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The effect of biochar on the growth of agricultural weed species

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THE EFFECT OF BIOCHAR ON THE GROWTH OF AGRICULTURAL WEED SPECIES

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Dedicated to my loving

partner, Steve Scott, and daughter, Dahlia Scott,

for their support and motivation.

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ABSTRACT

Mitchell, Karen A. M.S., Purdue University, May 2015. The Effect of Biochar on the Growth of Agricultural Weed Species. Major Professor: Kevin Gibson.

Biochar, a carbon-rich residue similar to charcoal, has been proposed as a soil amendment to improve soil quality and increase crop yields while simultaneously mitigating climate change by the sequestration of carbon. The beneficial effect of biochar on crops may extend to weed species and, although it is well known that weeds reduce crop yields, there is little published research on the effect of biochar on agricultural weed species. In a series of greenhouse and growth chamber experiments, three questions were addressed. First, how does nitrogen interact with biochar produced from a single feedstock to affect weeds? Second, how do differences in biochar feedstock affect root growth and root system architecture? Finally, how do differences in biochar feedstocks affect weed and crop growth? In the first experiment, three common weed species, barnyardgrass (*Echinochloa crus-galli* L. Beauv.), large crabgrass (*Digitaria sanguinalis* L. Scop.), and redroot pigweed (*Amaranthus retroflexus* L.), were grown to maturity under greenhouse conditions using a factorial design with biochar (0 and 2% of the soil dry weight) and nitrogen (0 and 14 g N m^{-2}) treatments. Nitrogen increased barnyardgrass and redroot pigweed total dry weight and large crabgrass panicle dry weight. Biochar increased barnyardgrass height by 22% and total dry weight by 47% but did not affect root : shoot biomass partitioning. Biochar reduced redroot pigweed

height by 30% but increased branch dry weight by 95%. Finally, biochar increased large crabgrass shoot dry weight by 34% but reduced root dry weight 30% suggesting that biochar allowed large crabgrass to partition more biomass to shoots than roots. In the second experiment, we examined the effects of two types of biochar on large crabgrass root system architecture using a rhizobox mesocosm. Root growth of large crabgrass varied with the type of biochar used; however, biochar did not affect total plant dry weight. The high-nutrient biochar increased above-ground dry weight and the low-nutrient biochar increased below-ground dry weight when compared to plants grown in the unamended soil. When given a choice between unamended and biochar-amended soil, large crabgrass roots grew preferentially in the biochar-amended soil, regardless of biochar type. In the final experiment, we examined the effect of two types of biochar on the growth of two crop and two weed species grown to maturity under greenhouse conditions. Biochar increased the growth of both crop species suggesting that the incorporation of biochar, especially high-nutrient biochar, into temperate agricultural soils may increase crop yields. However, biochar also increased the growth of both weed species, which may complicate current weed management practices. Overall, this research suggests that biochar has the potential to alter root system architecture and to increase the growth of common weed species. Biochar may therefore exacerbate weed problems in agricultural systems.

CHAPTER 1. PREFACE

Biochar is a carbon-rich product similar to charcoal that can be incorporated into the soil to improve soil properties while simultaneously sequestering carbon (Jeffery et al. 2011; Kookana et al. 2011). Black carbon, which is in the continuum of pyrogenic carbon with biochar, was discovered in the highly fertile *terra preta* soils of the Amazonian basin. It is believed that, prior to colonization, the Amazonian indigenous groups incorporated burned household and agricultural waste into the soil creating pockets of extremely fertile soils compared to the highly acidic, low fertility Oxisols and Ultisols that are common to the area (Neves et al. 2003). These fertile soils have piqued the interest of scientists for the past hundred years but it is only in the last twenty years that black carbon and biochar have gained global interest (Lehmann and Joseph 2009). Biochar has recently been referred as a 'win-win' solution for increasing crop productivity while mitigating climate change by sequestering carbon (Biederman and Harpole 2013). Two meta-analyses reported a mean increase of 10% to 11% in crop productivity after the incorporation of biochar into the soil (Liu et al. 2013; Jeffery et al. 2011). The increase in crop productivity is attributed to increased cation exchange capacity (Cornelissen et al. 2013), enhanced soil microbial diversity (Quilliam et al. 2012), and improved water holding capacity of the soil (Novak et al. 2012). However,

the physical and chemical properties of biochar are extremely variable and therefore, the effect of biochar on soil properties and crop productivity can be just as variable. Biochar is produced by pyrolysis, which is the anaerobic combustion of organic material (i.e. feedstock) at temperatures between 300 and 1000 C (Verheijen et al. 2010). The properties of biochar depend on the temperatures and type of feedstock used during production (Kloss et al. 2012; Schimmelpfenning and Glaser 2012).

Most biochar research has focused on interactions between crop productivity and soil properties. Although weeds are known to reduce yields, serve as a reservoir for pathogens, and interfere with cropping activities in agricultural systems, there is a limited amount of published research on the effect of biochar on the growth of weed species (Major et al. 2005; Quilliam et al. 2012). With studies reporting that biochar increases crop productivity, one might also expect that biochar would increase weed growth and competition with crops (Biederman and Harpole 2013; Liu et al. 2013; Jeffery et al. 2011). Major et al. (2005) conducted a field experiment in low fertility, highly acidic soils in the central Brazilian Amazon in which a variety of soil amendments were incorporated into the soil including biochar and fertilizer. Biochar alone did not increase weed cover but biochar plus an inorganic fertilizer increased weed cover more than the inorganic fertilizer alone (Major et al. 2005). Thus biochar has the potential to increase crop yields but may also significantly increase weed pressure when combined with fertilizer. With over 20 million tons of fertilizer applied annually in the United States alone (USDA 2012), the addition of biochar to agricultural soils has the potential to dramatically increase weed growth and competition with crops. In contrast, a study conducted on temperate agricultural soils suggests that biochar may have a short term

inhibitory effect on weed emergence (Quilliam et al. 2012). Quilliam et al. (2012) reported no long-term effect of biochar on weeds three years after biochar incorporation but weed emergence was reduced when biochar was reapplied. The authors were unable to explain the reduction in weed emergence but suggested that increased soil microbial activity might play a role (Quilliam et al. 2012). The varying results between the Major et al. (2005) and Quilliam et al. (2012) could be due to a number of differences between the studies including feedstocks, soil type, and climate and highlight the need for additional research on the interaction between biochar and weed species.

Although the root system is responsible for transferring benefits that biochar may provide to the rest of the plant, there is little research on the effect of biochar on root growth and root system architecture (RSA). Root morphology and architecture can vary greatly among species and has been shown to be affected by soil amendments including fertilizers (Fitter 1985). RSA can respond to soil conditions in several ways, including the growth of lateral roots, inhibition of primary root growth, formation of adventitious roots, or an increase in root hairs (Osmont et al. 2007). Root weight and length are the most commonly measured characteristics and the ratio of length to weight, or specific root length (SRL), can be used as an indicator of gross morphology. Fitter (1985) found that SRL is typically lower with the addition of fertilizer due to the ability of a plant to adjust its growth in response to nutrient and carbon supply. Plants respond to an imbalance in abiotic resources by allocating new biomass to organs involved in obtaining the needed resources. Plants can respond to a low nutrient supply by allocating more resources to lateral roots and root hairs (Hermans et al. 2006). Fitter and Stickland (1991) reported that the root systems of grasses exhibit a herringbone-like structure in

low-nutrient conditions for more efficient exploitation of the soil. This herringbone-like structure, characterized by a reduction in primary root growth and an increase in lateral root growth, is particularly associated with limited availability of phosphorus (Ingram and Malamy 2010). Nitrogen (N) availability has little effect on primary root growth, but an increase in lateral root growth is seen in N-limited soils (Ingram and Malamy 2010). However, if the entire root system is in N-limited conditions and a portion of the root system is exposed to high levels of N, the roots will proliferate only where there are high levels of N (Hodge 2004). The effect of biochar on soil fertility varies with the feedstock and production temperature but biochar has been shown to increase phosphorus and potassium availability, pH, CEC, and water holding capacity (Lehmann et al. 2003; Jeffery et al. 2011; Novak et al. 2012). The effect of biochar on N-availability is not well understood and evidence has been found suggesting that biochar can increase, decrease, or have no effect on N-availability (Lehmann et al. 2003; Atkinson et al. 2010; DeLuca et al. 2009). Currently, there are only two studies in which the effect of biochar on RSA was examined (Prendergast Miller et al. 2011, 2014). Prendergast-Miller et al. used rhizobox mesocosms to determine the effects of biochar on the root systems of wheat (*Triticum aestivum*) (2011) and spring barley (*Hordeum vulgare* L.) (2014) seedlings. Biochar had no significant effects on the total biomass or root architecture of wheat seedlings (Prendergast-Miller et al. 2011). However, the addition of biochar, produced from *Miscanthus x giganteus* straw, resulted in greater shoot and root biomass but reduced SRL of spring barley seedlings (Prendergast-Miller et al. 2014).

This research addressed three primary questions. First, how does nitrogen fertilizer interact with biochar produced from a single feedstock to affect weed growth?

Second, how do differences in biochar feedstock affect root growth and root system architecture? Finally, how do differences in biochar feedstock affect weed and crop growth?

