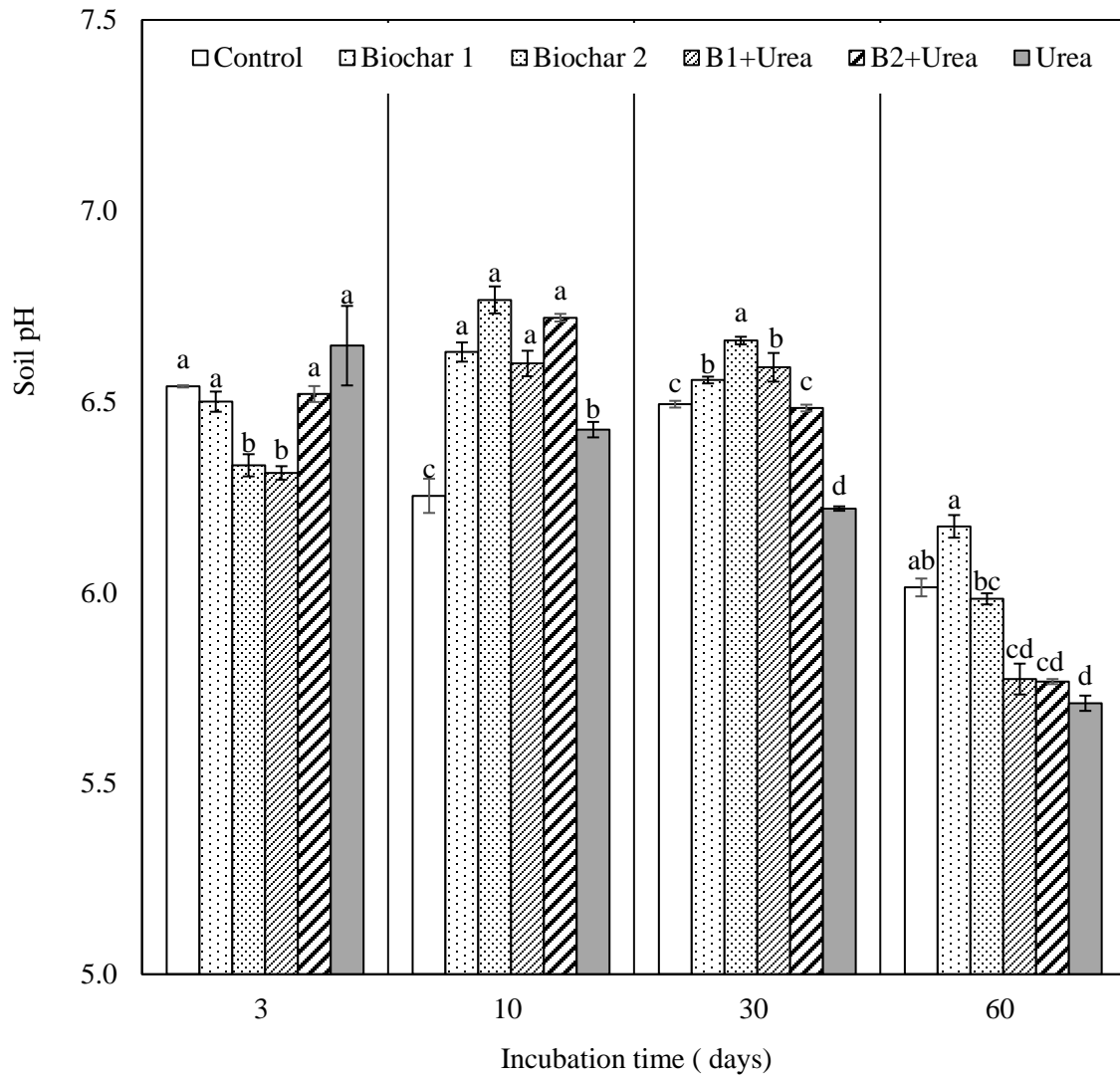
Figure A-6. Changes in soil pH concentration from all the treatments on day 3, day 10, day 30 and day 60 of incubation. Error bars represent standard error (n = 3). Different letters above the bars within each panel mean a significant difference between treatments ($p \leq 0.05$).

CHAPTER III – APPLICATION METHODS INFLUENCE BIOCHAR-NITROGEN FERTILIZER INTERACTION ON SOIL NITROGEN DYNAMICS

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Abstract

Applying nitrogen (N) fertilizer with biochar has the potential to reduce N losses, but the effect can vary with the application methods. The objective of this study was to evaluate the impact of two methods of biochar and N fertilizer application (surface placement and soil incorporation) on soil mineral N transformations in an Alfisol in Middle Tennessee. A 60-day aerobic incubation experiment was conducted with three treatments: 1) biochar (150 mg N g^{-1}), 2) urea (150 mg N g^{-1}), and 3) combination of biochar and urea (75 mg N g^{-1} biochar + 75 mg N g^{-1} urea). A control soil with no addition of urea or biochar was also included. Soil pH, mineral N content, and amoA gene transcripts were determined on days 3, 10, 30, and 60. Nitrous oxide (N_2O) production was measured on day 0, 1, 2, 4, 6, 8, 10, 13, 16, 21, 26, 31, 37, 43, 51 and 60. Compared to soil incorporation, surface application of urea and biochar alone resulted in 40% and 30% more mineral N, respectively, during the experiment. However, the incorporation method resulted in 104% more mineral N after 60 days of incubation than surface application method when biochar was co-applied with urea. The cumulative N_2O production was statistically significantly higher with incorporation compared to surface applications. More ammonia-oxidizing bacteria (AOB) activity was observed when urea was surface applied, evidenced by the higher number of amoA gene transcripts. The same trend was observed at day 60 when biochar and urea were co-applied. Biochar addition inhibited AOB activity during the early stage of incubation. The results also revealed that biochar incorporated with soil resulted in lower nitrification rate but higher N_2O emission than surface-applied. Surface application of urea alone stimulated more microbial activity than urea incorporated with soil, potentially resulting in more

NH_3 volatilization and NO_3^- leaching. In contrast, nitrification was enhanced when urea was incorporated with biochar.

Introduction

Nitrogen (N) fertilizer is widely used in agricultural production due to its essential role in improving crop yield (Galloway et al. 2008). However, excessive use of N fertilizers can cause N losses (Liu et al. 2016a), leading to serious environmental issues such as eutrophication, greenhouse gas emission and lake acidification (Galloway et al. 2003). Increasing crop yield without adversely affecting the environment is challenging and it is strongly influenced by the N transformation processes in the soil. Strategies to reduce N losses include selection of appropriate fertilizer types and application methods and the use of nitrification inhibitors (Cameron et al. 2013). Nonetheless, the off-site movement of N from agricultural systems is still a great concern. There is increasing evidence that biochar can increase soil N retention through increasing soil N pool as well as reducing soil N losses (Clough et al. 2013; Nguyen et al. 2017b).

Biochar is the byproduct when biomass is converted to biofuel under anaerobic conditions at high temperature (Lehmann 2007). Applying biochar has shown several merits including climate change mitigation, soil fertility improvement, and microbial activity increase (Stavi and Lal 2013; Gul et al. 2015; Ding et al. 2016a). So far, the effect of biochar amendment on carbon (C) sequestration has been widely studied while very little attention has been paid to its effect on soil N dynamics mainly because biochar, in general, contains very low N (0.1% - 8 %, depending on feedstock types and pyrolysis temperature) (Xu and Chan 2012; Weber and Quicker 2018). In addition, N in biochar is mainly in the form of stable heterocyclic structures containing pyrroles, pyrimidine, and indoles, which typically exhibit very low bioavailability (Knicker et al. 1996; Schulten and Schnitzer 1997; Almendros et al. 2003; Biederman et al. 2017). However, large specific surface area and high cation exchange capacity (CEC) of biochar materials can reduce N losses from excessive application of fertilizer N by the process of sorption (Clough et al. 2013). Sorption of organic N (e. g. urea) and ammonium (NH_4^+-N) decreases the accessibility of substrate to the microbes, resulting in a decreased rate of nitrification;

sorption of nitrate (NO_3^--N) decreases N loss through leaching and nitrous oxide (N_2O) emission (Laird et al. 2010; Wang et al. 2015a; Mandal et al. 2016; Zhao et al. 2017).

Biochar addition in the soil also influences the microbial activity and community structure, which has both positive and negative effects on N retention. Few studies reported that biochar promoted mineralization of stable N pool when the soil mineral N content was low (Nelissen et al. 2012; Nelissen et al. 2015) while other studies reported enhanced microbial N immobilization due to the labile C in biochar (Van Zwieten et al. 2010; Bruun et al. 2012; DeLuca et al. 2015). Biochar's effect on microbial activities is also attributed to its positive effect on pH regulation and water retention (Atkinson et al. 2010; Cayuela et al. 2014; Nguyen et al. 2017b; Teutscherova et al. 2017). Nitrification, the microbial process producing NO_3^-, is sensitive to pH changes (Norton and Stark 2011). Increased abundance of ammonia-oxidizing bacteria (AOB), especially *Nitrosomonas*, was observed with biochar addition, which was attributed to pH increase (Nicol et al. 2008; Song et al. 2014; Lin et al. 2017).

The potential of biochar to increase soil N retention, however, is influenced by the characteristics of biochar and soil, as well as the application methods (Blanco-Canqui 2017). The effect of biochar characteristics on soil N dynamics has been studied to some extent in different soils (Atkinson et al. 2010; Clough et al. 2013; Nguyen et al. 2017b), but that is not the case for the application methods. Inorganic N and/or biochar can either be surface applied or incorporated with soil. Subsurface placement or soil incorporation of N fertilizer has shown enhanced uptake of N by plants, and decreased loss of ammonia (NH_3) by volatilization, NO_3^- by leaching and N_2O by emission when compared to surface placed N fertilizers (Wiesler 1998; Nash et al. 2012; Ruidisch et al. 2013; Nkebiwe et al. 2016). Studies showed that surface application of biochar reduced water infiltration and accumulated more nutrients in the soil surface layer compared to biochar mixed with soil (Schnell et al. 2012; Page-Dumroese et al. 2015). When biochar was surface applied together with N fertilizer,

more NH_3 volatilization and less NO_3^- leaching were observed than when they were placed in deeper soil layer (Doydora et al. 2011; Li et al. 2018b). Past studies comparing N interaction with biochar based on how they were applied are limited: In most studies, amendments were incorporated into soil.

We investigated the effect of the surface application of biochar and urea on soil N retention in comparison with the more common method of soil incorporation, because in the no-till systems, amendments are usually applied on the soil surface. Although the subsurface placement of N fertilizers can efficiently reduce N losses from fertilizers, if the surface application of N fertilizer along with biochar could reduce the loss of N, it is highly beneficial as the surface application is a less energy intensive process. This is of particular importance to the agroecosystems of Tennessee as nearly 80% of row-crop producers in Tennessee follow no-till management. Several studies have shown strategies for improving soil N retention when N fertilizers were surface applied including irrigating immediately after application, applying the prilled form of fertilizers, and applying fertilizers with nitrification inhibitors (Sanz-Cobena et al. 2011; Mohammed et al. 2016; Schlossberg et al. 2018). Compared to these strategies, biochar application can be cost-effective and can provide multiple ancillary benefits to the system. Therefore, the objective of this study is to compare the soil N dynamics when N fertilizer was applied with and without biochar by two methods: surface application and soil incorporation. In this study, soil mineral N, N_2O emission and the abundance of AOB *amo*A gene transcripts were investigated based on a 60-day laboratory incubation of soil applied with N fertilizer alone and in combination with biochar. We hypothesized that surface application of biochar and urea would decrease the nitrification rate and N_2O emission compared to incorporation with soil.

Materials and methods

Soil collection

Soil samples were collected from a tall fescue (*Festuca arundinacea*) dominated pasture system in Lebanon city, TN, USA (36°11'45.3"N, 86°15'50.3"W). The mean annual temperature of this

location is 14.5°C and the total annual precipitation is 1342 mm. The soil is classified as Bradyville silt loam (fine, mixed, semiactive, thermic Typic Hapludalfs). In December 2017, soil samples were collected from 5 random locations in the pasture system from 0 to 15 cm depth using a soil auger and composited. Fresh samples were sieved soon after collection through a 2 mm sieve and a sub-sample was used for the soil moisture determination using gravimetric weight loss method. After storing another sub-sample (~ 4000 g) at 4°C for the incubation experiment, the rest was air-dried to measure soil physicochemical properties following standard protocols (Table B-1).

Biochar characterization

Biochar used in this study was produced from mixed hardwood chips with no bark through the gasification process at 700°C by Wastewater Treatment Plant in Lebanon City, TN. The basic properties of biochar are: moisture - 56.1 %; total ash - 3.17 %; pH - 10.4; CEC - 202 $cmol_c$ kg^{-1}; total C - 830 g kg^{-1}; total N - 10.5 g kg^{-1}, and surface area - 279 m^2 g^{-1}. Moisture and ash contents were determined according to ASTM D1762-84 (2007) and pH was determined by a pH meter using 1:20 biochar: deionized H_2O (w:v) (Rajkovich et al. 2012). Cation exchange capacity was determined according to Mukherjee et al. (2011). Total C and N concentrations were determined by the dry combustion method (Nelson and Sommers 1996) using Elementar Vario TOC cube CN analyzer. The surface area was determined based on CO_2 adsorption using the Brunauer-Emmet-Teller (BET) theory (Brunauer et al. 1938).

Microcosm set up

After pre-incubating fresh soils at room temperature in the dark for seven days, 35 g fresh soil was transferred into a specimen cup which was placed into a 500 mL mason jar for incubation. On day 0, soil moisture content was 26% and it was maintained constantly throughout the incubation (60 days) by adding Milli Q water with mini pipette every week after weighing the specimen cups. This experiment included four treatments with different biochar and urea ratios: control (no biochar, no

urea), urea alone (equivalent to 150 mg N kg^{-1} soil), co-application of biochar and urea (75 mg N kg^{-1} biochar + 75 mg N kg^{-1} urea), and biochar alone (150 mg N kg^{-1}). To determine the effect of application method on mineral N dynamics in soil, two methods were tested: (i) incorporation method by mixing urea/biochar with soil using a glass rod before incubation, and (ii) surface application method by applying urea/biochar on the soil surface. All jars were closed tightly and incubated at room temperature (26°C) in the dark. To maintain headspace O$_2$ level, jars were opened every 4-6 days and flushed with room air for 10 min by a small fan. Nitrous oxide measurements were conducted 16 times during the 60-days period, and four destructive samplings were done to determine soil mineral N and AOB activity. There were 84 jars in total with seven treatments, four destructive sampling points, and three replicates.

Measurement of nitrous oxide emission

Gas samples were collected on day 0, 1, 2, 4, 6, 8, 10, 13, 16, 21, 26, 31, 37, 43, 51 and 60 (16 time points) for N$_2$O analysis by injecting a polypropylene syringe in the sampling port on the center of the jar lids. 10 ml gas samples were used to flush the pre-evacuated 12 ml vial before completely filling it with gas for analysis. The concentration of N$_2$O was measured by a gas chromatograph (GC-2014, Shimadzu, Japan) with an electron capture detector (ECD). The amount of N$_2$O production on day i was calculated as below:

$$N_2O_{dayi} = [(N_2O_{sample.-dayi} - N_2O_{air-dayi}) \times V] / m \qquad (1)$$

where N$_2$O$_{sample-dayi}$ is the N$_2$O concentration (mg N L^{-1}) in the sample on day i, N$_2$O$_{air-dayi}$ is the N$_2$O concentration (mg N L^{-1}) in the atmosphere on day i, V is the headspace volume of the jar (L), and m is the dry mass of soils used for incubation (kg). Cumulative N$_2$O emission was calculated by adding the N$_2$O production from individual measurements.

Destructive sampling and soil analysis

Soils from replicated jars (n=3) were destructively sampled for the analysis of soil pH, mineral N

(NH_4^+-N and NO_3^--N), and AOB gene transcript abundances on days 0, 3, 10, 30 and 60. One part of the sample from each destructive sampling was immediately frozen at -80°C for RNA extraction and the other part was air-dried for soil pH and mineral N analysis. Soil pH was measured in 1:2 soil: water solution (w:w) using an electronic pH meter (Thomas 1996). Soil mineral N (NH_4^+-N and NO_3^--N) was measured using a Continuous Flow Analyzer (Skalar Analytical B.V., the Netherland) after extracting 5 g soil with 25 mL 2M KCl solution (Maynard et al., 1993).

