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CROP GROWTH AND NUTRIENT UPTAKE FROM AN INCEPTISOL AND VERTISOL WITH HIGH BIOCHAR APPLICATION RATES

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ABSTRACT

Soil biochar application effects on warm- and cool-season crops are not well understood. Corn (*Zea mays* L.), soybean (*Glycine max* L.), wheat (*Triticum aestivum* L.), cereal rye (*Secale cereal* L.), and alfalfa (*Medicago sativa* L.) were grown in a Marietta fine sandy loam (fine-loamy, siliceous, active, thermic Fluvaaquentic Eutrudept) and a Houston silty clay (very fine, smectitic, thermic Oxyaquic Haplaudert) amended with 0, 45, 90, and 180 Mg ha^{-1} of pine biochar and allowed to equilibrate for 56-d. To investigate a possible nitrogen effect, three nitrogen rates (0, 0.5 and 1.0X of the recommended rate for each crop) were added to non-legume species at planting. Legume crops were inoculated with Brady Rhizobia upon planting. All species were pre-germinated for 14-d in sand trays. After 21-d growth in the treatments, plants were harvested and shoots were analyzed for shoot dry weight, nutrient uptake and concentration, and forage quality. Decreases in uptake of nitrogen (N), phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) were found for corn, soybean and alfalfa in both soils at higher rates of biochar (90 and 180 Mg ha^{-1}). Changes in forage quality were specific to soil, crop and parameter and clear trends were not apparent. This study found biochar amendment decreased plant nutrient uptake, and affected forage quality. Thus, the appropriateness of biochar incorporation depends on the nature of the cropping system in which it is to be used.

Keywords: Crop Growth, Nutrient Uptake, Biochar Application

INTRODUCTION

The key to determining the role of soil application of biochar in climate change mitigation is contained in the linkage of energy production and carbon (C) sequestration (Woolf et al., 2009; Roberts et al., 2010; Bruckman and Klinglmüller, 2014; Shackley et al., 2012a). Some of the more important questions concerning the economic viability of biofuel-biochar systems reflect the challenges to positioning it within a nexus of food security concerns, which in turn reside within a confluence of global sustainability issues, including climate change, fresh water, arable land, population, and the economy (DeLong et al., 2010; Garrett, 2011; Princiotta and Loughlin, 2014; Seppelt et al., 2014; Smith, 2016). A number of additional effects of biochar applications on soil and environmental quality and yield improvements however, have shown mixed results (Kookana et al., 2011; Spokas et al., 2012). Various economic analyses of biofuel-biochar have selected different agronomic discounts. Roberts et al. (2010) for example, assigned values to biochar phosphorus (P) and potassium (K) content and improved fertilizer use efficiency, whereas Shackley et al. (2012b) used the price difference associated with yield increase over grain produced without addition of biochar to soil. Field et al. (2013) calculated cost offsets due to a liming effect of biochar and its associated displacement of nitrogen (N) fertilizer requirements. Focusing on crop production, i.e., biochar application, Galinato et al. (2010) also used its liming effect as an economic offset, while Blackwell et al. (2010) analyzed the financial performance of biochar using yield increase and a reduction in P fertilizer requirements. While there have been a number of different evaluations of biochar, until recently there has been little information available about possible tradeoffs in its use. Jerrery et al., (2015) provide an in-depth assessment of these tradeoffs when considering C sequestration, soil fertility, biofuel/energy production, pollutant fixation, and waste disposal.

Biochar is a carbon-rich by-product resulting from the burning of biomass in the presence of little to no oxygen. Typically, biochar is an organic, variable charge material that has a large surface area and is considerably porous. The conditions during pyrolysis along with the kind of feedstock biomass determine the physico-chemical properties of biochar (Enders et al., 2012). It appears that these biochar properties interact with soil properties in unpredictable ways such that inconsistencies in yield response can be difficult to explain (Van Zwieten et al., 2010; Rajkovich et al. 2012). Results for a specified set of studies, viewed in aggregate, showed an overall positive yield response to biochar addition for acid and neutral pH soils, and for coarse and medium soil textures (Jeffery et al., 2011). These suggest that the liming effect (Dai et al., 2014) and higher water holding capacity from biochar application are significant contributors to crop yield increases. More recently, Biederman and Harpole (2013) found that on average, biochar increased crop yield along with soil pH, soil total N, P, and K. However, close attention should be paid to the effects of biochar with specific soil types, especially farmland soils since though relatively few in number they can encompass significant portions of farming enterprises. Additionally, there is evidence that high biochar application rates can negatively affect soil fertility and crop biomass (Rondon et al., 2006; Krapfl et al., 2013; Stewart et al., 2013). Therefore, the approach prescribed by Enders et al. (2012), its efficacy supported by other findings (Galinato et al., 2010; Singh et al., 2010), is currently most appropriate. They

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determined that "… the most effective approach to predicting agronomic performance of biochars is to first define the predominant limiting factors of a particular soil-crop-climate situation and apply biochars likely to address growth constraints …" (Enders et al., 2012).