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CHAPTER 2. THE EFFECT OF BIOCHAR ON THREE COMMON AGRICULTURAL WEED SPECIES

2.1. Abstract

Biochar, a carbon-rich residue similar to charcoal, has been proposed as a soil amendment to improve soil quality and increase crop yields. The beneficial effect of biochar on crops may extend to weeds, which could increase weed pressure on crops. The objective of this experiment was to determine the effects of biochar and biochar plus a nitrogen fertilizer on three common agricultural weed species. Barnyardgrass, large crabgrass, and redroot pigweed were grown to maturity under greenhouse conditions using a factorial design with biochar (0 and 2% of the soil dry weight) and nitrogen (0 and 14 g N m^{-2}) treatments. Nitrogen increased barnyardgrass and redroot pigweed total dry weight by 48 and 23% respectively and large crabgrass panicle dry weight by 23%. Biochar increased barnyardgrass height by 22% and total dry weight by 47% but did not affect root : shoot biomass partitioning. Biochar increased large crabgrass shoot dry weight by 34% but reduced root dry weight by 30%. Finally, biochar reduced redroot pigweed height by 30% but increased branch dry weight by 95%. The diversity of weed species responses suggests that the addition of biochar to agricultural soils may complicate current weed management practices and emphasizes the need for further research on the interactions between biochar and weed species.

Nomenclature: Barnyardgrass, *Echinochloa crus-galli* L. Beauv.; large crabgrass, *Digitaria sanguinalis* L. Scop.; redroot pigweed, *Amaranthus retroflexus* L.

Keywords: Char, black carbon, biomass partitioning ratios, weeds.

2.2. Introduction

Biochar is a carbon-rich product formed through the pyrolysis of organic matter that can be incorporated into the soil to improve soil properties and sequester carbon (Jeffery et al. 2011). Although scientists have only recently examined the effects of biochar on soils and plants, the intentional use of black carbon or char as a soil amendment is not just a recent trend. In 1840, Justus von Liebig published the experimental observations of Edward Lucas in which he describes the benefits of incorporating charcoal powder made from fir and pine trees into soil. Lucas reported that *Thunbergia alata* and *Peireskiae aculeate* plants grown with charcoal powder developed faster and grew larger than plants grown without charcoal (von Liebig 1840). More recent studies have reported similar effects of biochar on crops. A meta-analysis examining the effect of biochar incorporation in the soil found a mean increase of 10% in crop productivity, i.e. yield or above-ground biomass (Jeffery et al. 2011). This increase in crop productivity has been attributed to greater nutrient retention (Cornelissen et al. 2013; Grossman et al. 2010), enhanced soil microbial diversity and activity (Solaiman et al. 2010; Quilliam et al. 2012; Lehmann et al. 2011), and improved water holding capacity (Novak et al. 2012). However, the chemical and physical properties of biochar

are variable and depend on the feedstock, i.e. the type of biomass used, and pyrolysis temperatures during production (Kloss et al. 2012; Schimmelpfennig and Glaser 2012).

Feedstock and production temperature are the main determining factors of the chemical and physical characteristics of biochar (Singh et al. 2010). For example, biochar produced at higher temperatures tends to have a higher specific surface area (SSA) than biochar produced at lower temperatures due to a reduction in organic compounds. This increase in porosity translates to an increase in water holding capacity (Kloss et al. 2012). In contrast, nutrient retention or cation exchange capacity (CEC) has been found to decrease with increasing production temperature (Gaskin et al. 2008); this has been attributed to the loss of negatively charged functional groups at higher temperatures. It is also important to note that these characteristics can change after application to the soil (Hale et al. 2011). Biochar consists of high molecular weight aromatic rings (Schmidt and Noack 2000) that allow it to persist in the soil for decades and possibly centuries (Lehmann 2007; McHenry 2009). For example, as biochar weathers in soil, the surface of the biochar becomes oxidized and CEC can increase (Cheng et al. 2008; Hammes and Schmidt 2009).

Although weeds are known to reduce crop yields, there is little published research on the effect of biochar on weeds (Major et al. 2005; Quilliam et al. 2012). Compared to a variety of soil amendments, biochar did not increase weed cover but biochar plus an inorganic fertilizer increased weed cover more than the inorganic fertilizer alone in low fertility, highly acidic soils of the central Brazilian Amazon (Major et al. 2005). In contrast, a study conducted on temperate agricultural soils suggests that biochar may have a short-term inhibitory effect on weed emergence (Quilliam et al. 2012). Biochar

produced from hardwoods at 450 C was incorporated into a sandy clay loam soil at a rate of 25 or 50 t ha^{-1} but produced no long-term effect on weeds three years after incorporation (Quilliam et al. 2012). The incorporation of biochar did not result in significant differences in the above-ground biomass or foliar nutrient content of the crop. However, weed emergence was reduced when biochar was reapplied. The authors were unable to explain the reduction in weed emergence but suggested that increased soil microbial activity might play a role (Quilliam et al. 2012). The conflicting results of Major et al. (2005) and Quilliam et al. (2012) could be due to a number of differences between the studies including biochar feedstock, soil type, and climate and highlight the need for smaller scale, controlled studies to better understand the effect of biochar on weed species.

Therefore, the objective of this work was to determine the effects of biochar and biochar plus a nitrogen fertilizer on three common agricultural weed species grown under greenhouse conditions. Redroot pigweed, large crabgrass, and barnyardgrass are summer annual weed species with the C4 photosynthetic pathway. All three are considered problematic in agriculture throughout the world due to their competitive nature and ability to act as an alternate host for crop diseases.

2.3. Materials and Methods

A randomized complete block design with three treatments (weed species, +/- biochar, and +/-nitrogen) in four blocks was used. Three weed species, redroot pigweed, large crabgrass, and barnyardgrass (Azlin Seed Service, 112 Lilac Drive, Leland, MS,

38756), were grown in 2.5 L soil-filled pots with four replicates of each treatment in two greenhouse trials in 2012.

2.3.1. Soil and Biochar Properties

Soil was obtained from a previous experiment (Adams et al. 2013). Biochar produced under slow-pyrolysis (450 C) from loblolly pine (*Pinus taeda* L.) and switchgrass (*Panicum virgatum* L. var. *virgatum*) by a commercial vendor (Eprida, Inc., 3020 Canton Road Suite 105, Marietta, GA 30066) was mixed with a field soil, Mahalasville series (sandy loam, mixed, superactive, mesic Typic Argiaquolls), at rates equivalent to 0 and 2% of the soil dry weight. Big bluestem (*Andropogon gerardii* Vitman) and sericea lespedeza (*Lespedeza cuneata* G. Don.) were grown together in the biochar-amended and unamended soils under greenhouse conditions (Adams et al. 2013). Plants were harvested after approximately six months and the soils were sieved to remove roots. Soil (+/- biochar) was stored in sealed containers separately at approximately 20 C until the start of the current experiment.

Total carbon (C) and nitrogen (N) were determined in quintuplicate by element analyzer (Table 1) (Thermo Scientific FlashEA 1112 series). Five 500 g samples of the amended and unamended soils and of the pure biochar were sent to a commercial laboratory for analysis of organic matter (OM), soil pH, CEC, and extractable Bray phosphorus (P), potassium (K), magnesium (Mg), and calcium (Ca) (Table 1.1.) (A&L Great Lakes Laboratories, 3505 Conestoga Drive, Fort Wayne, Indiana 46808). Loss-on-ignition of the dry mass at 360 C was used to measure percent OM content (Nelson and Sommers 1982). Plant available nutrients (K, Mg and Ca) were extracted using the

Mehlich III method and analyzed by inductively coupled plasma-atomic emission spectroscopy (Mehlich 1984). The CEC was measured using a modified ammonium-acetate compulsory displacement and pH was determined by a 1:1 ratio of soil : water (Sumner and Miller 1996).

2.3.2. Growth Conditions

A greenhouse trial was initiated on 20 June 2012 and repeated on 18 July 2012. Pots were thinned to a single plant within two weeks after seeding. Every two weeks, pots within each block were re-randomized to limit micro-climate effects. The N treatment was applied in the form of urea in three split applications, each equivalent to 45 kg N ha^{-1} at 0, 4, and 8 weeks after seeding the weeds. The N rate is recommended for Midwest fresh market tomato growers (Egel et al. 2012). Minimum and maximum air temperatures and humidity were recorded daily. Average minimum and maximum temperatures were 25.4 C SE±0.4 and 37.7 C SE±0.5 in the first trial and 22.7 C SE±0.3 and 33.8 C SE±0.4 in the second. Average minimum and maximum humidity were 36.5% SE±1.2 and 58.6% SE±1.7 in the first trial and 40.7% SE±1.5 and 60.2% SE±1.8 in the second. Plants were watered with tap water regularly to maintain soil water content near field capacity.