RNA extraction and amoA gene transcript determination

The abundance of AOB *amo*A gene transcripts was determined from the RNA extracted from the soil on day 10, 30 and 60 of incubation. Extraction of RNA was done from 2 g frozen soil (-80°C) using an RNeasy PowerSoil Total RNA Kit (Qiagen, Hilden, German) following the manufacturer's protocol. Polymerase chain reaction (PCR) amplification of the RNA templates was performed in 20 μL reaction mixture consisted of 3 μL RNA template with primer pairs *amo*A-1F/*amo*A-2R to check for remaining DNA (Rotthauwe et al. 1997; Haddad et al. 2007). The quality and quantity of RNA were determined using Nanodrop One[C] (Thermo Fisher, DE) to ensure high-quality RNA yield (nuclear acid concentration >30 ng μL^{-1}, A260/A230 >1.7 and A260/280 >1.8). SuperScript IV First-Strand Synthesis System (Thermo Fisher, MA) was used to synthesize cDNA with random hexamer primers (Invitrogen) according to the manufacturer's protocol. After synthesis, cDNA was stored at -20°C.

Quantitative real-time PCR (qPCR) was carried out to quantify the abundance of AOB *amo*A gene transcripts on a CFX Connect Real-Time PCR Detection System (Bio-Rad Laboratories Inc., Hercules, CA) using PowerUp SYBR Green Master Mix (Thermo Fisher, MA). A standard curve was generated from serial 10× dilutions of plasmid DNA from one representative clone containing the *amoA* gene. Triplicate analyses per sample were conducted in 20 μL reaction mixtures containing 10 μL of SYBR Green Master Mix, 0.5 μL of each primer, and 3 μL of cDNA template containing

approximately 20-25 ng of cDNA. The *amo*A genes were quantified using the primer pairs *amo*A-1F/*amo*A-2R (Rotthauwe et al. 1997). A negative no-template control was included in each run with sterilized distilled water as the template instead of a cDNA sample. The qPCR condition was controlled in the following order: 95°C for 3 min, 40 cycles of 60 s at 94°C, 45 s at 56°C, 60 s at 72°C, and 72°C for 10 min. The R^2 values of the standard curve were 0.991-0.997 and the primer efficiencies ranged from 71%-72%.

Calculations and statistical analysis

Net ammonification and net nitrification rates were calculated by Equation (2) and (3), respectively (Ste-Marie and ParC 1999; Wang et al. 2006) :

$$\text{NAR}= \left[c(\text{NH}_4^+\text{-N})_{i+1} - c(\text{NH}_4^+\text{-N})_i\right]/\triangle t \tag{2}$$

$$\text{NNR}= \left[c(\text{NO}_3^-\text{-N})_{i+1} - c(\text{NO}_3^-\text{-N})_i\right]/\triangle t \tag{3}$$

where NAR and NNR (mg kg^{-1} day^{-1}) are the net ammonification and net nitrification rates, respectively, i and $i+1$ are the initial and post-incubation time; $c(\text{NH}_4^+\text{-N})_i$ and $c(\text{NH}_4^+\text{-N})_{i+1}$ are the mean concentration of $\text{NH}_4^+\text{-N}$ in the initial and incubated samples, respectively; $c(\text{NO}_3^-\text{-N})_i$ and $c(\text{NO}_3^-\text{-N})_{i+1}$ are the mean concentration of $\text{NO}_3^-\text{-N}$ in the initial and incubated samples, respectively; $\triangle t$ is incubation time.

Concentrations of $\text{NH}_4^+\text{-N}$, $\text{NO}_3^-\text{-N}$, and cumulative N_2O emission after 60 days were analyzed by repeated measures two-way analysis of variance (ANOVA) using GLIMMIX procedure of SAS (version 9.4, Cary, NC) with application methods and fertilizer treatments as fixed effects and replication as well as interactions with replication as random effects. Subsequently, the effects of the treatments on the pH, gene transcript abundances, NAR, and NNR were analyzed for each time point separately using two-way ANOVA. Normality was tested by Kolmogorov-Smirnov test and homogeneity of variance were tested by Levene test before analysis. Statistical differences among treatments were determined by Fisher's protected least significant difference (LSD) at 5% confidence

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level ($p \leq 0.05$). Before analysis, the abundance of *amo*A gene transcripts was logarithm transformed.

Results

Soil ammonium nitrogen content

Urea addition, alone or in combination with biochar, significantly increased soil NH_4^+-N concentration until day 30 when compared to biochar alone and control treatments regardless of the method of application ($p < 0.05$) (Figure B-1). Surface application of urea alone resulted in higher soil NH_4^+-N than urea incorporation in soil throughout the 60-day incubation with the highest NH_4^+-N concentration was observed on day 10, which was 97.6 mg N kg^{-1} for surface application and 49.5 mg N kg^{-1} for incorporation method. Treatment differences were subtle at the end of the incubation (day 60) with the only notable difference was recorded from surface-applied urea alone treatment with soil NH_4^+-N content of 38.6 mg N kg^{-1}, which was >2 times higher than other treatments. No significant difference was observed when biochar was applied alone comparing to control, regardless of the application method.

Net ammonification rate, calculated using Equation (2), was also significantly higher with urea addition compared to the control until day 30 of the experiment while biochar alone showed no effect ($p < 0.05$) (Table B-2). The highest NAR value, 28.8 mg N kg^{-1} day^{-1}, was observed from the urea alone treatment on day 3. The surface application method had higher NAR than soil incorporation method until day 10, especially in the case of urea alone treatment. At day 60, the NAR was similar across the treatments.

Soil nitrate nitrogen content

Soil NO_3^--N content showed an increasing trend with time when urea was applied alone or in combination with biochar. When compared across the treatments, urea added treatments significantly increased NO_3^--N concentration while biochar alone treatment reduced NO_3^--N concentration compared to the control ($p < 0.05$) (Figure B-2). Similar to NH_4^+-N results, NO_3^--N was the highest

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with urea alone treatment throughout the incubation experiment, but application method had no effect on this treatment except on day 30, when surface-applied urea had significantly higher NO_3^--N concentration (265 mg N kg^{-1}) than incorporated urea (205 mg N kg^{-1}). Compare to control, biochar alone reduced NO_3^--N concentration by 21% to 45% during the experiment when incorporated with soil. Surface applied biochar showed no significant effect on NO_3^--N concentration except on day 30, when NO_3^--N concentration was reduced by 24% (54.2 mg N kg^{-1}). Co-application of biochar and urea reduced soil NO_3^--N concentration when compared to urea alone treatment. Surface applied urea-biochar mixture had higher NO_3^--N concentration than incorporated mixture until day 10, while on day 60, the NO_3^--N content from the incorporated urea-biochar mixture was 316 mg N kg^{-1}, which was 2 times as that from the surface-applied mixture.

Net nitrification rate, calculated using Equation (3), was the highest with urea alone treatment at all time points compared to other treatments (Table B-2). The highest NNR was observed with the surface applied urea alone treatment on day 30 (7.97 mg N kg^{-1} day^{-1}). Co-application of biochar with urea had lower NNR than urea alone treatment. Surface application of urea added treatments, in general, resulted in higher or similar NNR compare to incorporation except on day 60, when NNR from incorporated urea-biochar mixture was more than two times higher than NNR from the surface-applied mixture. In the case of biochar alone treatment, surface application had similar NNR compared to control while the incorporation had lower NNR than control on day 3 and day 10.

Cumulative nitrous oxide emission

Application of urea significantly increased cumulative N_2O emission while biochar alone reduced N_2O emission ($p < 0.05$) (Figure B-3). During the first 4 days, N_2O production from all the treatments was very similar, after that urea treatment significantly increased N_2O emission with the sharp increase was observed from day 4 to day 43. In general, surface application method showed lower N_2O emission than the corresponding soil incorporation method. Compared to control, biochar alone

treatment either showed similar N_2O emission (in the case of incorporated biochar) or lower N_2O

emission (in the case of surface applied biochar) throughout the experiment. At day 60, the highest

cumulative N_2O emission was observed from incorporated urea (0.146 mg N kg^{-1} soil) followed by

incorporated urea-biochar mixture (0.145 mg N kg^{-1} soil) and surface applied urea (0.139 mg N kg^{-1}

soil) and the lowest from surface-applied biochar (0.070 mg N kg^{-1} soil).

The abundance of amoA gene transcripts

The abundance of AOB *amoA* gene transcripts in the initial soil samples was 3.3×10^3 copies g^{-1}

dry soil. From day 10 to day 60, the abundance of AOB *amoA* gene transcripts was constant in the

control treatment, with mean values ranged from 3.3×10^4 and 8.3×10^4 copies g^{-1} dry soil (Figure B-4).

However, urea and biochar application affected *amo*A gene expression and the effect varied with the

application method. On day 10, *amo*A gene transcript abundance in urea incorporation treatment was

6.3×10^3 copies g^{-1} dry soil, which was one magnitude lower than control. On day 60, the abundance of

amoA gene transcripts increased to 1.5×10^5 copies g^{-1} dry soil when urea was incorporated with soil

while it was relatively stable (2.4×10^5 to 6.9×10^5 copies g^{-1} dry soil) with the surface application.

Incorporation of the urea-biochar mixture also increased *amoA* transcripts abundance (from 1.6×10^4

to 5.4×10^5 copies g^{-1} dry soil), however, surface application of the mixture decreased the *amo*A gene

expression from 1.1×10^5 copies g^{-1} dry soil on day 10 to 4.3×10^4 copies g^{-1} dry soil on day 60. The

*amo*A gene expression from biochar alone treatments was either similar or lower compared to control.

Discussion

Urea application and nitrogen mineralization

Urea, a common and quick release N source, significantly increased soil NH_4^+-N concentration

within a short time (Figure B-1, Table B-2). The peak concentrations of NH_4^+-N in urea treatments

were observed at day 10 then sharply decreased. This trend is consistent with another study, which

also showed complete urea hydrolysis in 10 days (Sommer et al. 2004). Urea addition also enhanced

nitrification, evidenced by increased NO_3^- concentration (Figure B-2), NNR (Table B-2), and N_2O production (Figure B-3), which could be due to the higher substrate (NH_4^+-N) and the ability of chemolithoautotrophic AOB to utilize CO_2 as C source (Denecke and Liebig 2003; Koops et al. 2006). Consequently, we found increased AOB *amo*A gene expression with urea addition compared to control (Figure B-4).

Surface application of urea caused higher NH_4^+-N and faster NAR than urea incorporation (Figure B-1, Table B-2), indicating more NH_3 volatilization from surface application, which is consistent with some past studies (Rochette et al. 2009b; Nkebiwe et al. 2016; Li et al. 2018a; Yao et al. 2018). Urea hydrolysis from urease activity leading to ammonification is influenced by multiple factors including urea concentration and organic matter content (Gould et al. 1973; Zantua et al. 1977). In our study, faster urea hydrolysis with the surface application is probably due to the elevated urease activity on the soil surface from the higher concentration of urea (Gould et al. 1973). The well aerated environment of the soil surface may also have contributed to the enhanced urease activity from surface-applied urea (Li et al. 2016). In a field study, faster urea hydrolysis from surface placed urea was also observed to be the result of higher organic matter content in the surface layer (Rochette et al. 2009a). Other environmental factors such as moisture could also impact urea hydrolysis. For example, Rochette et al. (2009c) reported slower urea hydrolysis from surface application than incorporation due to very low moisture at the soil surface in the dry and acidic soil.

Surface application of urea enhanced nitrification compared to soil incorporation (Figure B-2, Table B-2), which could be partly due to the higher substrate (NH_4^+-N) concentration. In addition, soil microbial activity pertaining to N transformation can be changed with the application method. In this study, we measured AOB activity, which controls ammonia oxidation process upon urea addition (Xiang et al. 2017). Our data showed that the expression of *amo*A gene transcripts, which encoded the enzyme catalyzing the ammonium oxidation step in the nitrification process, were inhibited by urea

incorporation on day 10 (Figure B-4). The negative effect of urea on *amo*A gene expression is likely

due to the toxicity of the excessive amount of NH_3 in the entire soil sample (Anthonisen et al. 1976;

Geisseler and Scow 2014). In contrast, surface-applied urea had higher *amo*A gene expression albeit

higher NH_4^+-N concentration. We attribute this to the difference in the distribution of urea with surface

application versus incorporation. In surface application, urea is concentrated on the soil surface,

therefore NH_3 and NH_4^+-N concentrations were also concentrated on the soil surface, leading to no

toxic level of NH_3 beneath the surface (Black et al. 1987; Malhi 1992). Singh and Beauchamp (1988)

also found that the nitrification was completely inhibited within 0 - 2 cm of urea placement.

Biochar application and nitrogen mineralization

Application of biochar alone application had a negative effect on soil mineral N, mainly due to

the inhibition of nitrification, evidenced by the same NH_4^+-N concentration as control and lower NO_3^--

N concentration (Figure B-1, Figure B-2, Table B-2). This result was in agreement with other studies,

which observed more N immobilization after biochar addition likely due to the high C: N ratio of

biochar (Van Zwieten et al. 2010; Bruun et al. 2012). Martin et al. (2015) reported that biochar can

limit substrate availability to nitrifiers by stimulating the competition of available NH_4^+-N between N-

immobilizing heterotrophs and nitrifiers. This negative effect occurred in the first 10 days,

demonstrated by the lower NNR during this period, which is consistent with the results of Bruun et al.

(2011). Some studies reported soil pH increase from biochar application as one of the reasons for the

increasing abundance of nitrifiers in acidic soil (Ulyett et al. 2014; Zhao et al. 2014; Teutscherova et

al. 2017). In the present study, soil pH was statistically higher in biochar added soils (6.68 with

incorporation and 6.63 with surface application, respectively) than that control soils (6.25) in the first

10 days (Figure B-5). AOB *amo*A gene expression, however, decreased in 10 days after biochar

application (Figure B-3), which indicates that pH change is probably not the dominant factor

influencing *amo*A gene expression in this study. The limited NH_4^+-N availability or the inhibition of

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microbial activity by some components of biochar (e. g. phenolic compounds and α-pinene), could be the reasons for the reduction in *amo*A gene expression (Sayavedra-Soto et al. 1996; Clough et al. 2010; Wang et al. 2015c). Besides, nitrification is also controlled by ammonia-oxidizing archaea (AOA) in N-poor soil (Wu et al. 2017; Zhang et al. 2018). There are reports of decreased expression of *amo*A gene in AOA with increased soil pH, resulting inhibition of nitrification (Nicol et al. 2008). The negative effect of biochar on nitrification was reduced when biochar was surface applied than incorporated (Figure B-1, Table B-2), which is probably due to less soil – biochar contact. The interface between biochar and soil, which is named as "charsphere" (Luo et al. 2013) or "charosphere" (Quilliam et al. 2013), is directly influenced by the physical and chemical properties of biochar. There is very limited research on the spatial heterogeneity of biochar's impact on soil N. Yu et al. (2019) found that the NH_4^+-N and NO_3^--N concentrations were reduced at the charosphere, which was attributed to high C and nutrient availability leading to N immobilization. Due to less soil to biochar contact, C and nutrients released from the surface applied biochar may have accumulated on the soil surface (Schnell et al. 2012), increasing N immobilization only at the soil surface. If the biochar used in this experiment contained components that can inhibit microbial activity, we can also expect a less negative effect on *amo*A gene expression from surface application of biochar than incorporation as the influence of these compounds would be limited to a thin layer of surface soil.

When biochar was applied with urea, soil NH_4^+-N and NO_3^--N concentrations, NAR, and NNR were increased compared to biochar alone treatment but decreased compared to urea alone treatment (Figure B-1, Figure B-2, Table B-2). This result indicated that urea, not biochar, was driving the N transformation in this experiment. Between the two methods of application of urea-biochar mixture, surface placement had higher mineral N concentration than soil incorporation during the first 10 days, and this was reversed at the end of the incubation. This contrasting trend was probably due to the sorption of urea on biochar. There is experimental evidence for strong physical adsorption of urea on

biochar surface, resulting in incomplete and delayed hydrolysis of urea (Saha et al. 2017). This sorption was found to weaken over time because microbes that occupy the surface of biochar begin the hydrolytic process, especially when urea is incorporated with soil (Mukherjee et al. 2014d; Dong et al. 2017). This could be the reason for more soil mineral N content after 30 days when the urea-biochar mixture was incorporated in the soil.