Renewable and low-cost carbon sources from biomass such as pinewood make an available and plentiful carbon precursor material for biochar (Yan et al., 2011). Some variation key biochar properties using pine feedstock have been reported (Enders et al., 2012; Kim et al., 2012). Enders et al. (2012) showed that oxygen (O) to C ratios for pine biochar changed from 0.36 to 0.1 as pyrolysis temperatures ranged from 350 to 600°C. They also observed hydrogen (H) to C ratios from 0.06 to 0.03 over the same temperatures. The highest O:C (0.36) and H:C (0.06) are slightly (O:C <0.4) and much (H:C <0.6) less than the thresholds recommended by Schimmelpfennig and Glaser (2012) for biochar suitable as a soil amendment and C sequestration. However, Kim et al. (2012) reported pitch pine biochar data with an O:C of 0.48 and H:C of 0.79 at a pyrolysis temperature of 300°C. These ratios fell below the Schimmelpfennig and Glaser criteria when pyrolysis was conducted ≥400°C. In addition, Kim et al. (2012) also showed that pine pitch biochar produced at 500°C had a BET-N_2 surface area of approximately 175 m^2g^{-1}, which exceeds the minimum value of 100 m^2g^{-1}recommended by Schimmelpfennig and Glaser (2012). Kim et al. (2012) obtained much smaller surface areas for lower pyrolysis temperatures. Very little fertilizer content was determined for pine biochars produced at 400 and 500°C (Gaskin et al. 2008). This finding was supported by the lack of positive yield effects from soil applied pine biochar with corn (Gaskin et al., 2010) and native grass (Krapfl, 2014).

While research has outlined the potential of biochar as an effective and viable soil amendment to degraded soils, sparking interest in the possibility of improving fertility on previously productive agricultural soils in the United States. At this point, it is not possible to draw conclusions on the effect of biochar that can be broadly applied, especially in temperate regions with younger soils compared to highly weathered soils in more tropical environments. This study was developed to address some of the uncertainties shown in previously reported results with respect to the effects on soil fertility and agronomic performance while utilizing a pine biochar on agricultural soils with varying properties. The objectives of this study were to determine the effect of biochar applications on (1) the soil fertility status as determined by a soil test extractant, pH, and C and N concentrations and (2) selected warm- and cool-season crop responses as determined by biomass production, nutrient availability, and forage quality.

MATERIALS AND METHODS

For these studies, two soils with different properties were used. Marietta (fine-loamy, siliceous, active, thermic Fluvaaquentic Eutrudept) and Houston (very fine, smectitic, thermic Oxyaquic Hapludert) soils were collected, air-dried and passed through a 2-mm sieve to remove debris.

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In addition, a locally produced pine biochar (pyrolyzed at 450ºC) was selected. Initial characterization of the biochar included total Si, Al, Ca, Mg and K using a modified dry ashing procedure followed by analysis via inductively coupled argon spectroscopy (ICP) (PerkinElmer Inc., Waltham, MA). Total surface area of the biochar was determined by gas phase adsorption (Pennell ,2002). Initial physical and chemical characterization of the soils and the biochar included pH in water (1:1 ratio and allowed to equilibrate until stable), Lancaster extractable Ca, Mg, Na, K and P via ICP (Thermo Fisher, Wlatham, MA) (Cox, 2001) and total C and N by dry combustion using a Vario EL *III* elemental analyzer (Elementar Americas Inc., Mt. Laurel, NJ) (Nelson and Sommers, 1996). Soil characterization also included soil water content at -0.03 MPa (representing "field capacity") (Dane and Hopmans, 2002) and soil texture by the hydrometer method (Gee and Orr, 2002).

Soil Fertility Status

An incubation study was conducted to evaluate the effects of biochar applications on soil test values, pH, and total C and N content for the two soils. The experiment used a completely randomized 4x2 factorial arrangement with three replications. Four treatments (0, 45, 90, and 180 Mg ha^{-1}) of biochar were thoroughly mixed with 3 kg of each soil. Water content at -0.03 MPa was determined for each soil/biochar combination. Each treatment was then brought to 80% of field capacity with deionized water. The treatments were placed in 3.8-L bags and incubated in the dark for a period of 56 days at 25°C. Treatments were thoroughly mixed each week and water content monitored. A sub-sample from each treatment replicate was analyzed as the initial soil (see above) at 0 and 56 days. Data were analyzed using PROC GLM of SAS 9.2. Comparisons were made by separation of means utilizing the Least Significant Difference (LSD) test at $\alpha = 0.05$.

Crop Response

Two greenhouse experiments, using the soil/biochar combinations from the soil incubation experiment, were conducted to investigate the effect of biochar addition on the growth and nutrient uptake of two warm-season crops (corn (*Zea Mays* L.) and soybean (*Glycine max* L.) and three cool-season crops (wheat (*Triticum aestivum* L.), cereal rye (*Secale cereal* L.) and alfalfa (*Medicago sativa* L.).

Each treatment combination was added volumetrically to lined, 3.8- L black plastic pots. In addition, three fertilizer treatments consisting of 0, 0.5 and 1.0X the recommended nitrogen fertilizer rates were applied to pots containing non-legume plants. This was equivalent to 0, 67.2, and 134 kg N ha^{-1} for corn and 0, 56, and 112 kg N ha^{-1} for wheat and rye (Oldham, 2012). Nitrogen treatments were applied as a liquid just prior to planting using ammonium nitrate (34-0-0) as the N source. The legume seeds, with the exception of the control, were inoculated with the

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appropriate Rhizobium strain (*Bradyrhizobium japonicum* for soybean and *Rhizobium meliloti* for alfalfa) prior to planting.