2.3.3. Harvest and Data Collection

Plants were grown to seed production and the onset of senescence. Grass inflorescences were bagged upon emergence to ensure that seeds were not lost to

shattering. Plant height was recorded along with the number of branches for redroot pigweed and the number of tillers for the grass species. Large crabgrass has a prostrate growth habit and the length of the longest tiller was measured rather than height. Barnyardgrass plants were harvested at 86 (±3) days after seeding (DAS) in the first trial and 75 (±2) DAS in the second trial. Large crabgrass plants were harvested at 98 (±2) and 84 (±2) DAS in the first and second trial respectively. Redroot pigweed plants were harvested at 92 (±3) and 81 (±3) DAS in the first and second trial respectively. Stems, roots, and reproductive structures were placed into separate paper bags and dried at 60 C to a constant weight. Branches and leaves of redroot pigweed were bagged and weighed separately from the stems. Biomass partitioning ratios were calculated. Shoot weight ratio (SWR) is above-ground dry weight (DW) divided by total plant DW. Root weight ratio (RWR) is below-ground DW divided by total plant DW. Root : shoot ratio (RSR) is below-ground DW divided by above-ground DW.

2.3.4. Statistical Analysis

Mixed model analyses of variance (ANOVA) were performed to evaluate the effects of biochar and N on plant variables. Block and trial were treated as random factors while species, biochar rate, and N application were considered fixed factors. Mean comparisons for all analyses were conducted using the Tukey-Kramer Honestly Significant Difference (HSD) adjusted to maintain a family-wise alpha level of 0.05. Data were tested for normality and heterogeneity of variance and square root or arcsine of the square root transformed as needed to comply with the assumptions of ANOVA. Data

were back-transformed for presentation. All statistical analyses were conducted using SAS 9.2 software package (SAS Institute Inc., Cary, NC, USA).

2.4. Results

2.4.1. Barnyardgrass

Biochar increased barnyardgrass height, shoot DW, seed DW, and total DW (Table 1.2.). Root DW and the number of barnyardgrass tillers were not affected by biochar. Nitrogen increased the total DW of barnyardgrass but did not affect height or the number of tillers (Table 1.2.). Biomass partitioning (SWR, RWR, RSR) was not affected by biochar or by N (Figure 1.1.). Biomass was primarily partitioned to shoots and seeds; RWR was less than 0.35 for both biochar and N treatments. Interaction between N and biochar was not detected for any variable of any of the species. This suggests that, unlike Major et al. (2005), biochar did not interact with N to affect weed growth.

2.4.2. Large crabgrass

Interaction between trial and biochar was detected for large crabgrass height and shoot DW so data were separated by trial and reanalyzed. Trial affected the magnitude of large crabgrass responses to biochar but not the direction, i.e. p-values for stem length and shoot DW were < 0.05 in the first trial but > 0.05 in the second trial. Since trial only affected the magnitude of biochar effects on stem length and shoot DW, results from the

full model analyses are presented. Biochar did not affect total DW, the number of tillers, or panicle DW but biochar did increase stem length and shoot DW (Table 1.3.). Biochar reduced root DW (Table 1.3.). Biochar increased partitioning of biomass to shoots from roots; SWR was greater for large crabgrass grown with than without biochar (Figure 1.2.). Biochar decreased RWR (Figure 1.2.). Large crabgrass responded to N with increased panicle DW (Table 1.3.); N did not affect biomass partitioning of large crabgrass (Figure 1.2.).

2.4.3. Redroot pigweed

Nitrogen increased branch DW and total DW of redroot pigweed but did not affect other redroot pigweed variables (Table 1.4.). Biochar reduced redroot pigweed height, stem DW, and leaf DW but increased branch DW (Table 1.4.). There were no significant differences in the number of branches between treatments. Biomass was partitioned to branches at the expense of stem biomass when redroot pigweed was grown with biochar, but did not affect SWR, RWR, or RSR (Figure 1.3.). Interaction between N and biochar was not detected for any plant variable.

Table 2.1. Characteristics[a] of unamended soil (Soil), biochar, and soil amended with 2% biochar (BC soil) from samples collected at the start of the experiment. Values are means of five 500 g subsamples; parentheses enclose standard error of the mean.

	pH	C:N	OM	CEC	N	P	K	Mg	Ca
			%	meq /100 g	ppm				
Soil[b]	7.10	13.3	2.2	10.4	1,165	48	95	411	1,338
	(<0.01)	(0.8)	(0.1)	(0.1)	(10.9)	(0.7)	(1.3)	(2.4)	(12.5)
Biochar[c]	7.08	45.0	64.4	15.5	13,411	296	3,742	361	588
	(0.03)	(1.3)	(0.8)	(0.2)	(430.8)	(6.0)	(66.5)	(2.4)	(12.5)
BC Soil	6.83	15.3	3.4	10.0	1,486	65	280	374	1,175
	(0.03)	(0.3)	(0.1)	(0.1)	(38.3)	(0.4)	(1.9)	(2.4)	(14.4)

[a]Abbreviations: OM, organic matter; CEC, cation exchange capacity; meq/ 100 g, milliequivalent per 100 grams of dry soil; ppm, parts per million.

[b]Mahalasville series (sandy loam, mixed, superactive, mesic Typic Argiaquolls) consisting of approximately 60% sand, 28% silt and 12% clay.

[c]Biochar was produced at 450 C under slow pyrolysis from loblolly pine and switchgrass.

Table 2.2. Effects of biochar (BC) and nitrogen (N) on barnyardgrass. Biochar was incorporated into the soil at a rate of 2% of the soil dry weight. Nitrogen, in the form of urea, was applied at a rate of 14 g N m^{-2}. Values are means (n=15 to 16); parentheses enclose standard error of the mean. Within each treatment, values in columns with different letters indicate significant differences were detected (P<0.05).

	Height	Tiller Count	Root DW	Shoot DW	Seed DW	Total DW
	cm		g			
-BC	102.0 (4.6)**b**	11.6 (1.0)**a**	16.1 (4.2)**a**	21.3 (2.7)**b**	6.3 (0.5)**b**	43.8 (6.2)**b**
+BC	124.1 (4.4)**a**	11.8 (0.9)**a**	25.1 (6.1)**a**	30.6 (3.3)**a**	8.7 (0.6)**a**	64.4 (9.2)**a**
-N	110.1 (5.4)**a**	11.1 (1.0)**a**	14.6 (2.8)**a**	22.4 (2.8)**a**	6.6 (0.7)**a**	43.6 (5.4)**b**
+N	116.5 (5.1)**a**	12.2 (0.8)**a**	26.6 (6.6)**a**	29.6 (3.4)**a**	8.5 (0.5)**a**	64.6 (9.6)**a**

Abbreviations: DW, dry weight; -BC, no biochar; +BC, soil amended with 2% biochar; -N, no nitrogen; +N, nitrogen applied.

Table 2.3. Effects of biochar (BC) and nitrogen (N) on large crabgrass. Biochar was incorporated into the soil at a rate of 2% of the soil dry weight. Nitrogen, in the form of urea, was applied at a rate of 14 g N m^{-2}. Values are means (n=15 to 16); parentheses enclose standard error of the mean. Within each treatment, values in columns with different letters indicate significant differences were detected (P<0.05).

	Tiller Length	Tiller Count	Root DW	Shoot DW	Panicle DW	Total DW
	cm		g			
-BC	125.0 (6.0)**b**	13.2 (0.7)**a**	17.7 (2.8)**a**	29.7 (3.2)**b**	6.9 (0.4)**a**	54.3 (5.2)**a**
+BC	139.4 (7.8)**a**	12.7 (0.7)**a**	12.4 (1.4)**b**	39.9 (4.9)**a**	7.4 (0.5)**a**	59.7 (6.0)**a**
-N	132.5 (8.3)**a**	12.9 (0.8)**a**	12.1 (1.4)**a**	32.4 (4.6)**a**	6.5 (0.5)**b**	51.0 (5.5)**a**
+N	130.7 (7.0)**a**	12.7 (0.6)**a**	14.7 (2.3)**a**	35.6 (4.7)**a**	8.0 (0.4)**a**	58.4 (5.9)**a**

Abbreviations: DW, dry weight; -BC, no biochar; +BC, soil amended with 2% biochar; -N, no nitrogen; +N, nitrogen applied.

Table 2.4. Effects of biochar (BC) and nitrogen (N) on redroot pigweed. Biochar was incorporated into the soil at a rate of 2% of the soil dry weight. Nitrogen, in the form of urea, was applied at a rate of 14 g N m^{-2}. Values are means (n=14 to 15); parentheses enclose standard error of the mean. Within each treatment, values in columns with different letters indicate significant differences were detected (P<0.05).

	Height	Branch Count	Root DW	Stem DW	Leaf DW	Branch DW	Seed DW	Total DW
	cm		g					
-BC	86.4 (10.0)**a**	31.9 (3.4)**a**	3.9 (0.5)**a**	6.6 (1.0)**a**	5.5 (0.6)**a**	2.0 (0.3)**b**	11.6 (1.1)**a**	33.5 (2.1)**a**
+BC	60.1 (8.3)**b**	27.3 (2.7)**a**	3.2 (0.4)**a**	3.8 (0.7)**b**	4.1 (0.5)**b**	3.9 (0.5)**a**	11.8 (0.9)**a**	26.7 (2.6)**a**
-N	73.4 (10.4)**a**	29.7 (3.3)**a**	3.2 (0.5)**a**	4.6 (0.9)**a**	4.1 (0.5)**a**	2.5 (0.4)**b**	10.6 (0.9)**a**	25.0 (2.6)**b**
+N	72.2 (9.1)**a**	28.9 (2.7)**a**	3.8 (0.4)**a**	5.1 (0.9)**a**	5.3 (0.6)**a**	3.7 (0.5)**a**	12.8 (1.0)**a**	30.7 (2.9)**a**

Abbreviations: DW, dry weight; -BC, no biochar; +BC, soil amended with 2% biochar; -N, no nitrogen; +N, nitrogen applied.