Biochar application and nitrous oxide emission

Our results showed that urea application significantly increased N_2O emission and the co-application of biochar and urea had less N_2O emission than urea alone (Figure B-3). Similar to our results, Grutzmacher et al. (2018) also reported that the application of biochar and N fertilizer together decreased N_2O emission by 67-95 %. The favorable effect of biochar in reducing N_2O emission could be attributed to biochar's ability to inhibit nitrification (as described in the previous section) and/or stimulate complete denitrification process (Butterbach-Bahl et al. 2013; Van Zwieten et al. 2014). The enzyme that catalyzes the last step of complete denitrification, which is the reduction of N_2O to N_2, is encoded by *nos*Z gene (Levy-Booth et al. 2014). Past studies showed that addition of biochar can increase the abundance and expression of *nos*Z gene, indicating complete denitrification can be enhanced by biochar application (Harter et al. 2017; Feng et al. 2018). Besides, metal ion or other organic radicals in biochar can enhance N_2O reduction by transferring electrons to denitrifies (Cayuela et al. 2015; Grutzmacher et al. 2018). Another study attributed biochar-induced reduction in N_2O emission to the entrapment of N_2O in the pore space of biochar (Harter et al. 2016).

Soil incorporation of urea and urea-biochar mixture resulted in greater N_2O production than surface application, particularly during the later stages of incubation (Figure B-3), which was consistent with the findings of Engel et al. (2010) and Halvorson and Del Grosso (2013). Drury et al. (2006) found shallow band placement (2 cm) of ammonium nitrate fertilizer resulted in 26% less N_2O emission compared to deep band placement (10 cm) both in the no-tilled and conventional-tilled

system. However, Nash et al. (2012) reported more N_2O production from the surface application of urea in a no-till system compared to deep banding (15 cm). Venterea and Stanenas (2008) also reported that greater N_2O production from near the soil surface. Linquist et al. (2009) attributed lower N_2O production in the sub-surface soil layer to the limited oxygen availability that hinders the rate of nitrification. In the case of biochar applied alone, surface application reduced N_2O emission compared to the incorporated biochar, the mechanistic understanding of this result needs further investigation.

Conclusions

In this study, the effect of two methods of application of urea, biochar, and mixture of urea and biochar on N dynamics was investigated through a 60-days incubation experiment. We found that the effect of application methods on soil mineral N dynamics varied with N source. When urea was applied alone, surface application enhanced ammonification and nitrification processes more than incorporation, evidenced by higher soil mineral N concentration and more AOB *amo*A gene transcripts. The opposite trend was observed when urea and biochar were applied together. In this case, incorporation stimulated N mineralization, perhaps due to the slow release of urea sorbed onto the biochar surface. In general, surface application of biochar and urea reduced N_2O production compared to incorporation, but the mechanism is not clearly understood. Future studies are needed to understand the mechanisms controlling the differential response of fertilizer application methods on soil N dynamics.

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Appendix B: Tables and Figures

Table B-1. Properties of the soil used for the incubation experiment

Soil property	Unit	Mean value
pH (H_2O)		6.3
Moisture content	%	26
Total organic C	g C kg^{-1}	18.9
Total N	g N kg^{-1}	1.7
C: N ratio		11:1
NH_4^+-N	mg N kg^{-1}	4.3
NO_3^--N	mg N kg^{-1}	26.4
P	mg kg^{-1}	1.91
K	mg kg^{-1}	176
Ca	mg kg^{-1}	1195
Na	mg kg^{-1}	1.34
Mg	mg kg^{-1}	177
Al	mg kg^{-1}	154
Cu	mg kg^{-1}	0.58
Fe	mg kg^{-1}	3.27

Table B-2. Effect of application methods and fertilizer types on net ammonification rate (NAR) and net nitrification rate (NNR). Different letters mean significant difference across treatments in each sampling time point.

Time	Control	Biochar		Biochar + Urea		Urea	
		Incorporated	Surface placed	Incorporated	Surface placed	Incorporated	Surface placed
			Net ammonification rate (mg N kg^{-1} day^{-1})				
Day 3	-0.32^g	-0.21^f	0.21^e	1.27^d	5.46^c	11.2^b	28.4^a
Day 10	0.36^e	0.35^e	0.15^f	2.22^c	1.13^d	4.52^b	8.55^a
Day 30	0.01^d	0.05^c	0.01^d	0.05^c	0.07^c	1.20^b	1.55^a
Day 60	0.07^e	0.10^{de}	0.14^{bcd}	0.20^b	0.12^{cd}	0.18^{bc}	0.57^a
			Net nitrification rate (mg N kg^{-1} day^{-1})				
Day 3	-1.22^d	-2.83^e	-1.75^d	0.28^c	1.85^b	3.94^a	3.80^a
Day 10	0.56^e	-0.25^f	0.72^e	3.16^d	4.53^c	5.63^b	6.59^a
Day 30	1.50^b	0.43^d	0.93^c	1.71^b	1.35^b	5.94^a	7.97^a
Day 60	0.77^d	0.41^e	0.81^d	4.83^b	2.08^c	5.75^a	5.78^a
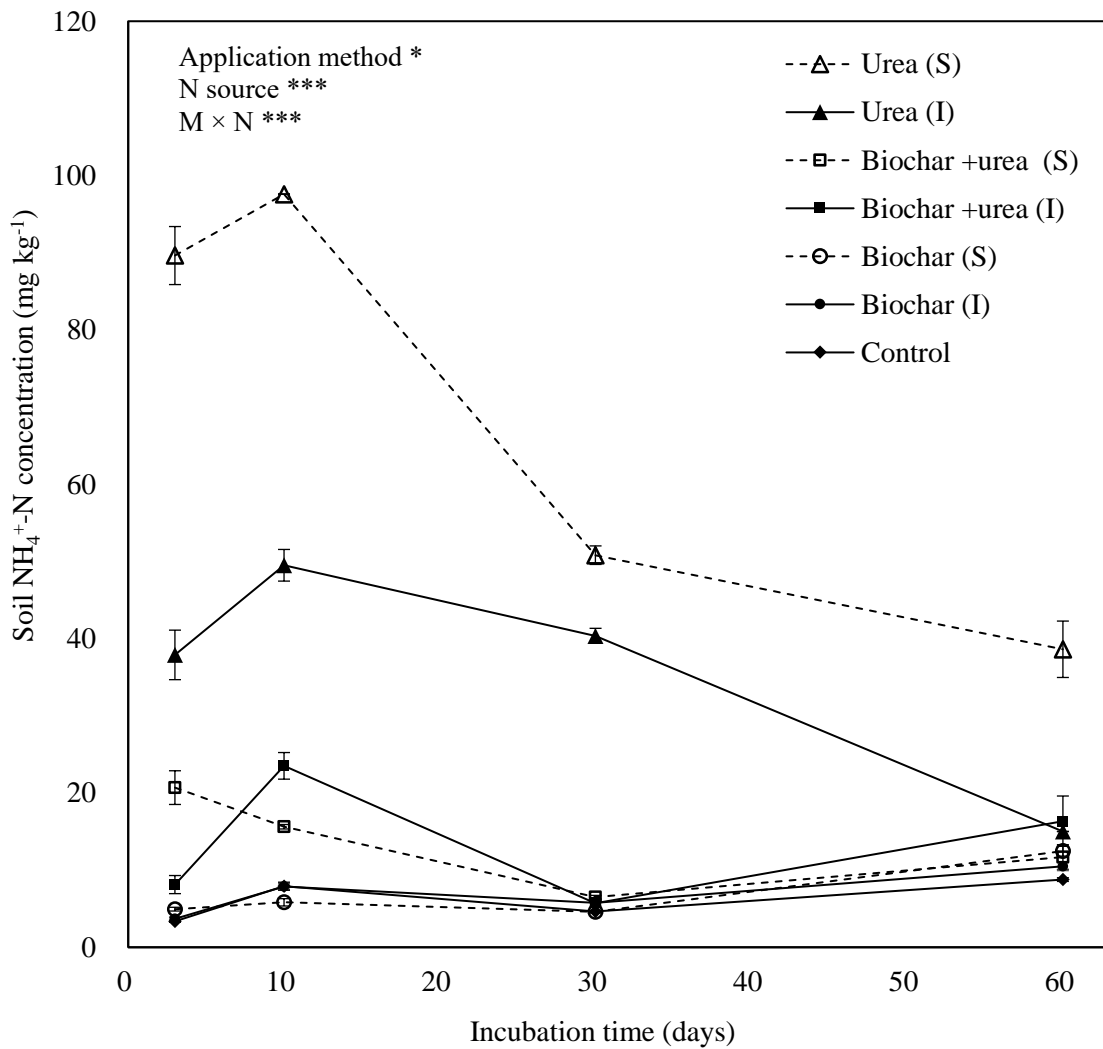
Figure B-1. Soil NH_4^+-N contents in response to two methods of application of urea and biochar. S means surface application method and I means incorporation method. M means application method and N means N source. Error bars presents the standard error of the means (n=3). Asterisks show significant levels (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).
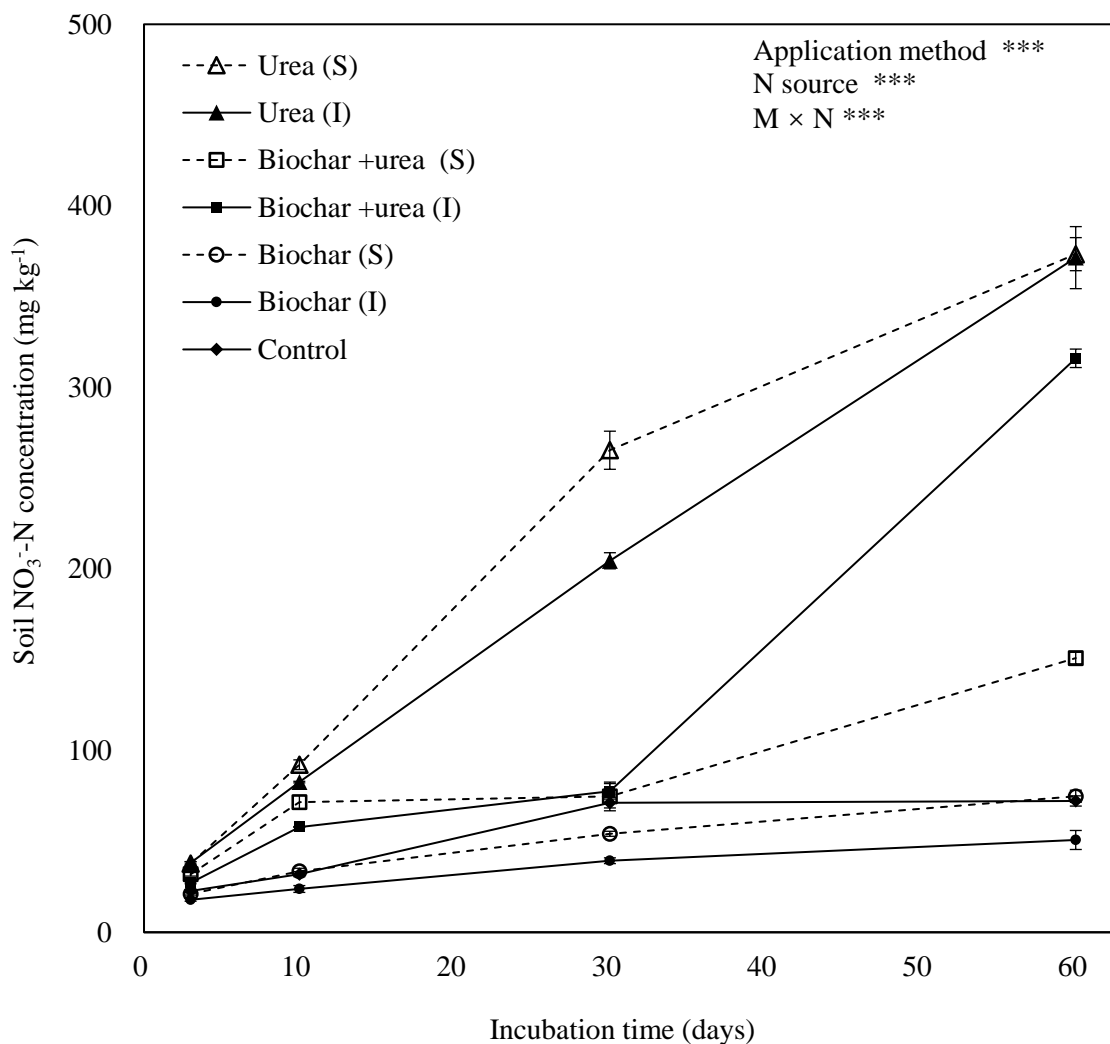
Figure B-2. Soil NO_3^--N contents in response to two methods of application of urea and biochar. S means surface application method and I means incorporation method. M means application method and N means N source. Error bars presents the standard error of the means (n=3). Asterisks show significant levels (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).
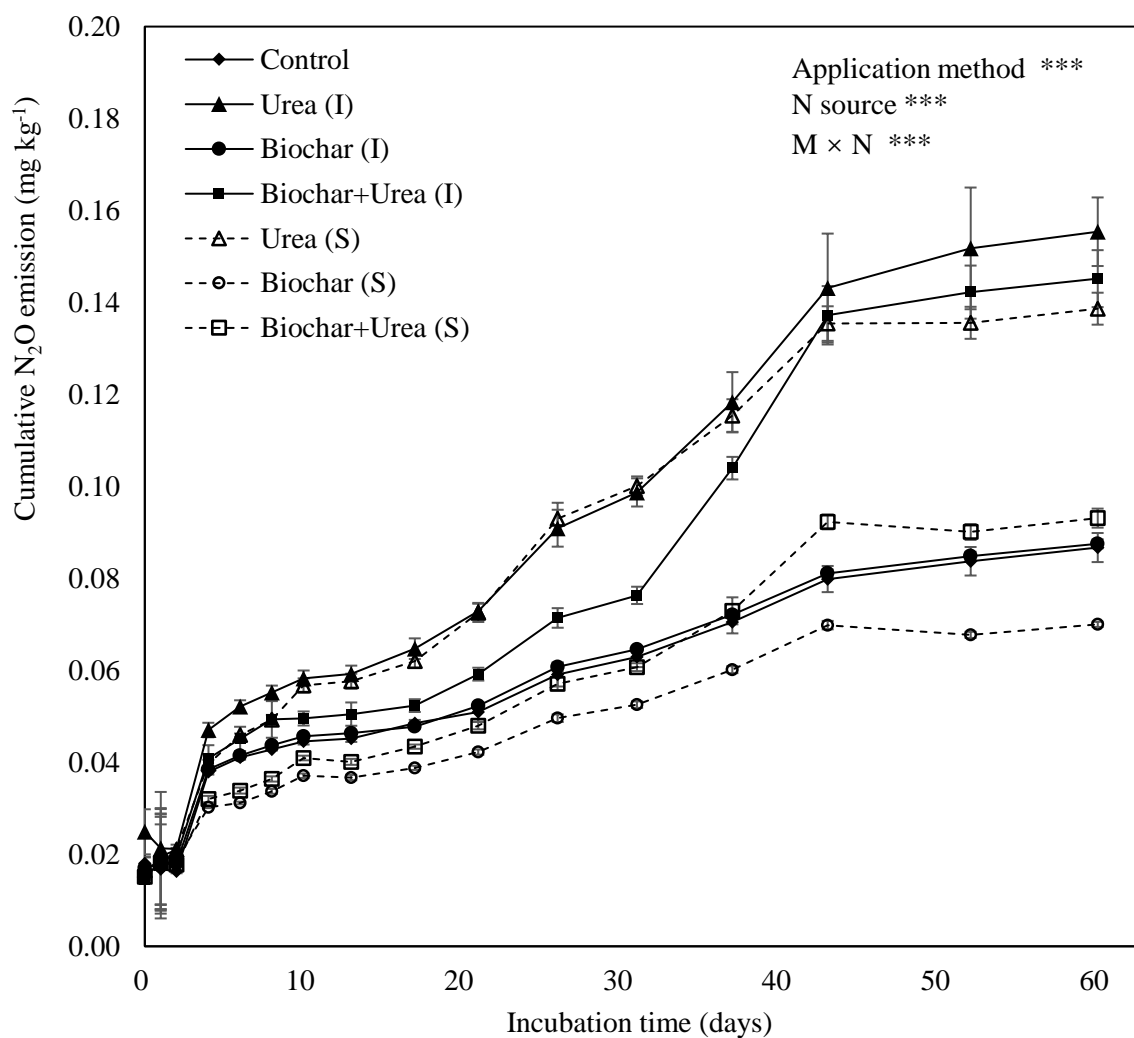
Figure B-3. The cumulative N$_2$O emission in response to two methods of application of urea and biochar. S means surface application method and I means incorporation method. M means application method and N means N source. Error bars represent standard error of the means (n=3). Asterisks show significant levels. (*** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$).
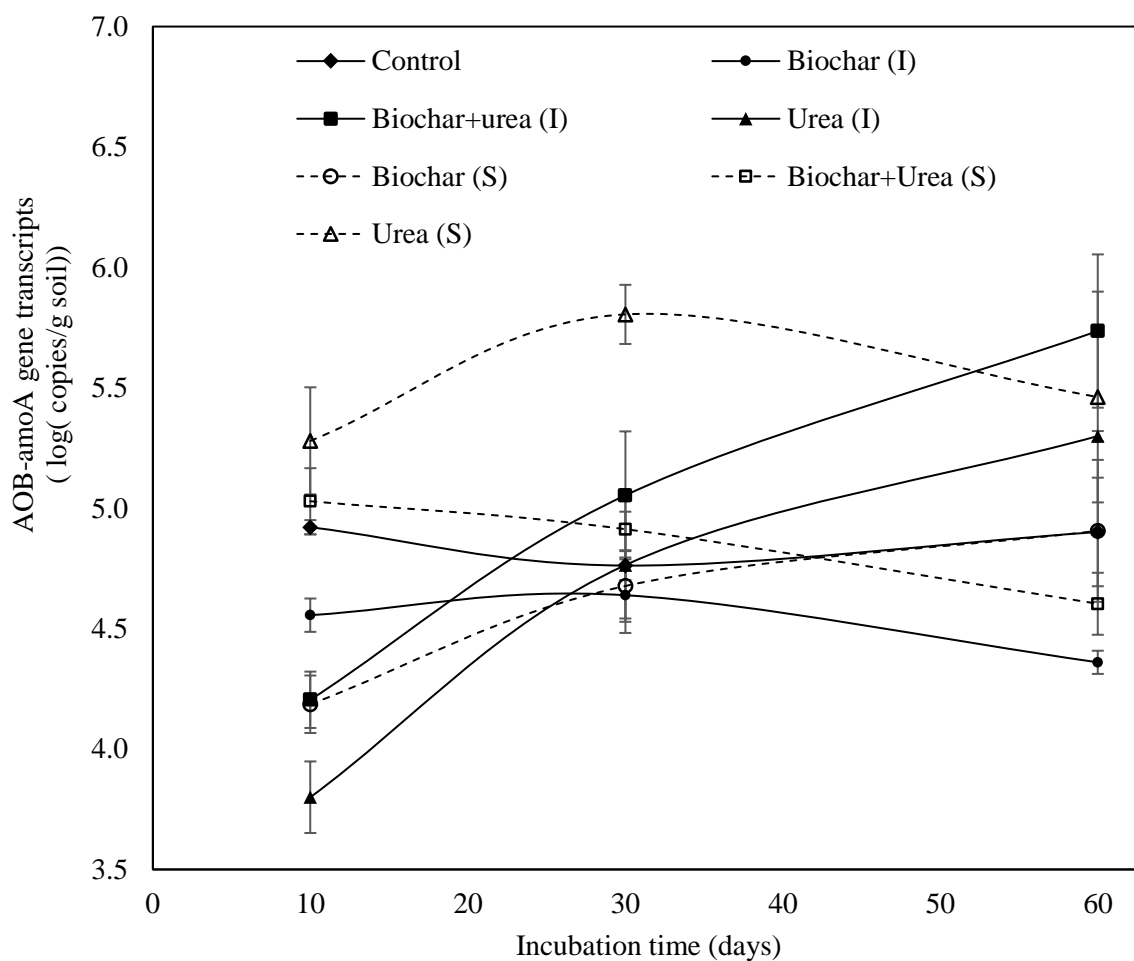
Figure B-4. The abundance of AOB *amo*A gene transcripts copies on day 10, 30, and 60 of the incubation. S means surface application method and I means incorporation method. Error bars represent standard deviation of the means (n=3).
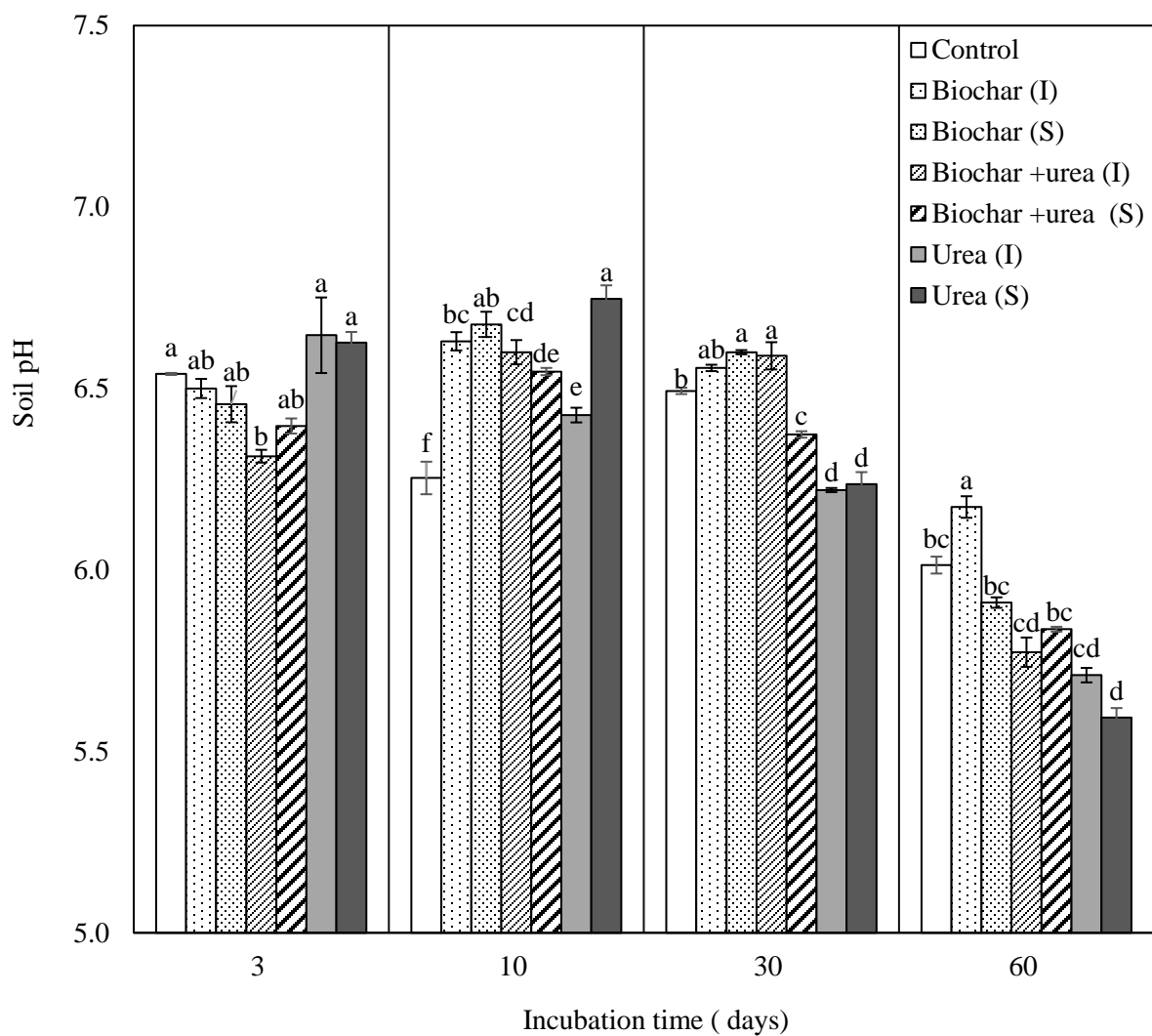
Figure B-5. Changes in soil pH concentration from all the treatments on day 3, day 10, day 30 and day 60 of incubation. Error bars represent standard error (n = 3). Different letters above the bars within each panel mean significant difference between treatments (p ≤ 0.05).