Seeds for all species were germinated at 25˚ C in sand trays prior to planting in the biochar treated soil. Seven days after emergence, two plants were transplanted into each soil/biochar treatment. Plants were grown in the greenhouse for a period of 21 days after planting using a randomized complete block design. Pots were weighed and watered with deionized water daily to maintain a moisture content equivalent to 80% field capacity. After watering, all pots were re-randomized within blocks. Greenhouse conditions for corn and soybean (warm season species) consisted of average daytime temperatures of 43° C and average nighttime temperatures of 27° C while conditions for the wheat, rye and alfalfa (cool- season species) consisted of average daytime temperatures of 27° C and nighttime temperatures of 16° C. After 21 days of growth, shoots were harvested to the soil level. Shoot tissues were dried at 65˚ C for a period of 24 hours, weighed and ground to pass a 1-mm screen. Ground samples were used to determine nutritive value crude protein (CP), acid detergent fiber (ADF), and neutral detergent fiber (NDF)] using the Foss 6500 NIRS instrument (Foss North America, Eden Prairie, MN) and using the legume and hay equations developed by the NIRS Forage and Feed Testing Consortium (NIRS C, Hillsboro, WI).

Shoot tissue concentrations of Ca, Mg, K and P were determined by dry ashing and analyzed using inductively coupled argon plasma spectroscopy (Jones, 2001). Samples were also analyzed for total C and N using a Vario EL III elemental analyzer (Elementar Americas Inc., Mt. Laurel, NJ) (Nelson and Sommers, 1996).

To evaluate the effects of the biochar/N treatments on biomass yield, tissue nutrient concentration, crop nutrient uptake, ADF, NDF, and lignin data were analyzed using PROC GLM of SAS 9.2, and comparisons were made by separation of means utilizing the Least Significant Difference (LSD) test at $\alpha = 0.05$.

RESULTS AND DISCUSSION

Biochar and Soil Characteristics

The initial evaluation of the pine biochar established physical and chemical characteristics of the amendment material. Mineral analysis results demonstrate that the biochar samples contain 3.5% wt Si, 0.7% wt Al, 0.5% wt Ca, 0.3% wt Mg, and 0.1% wt K while the BET surface area of biochar was 12.5 m^2/g (Yu, personal communication). The biochar had a total carbon content of approximately 645 g kg^{-1} (Table 1). This amount of carbon was slightly lower than expected for the pine biochar. Previous research found C contents of pine biochar around 735 g kg^{-1} (Harris et al., 2013, Mukome et al., 2013, Yargicoglu et al., 2014). However, pine biochar C as low as

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245 g kg^{-1} and as high as 863 g kg^{-1} has been reported (Brantly et al., 2015, Nelissen et al., 2014). Total nitrogen content of the biochar was below detectable limits. This also, is consistent with other research showing pine biochar to be low in nitrogen. Total N in pine biochar has been reported to range from about 2 g kg^{-1} to about 10 g kg^{-1} (Mukome et al., 2013, Nelissen et al., 2014)

Initial biochar concentrations of extractable Ca, K, Mg, and P were 217, 115, 30, and 7.8 mg kg^{-1}, respectively (Table 1). The biochar in this study contained levels of extractable nutrients consistent with previous research by Laird et al. (2010a). Laird et al. (2010b) determined plant available nutrients in a mixed hardwood biochar using the Mehlich 3 method and analyzed via ICP analysis (Mehlich, 1984). While soil tests to determine plant available nutrients are an index based on extractant and values of the nutrients may vary among extracts, values for Ca, Mg, and K determined with Mehlich 3 and Lancaster extractants have been shown to be in general agreement (Cox, 2001). Biochar pH was determined to be 6.3 (Table 1). A significant amount of research points toward biochars as having a neutral to basic pH (Chan and Xu, 2009; Downie et al., 2009; Singh et al., 2010), however, both Yargicoglu et al., (2014) and Nelissen et al. (2014) reported pine char pH around 6.5.

Like the biochar, both soils collected for this study were analyzed for initial total C and N, extractable nutrient values and pH. Soil test categories based on Mississippi State University Extension Service (MSU-ES) recommendations for K and P were also determined (Oldham, 2012). These categories are an important aspect of this study in that, biochar addition to the soil must change the category in order to affect nutrient management decisions.

The Marietta silt loam had a very high pH when compared to the biochar and the Houston silty clay loam and extractable nutrient levels were in the high category for K, while P fell into the medium category based on the MSUES recommendations (Oldham, 2012). Total C in this soil was very low and total N was below detectable limits (Table 1). In addition, the clay content of the Marietta soil was approximately half that of the Houston while the sand content was double ensuring the physical and chemical characteristics of the two soils would be very different. The pH of the Houston soil was similar to the pH found in the char while extractable nutrient levels ranged from the low category for P to the medium category for K (Table 1). Total C and N levels in this soil were considered normal.