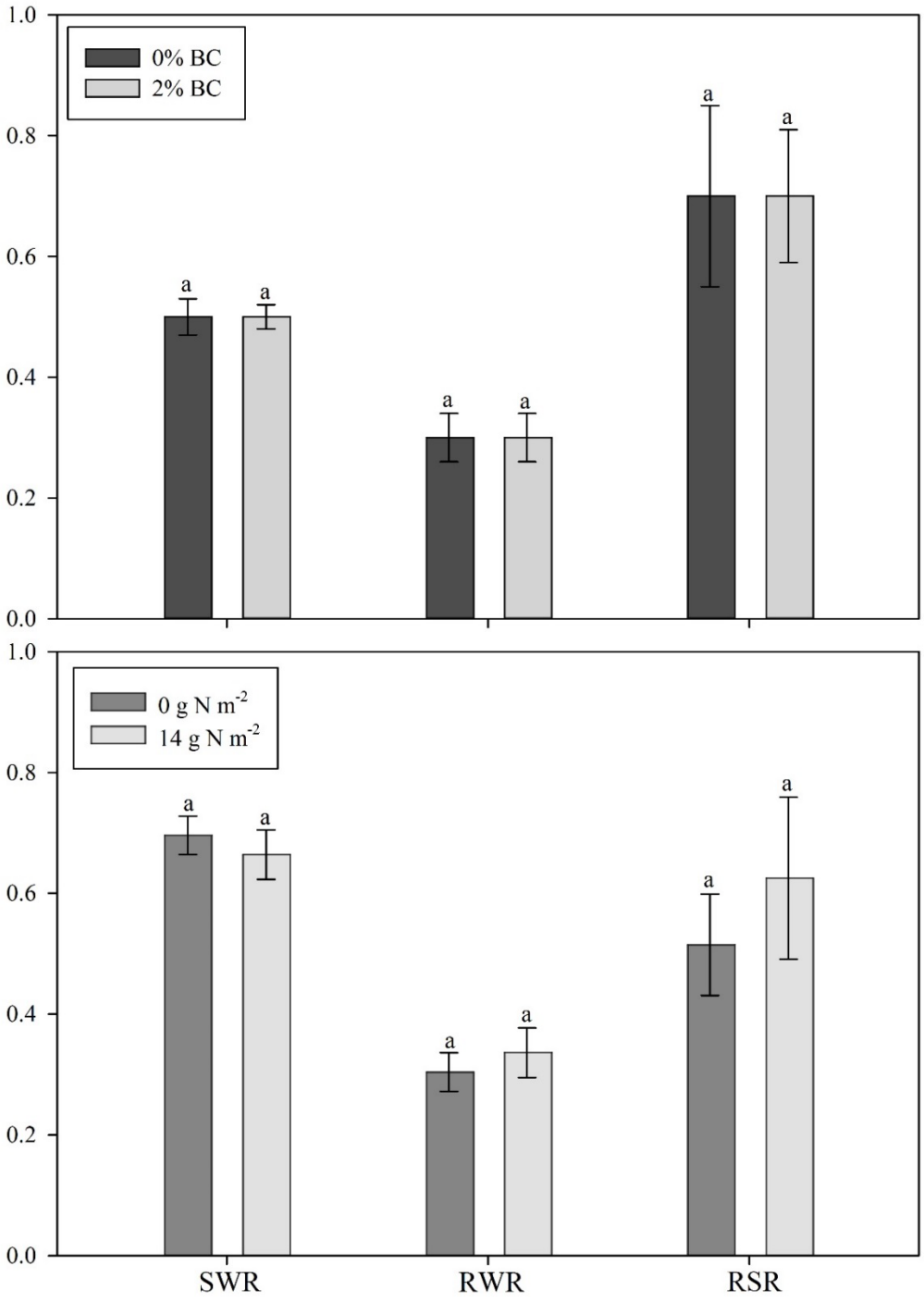

Figure 2.1. Effects of biochar (BC) and nitrogen (N) on barnyardgrass shoot weight ratio (SWR), root weight ratio (RWR), and root : shoot ratio (RSR). Columns represent means (n=15 to 16); error bars represent standard error of the mean. Within a treatment, means with the same letter were not significantly different (P<0.05).

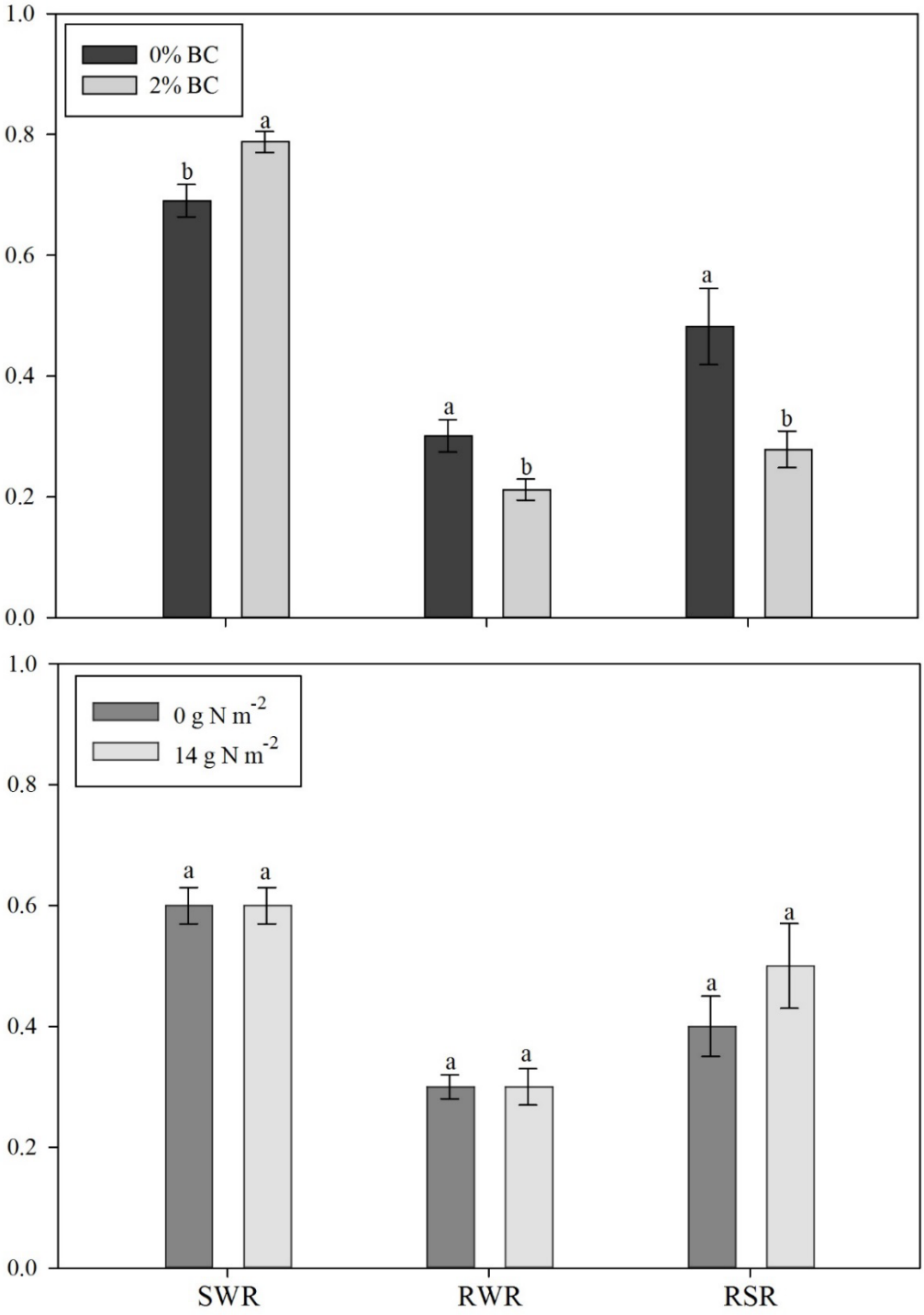

Figure 2.2. Effects of biochar (BC) and nitrogen (N) on large crabgrass shoot weight ratio (SWR), root weight ratio (RWR), and root : shoot ratio (RSR). Columns represent means (n=15 to 16); error bars represent standard error of the mean. Within a treatment, means with the same letter were not significantly different (P<0.05).