CHAPTER IV - IN-FIELD IMPROVEMENT OF SOIL PROPERTIES IN THE FORAGE PRODUCTION SYTEM BY BIOCHAR APPLICATION

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Abstract

Biochar is considered as an amendment to improve soil properties in agricultural systems; however, the response varies with several factors, including biochar application time and rate. To determine the effect of different rates of biochar on soil and plant attributes in a no-till system, we started a two-year field experiment in tall fescue (*Festuca arundinacea*) dominated forage system in Lebanon, TN. The soil at the study site belongs to Bradyville silt loam. Biochar used in this study was produced from 97% hardwood woodchips and 3% biosolids at 700°C. Treatments included six rates of biochar (0, 4.5, 9, 13.5, 18, and 22.5 Mg ha^{-1}) laid out in a randomized complete block design with four replications. Biochar was applied on the soil surface in April 2017. Soil samples were collected from 0 to 15 cm depth biannually from June 2017 to December 2018, and plant harvest was done in May 2017 and 2018. We determined plant yield and nutrient uptake as well as soil properties such as pH, gravimetric moisture content, organic carbon, microbial biomass, total nitrogen, and available nutrients. Results showed that, across different rates, biochar addition reduced soil mineral nitrogen by 38 to 53% within six months. Also, over the two years, 16 to 22% the increase in soil organic carbon concentration and 12 to 21% the increase in extractable phosphorus content were observed with different rates of biochar application compared to control. Different biochar rates also increased plant potassium uptake by 16 to 26% in the first year but showed no influence on biomass yield. Application of 9 Mg ha^{-1} biochar showed the maximum economic profit based on the cost-benefit analysis. Our results showed that biochar is a promising soil amendment in improving soil health in the no-till production systems of Tennessee.

Introduction

Biochar, the co-product of the thermochemical conversion of biomass to fuel and energy, is gaining a lot of attention as a soil amendment in recent years (Lehmann 2007; Gul et al. 2015; Kameyama et al. 2017). Biochar has been regarded as one of the best management practices to remediate low fertility soils because of its potential in improving soil organic carbon (SOC) content, nutrients availability, and crop productivity (Agegnehu et al. 2017; El-Naggar et al. 2019). Biochar can increase SOC due to its high carbon (C) content and increased C stability. Compared to the feedstock, C concentration in the biochar increases after pyrolysis because water and volatile matters in the feedstock are removed (Weber and Quicker 2018). The total C content in biochar ranges from 50% - 95%, depending on the feedstock type and pyrolysis temperature (Krull et al. 2009). More than 90% of C in the biochar is recalcitrant due to the aromatic structure, increasing the resistance of biochar to microbial decomposition (Wang et al. 2016). Woolf and Lehmann (2012) predicted that long term biochar application could increase SOC stock by 30% - 60 %. Since SOC content plays an important role in the aggregate formation, water infiltration, and nutrient retention, biochar addition can ultimately improve soil quality (Franzluebbers 2002; Powlson et al. 2012; Kay 2018).

In addition to SOC sequestration, biochar can improve soil fertility by supplying nutrients, modifying soil pH, and reducing nutrient losses. Biochar produced from nutrient-rich feedstocks such as manure provided P and K for plant uptake (Cantrell et al. 2012; Domingues et al. 2017). On the other hand, biochar exhibits a strong affinity for the sorption of nutrients because of the large surface area and high cation exchange capacity (CEC), ultimately reduces the nutrient leaching (Laird et al. 2010; Clough et al. 2013). In addition, biochar influences nutrient transformations in soil by regulating soil pH (Andersson and Siman 1991; Nicol et al. 2008; Devau et al. 2009; Dai et al. 2017). Several studies attributed the liming effect of biochar to the consumption of proton (H^+) and aluminum (Al^{3+}) by the chemical constituents in biochar, including but not limited to, the carbonate minerals, alkaline

oxides, and the surface functional groups such as carboxyl (–COO⁻) and hydroxyl (–O⁻) (Yuan et al. 2011; Dai et al. 2017).

Biochar addition has the potential to increase crop yield due to the improvement in soil physical, chemical, and biological properties (Palansooriya et al. 2019). About 10% crop yield increase with biochar addition was observed globally, however, the impact varied widely (−28% to 39 %) depending on soil and biochar properties (Jeffery et al. 2011). According to Biederman and Harpole (2013), the increase in productivity is mainly attributed to the ability of biochar for pH regulation and nutrient availability. As a result, the benefit from biochar addition could be more pronounced from tropical than temperate soils as tropical soils generally exhibit lower pH and fertility (Jeffery et al. 2017). In general, biochar is characterized by large surface area, high CEC, and high nutrient contents, which support improved soil condition and crop yield. However, biochar properties are highly dependent on the feedstock types and pyrolysis processing conditions (Mukome et al. 2013; Fryda and Visser 2015; Weber and Quicker 2018). Biochar properties also depend on amendment time because with time, breakdown of biochar occurs which decreases particle size, increases microporosity and surface area, improves exchange capacity, and reduces nutrient concentration (Hammes et al. 2008; Mukherjee et al. 2014c; Dong et al. 2017). Temporal effect of biochar after field application on soil properties and plant growth has not been studied widely, especially in the southeastern U.S. (Ballantine et al. 2012; Mukherjee et al. 2014b; Laird et al. 2017; Pandit et al. 2018).

Biochar application method also influences soil and crop responses because biochar mostly influences soil properties in a limited area around the location of application, which is termed "chromosphere" (Yu et al. 2019). Therefore, surface application or broadcasting of biochar results in a smaller chromosphere compared to mixing biochar with soil. As a result, surface placed biochar is expected to have limited impact on the soil-plant system. This expectation is challenged by some recent studies that showed that surface placed biochar can decrease nutrient loss through surface

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runoff, reduce water infiltration, and increase surface albedo compared to soil incorporated biochar (Schnell et al. 2012; Verheijen et al. 2013; Page-Dumroese et al. 2015; Ashworth et al. 2016).

The surface application also leads to minimal soil disturbance. Since agricultural soils in Tennessee are vulnerable to erosion (Denton and Typler 2002; Graveel et al. 2002), most producers follow no-till management to prevent further soil loss. Therefore, the surface application is the preferred method for any input application. However, most biochar field studies conducted across the U.S. had incorporated biochar with the soil, and studies based on surface application are minimal. In addition, although biochar has multiple agronomic benefits, the economic benefit is currently uncertain (Dumbrell et al. 2016). Studies focusing on the feasibility of biochar application are very rare. Therefore, a field study was conducted in Middle Tennessee to, 1) determine the optimum rate of biochar needed for desirable soil and crop attributes in no-tillage agriculture, and 2) evaluate the temporal effect of biochar amendment on soil properties and crop growth over two years. Our hypotheses are, 1) surface placed biochar can improve soil quality and plant growth in a no-till system, 2) a positive relationship exists between biochar application rate and soil and plant benefits, and 3) the positive effects from biochar amendment will be reduced over time from the field with time.

Materials and methods

Field experiment set up

A field experiment was established in a pasture system at the James E. Ward Agriculture Center in Lebanon city, TN, USA (36°11'45.3"N, 86°15'50.3"W). The site received average annual precipitation of ~1300 mm and average annual temperature of 17°C for the years 1895 to 2018 (NOAA 2019). The monthly temperature during the experimental period was closer to the long-term mean monthly temperature while the monthly precipitation during the experimental period was slightly higher than the long-term mean monthly precipitation (Figure C-1). The natural vegetation is dominated by tall fescue (*Festuca arundinacea*). The soil at the experimental site is Bradyville silt

loam (fine, mixed, semiactive, thermic Typic Hapludalfs). The experiment consists of six biochar rates

as treatments and four replications in a randomized complete block design with 24 plots in total. Each

plot was about 40 m^2 in size (13.3 m length $\times$ 3.0 m width). The six biochar rates are 0, 4.5, 9, 13.5, 18,

and 22.5 Mg ha^{-1}. Biochar was surface applied by hand in April 2017 when the fescue plants were in

the beginning stage of growth.

The biochar used in the experiment was produced from 97% mixed hardwood chips without bark

and 3% biosolid through gasification process at 700°C by the Wastewater Treatment Plant in Lebanon

City, TN. Biochar characterization was done by the Control Laboratories Inc., CA, and the physical

and chemical properties are listed in Table C-1.

Soil sampling and analysis

Soil samples were collected in June 2017, December 2017, May 2018, and December 2018.

Samples from 0-15 cm depth were collected from 20-25 random locations per plot using a 2.5 cm

diameter soil probe. Samples from each plot were composited, transferred to the zipped bags, and

transported to the research lab on the same day. The field-moist samples were sieved through a 4 mm

sieve to remove rocks and large plant residues and then divided into two subsamples. One subsample

was used for gravimetric moisture content (Black 1965) and microbial biomass analysis, and the other

was air-dried and sieved through a 2 mm sieve for soil physical and chemical analysis.

Soil pH was determined on a 1:2 soil: water suspension (w:w) with a pH meter (Thomas 1996).