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Table 1: Mean values (n=9) for initial total C and N, pH, and extractable nutrient levels in pine biochar, Marietta and Houston soils

	C	N	pH	Ca	K	Mg	P	Sand	Silt	Clay
	g kg^{-1}				mg kg^{-1}				g kg^{-1}	
Char	645.4	BDL†	6.3	216.5	115.2	30.0	7.8	-	-	-
Marietta	3.97	BDL	8.0	4206.7	87.7	48.4	22.0	284.6	532.6	182.8
Houston	18.18	1.14	6.5	1552.3	80.6	63.0	13.0	141.6	492.9	365.5

†Below Detectable Limits

Soil Incubation Study

Although the Total C and N increased with increasing biochar application rate, the Total C and N in the Marietta sandy loam did not change within any rate of biochar over the 56-d incubation period (Table 2). All of the extractable nutrient concentrations increased over the incubation period in all biochar treatments (Table 2) which is consistent with other research (Chan et al., 2008). Their study found significant increases in exchangeable nutrients over a six-week incubation period with the addition of biochar at levels of 10, 50 and 100 Mg ha^{-1} (Chan et al., 2008). However, despite statistical significance, the increases in extractable P and K were not enough to change the soil test category, thus soil fertility management would not be influenced by biochar addition (Oldham, 2012). After the various additions of biochar and over the course of a 56 day incubation, there were no significant changes in pH at any biochar rate. The pH influence shown in other studies was not evident here given the initial, slightly acidic pH of the biochar and the already alkaline pH of the Marietta soil. While Laird et al. (2010b) found increases in pH on a degraded agricultural soil amended with biochar and Chan et al. (2007) also found increases of 0.61 to 1.22 pH units on soil amended with biochar, this study showed no significant differences in pH with biochar addition in the Marietta soil.

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Table 2: Mean values (n=9) for initial total C and N, pH, and extractable nutrient levels in the Marietta soil after four rates of biochar and at 0 and 56 days after treatment

Char rate	DAT†	C	N	pH	Ca	K	Mg	P
Mg ha^{-1}		g kg^{-1}			mg kg^{-1}			
0.0	0	3.97	BDL††	7.95a§	4206.78a	87.71a	48.40b	21.10a
	56	3.99	BDL	8.06a	4318.44a	87.90a	55.52a	22.33a
45	0	19.90	0.04	8.09a	4421.17b	91.21b	50.36b	22.55b
	56	20.95	0.04	8.05a	4596.28a	97.46a	55.83a	24.93a
90	0	39.10	0.14	8.12a	3843.77b	79.21b	44.32b	20.13b
	56	40.56	0.15	8.07a	4454.70a	94.84a	54.47a	24.64a
180	0	40.76	0.19	8.17a	3815.07b	79.60b	44.05b	20.98b
	56	48.98	0.24	8.15a	4298.97a	93.42a	53.89a	24.75a

†Days after treatment

†† Below Detectable Limits

§Different letters within column and char rate indicate significant difference at α=0.05

While all of the extractable soil nutrients increased with biochar addition in the Marietta soil, there were no significant changes in these parameters or pH in the Houston soil at any rate of biochar addition with the exception of a K increase at the highest biochar rate. This extractable K increase would not have changed the soil test categoryhence, soil fertility was not affected and nutrient management decisions would not change at any rate (Table 3). This is consistent with other studies where biochars were produced at temperatures less than 500º C did not have a major impact on soil fertility (Steinbeiss et al., 2009). Total C and N in this soil followed the same pattern found in the Marietta soil. There were no differences in total C or total N at any rate of biochar after the incubation period. The results from both soils are consistent with other research. The increases in the extractable nutrients found in the Marietta soil support the findings of Chan et al. (2007) and Novak et al. (2009) while the lack of change found in the Houston soil support the results of Steinbeiss et al. (2009). These differences suggest the effect of biochar amendments on nutrient availability is highly dependent on the soil chemical and physical properties. It is interesting to note that the soil texture of the Norfolk (fine-loamy,

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kaolinitic, thermic Typic Kandiudult) used in Novak et al.'s (2009) study has a low clay content similar to the Marietta used in this study. More research is needed to determine when biochar addition will be beneficial.

Table 3: Mean values (n=9) for initial total C and N, pH, and extractable nutrient levels in the Houston soil after four rates of biochar and at 0 and 56 days after treatment

Char rate	DAT†	C	N	pH	Ca	K	Mg	P
$Mg\ ha^{-1}$		$g\ kg^{-1}$			$mg\ kg^{-1}$			
0	0	18.18	1.10	6.51a††	1552.3a	80.62a	62.98a	13.01a
	56	18.90	1.14	6.02a	1495.2a	78.78a	62.71a	12.75a
45	0	28.80	1.19	6.55a	1542.50a	83.79a	63.59a	13.00a
	56	29.93	1.22	6.34a	1377.30a	78.14a	58.41a	12.67a
90	0	32.74	1.26	6.48a	1535.23a	85.07a	64.22a	12.34a
	56	32.95	1.30	6.09a	1502.73a	86.51a	63.20a	12.36a
180	0	62.87	1.55	6.47a	1540.53a	88.27b	65.20a	13.12a
	56	63.20	1.52	6.19a	1522.17a	96.75a	63.61a	12.30a

†Days after treatment

††Different letters within column and char rate indicate significant difference at α=0.05

Plant growth

Marietta Soil

Shoot biomass was determined for all crops as a general measure of yield response to biochar application. Yield responses were crop and soil specific and were dependent on biochar rate as well. There was no significant effect of N fertilizer on any of the measured plant characteristics of any crop at any biochar application rate nor any significant interaction between biochar and N fertilizer application, hence, data were pooled for all N treatments

Shoot dry weight declined significantly in all crop species grown in the Marietta soil (Table 4). However, the biochar rate where the decrease become significant varied among the species.