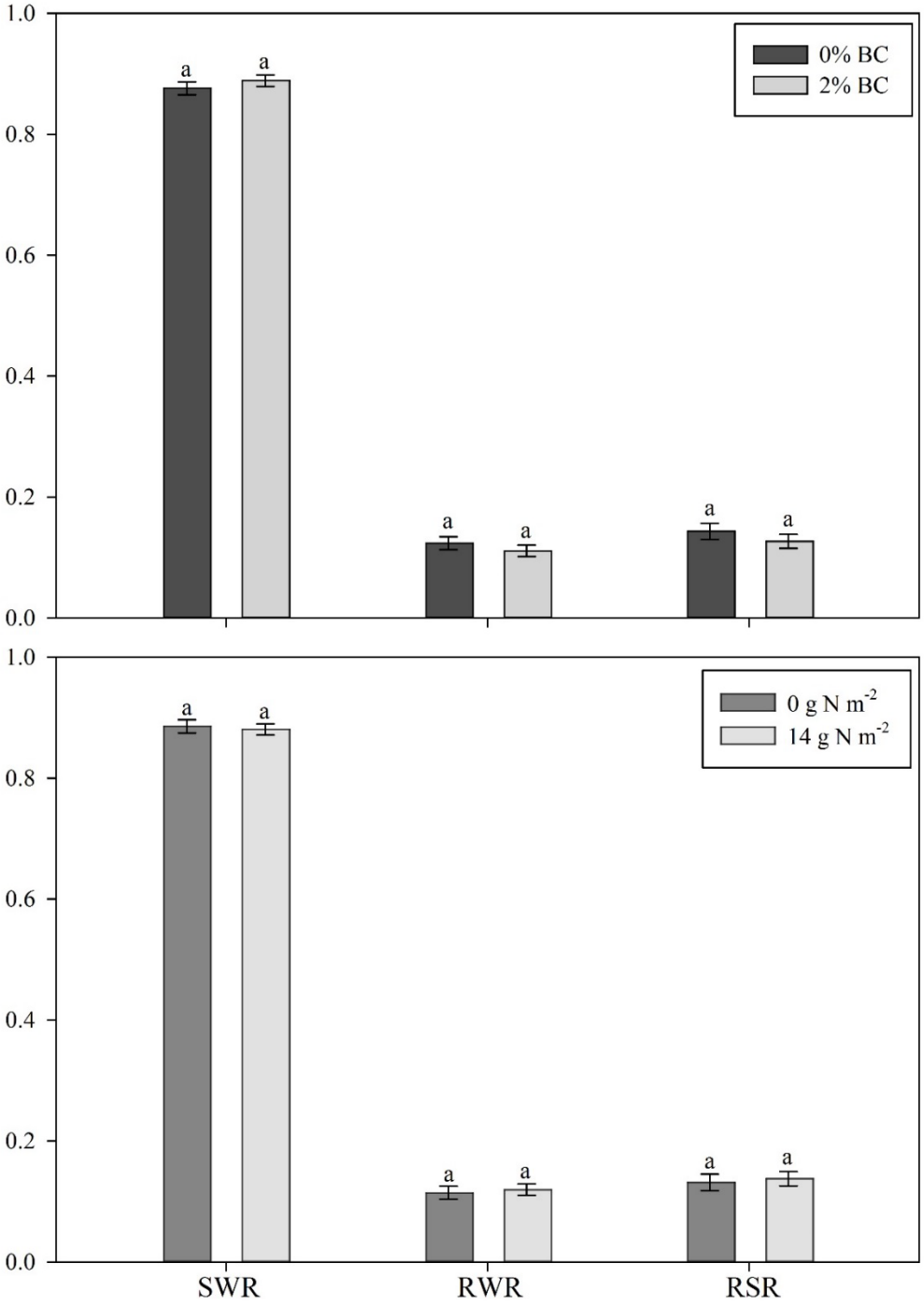

Figure 2.3. Effects of biochar (BC) and nitrogen (N) on redroot pigweed shoot weight ratio (SWR), root weight ratio (RWR), and root : shoot ratio (RSR). Columns represent means (n=14 to 15); error bars represent standard error of the mean. Within a treatment, means with the same letter were not significantly different (P<0.05).

2.5. Discussion

According to the optimal partitioning theory (OPT), plants allocate biomass to the organ associated with gathering the most limiting resource (McCarthy and Enquist 2007). The three most common limiting factors are mineral nutrition, water supply, and carbohydrate supply. Variations in the amount of these factors may influence allocation patterns of the plant (Brouwer 1962). The use of biomass partitioning ratios (SWR, RWR, and RSR) and the OPT have been criticized for not taking plant size or stage of development into consideration, i.e. for attributing differences in partitioning to treatment effects rather than to differences in developmental stages (Weiner 2004). However, biomass partitioning ratios are appropriate for this experiment because plants were compared at a similar developmental stage, i.e. reproductive maturity. Our results suggest that biochar increased partitioning of large crabgrass biomass from roots to shoots that resulted in longer stems; therefore, biochar may increase the ability of large crabgrass to spread above-ground.

The increased partitioning of redroot pigweed to branch DW at the expense of height, stem DW, and leaf DW cannot easily be explained by OPT. Biochar can have effects on soils and plants that go beyond simple fertilizer effects (Solaiman et al. 2010; Lehmann et al. 2011; Spokas 2010; Meller Harel et al. 2012; Elad et al. 2011). Spokas (2010) reported that five types of biochar produced ethylene in the dry state without the addition of soil or microbial inoculums and ten types of biochar produced ethylene after the addition of water. Ethylene has been shown to reduce the height of redroot pigweed seedlings (Raskin and Beyer 1989), which would be consistent with redroot pigweed

growth in our experiment. However, no research has been conducted on the effect of prolonged exposure to ethylene on redroot pigweed and our field soil and biochar were not tested for ethylene.

In the past two decades, the use of biochar as a soil amendment has grown in popularity due to its ability to increase soil fertility while simultaneously sequestering carbon. However, it is important to note that due to its strong adsorption properties, biochar has been shown to reduce the efficacy of some soil-applied herbicides (Graber et al. 2012). This study suggests that biochar may increase weed pressure either by increasing plant size or by allowing for increased spread. The response of redroot pigweed in this experiment also raises concern for the possibility of complex interactions among plant species, soils, and biochar suggesting that the application of biochar may complicate current weed management practices and highlighting the need for further research.

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CHAPTER 3. ROOT SYSTEM ANALYSIS OF LARGE CRABGRASS GROWN WITH TWO TYPES OF BIOCHAR

3.1. Abstract

Background and aims Biochar, a soil amendment similar to charcoal, may increase crop productivity by improving soil properties while simultaneously sequestering carbon. Although the root system is responsible for the benefits that biochar may provide to the plant, little research has been published on the effect of biochar on root system architecture. The objective of this study was to examine the effect of two types of biochar on the root growth and root system architecture of large crabgrass, a common and problematic weed species.

Methods Large crabgrass was grown in rhizoboxes filled with a sandy loam field soil +/- biochar (2% wt wt^{-1}). Two types of biochar produced by slow pyrolysis at 450°C were used: a low-nutrient biochar produced from a mixture of softwoods and a high-nutrient biochar produced from loblolly pine and switchgrass. Two soil patterns were used: solid (rhizobox filled uniformly with field soil +/- biochar) and split (unamended and amended field soil, each occupying half of the rhizobox vertically). Plants were completely randomized in two growth chambers and grown for 38 days after transplanting. Root

systems were scanned with a flatbed PC scanner and images were analyzed using ImageJ with SmartRoot. Plant biomass was dried and weighed.

Results Solid-pattern: Root growth of large crabgrass varied with the type of biochar used; however, biochar did not affect total plant dry weight. High-nutrient biochar increased above-ground dry weight and low-nutrient biochar increased below-ground dry weight when compared to plants grown in the unamended soil.

Split-pattern: Large crabgrass roots grew preferentially in the half of the rhizobox amended with biochar regardless of type. Root biomass was 74% and 79% greater in soil with low-nutrient and high-nutrient biochar, respectively, than in the unamended soil.

Conclusions This study suggests that the addition of biochar to soils, regardless of feedstock or nutrient content, will likely increase the ability of large crabgrass to spread either above-ground or below-ground by increased root growth. Large crabgrass roots develop more extensively in biochar-enriched soils.

Nomenclature: Large crabgrass, *Digitaria sanguinalis* L. Scop.

Keywords: Char, black carbon, flat rhizotron, rhizobox, root system architecture, weed.

3.2. Introduction

Biochar is a carbon-rich product formed through the pyrolysis of organic matter that has been proposed as a soil amendment to sequester carbon and improve soil properties and crop yields. A recent meta-analysis of 371 independent studies from 114 published manuscripts found that the addition of biochar to soils resulted in increased

crop yields, soil microbial biomass, rhizobia nodulation, soil phosphorus (P), soil potassium (K), and total nitrogen (N) and carbon (C) (Biederman and Harpole 2013). Plant tissue K concentration was also increased by biochar addition to the soil. Similarly, a meta-analysis conducted by Jeffrey et al. (2011) found a mean increase of 10% in crop productivity following biochar additions to the soil. Both meta-analyses found the greatest effect of biochar in acidic soils. Biederman and Harpole (2013) found that annual plants responded to biochar with increased below-ground growth but they did not detect an effect of biochar on biomass partitioning between root and shoot. They therefore suggested that, in annuals, biochar increases both above- and below-ground growth. However, this conclusion was based on relatively few studies (n=10) (Biederman and Harpole 2013) and we are aware of only two studies in which the effect of biochar on root system architecture, the spatial arrangement or topology of plant roots, was examined (Prendergast-Miller et al. 2011, 2014). Although the root system is responsible for transferring benefits that biochar may provide to the plant, there is little research on the effect of biochar on root growth and root system architecture (RSA).