For determining soil mineral nitrogen (nitrate-N (NO_3^--N) and ammonium-N (NH_4^+-N)) concentration,

5 g air-dried soil was extracted with 25 ml of 2 M KCl by shaking on a shaker (Thermal scientific

MaxQ 2000) at 180 rpm for 10 minutes. The filtrate was collected after filtering through Whatman No.

2 filter paper and analyzed using a Skalar Continuous Flow Analyzer (Maynard et al. 1993). Other

extractable nutrients including phosphorous (P), potassium (K), calcium (Ca), and magnesium (Mg)

were extracted by shaking 5 g soil and 20 ml Mehlich-1 solution (Mehlich 1953) at 180 rpm for five

minutes followed by filtering the extract using Whatman No.2 filter paper. The filtrate was analyzed using an Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) (Spectro Ciros CCD). Soil organic carbon and total nitrogen (TN) were determined by the dry combustion method (Nelson and Sommers 1996) using Elementar Vario TOC cube CN analyzer in solid mode. Soil microbial biomass carbon and nitrogen (MBC and MBN) were determined (first and last sampling only) by chloroform fumigation-extraction method (Vance et al. 1987). Approximately 10 g of fresh soil was fumigated with 40 ml chloroform in a desiccator in the dark for 48 hours. After fumigation, the samples were extracted with 45 ml of 0.5 M K_2SO_4. Another 10 g fresh unfumigated sample was also extracted similarly when chloroform fumigation began. The C and N contents in the fumigated and unfumigated extracts were determined using Elementar Vario TOC cube CN analyzer in liquid mode. MBC was calculated by Equation (1):

$$\text{MBC} = 0.45 \times \left(\frac{C_{T=48} \times 0.045}{\text{Weight of dry soil}} - \frac{C_{T=0} \times 0.045}{\text{Weight of dry soil}} \right) \tag{1}$$

where $C_{T=48}$ means C concentration of the fumigated sample, $C_{T=0}$ means C concentration of the unfumigated sample, 0.045 was the volume (L) of 0.5 M K_2SO_4 used for extraction, and 0.45 is a constant used to represent the efficiency of extraction. The same equation was used to calculate MBN.

Plant analysis

Plant samples were annually harvested by a sickle bar mower (1.9 m long) in May. The aboveground biomass from each plot was collected in a bag and weighed immediately after harvest in the field. Subsamples were brought back to the research lab in paper bags to determine the moisture content by oven drying at 60°C for 72 hours. The biomass yield was calculated by Equation (2).

$$\text{Yield} = M_{\text{fresh}} \times \left(1 - \frac{m_{\text{fresh}} - m_{\text{dry}}}{m_{\text{dry}}} \right) \tag{2}$$

where M_{fresh} means the total weight of aboveground biomass in each plot, m_{fresh} means the weight of subsample before oven drying, and m_{dry} means the weight of subsample after oven drying.

Plant tissue analysis was conducted at the University of Tennessee's Beef and Forage Center in Knoxville using Near Infrared (NIR) Spectroscopy technique. The analysis included P, K, Ca, Mg, crude protein (CP), acid detergent fiber (ADF), neutral detergent fiber (NDF), fat, lignin, sugar, fructan, water soluble carbohydrates (WSC) and digestible energy (DE).

Economic analysis

A cost-benefit analysis was performed on the basis of (i) forage yield, (ii) nutrient supplying potential of biochar, and (iii) SOC storage (Roberts et al. 2009; Pandit et al. 2018). Since the site is an unmanaged pasture land for many years, the forage yield was expected to be low. The revenue from the forage yield was calculated based on the two-year average yield from each treatment and the average hay price in Tennessee in 2018 ($143 Mg^{-1}) (USDA 2019). Prior to the experiment, the study site had low to medium soil P and K concentrations and sufficient micronutrients for plant growth. Therefore, the fertilizer cost saving for the farmer from biochar application was mainly derived from P and K suppliment. The total amount of P and K received by each plot was calculated from the biochar rate, the concentration of total P and K in the biochar, and the study area. Assuming that P and K present in the biochar is 100% bio-available (Roberts et al. 2009), the revenue from P and K saving was calculated based on the amount of total P and K, and the price of P and K fertilizer. The average price of superphosphate containing 45% phosphate (P_2O_5) was $3458 Mg^{-1} P and the price of potassium chloride containing 60% potassium was $1104 Mg^{-1} K in 2014 in USA (USDA 2018). The revenue from SOC storage by biochar application was calculated based on the increased SOC content and the lowest C-price recommended by the world bank to achieve the Paris Agreement temperature target in 2020, which is $40 Mg^{-1} CO_2 (equivalent to $538 Mg^{-1} C) (Stiglitz et al. 2017). The increased SOC content is calculated as the difference of SOC content between biochar treatments and control. Total amount of SOC corresponding to each treatment was calculated based on the concentration of SOC from the last sampling and the weight of soil. The weight of soil in the study area at 0-15 cm was 87 kg

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(0.15 m×40 m^2 ×1450 kg m^{-3}). The biochar input cost includes costs for feedstock, pyrolysis, and transportation (Pandit et al. 2018). The cost of feedstock in this experiment was very low because it was actually waste materials such as wooden pallets and biosolids, which were otherwise disposed in a landfill. According to Filiberto and Gaunt (2013), the cost of biochar produced from wastes could be as low as $50 ton^{-1} (equivalent to $55 Mg^{-1}) in the USA. The feedstock transportation cost is negligible in this study because the pallets were brought to the facility by the industries. The cost for the transportation of biochar to the study site is also negligible because the study site is only four miles away from the biochar production facility. The gross profit of biochar-inclusive farming was calculated as total revenue - total cost.

Statistical analysis

Treatment effect on soil properties, forage yield and forage quality were tested by repeated measures one-way analysis of variance (ANOVA) using GLIMMIX procedure of SAS (version 9.4, Cary, NC) with biochar rate, sampling time, and their interaction as fixed effects and block as random effect. Normality was tested by Kolmogorov-Smirnov test and homogeneity of variance was tested by Levene test before ANOVA. If the assumption of the equality of variance was not valid, logarithm or square root transformation was used. Fisher's protected least significant difference (LSD) was performed to determine significant differences among treatment means at 0.05 level of probability. Pearson correlation was conducted to relate soil pH with Ca, Mg, Na, and K concentrations.

Results

Forage yield and quality

Forage yield and quality showed no response to biochar addition but were significantly different between 2017 and 2018 (Table C-2). In 2017, the average forage yield across all treatments was 1879 kg ha^{-1}, which was more than two times higher than that in 2018 (773 kg ha^{-1}). The same trend was also observed for forage quality parameters such as ADF, NDF as well as for Ca, P, and Mg

concentrations in the plant tissue. However, other forage quality parameters such as CP, fat, lignin, sugar, fructan, ESC and DE were higher in 2018 compared to 2017. Plant K concentration was the only plant property that varied with biochar application rate. Significant increase in K concentration (12.8 mg g^{-1}) was observed with the application of 18 Mg ha^{-1} biochar, which was 15% greater than that in control. However, this positive response of biochar was only observed in 2017.

Soil pH

In the second sampling (240 days after addition), 18 Mg ha^{-1} biochar significantly increased soil pH with a mean value of 6.61, which was 0.3 unit higher than control (Figure C-2). Other biochar application rates did not change soil pH. In the third sampling (one year after biochar addition), soil pH was significantly increased with the application of >9 Mg ha^{-1} biochar with mean values ranged from 6.56 to 6.63 compared to 6.37 for control. However, soil pH showed a general decrease in the fourth sampling with no significant difference across the treatments was observed.

Soil organic carbon and total nitrogen

Soil organic carbon concentration was significantly increased from first sampling (18.8 g kg^{-1}) to second sampling (23.4 g kg^{-1}) by the highest biochar rate of 22.5 Mg ha^{-1} ($p < 0.05$, Figure C-3). After 20 months, compared to control, SOC increase of 3.24 g kg^{-1} to 3.75 g kg^{-1} was observed with the application of >9 Mg ha^{-1} biochar. Similar to SOC, soil TN was also increased with biochar addition ($p < 0.05$, Figure C-4). A significant difference was first observed in the second sampling, with 25 to 30% increase by the application of >13.5 Mg ha^{-1} biochar. At the end of the experiment, the mean TN values for 4.5, 9, 13.5, 18, and 22.5 Mg ha^{-1} treatments were 2.84, 2.91, 2.60, 2.58, and 2.63 g kg^{-1}, respectively, which were significantly higher than the control (2.21 g kg^{-1}). No significant difference was observed in the MBC and MBN data across the biochar treatments, but MBC and MBN increased with time (Table C-3, Table C-4). In June 2017, the average MBC and MBN across all the treatments were 363 mg kg^{-1} and 90.8 mg kg^{-1}, respectively, which increased to 417 mg kg^{-1} and 117 mg kg^{-1},

respectively in December 2018.

Soil nutrients

Soil nutrient concentrations were influenced by biochar application (Table C-3). The response of NH$_4^+$-N and NO$_3^-$-N, the two mineral forms of N, to biochar application was opposite. Soil NH$_4^+$-N significantly decreased with biochar addition in the first two samplings and this negative effect gradually disappeared in the third and fourth sampling ($p < 0.05$, Figure C-5). The effect of biochar on NH$_4^+$-N concentration was most evident in the first sampling with a decreasing trend with increasing biochar rates except for the rate of 22.5 Mg ha^{-1}. The lowest concentration of 7.4 mg kg^{-1} was observed for 18 Mg ha^{-1} treatment and the highest of 23.2 mg kg^{-1} was observed for the control treatment. In the second sampling, this negative effect was only observed when biochar application rate was between 9 and 18 Mg ha^{-1} with concentrations ranging from 5.6 to 7.6 mg kg^{-1}, which were statistically lower than control (9.0 mg kg^{-1}). In contrast to NH$_4^+$-N, soil NO$_3^-$-N concentration increased with >9 Mg ha^{-1} biochar addition in the first two samplings ($p < 0.05$, Figure C-6). Compared to the control, the increase in NO$_3^-$-N across the biochar rates in the first and second samplings was 200% to 360% and 46% to 175 %, respectively. In the third sampling, soil NO$_3^-$ in the control treatment was 1.25 mg kg^{-1}, which was statistically higher than most of the biochar treatments except 22.5 Mg ha^{-1}. No significant treatment effect was observed in the fourth sampling. Soil extractable P also increased with biochar addition but only in the first and third sampling ($p < 0.05$, Figure C-7). In the first sampling, 22.5 Mg ha^{-1} biochar significantly increased soil P (1.89 mg kg^{-1}) compared to control (1.35 mg kg^{-1}), and in the third sampling, both 18 Mg ha^{-1} and 22.5 Mg ha^{-1} biochar rates increased soil P with mean values of 1.91 mg kg^{-1}and 2.32 mg kg^{-1}, respectively. The concentration of soil Ca increased by 8% to 22% with the addition of 9 to 18 Mg ha^{-1} biochar only in the last two samplings ($p < 0.05$, Figure C-8). Although soil K and Mg concentrations increased over time, no significant effect of biochar rates was found (Table C-3).

Economic valuation

The cost-benefit analysis revealed the gross economic return from the application of different rates of biochar (Table C-5). The cost for the amount of biochar used in this study ranged from $0.99~ $4.95. The total revenue was increased from no biochar to 9 Mg ha^{-1} biochar rate and then decreased with the lowest gross profit of $2.89 was observed with 22.5 Mg ha^{-1} and the highest profit of $3.99 was observed with 9 Mg ha^{-1}. Soil C increase contributed more than 56% of the total income.

Discussion

The results showed that surface application of biochar in an unmanaged forage system increased SOC and most soil nutrients as well as modified soil pH. Though several past studies found a similar positive effect of biochar amendment on soil properties, the method of application was different: biochar was incorporated into soil in most of the past studies (Revell et al. 2012; Verhoeven and Six 2014; Xiu et al. 2019) while it was applied on soil surface in the present study. The increase in soil extractable Ca and P from the biochar added plots may be caused by the release of these nutrients from the biochar itself. Biochar could also adsorb nutrients from soil soon after contact with soil and become slowly available as time progresses (Brockhoff et al. 2010; Farrar et al. 2019). Biochar used in this study was rich in P (Table C-1). Although extractable Ca was not measured from the biochar used in this study, previous studies showed that biochar produced from wood at 700°C is typically rich in Ca and the average extractable Ca in the biochar is ~10,000 mg kg^{-1} (Gezahegn et al. 2019). Assuming that nutrient release rate was similar for all the elements, it would take more time for a detectable difference in Ca than P because the soil at the study site contains relatively higher Ca than P concentrations. Cations such as K, Ca and Mg present in biochar are mainly in the form of carbonate, oxides, or other metal minerals (Vassilev et al. 2013), which can react with soil H$^+$ or Al^{3+} and increase soil pH. In this study, pH was positively related to soil Ca concentration (Figure C-1, Table C-6). This result is in agreement with Yuan and Xu (2011) and Fidel et al. (2017), who attributed the liming effect to the biochar alkalinity, which is

strongly correlated with the base cation concentration of biochar. At the end of the experiment, pH and P showed no significant difference across the treatments, indicating that annual application of biochar may be needed to maintain soil fertility.

It was reported that biochar amendment can cause N deficiency to plants due to its high C:N ratio (Atkinson et al. 2010; Cayuela et al. 2014). In this study, a decrease in NH_4^+-N was observed in all biochar treatments in the first two sampling periods (Figure C-5), which can be attributed to one or more of N loss pathways such as NH_3 volatilization, immobilization, and nitrification. Cameron et al. (2013) summarized that high soil pH and temperature, and low soil moisture could increase NH_3 volatilization. However, soil pH and moisture changes were not observed in this study when NH_4^+-N was decreasing. We did not measure soil temperature from the field, however, a previous study reported that biochar can reduce daytime soil temperature and increase night temperature without changing the average temperature significantly (Blanco-Canqui 2017). In addition, surface placed biochar can reduce albedo (Verheijen et al. 2013) and consequently evaporation (Blanco-Canqui 2017). So, we believe that NH_3 volatilization was not the main mechanism for the decreased concentration of soil NH_4^+-N. Total nitrogen increased with biochar rates and time since application in this experiment (Figure C-4). Some studies contributed this increased TN with biochar application to enhanced N immobilization (Jones et al. 2011; Borchard et al. 2014; Mukherjee et al. 2014c). However, MBN, a pool of N immobilized in microbial bodies, was not increased by biochar addition (Table C-3), which indicates that immobilization did not contribute substantially to the decreased NH_4^+-N concentration. Increased nitrification could be another reason for the decrease in NH_4^+-N. We found that NO_3^--N concentration was increased when NH_4^+-N decreased (Figure C-5). However, our incubation experiment (Chapter 2) revealed that biochar application reduced the expression of ammonia-oxidizing bacteria *amo*A gene, indicating that the activity of nitrification was inhibited by biochar. The increased NO_3^--N concentration could be caused by the greater retention of NO_3^--N in the biochar added plots by physical or chemical

sorption due to the large surface area and presence of active functional groups on the surface of biochar (Clough et al. 2013; Mukherjee et al. 2014c; Zhao et al. 2017).

As expected, SOC and TN were significantly increased with biochar addition, and the increase was clear since the second sampling (Figure C-3, Figure C-4). Compared to the past studies, the positive effect of biochar amendment on SOC and TN was delayed in our study, which is attributed to the method of biochar application. Biochar was surface applied in our study, versus incorporated with soil in most other studies. In a lab experiment, Novak et al. (2009) found that SOC and TN were increased immediately with >20 Mg ha^{-1} biochar addition. Gao et al. (2016) reported SOC was increased by 32% four months after the application of 20 Mg ha^{-1} wood biochar in the San Juan Islands, USA. Mukherjee et al. (2014a) reported that application of 7.5 Mg ha^{-1} biochar increased SOC in a 115-day field experiment in Ohio, USA.