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Corn, wheat, and alfalfa did not have significant reductions until the highest (180 Mg biochar ha^{-1}) rate, while significant decreases began at 45 Mg biochar ha^{-1} in soybean and at 90 Mg biochar ha^{-1} in rye. Dry weights tended to decrease prior to these treatments in each crop, however the reductions were not significant.

Nutrient uptake across all crops follow the same trend as shoot dry weight production. There was no significant effect of N fertilizer on nutrient uptake or an interaction effect of biochar by fertilizer for any of the non-legume crops, hence, these data were pooled for analysis. However, the biochar rate where these decreases became significant were nutrient, biochar rate, and species dependent. Nutrient uptake by the corn seedlings steadily declined as biochar application rates increased, however, a significant decrease in K, Mg, P, and N uptake did not occur until biochar rate reached 180 Mg biochar ha^{-1} with the exception of Ca. Significantly lower Ca uptake occurred at the 90 Mg biochar ha^{-1} rate when compared to the control (Table 4). These results suggest corn may have some resistance to any antagonistic effects of the biochar but, at some point, plant nutrition will be affected. In soybean, a significant reduction in uptake for all nutrients was also found. However, the reduced uptake began at the lowest rate of biochar (45 Mg biochar ha^{-1}) and continued as rates increased (Table 4). Wheat also had reductions in nutrient uptake when grown in biochar amended soils. Calcium, Mg, and N had significant decreases only at the highest level of biochar applied (180 Mg biochar ha^{-1}) (Table 4). Potassium and P did change at any rate of biochar application. No change was found in nutrient levels in Rye with the exception of Ca (Table 4). The lowest application of biochar (45 Mg biochar ha^{-1}) resulted in a significant decrease in Ca uptake in rye to 3.09 mg pot^{-1}. However, Calcium uptake appeared to recover at 90 and 180 Mg biochar ha^{-1} where uptake rates were not different from the control. Biochar application significantly reduced the amount of nutrient uptake for alfalfa in all measured nutrients (Table 4). Alfalfa K, P, and N uptake decreased with all levels of biochar application while Ca and Mg uptake was only significantly reduced at the lowest (45 Mg biochar ha^{-1}) and highest levels (180 Mg biochar ha^{-1}) biochar treatment.

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Table 4: Mean values (n=9) for shoot dry weight and nutrient uptake of 21-day-old corn, soybean, wheat, rye and alfalfa grown in Marietta soil.

Species	Char rate	Shoot dry wt.	Ca	K	Mg	P	N
	$Mg\ ha^{-1}$	g	mg pot^{-1}				
Corn	0	1.22a†	21.16a	47.78a	2.09a	1.72a	37.46a
	45	1.17a	21.14a	45.88a	2.10a	1.48a	34.91a
	90	0.99a	16.89b	35.07a	1.68a	1.27a	29.37a
	180	0.66b	12.71b	21.31b	1.20b	0.71b	17.89b
Soybean	0	1.64a	37.79a	20.09a	4.86a	2.31a	60.54a
	45	1.25b	26.39b	13.94b	3.30b	1.22b	41.16b
	90	1.06bc	19.53c	11.64b	2.18b	0.85c	31.81b
	180	0.93c	15.01c	10.91b	1.73c	0.77c	24.49b
Wheat	0	0.52a	4.97a	27.88a	0.77a	2.46a	28.69a
	45	0.47a	4.36a	25.32a	1.71a	2.02a	25.83a
	90	0.46a	4.07a	24.88a	1.63a	2.15a	25.47a
	180	0.38b	3.80a	21.79a	0.52b	1.97a	20.52b
Rye	0	0.46a	5.23a	20.73a	0.75a	2.10a	26.49a
	45	0.46a	3.09b	14.35a	0.53a	1.26a	16.94a
	90	0.39b	3.97a	14.00a	0.62a	1.50a	18.44a
	180	0.25c	4.07a	14.66a	0.59a	1.55a	19.63a
Alfalfa	0	0.24a	6.67a	8.35a	0.59a	0.62a	11.02a
	45	0.20a	4.08b	4.95b	0.33b	0.29b	6.05b
	90	0.22a	5.78a	5.28b	0.47a	0.28b	6.92b
	180	0.14b	3.78b	4.55b	0.34b	0.36b	6.89b

†Different letters within column and species indicate significant difference at α=0.05

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These reductions in nutrient uptake across all crops follow the same general trend as shoot dry weight production. The significant reduction in uptake are believed to be linked to smaller plants at high biochar rates. To determine if the reductions in nutrient uptake due to smaller plants or an effect on root/plant uptake efficiency, we also compared tissue concentrations of each nutrient for each species.