Root system structure, the overall morphology of plant roots, is determined by the plant species and genotype, i.e. grasses tend to have complex fibrous root systems. However, RSA is plastic and plants can alter their RSA in response to environmental cues, such as drought or nutrient availability. For example, Linkohr et al. (2002) reported that the primary and lateral root length of *Arabidopsis* was inversely correlated with N supply. While *Arabidopsis* primary root length increased and lateral root length decreased with increasing levels of inorganic phosphate (Linkohr et al. 2002). Research on barley (*Hordeum vulgare* cv. Procter) found that manipulated levels of N, P, and K in a

localized section of the root zone resulted in the proliferation of barley roots in the area with a high nutrient level (Drew 1975). These changes in root development are evidence that roots are able to perceive and react to the surrounding soil environment. It is this plasticity of root growth that has made it difficult to develop a RSA classification system (Fitter 1987).

To better understand how biochar in the soil affects root growth and subsequently plant performance, Prendergast-Miller et al. (2014) used a rhizobox mesocosm to grow spring barley (*H. vulgare* L. var. Waggon) with and without biochar. Biochar increased shoot and root biomass of spring barley but did not affect total root length and also resulted in smaller rhizosheaths (soil bound to the root system) than the control. Rhizosheaths are an indicator of root exudates and root hair development. Larger rhizosheaths may develop under P-limited conditions. The reduction of the rhizosheaths with biochar suggests that biochar may be a direct source of soluble P. This evidence supports the hypothesis that biochar amendment has direct effects on plant nutrient acquisition (Prendergast-Miller et al. 2014). However, Haling et al. (2014) found that other soil properties such as bulk density and soil moisture may also have an effect on root hair and rhizosheaths development.

Although weeds are known to reduce crop yields, there is little published research on the effect of biochar on weeds (Major et al. 2005; Quilliam et al. 2012). Compared to a variety of soil amendments, biochar did not increase weed cover but biochar plus an inorganic fertilizer increased weed cover more than the inorganic fertilizer alone in low fertility, highly acidic soils of the central Brazilian Amazon (Major et al. 2005). In contrast, a study conducted on temperate agricultural soils suggests that biochar may

have a short-term inhibitory effect on weed emergence (Quilliam et al. 2012). Biochar produced from hardwoods at 450°C was incorporated into a sandy clay loam soil at a rate of 25 or 50 t ha^{-1} but produced no long-term effect on weeds three years after incorporation (Quilliam et al. 2012). However, weed emergence was reduced when biochar was reapplied. The incorporation of biochar did not result in significant differences in the above-ground biomass or foliar nutrient content of the crop. The authors were unable to explain the reduction in weed emergence but suggested that increased soil microbial activity might play a role (Quilliam et al. 2012). The conflicting results of Major et al. (2005) and Quilliam et al. (2012) could be due to a number of differences between the studies including biochar feedstock, soil type, and prevailing growth conditions, and highlight the need for additional controlled studies to better understand the effect of biochar on weed species.

Large crabgrass is a common and problematic annual weed native to Europe but found in most temperate and tropical regions (Mitich 1988). Large crabgrass has fibrous roots and an often prostrate growth habit. It can produce adventitious roots at stem nodes, and form thick mats of shoots (Mitich 1988). Crabgrass appears to have relatively high P and K requirements (Peters and Dunn 1971) and reduction in soil P and K availability can significantly reduce large crabgrass growth (Hoveland et al. 1976). In a greenhouse experiment, three weed species, including large crabgrass, were grown with and without biochar (Mitchell et al. in prep.). Biochar increased shoot dry weight (DW) of large crabgrass by 34% but reduced root DW by 30% suggesting that biochar allowed large crabgrass to partition more biomass to shoots than roots. However, Mitchell et al. (in prep.) only examined plant dry weights and could not elucidate on changes to RSA.

Therefore, the objective of this study is to examine the effects of two types of biochar on root growth and RSA of large crabgrass using a rhizobox mesocosm.

3.3. Materials and Methods

3.3.1. Biochar and Soil Properties

Biochar produced at the same temperature (450°C) but from two different feedstocks was used in this experiment. The high-nutrient biochar (HNB) was produced from a mixture of loblolly pine (*Pinus taeda*) and switchgrass (*Panicum virgatum*) by a commercial producer (Eprida, Inc., 3020 Canton Road Suite 105, Marietta, GA 30066, US). The low-nutrient biochar (LNB) was produced from a mixture of fir, pine, and spruce by a commercial producer (Diacarbon Energy, Inc., 2250 Boundary Road 120, Burnaby, BC V5M 3Z3, Canada). A sandy loam field soil, Desker series (coarse-loamy, mixed, superactive, mesic Mollic Hapludalfs), was collected in June 2013 from the top 10 cm of a conventional agricultural field located at Throckmorton Purdue Agricultural Center (8343 South US 231, Lafayette, IN 47909, US; 40°17’42.0”N 86°54’33.8”W). Soil was pulverized using a Model 112 Royer Shredder-Mixer (Royer, Ind., 6856 Howlett Road, Oshkosh, WI 54902, US). The biochar and field soil were passed through a 4-mm mesh sieve separately to achieve uniform particle size. Field soil was amended with one of the two types of biochar at a rate of 2% of the soil dry DW and thoroughly mixed together in a 50 L electric concrete mixer for 2 h. Unamended field soil was also mixed for 2 h and was used as the control.

Total C and N were determined for all treatments in quintuplicate by element analyzer (Thermo Scientific FlashEA 1112 series). Organic matter (OM), pH, cation exchange capacity (CEC), and extractable Bray 2-phosphorus, K, magnesium (Mg), and calcium (Ca) were determined for all treatments by a commercial laboratory (A&L Great Lakes Laboratories, 3505 Conestoga Drive, Fort Wayne, Indiana 46808, US). Loss-on-ignition of the dry mass at 360°C was used to measure percent OM content (Nelson and Sommers 1996). Plant available nutrients (K, Mg, and Ca) were extracted using the Mehlich III method and analyzed by inductively coupled plasma-atomic emission spectroscopy (Mehlich 1984). The CEC was measured using a modified ammonium-acetate compulsory displacement and pH was determined by a 1:1 ratio of soil : water (Sumner and Miller 1996).

3.3.2. Growth Conditions

Large crabgrass was grown in rhizoboxes to examine the effect of two types of biochar on root growth and RSA. Rhizoboxes were constructed using two transparent acrylic sheets and three wooden spacers held together with medium binder clips to make interior dimensions of 24×20×0.5 cm (H×W×D; Figure 2.1.). Each rhizobox was filled with 240 mL of soil (+/- biochar) to a thickness of 0.5 cm. Two soil patterns were used: solid and split. The solid-pattern consisted of unamended *or* amended soil positioned uniformly throughout the rhizobox and the split pattern consisted of unamended *and* amended soil, each occupying half of the rhizobox vertically (Figure 2.1.). Large crabgrass seeds were germinated on moist filter paper (Azlin Seed Service, 112 Lilac

Drive, Leland, MS, 38756) and seedlings with a 20-mm radicle were carefully placed in the top center of the rhizobox. Aluminum foil was wrapped around the rhizoboxes to exclude light and the rhizoboxes were placed in a growth chamber (Conviron PGR15, 590 Berry Street, Winnipeg, Manitoba, Canada R3H 0R9). The rhizoboxes were kept at a 65° angle to force roots to grow against the acrylic sheet (Brennan et al. 2014). Plants were grown for 38 d at 28 / 18°C day/night temperatures respectively with a 15 h photoperiod (9 h night) to imitate average Indiana summer conditions. Photo-synthetically active radiation (PAR) in the growth chambers was 500 µmol m^{-2} s^{-1} (AccuPAR LP-80 PAR/LAI Ceptometer, Decagon Devices, Inc., 2365 NE Hopkins Court, Pullman, WA 99163, USA). Plants were watered daily with 20 mL of distilled (DI) water.

3.3.3. Plant analyses

At 38 days after transplant (DAT), rhizoboxes were scanned with an Epson Perfection V37 desktop scanner at 600 dpi. Height and number of tillers were recorded before plants were harvested by cutting the stem at the soil surface. Leaves and stems were placed separately in paper bags and dried at 60°C to a constant weight. The solid-pattern rhizoboxes were taken apart and roots were carefully removed from the soil using dissecting forceps. The split pattern rhizoboxes were also taken apart; however, the root system was cut with a straight blade down the center before the roots were removed. Roots were gently washed in DI water and dried at 60°C to a constant weight.