In this study, plant K concentration increased with biochar rate in 2017, but not in 2018 (Table C-2). This increase can be attributed to the K released from the biochar. The result is in agreement with Gaskin et al. (2010), who also found that K concentration in the surface soil and in corn tissues was linearly increased with biochar rate in the first year after biochar addition in Georgia. Although biochar used in our study is rich in P and soil extractable P was significantly increased by the addition of 22.5 Mg ha^{-1} biochar in the first sampling, the tissue P concentration was not increased with biochar rate (Table C-2). This different P and K uptake response to biochar addition is probably due to the form of nutrients in the biochar. Liu et al. (2019) summarized that biochar increase soil nutrient concentration by providing both soluble and mineral forms of nutrients. The soluble form could be used by the plant immediately after biochar addition while the mineral forms are relatively stable and are supposed to provide longer-term fertility for the plant growth. Liu et al (2019) reported that more than 50% of total K in biochar is water soluble even at a higher processing temperature of 700°C. However, P in biomass is converted to less soluble form when produced above 600°C, which is helpful in increasing long-term

plant-available P pool but not for the short-term increase in the extractable P content of soil (Qian and Jiang 2014). Xu et al. (2016) reported that less than 10% of the total P in the biochar is water-soluble P when biochar was produced from crop residues at 600°C. Other forage quality parameters showed no response to biochar rates, but significantly different in 2017 and 2018 (Table C-2). This temporal effect is probably caused by the difference in harvest time. Harvesting was done 20 days earlier in 2018 than in 2017. According to Hannaway et al. (1999), early harvesting of tall fescue could increase the nutritive value of forage but reduce yield, which is found to be true in this study because CP, fat, lignin, sugar, and fructan were higher and yield was lower in 2018 compared to 2017.

The economic assessment, although an estimate, presented the evidence of potential revenue from biochar addition (Table C-5). The result showed that the maximum gross profit ($3.99) was observed at 9 Mg ha^{-1} biochar application rate. This trend is similar to Pandit et al. (2018), who conducted a similar economic analysis of a three-year sequential corn (*Zea mays*) and mustard (*Brassica spp.*) system with five biochar rates ranging from 0 to 40 Mg ha^{-1} and found that the highest gross margin was observed with 15 Mg ha^{-1}. The highest rate of 40 Mg ha^{-1} was not profitable due to the higher cost of biochar. In our study, biochar cost was minimal because feedstock was freely available and the biochar transportation cost was negligible, However, it is important to keep in mind that we intend to conduct only an approximate evaluation and it is associated with several uncertainties. For example, the revenue savings from non-use of P and K fertilizers may be overstated because we are unsure the actual bioavailability of P and K present in biochar. We assumed that 100% is available for plant uptake. Other positive effects such as reducing soil acidity, decreasing greenhouse gas emission, removing contaminants, or improving soil microbial activity are not included. The potential negative effects such as retention of permanent organic pollutants, deficiency of plant available N, and potential to increase salinity also need to be considered (Cayuela et al. 2014; Wang et al. 2015; Zhang et al. 2016). Surface placed biochar could result in other adverse impacts such as decreased water infiltration (Page-

Dumroese et al. (2015), increased denitrification (Cardenas et al. 2017) and increased risk of nutrient runoff (Saarnio et al. 2018).

Conclusions

In this study, we found that surface applied biochar improved soil pH, nutrient content, SOC and TN in a pasture system in Tennessee. These positive effects varied with the time since biochar applied. The influence of biochar on SOC and TN enhanced over time while the influence on soil pH and nutrient concentration weakened, except for Ca. No significant effect of biochar on forage quality was observed in this experiment, except for the plant K concentration. The greatest effect from biochar application was observed at a rate of 18 Mg ha^{-1} while the economic analysis revealed that 9 Mg ha^{-1} is the profit-maximizing rate due to the balance between cost (biochar cost) and benefit (available nutrients and carbon sequestration).

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Appendix C: Tables and Figures

Table C-1 Properties of the biochar used in the study

Property	Unit	Mean value
Bulk density	g cm^{-3}	0.2
Moisture	%	61.2
Ash content	%	5.2
Surface area	m^2 g^{-1}	263
Volatile matter	%	8.4
pH (H$_2$O)		9.25
Electrical conductivity	dS m^{-1}	0.24
Liming effect	% CaCO$_3$	7.30
Organic carbon	g kg^{-1}	837
Total nitrogen	g kg^{-1}	10.5
Ammonium (NH$_4^+$-N)	mg kg^{-1}	9.6
Nitrate (NO$_3^-$-N)	mg kg^{-1}	2.0
Total P	mg kg^{-1}	3164
Total K	mg kg^{-1}	2989
Carbon / Nitrogen		79:1
Hydrogen / Carbon		0.36 - 0.7

Table C-2. Effect of biochar application rate and time on forage yield and quality (mean (SE)).

Treatment	Unit	T1[§]	T2	T3	T4	T5	T6
				2017			
Yield	kg ha^{-1}	1703 (228)	1996 (271)	1718 (391)	1902 (242)	1888 (319)	2062 (206)
CP[†]	%	8.60 (0.54)	9.30 (0.51)	9.20 (0.81)	9.50 (0.26)	9.50 (0.81)	8.50 (1.28)
ADF	%	37.3 (1.10)	37.0 (0.73)	38.5 (0.52)	37.3 (0.50)	37.2 (1.27)	38.9 (0.97)
NDF	%	58.6 (1.40)	57.8 (1.05)	59.4 (0.40)	58.0 (1.12)	59.7 (1.65)	60.5 (0.85)
Ca	g kg^{-1}	5.80 (0.05)	6.10 (0.43)	5.60 (0.27)	5.90 (0.42)	5.30 (0.28)	4.80 (0.47)
P	g kg^{-1}	1.60 (0.00)	1.70 (0.03)	1.80 (0.08)	1.70 (0.06)	1.70 (0.07)	1.70 (0.06)
K	g kg^{-1}	11.1 (0.67)	12.0 (0.30)	12.4 (0.54)	12.6 (0.66)	12.8 (0.37)	14.0 (0.54)
Mg	g kg^{-1}	2.80 (0.13)	2.80 (0.19)	2.80 (0.13)	2.80 (0.14)	2.70 (0.13)	2.70 (0.15)
Fat	%	1.82 (0.08)	1.87 (0.07)	1.81 (0.05)	1.90 (0.04)	1.84 (0.10)	1.74 (0.06)
Lignin	%	1.74 (0.12)	1.75 (0.30)	2.74 (0.20)	2.37 (0.29)	2.54 (0.40)	2.84 (0.45)
Sugar	%	7.17 (0.45)	6.68 (0.35)	6.39 (0.33)	6.90 (0.26)	6.25 (0.53)	6.41 (0.23)
Fructan	%	1.80 (0.16)	1.79 (0.12)	1.93 (0.08)	1.54 (0.05)	1.68 (0.21)	1.84 (0.03)
WSC	%	8.21 (0.51)	7.64 (0.44)	7.57 (0.47)	7.55 (0.21)	7.13 (0.62)	7.61 (0.36)
DE	%	1.96 (0.04)	2.05 (1.64)	1.93 (0.90)	1.96 (0.86)	2.03 (0.64)	1.89 (0.68)
				2018			
Yield	kg ha^{-1}	680 (349)	438 (115)	345 (32.1)	425 (6.12)	591 (66.1)	903 (194)
CP	%	10.6 (0.51)	10.1 (0.41)	10.5 (0.49)	9.10 (0.46)	10.0 (0.63)	9.70 (0.16)
ADF	%	35.4 (0.26)	35.7 (0.73)	34.9 (0.49)	35.3 (1.30)	34.6 (0.43)	34.8 (0.84)
NDF	%	56.0 (0.93)	56.3 (0.81)	56.3 (1.02)	58.4 (1.61)	56.2 (1.36)	56.7 (1.07)
Ca	g kg^{-1}	5.00 (0.51)	4.80 (0.40)	4.70 (0.48)	4.50 (0.27)	4.60 (0.32)	4.50 (0.19)
P	g kg^{-1}	1.50 (0.09)	1.50 (0.05)	1.50(0.09)	1.50 (0.13)	1.60 (0.09)	1.50 (0.05)
K	g kg^{-1}	16.5 (0.68)	16.2 (0.48)	16.6 (0.29)	13.9 (0.93)	16.6 (0.48)	16.4 (0.64)
Mg	g kg^{-1}	1.50 (0.14)	1.40 (0.11)	1.50 (0.23)	1.80 (0.38)	1.60 (0.23)	1.50 (0.07)
Fat	%	2.21 (0.07)	2.15 (0.04)	2.19 (0.04)	2.04 (0.05)	2.17 (0.05)	2.16 (0.05)
Lignin	%	6.05 (0.19)	5.87 (0.12)	5.71 (0.21)	5.75 (0.14)	5.54 (0.11)	5.40 (0.15)
Sugar	%	7.69 (0.62)	7.54 (0.77)	8.07 (0.49)	7.35 (0.50)	7.79 (0.32)	8.17 (0.41)
Fructan	%	2.11 (0.21)	2.11 (0.14)	2.19 (0.15)	2.48 (0.16)	2.42 (0.19)	2.37 (0.15)
WSC	%	11.0 (0.37)	10.8 (0.73)	11.6 (0.52)	10.9 (0.58)	11.4 (0.20)	11.7 (0.52)
DE	%	2.08 (0.02)	2.06 (0.03)	2.09 (0.03)	2.04 (0.05)	2.09 (0.03)	2.07 (0.03)

Table C-2 (continued)

Treatment	Unit	T1[§]	T2	T3	T4	T5	T6
				ANOVA table			
		Rate		Time		Rate × Time	
Yield	kg ha⁻¹	0.20		<0.001*		0.80	
CP	%	0.20		0.008*		0.38	
ADF	%	0.81		<0.001*		0.35	
NDF	%	0.72		<0.01*		0.40	
Ca	g kg⁻¹	0.37		<0.001*		0.76	
P	g kg⁻¹	0.75		<0.001*		0.82	
K	g kg⁻¹	0.03		<0.001*		0.02*	
Mg	g kg⁻¹	0.84		<0.001*		0.91	
Fat	%	0.86		<0.001*		0.19	
Lignin	%	0.78		<0.001*		0.16	
Sugar	%	0.95		<0.01*		0.48	
Fructan	%	0.88		<0.001*		0.16	
WSC	%	0.81		<0.001*		0.51	
DE	%	0.84		<0.001*		0.59	

§ Biochar rate T1 = 0 Mg ha⁻¹, T2 = 4.5 Mg ha⁻¹, T3 = 9 Mg ha⁻¹, T4 = 13.5 Mg ha⁻¹, T5 = 18 Mg ha⁻¹, T6 = 22.5 Mg ha⁻¹.

† CP means crude protein, ADF means acid detergent fiber, NDF means neutral detergent fiber, WSC means water soluble carbohydrates, and DE means digestible energy.

* means significantly different at $p = 0.05$.

Table C-3. Effect of biochar application rate and time on soil pH, moisture content, soil organic carbon (SOC), total nitrogen (TN), ammonium nitrogen (NH_4^+-N), nitrate nitrogen (NO_3^--N), extractable phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), microbial biomass carbon (MBC) and microbial biomass nitrogen (MBN).

		Rate	Time	Rate × Time
pH		*	*	*
Moisture	(%)	*	*	n.s.
SOC	(g kg^{-1})	*	*	*
TN	(g kg^{-1})	*	*	n.s.
NH_4^+-N	(mg kg^{-1})	*	*	*
NO_3^--N	(mg kg^{-1})	*	*	*
P	(mg kg^{-1})	*	*	*
K	(mg kg^{-1})	n.s.	*	n.s.
Ca	(mg kg^{-1})	*	*	n.s.
Mg	(mg kg^{-1})	n.s.	*	n.s.
MBC	(mg kg^{-1})	n.s.	*	n.s.
MBN	(mg kg^{-1})	n.s.	*	n.s.

* means significantly different at $p = 0.05$

n.s. means not significantly different at $p = 0.05$

Table C-4. Effect of biochar application rate and time on soil microbial biomass (mean (SE)).*

Biochar rate (Mg ha^{-1})	Microbial biomass carbon (MBC) mg kg^{-1}		Microbial biomass nitrogen (MBN) mg kg^{-1}	
	1st sampling	4th sampling	1st sampling	4th sampling
0	371 (44)	406 (29)	87 (2)	113 (6)
4.5	367 (19)	401 (20)	77 (9)	112 (6)
9	360 (54)	418 (40)	93 (12)	115 (8)
13.5	406 (49)	456 (68)	89 (9)	124 (13)
18	345 (23)	400 (45)	101 (9)	115 (11)
22.5	329 (20)	418 (30)	99 (14)	124 (6)

* $p > 0.05$

Table C-5. Economic analysis (US$) after 20 months of biochar application relative to no biochar addition in a pasture system (Area = 0.004 ha^{-1})

	Biochar rate (Mg ha^{-1})						Note
	0	4.5	9	13.5	18	22.5	
Amount of biochar (Mg)	0.00	0.018	0.036	0.054	0.072	0.090	
Gross cost of biochar ($)	0.00	0.99	1.98	2.97	3.96	4.95	$55 Mg^{-1} biochar
2017 Forgae yield ($)	0.97	1.14	0.98	1.09	1.08	1.18	$143 Mg^{-1} hay
2018 Forgae yield ($)	0.43	0.35	0.34	0.50	0.43	0.60	
P fertilizer ($)[a]	0.00	0.20	0.39	0.59	0.79	0.98	$3483 Mg^{-1} P
K fertilizer ($)[b]	0.00	0.06	0.12	0.18	0.24	0.30	$1104 Mg^{-1} K
C storage ($)	0.00	2.24	4.14	3.57	4.42	4.79	$538 Mg^{-1} C
Total revenue ($)	1.40	3.99	5.97	5.93	6.96	7.84	
Gross profit ($)	1.40	3.00	3.99	2.96	3.00	2.89	

a. The price of 45% phosphate (P$_2$O$_5$) is $685. Since the percentage of phosphorus in the phosphate is 44 %, so the price of phosphorus is $3483 Mg^{-1}. The total phosphorus concentration in the biochar is 3.16 g kg^{-1}.

b. The total potassium concentration in the biochar is 2.99 g kg^{-1}.

Table C-6. Person correlation coefficients of pH with cations

Time	Ca	Mg	K
1st sampling	0.69***	0.69***	0.56**
2nd sampling	0.57**	0.30	0.41
3rd sampling	0.74***	0.32	-0.15
4th sampling	0.23	-0.09	-0.08

* means the correlation is statistically significant at $p \leq 0.05$;

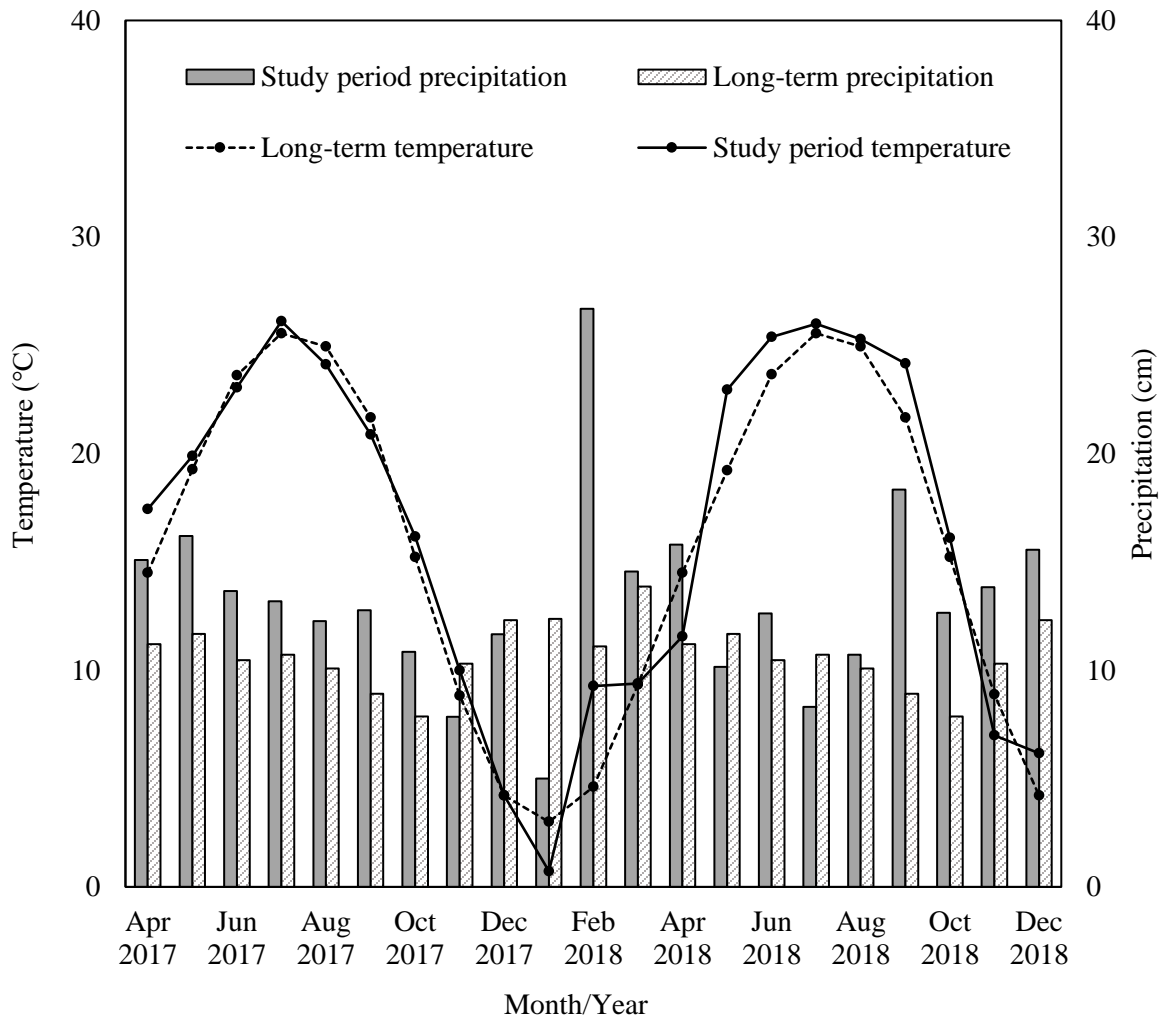** means the correlation is statistically significant at $p \leq 0.01$;