A significant decrease in corn plant tissue K was found in the 180 Mg biochar ha^{-1} treatment (32.12 mg g^{-1}) when compared to 37.85 mg g^{-1} in the control (Table 5). It is interesting that, in the soil incubation study, extractable K increased in this soil after 56 days (Table 2). This suggests that the addition of biochar is negatively affecting the plant and its ability to absorb K. It also appears soybean's nutrient uptake was negatively affected by biochar application. Soybean had decreased Ca and N at 180 Mg biochar ha^{-1} when compared to the control while Mg and P levels in soybean decreased at all levels of biochar addition (Table 5). In wheat, Mg decreased at 90 Mg biochar ha^{-1} and 1.25 mg g^{-1} at 180 Mg biochar ha^{-1} (Table 5) while N decreased at 180 Mg biochar ha^{-1} (Table 5). Phosphorus also appeared to be affected by the biochar, however, the effects are not clear. After a reduction at 45 Mg biochar ha^{-1}, tissue P appeared to recover at higher biochar rates. We might speculate that this is a plant response to stress conditions. Tissue nutrient concentration responses in rye were also highly variable. Similar to P in wheat, Ca in rye significantly decreased from the control to 45 Mg biochar ha^{-1} and then appeared to recover at higher biochar rates (Table 5). Magnesium increased from the control to 90 Mg biochar ha^{-1} and continued to increase at 180 Mg biochar ha^{-1}; and N significantly decreased the control to 180 Mg biochar ha^{-1} (Table 5). Calcium in alfalfa significantly decreased from the control to 180 Mg biochar ha^{-1}, and N significantly decreased from the control to the 45 Mg biochar ha^{-1} (Table 4.1).

The changes in nutrient concentration in the plant tissues in this soil and the variability of those changes suggest that the addition of the biochar is affecting each species. There appears to be a decrease in the efficiency of nutrient uptake resulting in lower tissue concentrations in all of the species. There also appears to be a plant response in the treatments where nutrient concentrations returned to levels equal to the control. These responses need further evaluation which is beyond the scope of this study prior to any conclusions.

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Table 5: Mean values (n=9) for shoot dry weight and nutrient concentration of 21-day-old corn, soybean, wheat, rye and alfalfa grown in Marietta soil.

Species	Char rate	Shoot dry wt.	Ca	K	Mg	P	N
	Mg ha^{-1}	g	mg g^{-1}				
Corn	0	1.22a†	18.42a	37.85a	1.70a	1.29a	31.56a
	45	1.17a	18.55a	38.12a	1.81a	1.27a	30.24a
	90	0.99a	17.68a	35.37ab	1.73a	1.26a	29.44a
	180	0.66b	19.56a	32.12b	1.86a	1.09a	27.52a
Soybean	0	1.64a	22.87a	12.05a	2.91a	1.32a	36.25a
	45	1.25b	20.99a	11.36a	2.62a	0.97b	32.79ab
	90	1.06bc	18.32b	11.07a	2.03b	0.82b	29.58bc
	180	0.93c	15.85c	10.98a	1.79b	0.80b	26.32c
Wheat	0	0.52a	9.36a	52.35a	1.46a	4.62ab	53.98a
	45	0.47a	9.08a	51.42a	1.45a	4.14b	52.88a
	90	0.46a	8.17a	50.06a	1.28b	4.36ab	51.60ab
	180	0.38b	9.33a	52.81a	1.25b	4.79a	49.40b
Rye	0	0.46a	12.49a	39.89a	1.55b	3.93a	54.21a
	45	0.46a	9.72b	44.49a	1.61ab	3.98a	53.62a
	90	0.39b	11.37ab	40.69a	1.77a	4.42a	53.76a
	180	0.25c	11.76a	43.27a	1.69ab	4.56a	51.47b
Alfalfa	0	0.24a	27.78ab	34.57a	2.44a	2.54a	45.49a
	45	0.20a	25.95ab	31.13a	2.15a	1.77a	38.35b
	90	0.22a	30.98a	30.43a	2.56a	1.76a	42.84ab
	180	0.14b	24.80b	29.13a	2.26a	2.51a	44.49a

†Different letters within column and species indicate significant difference at α=0.05

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Houston Soil

Similar to the results in the Marietta soil, shoot dry weight decreased significantly with increasing biochar rate with the exceptions of rye and alfalfa in the Houston soil (Table 6). Corn shoot dry weight was reduced in the 90 and 180 Mg biochar ha^{-1} rates while soybean dry weight reduction did not become significant until the 180 Mg biochar ha^{-1} rate (Table 6). Wheat shoot dry weight was less than the control at all rates of biochar. No significant differences in shoot dry weight were found for either rye or alfalfa at 45 or 180 Mg biochar ha^{-1}, however shoot dry weight in rye was less than the control in the 90 Mg biochar ha^{-1} treatment.

Significant reductions in all nutrient uptake in corn and wheat were found for plants grown in pots amended with biochar rates of 90 and 180 Mg biochar ha^{-1} for all measured nutrients (Table 6). Total nitrogen uptake by the corn seedlings was significantly reduced for all biochar rates. As with the plants grown in the Marietta soil, nutrient concentrations were also compared to determine if plant uptake efficiency was affected by biochar application. In corn and wheat, tissue P decreased with biochar application at the 180 Mg biochar ha^{-1} rate while K increased in all biochar application rates when compared to the control (Table 7). This K response may once again suggest a plant stress reaction as was speculated in the Marietta soil. Nitrogen concentrations in wheat may have also shown a slight response. In the 45 Mg biochar ha^{-1} treatment, N in plants tissues increased above that of the control (Table 7). Results similar to those seen in corn and wheat are shown in Table 6 for soybeans grown in biochar amendments. Significant reductions in all nutrient uptake were found. Significant reductions in Ca and N were found at the lowest (45 Mg biochar ha^{-1}) while decreases in P, K, and Mg were not found until the highest rate of biochar application (180 Mg biochar ha^{-1}) (Table 6). Nutrient concentrations were reduced only at the highest rate of biochar for K, Mg P, and N (Table 7).