Root images were analyzed using ImageJ 1.41o (Schneider et al. 2012) with SmartRoot (Lobet et al. 2011) plug-in for root length, root diameter, number of roots within each root order, and insertion angle of laterals. The roots were not completely visible at the top of the rhizobox therefore root analysis was started two cm from the soil surface. Large crabgrass has a complex fibrous root system with multiple seminal roots. In this study, roots originating in the top two cm of the soil are referred to as primary roots. Laterals branching from the primary roots are referred to as secondary roots, laterals branching from the secondary roots are referred to as tertiary roots and so forth. Three estimates of root length were calculated: total root system length, total root length, and individual root length. Total root system length is the sum of all roots in the entire root system, disregarding root order (primary, secondary, tertiary). Total root length is the sum of all roots within a root order. Individual root length is the average length of a single root within a root order. Plant biomass partitioning and root system architecture ratios were calculated. Root : shoot ratio (RSR) is root DW divided by shoot DW. Root weight ratio (RWR) is root DW divided by total plant DW. Shoot weight ratio (SWR) is shoot DW divided by total plant DW. Root mass density (RMD) is root DW divided by soil volume. Root length density (RLD) is total root system length divided by soil volume. Specific root length (SRL) is total root system length divided by root DW.

3.3.4. Statistical Analysis

The rhizoboxes were completely randomized in two growth chambers with four replicates of each treatment in each growth chamber. Statistical analyses of all the results

were completed using SAS 9.3 (SAS Institute Inc., Cary, NC, USA). All data were tested for normality and homogeneity of variances. Transformations were not necessary. Analysis of variance (ANOVA) was used to determine significant differences in the soil and biochar analyses results. A Fisher's least significant difference (LSD) test was used to determine significant differences between the two amended soils and the unamended soil.

ANOVA was also used to determine significant differences between treatments. There were no biochar by growth chamber interactions, therefore data from both growth chambers were pooled. Each soil pattern was analyzed separately. Data from the split-pattern rhizoboxes were analyzed to allow comparisons of growth within a rhizobox, i.e. between amended and unamended halves, and comparisons between rhizoboxes containing the two biochars. The latter analyses were conducted by comparing growth in the unamended LNB half to growth in the unamended HNB half and by comparing growth in the LNB-amended half to growth in the HNB-amended half. Least-square means tests were completed for mean comparisons of all dependent variables.

3.4. Results

3.4.1. Biochar and Soil Analyses

The pH and C:N ratio were greater in the LNB than in the HNB (Table 2.1.). However, total C and N, as well as CEC, was greater in HNB than in the LNB. Plant nutrients (K, Mg, and Ca) and extractable Bray phosphorus were also greater in HNB than in the LNB. The OM did not differ between biochars. The pH and C:N ratio were

greater for the LNB-amended soil than for the control and the HNB-amended soil. The pH did not differ between the control soil and the HNB-amended soil but C:N ratios were greater in the HNB soil than in the control soil. Percent C was greater in both biochar-amended soils than in the control soil. Percent N was greater in the control and the HNB-amended soils than in the LNB-amended soil. The OM was greatest in the HNB-amended soil and lowest in the control soil. No differences were detected among soils for CEC or Ca. Soil test K and Bray phosphorus were greater in the HNB-amended soil than in the control soil or LNB-amended soil. Available Mg was greater in the control and HNB-amended soils than in the LNB-amended soil (Table 2.1.).

3.4.2. Solid-pattern

Biochar did not affect large crabgrass total DW (Figure 2.2.). However, shoot DW was 55% greater for the HNB treatment than for the control. Shoot DW did not differ between biochar types. The LNB increased root DW by 66% when compared to the control and by 99% when compared to the HNB treatment (Figure 2.2.). Both types of biochar increased tillering (Figure 2.3.). Plants grown with HNB produced more than twice as many tillers as the plants grown in the control soil. Tiller production was similar between the LNB and HNB treatments (Figure 2.3.).

Biochar affected biomass partitioning and root densities (Table 2.2.). The RSR and RWR were lower for plants grown in HNB soil then for plants grown in the control or LNB soils. The SWR was greater for plants grown in HNB soil then for plants grown in the control or LNB soils. No differences in RSR, RWR, or SWR were detected

between the LNB and control treatments. The RMD and RLD were greater for the plants grown in LNB soil than in the HNB or control soils. The RLD was lower in the HNB than in the control treatment. The SRL did not differ among the three treatments (Table 2.2.).

The RSA of large crabgrass responded differently to each treatment (Figure 2.4.). Biochar did not affect the total length of primary roots but the individual length of primary roots was greater for plants grown in LNB soil than for plants grown in the HNB or the control soils (Figure 2.5.). Total and individual root length of secondary and tertiary roots was greater for plants grown in LNB soil than for plants grown in the HNB or control treatments. No differences were detected in the individual root length of primary or secondary roots between the HNB and control treatments. However, the individual root length of tertiary roots was greater in the control than in the HNB treatment (Figure 2.5. (b)).

Primary root diameter was greater with LNB than the HNB or control treatments with no differences detected between the control and HNB treatments. However, biochar increased secondary and tertiary root diameters regardless of biochar type (Figure 2.6.). Differences in secondary and tertiary root diameter were not detected between the HNB and LNB treatments. Insertion angles were greater for the control treatment than for the HNB treatment, regardless of root order (Figure 2.7.). Both the control and HNB had a greater insertion angle than LNB but only for secondary and quaternary roots (Figure 2.7.).

3.4.3. Split-pattern

Shoot dry weights for the split-pattern rhizoboxes were 64.9 SE ±8.9 and 72.3 SE ±7.8 mg plant^{-1} for large crabgrass grown with LNB and HNB respectively. When given a choice between unamended field soil (control) and field soil amended with 2% biochar, large crabgrass roots grew preferentially in the half of the rhizobox amended with biochar regardless of the type of biochar (Figure 2.8). Root DW was 74% and 79% greater in the rhizobox half amended with LNB and HNB respectively than in the control half (Figure 2.9.). The total root system length was 640% and 243% greater in the half amended with LNB and HNB respectively than in the control half (Figure 2.10.). Individual and total root lengths as well as RLD and SRL were greater in the LNB soil than in the HNB soil (Figure 2.8., Table 2.3.). Biochar did not affect RMD in the split-pattern rhizobox (Table 2.3.). No differences were detected between the two control halves for any dependent variable (data not shown).

Table 3.1. Characteristics of unamended field soil (Control)[a], low-nutrient biochar (LNB)[b], high-nutrient biochar (HNB)[c], and the amended soils prior to conducting experiment. Values are means of four samples; parentheses enclose standard error of the mean. Values with different letters indicate significant differences were detected (P<0.05).[d]

	pH	C : N	Total C	Total N	OM	CEC	K	Mg	Ca	P
			%	%	%	meq 100 g^{-1}	ppm			
LNB	9.52 **a**	259.0 **a**	55.2 **b**	0.21 **b**	65.4 **a**	0.8 **b**	135 **b**	14 **b**	60 **b**	1 **b**
	(0.14)	(14.2)	(1.0)	(0.01)	(0.3)	(0.1)	(14.1)	(2.9)	(10.0)	(0.2)
HNB	7.08 **b**	45.0 **b**	61.4 **a**	1.36 **a**	64.4 **a**	15.5 **a**	3,742 **a**	361 **a**	588 **a**	296 **a**
	(0.03)	(1.3)	(1.2)	(0.05)	(0.8)	(0.2)	(66.5)	(2.4)	(12.5)	(6.0)
Control	7.03 **b**	11.7 **c**	2.1 **b**	0.18 **a**	2.7 **c**	8.9 **a**	175 **b**	236 **a**	1,300 **a**	55 **b**
	(0.03)	(0.1)	(0.1)	(<0.01)	(0.04)	(0.2)	(3.4)	(3.2)	(20.4)	(0.6)
Soil with 2% LNB	7.25 **a**	21.0 **a**	3.2 **a**	0.15 **b**	2.9 **b**	8.7 **a**	191 **b**	223 **b**	1,263 **a**	54 **b**
	(0.03)	(0.5)	(0.1)	(<0.01)	(0.1)	(0.2)	(2.8)	(4.3)	(31.5)	(0.9)
Soil with 2% HNB	7.08 **b**	15.5 **b**	3.0 **a**	0.19 **a**	3.7 **a**	9.3 **a**	365 **a**	244 **a**	1,275 **a**	67 **a**
	(0.03)	(0.4)	(0.1)	(<0.01)	(0.03)	(0.2)	(7.6)	(5.2)	(32.3)	(1.1)

[a]Desker series (coarse-loamy, mixed, superactive, mesic Mollic Hapludalfs) consisting of approximately 68% sand, 22% silt, and 10% clay.

[b]Low-nutrient biochar (LNB) was produced by slow-pyrolysis at 450°C from a mixture of softwoods: fir, pine, and spruce.

[c]High-nutrient biochar (HNB) was produced by slow-pyrolysis at 450°C from loblolly pine and switchgrass.

[d]Abbreviations: C : N, carbon : nitrogen ratio; OM, organic matter; CEC, cation exchange capacity; meq 100 g^{-1}, milliequivalent per 100 grams of dry soil; ppm, parts per million.