*** means the correlation is statistically significant at $p \leq 0.001$

Figure C-1. Mean monthly air temperature and precipitation (long-term average and during the study period) at the study site.
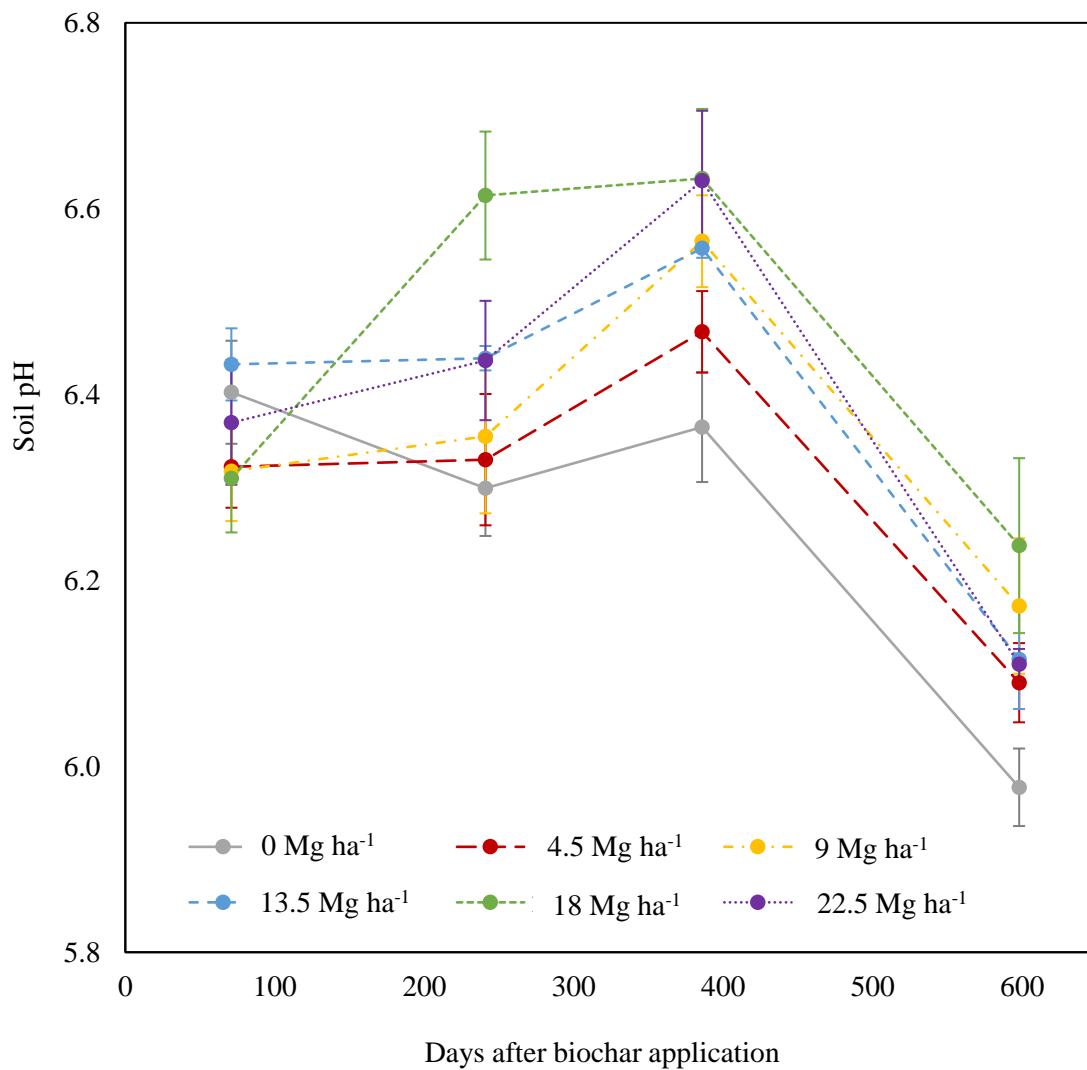
Figure C-2. Soil pH at 0-15 cm depth at different sampling time. Error bars represent standard error (n = 4).
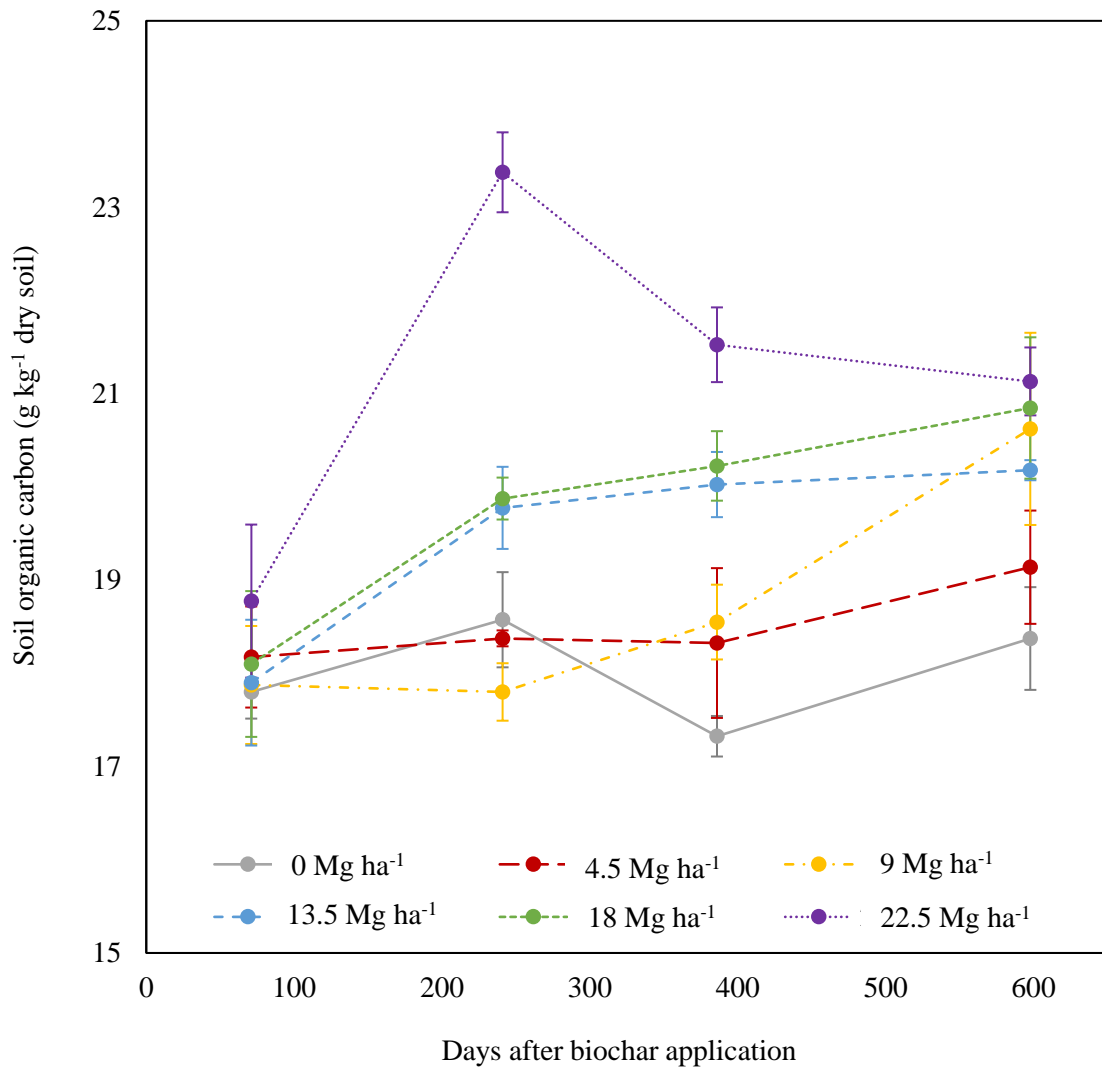
Figure C-3. The concentration of soil organic carbon at 0-15 cm depth at different sampling time.

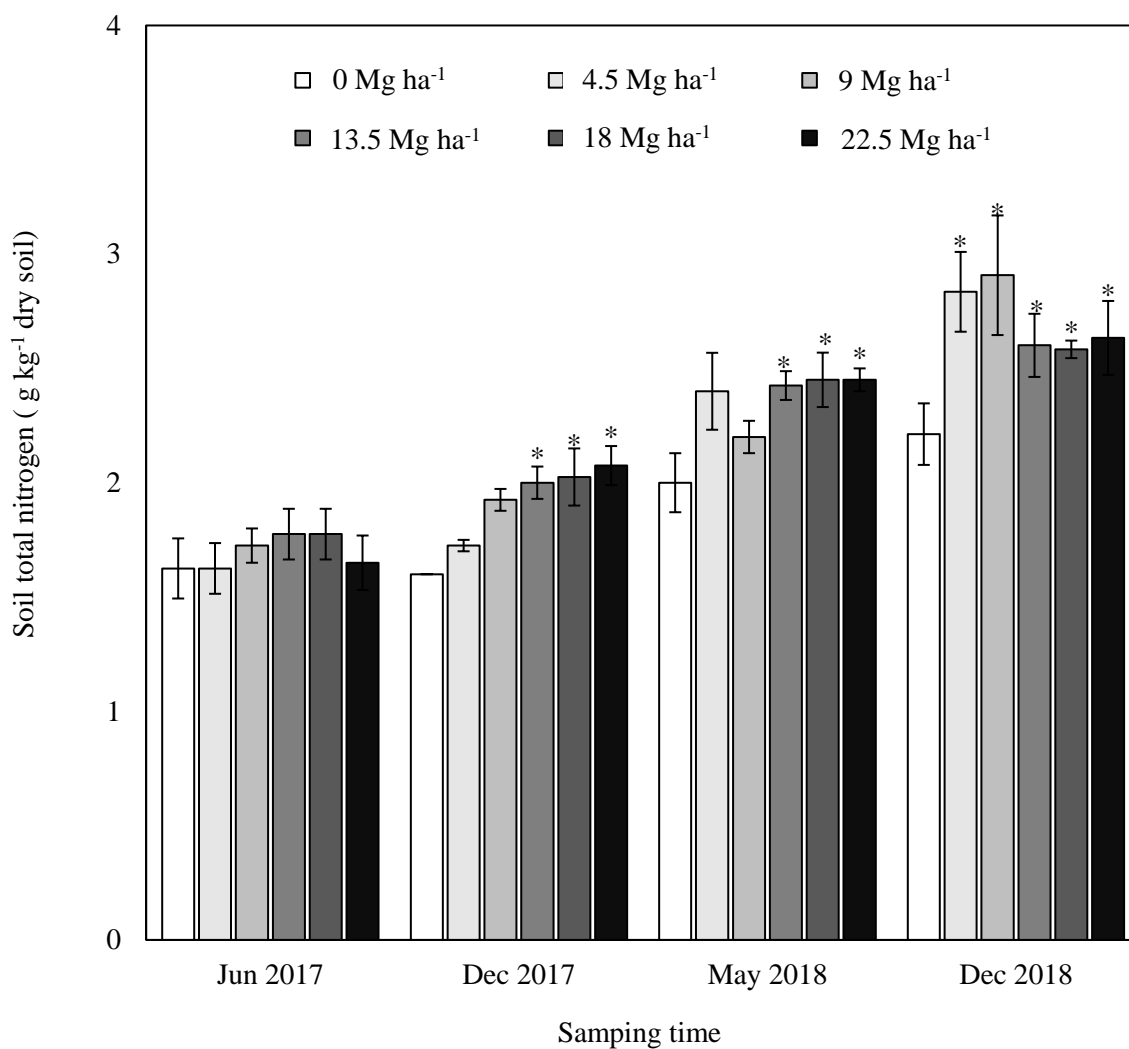Error bars represent standard error (n = 4).

Figure C-4. The concentration of soil total nitrogen at 0-15 cm depth at different sampling time. *
means significantly different from control based on the LSD test (p < 0.05). Error bars represent
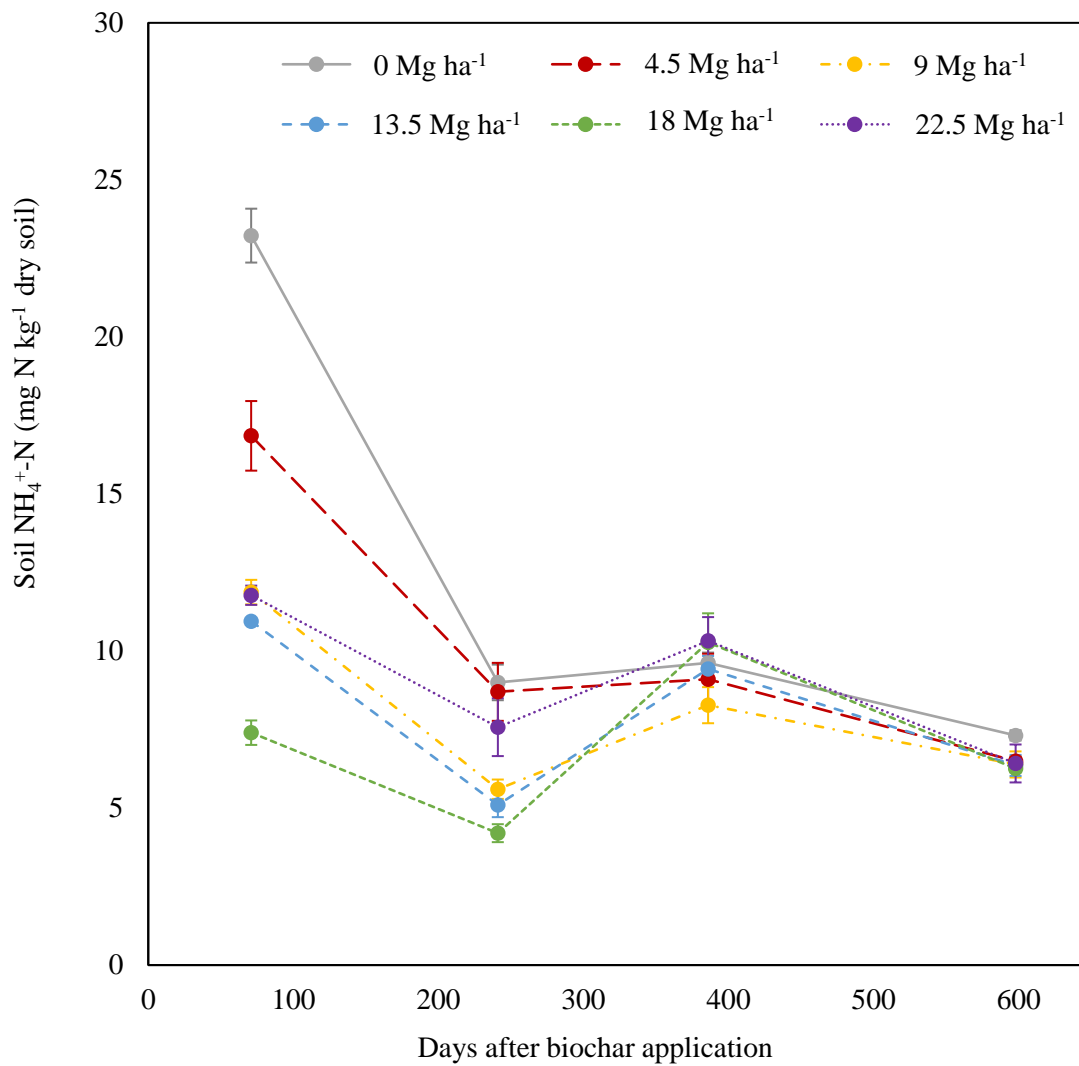standard error (n = 4).