Opposite from the results seen in rye for Marietta soil, rye grown in the Houston soil amended with the least amount of biochar (45 Mg biochar ha^{-1}) resulted in an increase in uptake for all measured nutrients with the exception of Ca (Table 6). Only Ca and N concentrations differed with biochar application in this species. Ca levels in the 90 Mg biochar ha^{-1} rate and N levels at the 45 and 90 Mg biochar ha^{-1} rate (Table 7). Alfalfa grown in biochar amended Houston soil results show a significant decrease in uptake of all measured nutrients at only the highest rates of application (90 and 180 Mg biochar ha^{-1}) with the exception of P where reduced levels were found at all biochar addition rates (Table 6). Reduced nutrient concentrations in this species were only apparent for Ca, and P (Table 7).

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Table 6: Mean values (n=9) for shoot dry weight and nutrient uptake of 21-day-old corn, soybean, wheat, rye and alfalfa grown in Houston soil.

Species	Char rate	Shoot dry wt.	Ca	K	Mg	P	N
	Mg ha^{-1}	g	mg pot^{-1}				
Corn	0	6.22a†	75.37a	238.87a	16.45a	13.66a	224.69a
	45	5.21a	59.57a	216.39a	13.67a	12.54a	181.99b
	90	2.90b	36.93b	123.70b	6.95b	4.81b	109.47b
	180	2.40b	31.24b	107.04b	5.58b	4.43b	86.77c
Soybean	0	3.85a	69.97a	69.09a	14.03a	9.16a	186.02a
	45	3.40a	56.44b	65.44a	11.83a	7.46a	142.42b
	90	2.97a	50.26b	52.13a	10.35a	6.14a	133.35b
	180	1.58b	28.41c	22.71b	4.89b	1.79b	67.76c
Wheat	0	0.89a	5.44a	32.45a	1.32a	3.37a	38.90a
	45	0.62b	4.58ab	27.38a	1.11a	2.48b	29.45b
	90	0.58bc	4.02b	26.31a	1.02b	2.38b	29.26b
	180	0.44c	3.87b	25.90a	0.93b	2.12b	31.59b
Rye	0	9.51a	5.02a	24.67b	0.94b	2.20b	27.68c
	45	8.21ab	7.38a	37.69a	1.51a	3.58a	49.37a
	90	7.73b	5.09a	27.92b	1.17b	2.70b	38.55b
	180	8.26ab	4.66a	22.94b	1.01b	2.13b	31.34c
Alfalfa	0	0.32a	9.11a	18.32a	1.06a	1.65a	26.00a
	45	0.33a	7.09a	14.29a	0.84a	1.23b	21.17a
	90	0.37a	4.94b	11.59b	0.62b	0.97b	16.89b
	180	0.31a	3.20b	6.76c	0.41b	0.57b	10.62b

†Different letters within column and species indicate significant difference at α=0.05

Table 7: Mean values (n=9) for shoot dry weight and nutrient concentration of 21-day-old corn, soybean, wheat, rye and alfalfa grown in Houston soil.

Species	Char rate Mg ha^{-1}	Shoot dry wt. g	Ca	K	Mg	P	N
					mg g^{-1}		
Corn	0	6.22a†	12.23a	38.69b	2.62a	2.21ab	36.63a
	45	5.21a	13.01a	41.25ab	2.59a	2.37a	34.55a
	90	2.90b	12.69a	41.98ab	2.40a	1.84bc	38.24a
	180	2.40b	13.05a	44.48a	2.33a	1.66c	36.52a
Soybean	0	3.85a	18.93a	17.22ab	3.65a	2.26a	49.17a
	45	3.40a	18.01a	17.76a	3.44a	2.01a	abo
	90	2.97a	17.70a	17.21ab	3.50a	1.96a	46.44ab
	180	1.58b	17.38a	14.45b	3.06b	1.11b	42.58b
Wheat	0	0.89a	7.39b	44.01b	1.76b	4.56a	52.47b
	45	0.62b	8.77a	51.12a	2.10a	4.65a	56.07a
	90	0.58bc	7.57ab	49.73ab	1.92ab	4.50a	55.08ab
	180	0.44c	6.67b	44.53ab	1.62b	3.66b	55.13ab
Rye	0	9.51a	9.51a	46.79a	1.95a	4.21a	55.77b
	45	8.21ab	8.21ab	42.71a	1.84a	4.11a	57.08ab
	90	7.73b	7.73b	43.05a	1.80a	4.11a	58.32a
	180	8.26ab	8.26ab	40.20a	1.73a	3.77a	55.02b
Alfalfa	0	0.32a	18.47a	36.25a	2.11a	3.27a	50.80a
	45	0.33a	17.66a	35.84a	2.10a	2.98ab	53.10a
	90	0.37a	15.23b	35.90a	1.93a	3.01a	52.41a
	180	0.31a	15.64b	32.31a	1.98a	2.64b	51.72a

†Different letters within column and species indicate significant difference at α=0.05

The changes in shoot dry weights, nutrient uptake, and nutrient concentration found in this soil appear to support the results found in the Marietta soil. Plant growth and uptake efficiency affected by biochar rate on a species by species basis for both soils. This suggests that biochar application may have an adverse effect on plant growth and care should be used in its application to soils. At no char rate in either soil did our N, P, or K nutrient use efficiency reach 7.2 % as reported by Roberts et al. (2010).

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Forage Quality

Forage quality is defined in terms of animal performance, such as daily gain, milk production, wool production or reproduction . Forage quality of wheat, rye, and alfalfa was analyzed as these are commonly used as forage crops. Common measures of forage quality are crude protein (CP), acid detergent fiber (NDF), neutral detergent fiber (NDF), and lignin.

Marietta Soil

Biochar application did not have a significant change in lignin content for wheat, while CP measurements were significantly reduced at all levels of application beginning with 45 Mg biochar ha^{-1} and continuing through 180 Mg biochar ha^{-1} (Table 8). Results also indicated a significant increase in ADF and NDF levels for wheat grown in biochar amended soil with the highest level of application (180 Mg biochar ha^{-1}).

Rye measurements of ADF and NDF levels both show a significant increase only at the highest level of biochar application (180 Mg biochar ha^{-1}) in Marietta soil (Table 8). Results show no significant change in CP or lignin content at any level of biochar application.

Alfalfa shows a significant decrease in CP at the rate of 45 Mg biochar ha^{-1} only, with no significant changes noted in ADF, NDF, or lignin content (Table 8).

Table 8: Mean values (n=9) for forage quality of 21-day-old wheat, rye and alfalfa grown in Marietta soil.

Species	Char rate	CP	ADF	NDF	Lignin
	Mg ha^{-1}	%			
Wheat	0	31.2a	20.1b	40.2b	5.5a
	45	29.8b	21.2b	41.6b	5.3a
	90	29.9b	20.8b	41.2b	5.5a
	180	28.3b	21.5a	42.1a	5.8a
Rye	0	32.9a	15.6b	34.4b	4.7a
	45	31.9a	16.6b	35.9b	5.3a
	90	33.1a	15.9b	35.6b	5.1a
	180	31.5a	17.4a	37.5a	5.0a

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Alfalfa	0	33.4a	19.1a	26.0a	4.3a
	45	30.5b	22.1a	28.4a	5.1a
	90	32.9a	19.5a	25.3a	4.7a
	180	32.6a	19.7a	25.7a	4.6a

†Different letters within column and species indicate significant difference at α=0.05

Houston Soil

In wheat, CP shows a significant increase only at the lowest rate of biochar application (45 Mg biochar ha^{-1}), while ADF levels show a significant increase only at the highest rate of application (180 Mg biochar ha^{-1}). Biochar both significantly reduced and increased NDF levels depending on the rate of application. Results show a decrease in NDF at 45 Mg biochar ha^{-1} of biochar and an increase in NDF at 180 Mg biochar ha^{-1} of biochar (Table 9).

Table 9: Mean values (n=9) for forage quality of 21-day-old corn, soybean, wheat, rye and alfalfa grown in Houston soil.

Species	Char rate	CP	ADF	NDF	Lignin
	Mg ha^{-1}	%			
Wheat	0	30.6b	19.9b	39.0a	6.4a
	45	32.3a	18.9b	38.1b	6.3a
	90	30.7b	20.9b	40.0a	5.2a
	180	30.4b	21.1a	40.8a	6.3a
Rye	0	33.9a	15.4a	34.6a	5.2a
	45	33.1a	15.0a	34.2a	5.3a
	90	32.9a	13.3b	32.4b	5.7a
	180	32.3a	14.6a	33.8a	5.6a
Alfalfa	0	34.1a	19.7a	28.1a	3.2a

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45	35.0a	18.9a	28.0a	2.3a
90	35.5a	18.0a	26.5a	2.5a
180	34.8a	19.0a	27.9a	2.5a

†Different letters within column and species indicate significant difference at α=0.05

Rye results show no significant changes in CP or lignin content levels for Houston soil, but a significant decrease in both ADF and NDF levels was seen at the 90 Mg biochar ha^{-1} application of biochar (Table 9).

Alfalfa grown in Houston soil amended with biochar did not have a significant effect on any of the measured elements of our study (Table 9). Levels of CP, ADF, NDF, and lignin content all remained consistent throughout all levels of biochar application rates.

CONCLUSION

In summary, the physical and chemical characteristics of the pine biochar were not appreciably different from characteristics found in other studies with the exception of pH. While other studies have found biochar to have a basic pH leading to a liming effect when added to soil, our study found a slightly acid pH. In addition, the pine biochar was also found to have appreciable levels of extractable Ca, Mg, K, and P. Which could lead to an increase in soil fertility upon addition to the soil. After 56 days, total C and N did not change within any biochar rate while extractable Ca, K, Mg, and P increased within biochar rate for all rates in the Marietta soil but not the Houston which has twice the amount of clay when compared to the Marietta. This suggests that the application of pine biochar may serve to release bound nutrients into a more available form in some soils over time. The addition of pine biochar also appeared to reduce shoot dry weight in both soils. In the Marietta soil, these pine biochar additions also reduced nutrient uptake and concentration in the plants, however, extent of these reductions varied with plant species. Generally, nutrient uptake and concentrations for those plants grown in the Houston soil were reduced with biochar addition, however the extent of effects were much more variable than those in the Marietta. Changes in forage quality were specific to soil, crop and parameter. Acid detergent fiber and NDF levels increased in wheat and rye with biochar addition in the Marietta soil, however these increases were rate specific. Forage quality of plants grown in the Houston soil were highly variable and no clear trends were apparent.

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