Figure C-5. The concentration of soil ammonium at 0-15 cm depth at different sampling time. Error bars represent standard error (n = 4).
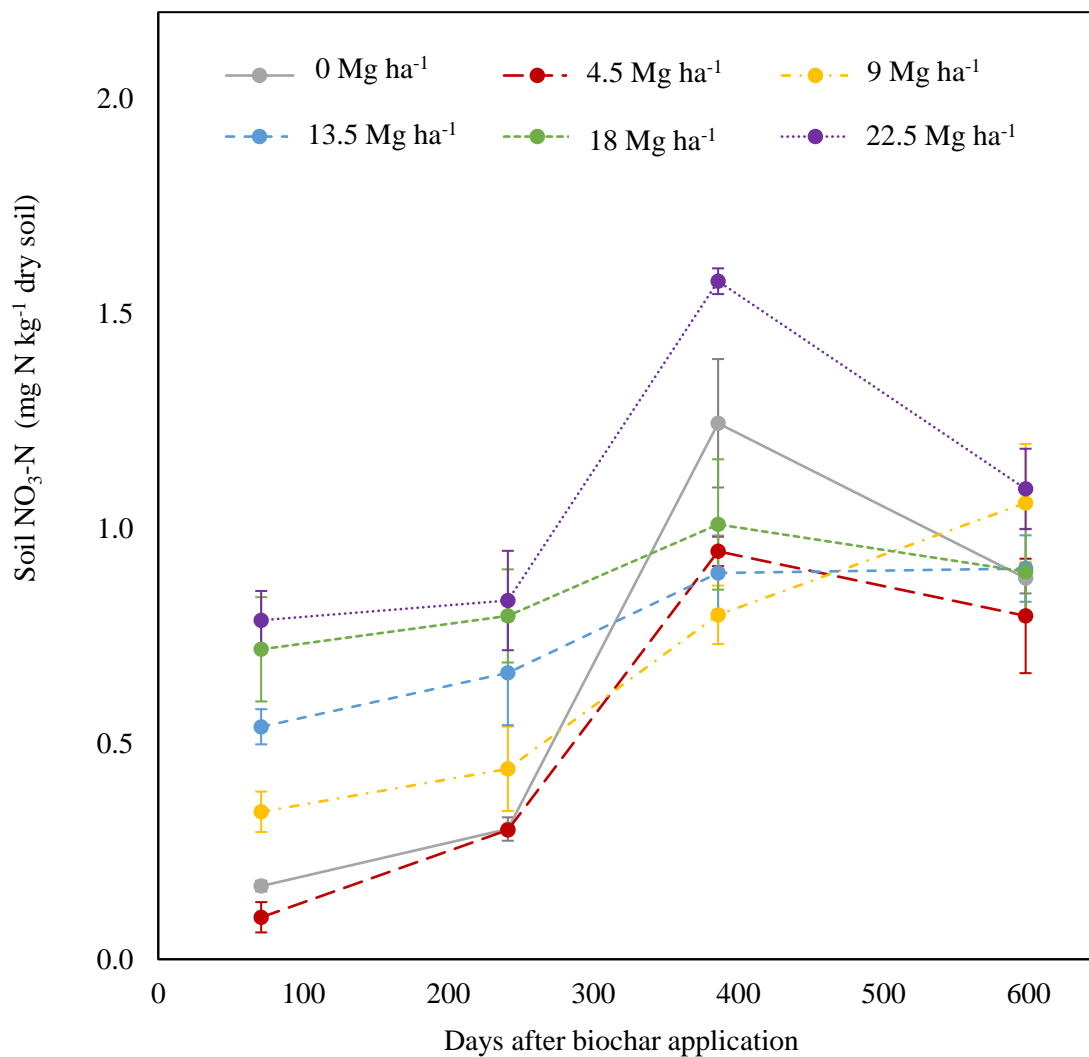
Figure C-6. The concentration of soil nitrate at 0-15 cm depth at different sampling time. Error bars represent standard error (n = 4).
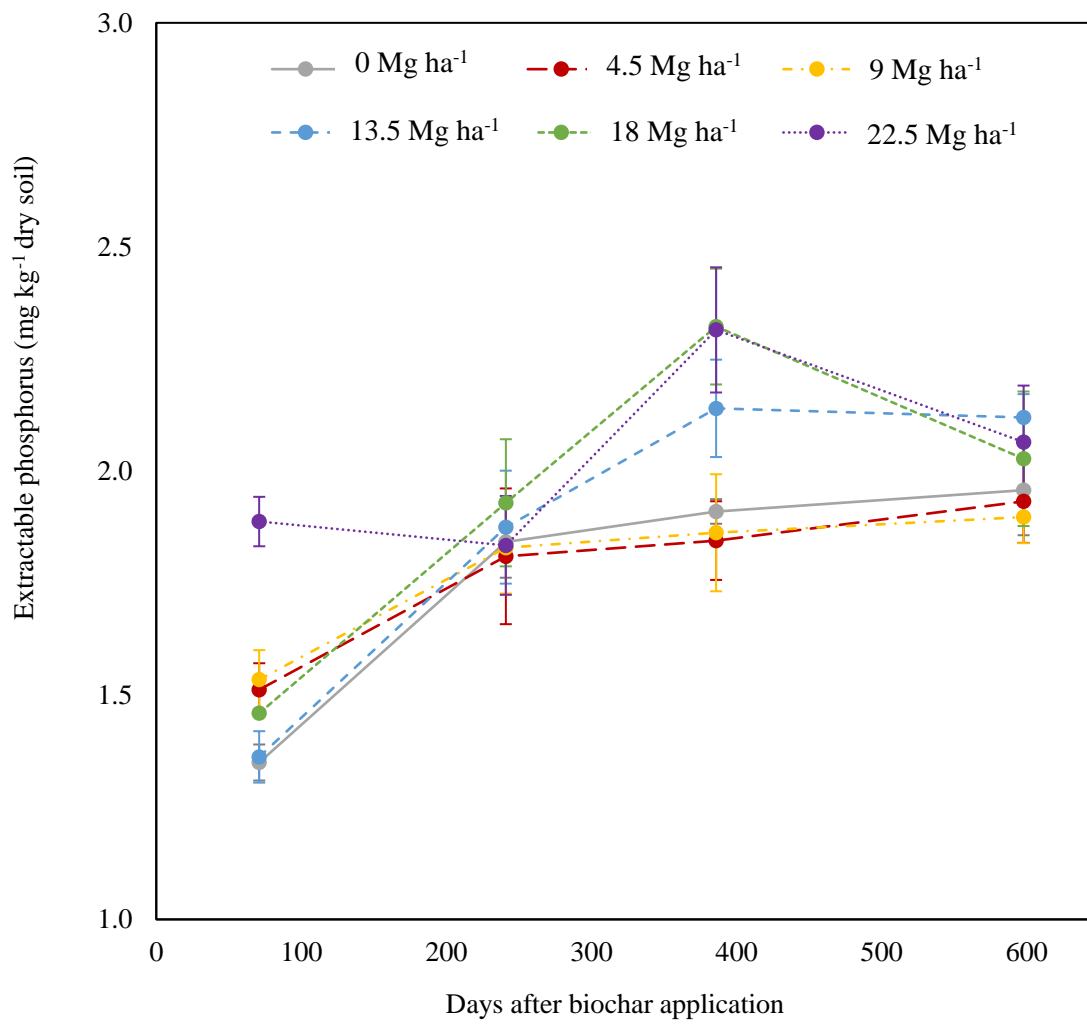
Figure C-7. The concentration of soil extractable phosphorus at 0-15 cm depth at different sampling time. Error bars represent standard error (n = 4).
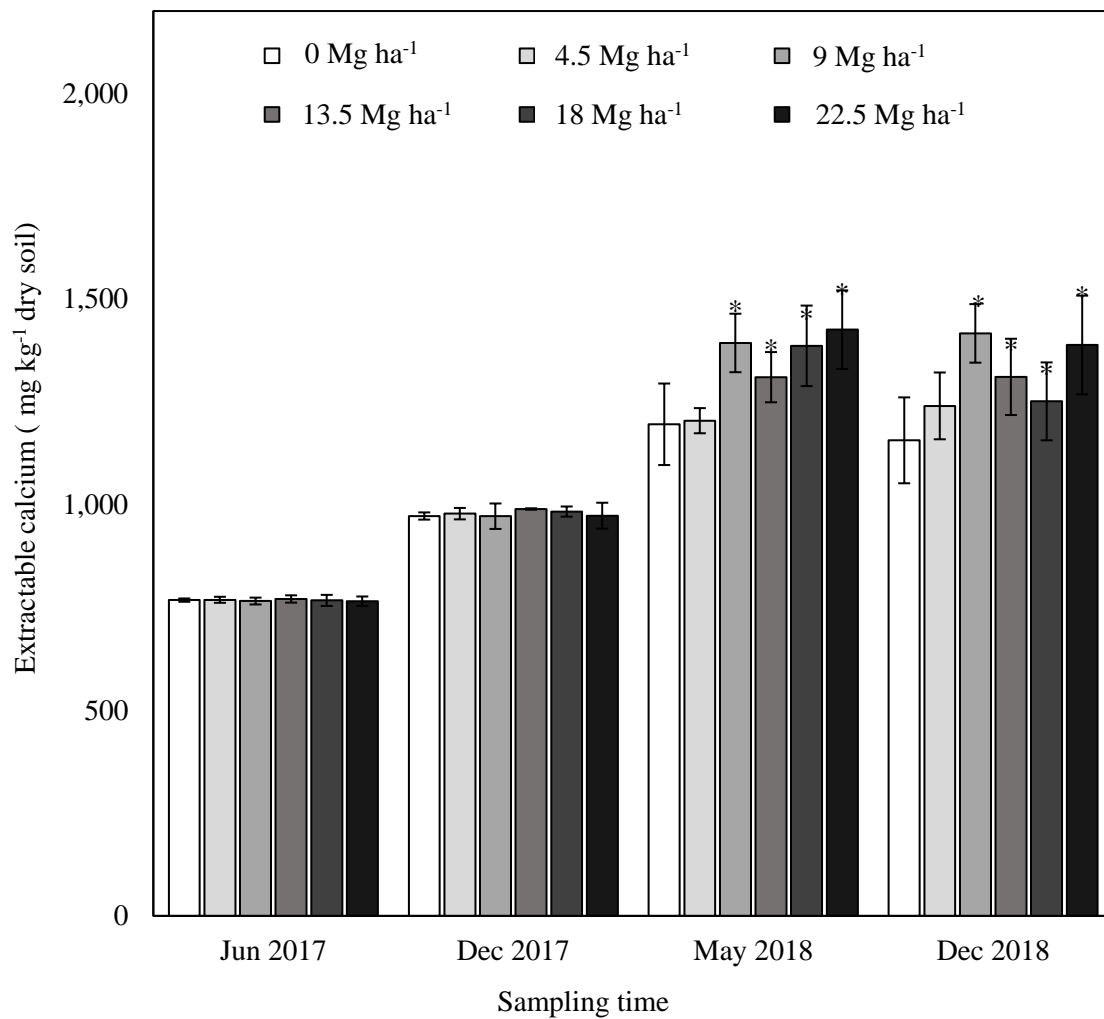
Figure C-8. The concentration of soil calcium at 0-15 cm depth at different sampling time. * means significantly different from control based on the LSD test ($p < 0.05$). Error bars represent standard error (n = 4).

CHAPTER V – SUMMARY AND FUTURE RESEARCH DIRECTIONS

This research includes both laboratory- and field-scale investigation of the effect of biochar types, rates, and application methods on soil properties and plant growth in general, and soil nitrogen dynamics in particular.

A 60-day laboratory incubation was conducted and soil mineral nitrogen content, nitrous oxide emission, and ammonia-oxidizing bacteria *amo*A gene transcripts were measured to determine the effect of two types of biochar on soil nitrogen dynamics, with or without the co-application of urea. Two types of hardwood biochar differing in moisture content, ash content and cation exchange capacity were used (B1 and B2). Compared to control (soil with no biochar and urea addition), B1 decreased soil nitrate nitrogen concentration by 21-45% and *amo*A gene transcripts by 57-73% while no significant differences were observed with B2 addition. Although soil nitrate nitrogen concentration was significantly increased in biochar plus urea treatments, this positive effect was lower for B1 + urea treatment compared to B2 + urea treatment. Meanwhile, the cumulative nitrous oxide emission from B2 + urea treatment was 36% lower than that from B1 + urea treatment after 60 days. These results indicate that biochar with higher cation exchange capacity has the potential to decrease nitrification, while biochar with higher ash content effectively reduces nitrous oxide emission when co-applied with urea.

When the application methods (surface placement and soil incorporation) were compared, results revealed that surface application of biochar or urea alone stimulated nitrification than incorporation method, evidenced by higher soil mineral nitrogen concentration and more *amo*A gene transcripts. However, nitrification was enhanced by the incorporation method when biochar was co-applied with urea. Besides, lower nitrous oxide emission was observed when fertilizers were surface placed compared to mixed with soil, regardless of the amendment type.

The *in-situ* response of soil and plant to different rates of biochar ranging from 0 to 22.5 Mg ha^{-1}

was monitored in a pasture system in Middle Tennessee. We measured aboveground biomass yield, plant nutrient concentration, and other plant chemical constituents as well as soil properties such as pH, gravimetric moisture content, organic carbon, microbial biomass carbon and nitrogen, total nitrogen, and plant-available nutrients. The results revealed the following findings: (1) general positive relationship between biochar rate and soil quality parameters - some properties including organic carbon, total nitrogen, and soil pH increased with biochar rate and the effect lasted longer, (2) the temporal effect of surface placed biochar on plant growth and soil quality in this unmanaged pasture system varied with the plant and soil variables – the effect on plant-available nutrients was significant only until two months after application while the effect on soil organic carbon and total nitrogen were significant after eight months of application.

Overall these studies show that biochar has a positive effect in improving soil mineral nitrogen retention and overall soil quality. We confirmed that biochar characteristics, application method, and biochar rates control the effect of biochar on soil quality, especially on soil nitrogen, but more work is needed to obtain a better understanding of how biochar influence nitrogen related chemical and biological processes.

Several recommendations for future work are proposed based on the results of this research. We attributed the decrease of nitrate nitrogen concentration and *amo*A gene transcripts with biochar application to the sorption of substrates to biochar surface. However, this conclusion can be challenged because the inhibition of nitrification can be caused by other reasons such as toxic heavy metals or organic components (e. g. phenolic compounds) in the biochar. Since the information about biochar properties is limited, we cannot exclude other possibilities. Therefore, it is of critical importance to measure these biochar and soil properties in future experiments. Besides, the sorption of urea to biochar surface due to its high CEC is also considered as the main reason inhibiting ammonification and nitrification processes. However, no direct evidence is found in this research. To confirm this

assumption, direct measurement of urea from the soil and biochar is warranted.

We also found that biochar addition via surface application reduced nitrous oxide emission, which was attributed to enhanced denitrification, reduced nitrification, or physical sorption, however, the mechanisms are still not very clear. Thus, future research should evaluate the biochar-induced change in soil properties such as total porosity, redox status, enzyme activity, and microbial activity, especially the abundance and activity of denitrifiers.

The economic analysis in Chapter IV presents the potential economic benefit from biochar amendment. However, the uncertainty cannot be ignored. To fully estimate the revenue from biochar amendment, we suggest two future research directions. One is establishing a field experiment in a cropping system to compare biochar with other soil amendments such as synthetic fertilizers and organic manure. The second future direction is quantifying all possible ecosystem benefits from biochar application in addition to C sequestration. Since biochar is not a cheap soil amendment compared to others, calculating the costs and benefits is very important while deciding on the appropriate soil amendment.

Overall, this research reveals that biochar has the potential to improve soil quality and reduce nitrogen loss of southeastern cropping systems, but more research need to be done on different soil types and cropping systems before making robust recommendations on the feasibility of biochar as a potential soil amendment to build and maintain ecosystem sustainability.

VITA

Xiuwen Li was born in Loudi, China in 1990. She attended San Yat-sun University in Guangdong, China where she got a bachelor's degree in Ecology in 2012. After that Xiuwen got a master's degree in Geology (Urban and Regional Planning) from Peking University in 2015. In 2017, Xiuwen joined as an MS student in the Department of Biosystems Engineering and Soil Science at the University of Tennessee under the supervision of Dr. Sindhu Jagadamma. Xiuwen received the Maxwell/Jean springer soil science assistantship which partially covered her MS program and received the department's promising graduate student award in 2019. She graduated in July 2019 with a MS degree in Environmental and Soil Science.