Table 3.2. Plant resource allocation and root system architecture ratios of large crabgrass grown in a solid-pattern rhizobox filled uniformly with unamended field soil (Control)[a], field soil with 2% low-nutrient biochar (LNB)[b], or field soil with 2% high-nutrient biochar (HNB)[c]. Values are means (n=7 to 8); parentheses enclose standard error of the mean. Values with different letters indicate significant differences were detected (P<0.05).[d]

Plant trait	Abbreviation	Equation (units)	Control	LNB	HNB
Root shoot ratio	RSR	Root DW/shoot DW (mg mg^{-1})	1.17 (0.13) **a**	1.36 (0.22) **a**	0.57 (0.08) **b**
Root weight ratio	RWR	Root DW/plant DW (mg mg^{-1})	0.53 (0.03) **a**	0.56 (0.03) **a**	0.35 (0.03) **b**
Shoot weight ratio	SWR	Shoot DW/plant DW (mg mg^{-1})	0.47 (0.03) **b**	0.44 (0.03) **b**	0.65 (0.03) **a**
Root mass density	RMD	Root DW/soil volume (mg cm^{-3})	0.24 (0.04) **b**	0.40 (0.07) **a**	0.20 (0.03) **b**
Root length density	RLD	Root length/soil volume (cm cm^{-3})	1.13 (0.06) **b**	1.45 (0.08) **a**	0.79 (0.02) **c**
Specific root length	SRL	Root length/root DW (cm mg^{-1})	6.03 (1.39) **a**	4.29 (0.65) **a**	4.75 (0.83) **a**

[a]Desker series (coarse-loamy, mixed, superactive, mesic Mollic Hapludalfs) consisting of 68% sand, 22% silt, and 10% clay.

[b]Low-nutrient biochar (LNB) was produced by slow-pyrolysis at 450°C from a mixture of fir, pine, and spruce.

[c]High-nutrient biochar (HNB) was produced by slow-pyrolysis at 450°C from loblolly pine and switchgrass.

[d]Abbreviations: DW, dry weight.

Table 3.3. Root system architecture ratios of large crabgrass grown in a split-pattern rhizobox filled with unamended field soil (Control)[a] and field soil amended with either 2% low-nutrient biochar (LNB)[b] or 2% high-nutrient biochar (HNB)[c], each occupying half of the rhizobox vertically. Values are means (n=8); parentheses enclose standard error of the mean. No differences were detected between the two control halves (data not shown). Values with different letters indicate significant differences were detected ($P<0.05$).[d]

Plant Trait	Abbreviation	Equation (units)	LNB	HNB
Root mass density	RMD	Root DW/ soil volume (mg cm^{-3})	0.13 (0.02) **a**	0.12 (0.02) **a**
Root length density	RLD	Root length/ soil volume (cm cm^{-3})	1.23 (0.12) **a**	0.52 (0.07) **b**
Specific root length	SRL	Root length/ root DW (cm mg^{-1})	10.84 (1.38) **a**	4.76 (0.69) **b**

[a]Desker series (coarse-loamy, mixed, superactive, mesic Mollic Hapludalfs) consisting of 68% sand, 22% silt, and 10% clay.

[b]Low-nutrient biochar (LNB) was produced by slow-pyrolysis at 450°C from a mixture of fir, pine, and spruce.

[c]High-nutrient biochar (HNB) was produced by slow-pyrolysis at 450°C from loblolly pine and switchgrass.

[d]Abbreviations: DW, dry weight.

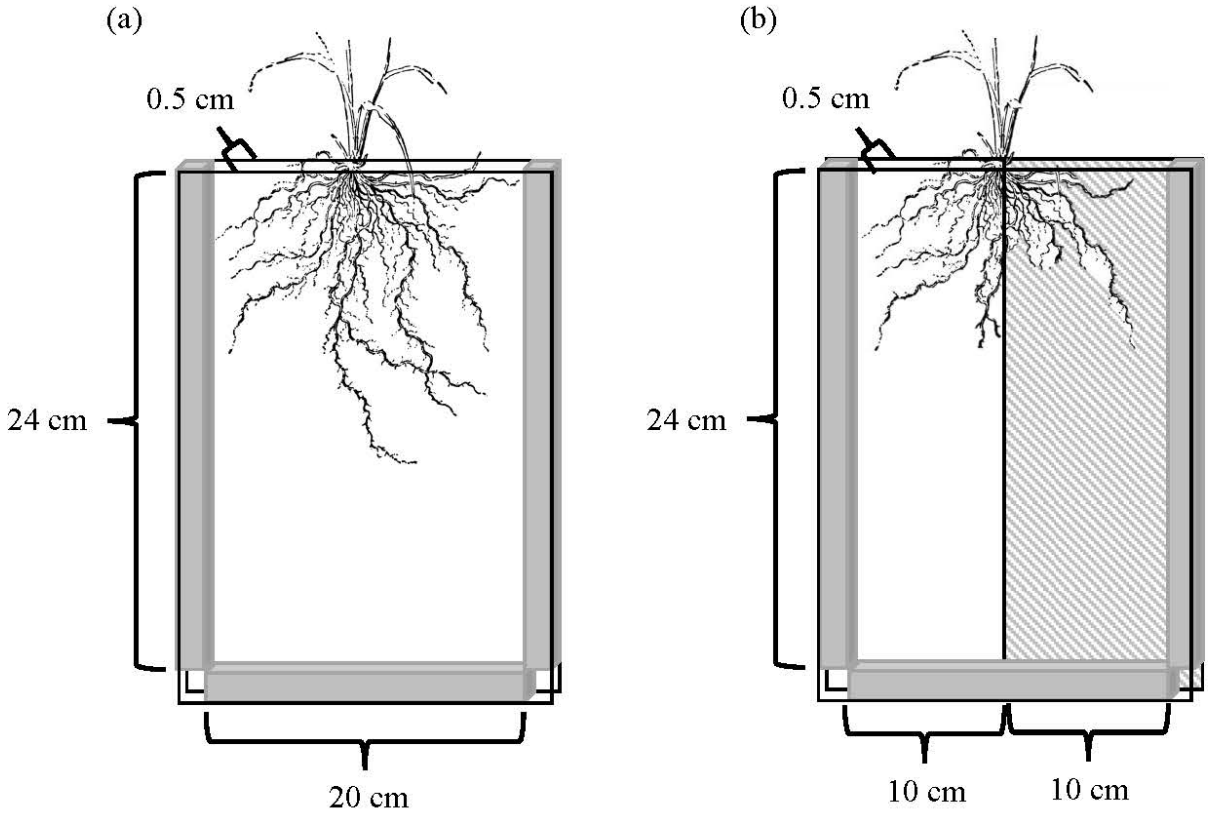

Figure 3.1. A diagram of rhizobox construction and soil patterns. Rhizoboxes were made from two transparent acrylic sheets (0.3 cm thick) with wood spacers (0.5 cm thick) in-between the acrylic sheets on both sides and bottom (shown in pale grey) and held together with medium binder clips. The solid-pattern (a) is uniformly filled with field soil (+/- 2% biochar). The split-pattern (b) is filled with unamended field soil and field soil amended with 2% biochar, each occupying half of the rhizobox vertically.

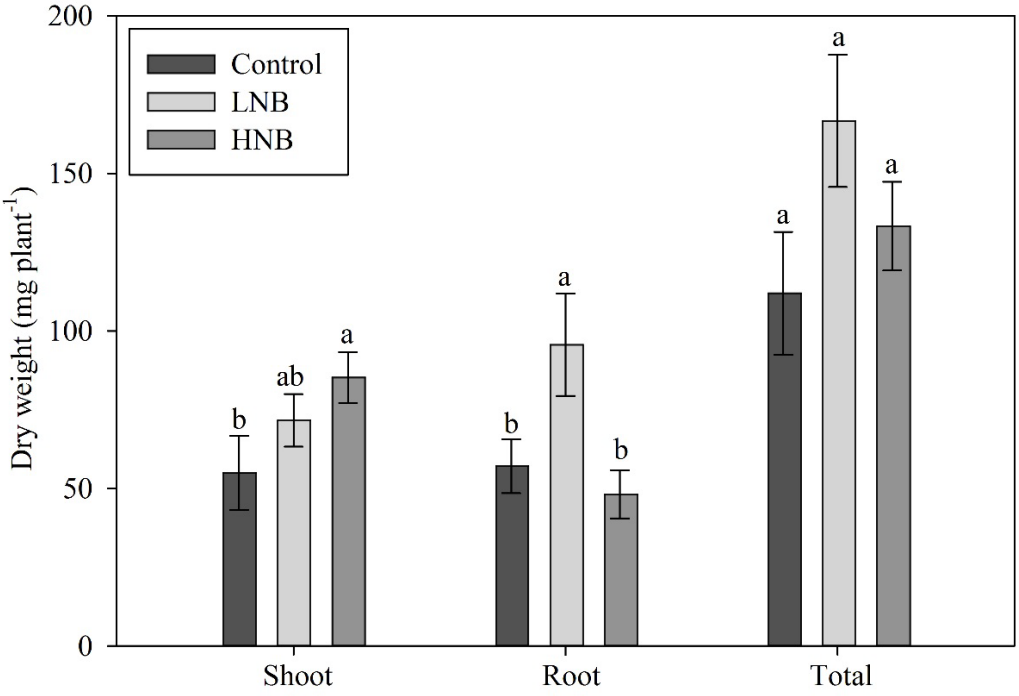

Figure 3.2. Shoot, root, and total plant dry weight (DW) of large crabgrass grown in solid-pattern rhizobox with unamended field soil (Control), field soil with 2% low-nutrient biochar (LNB), or field soil with 2% high-nutrient biochar (HNB). Columns represent means (n=7 to 8); error bars represent standard error of the mean. Means with different letters indicate significant statistical difference (P<0.05).

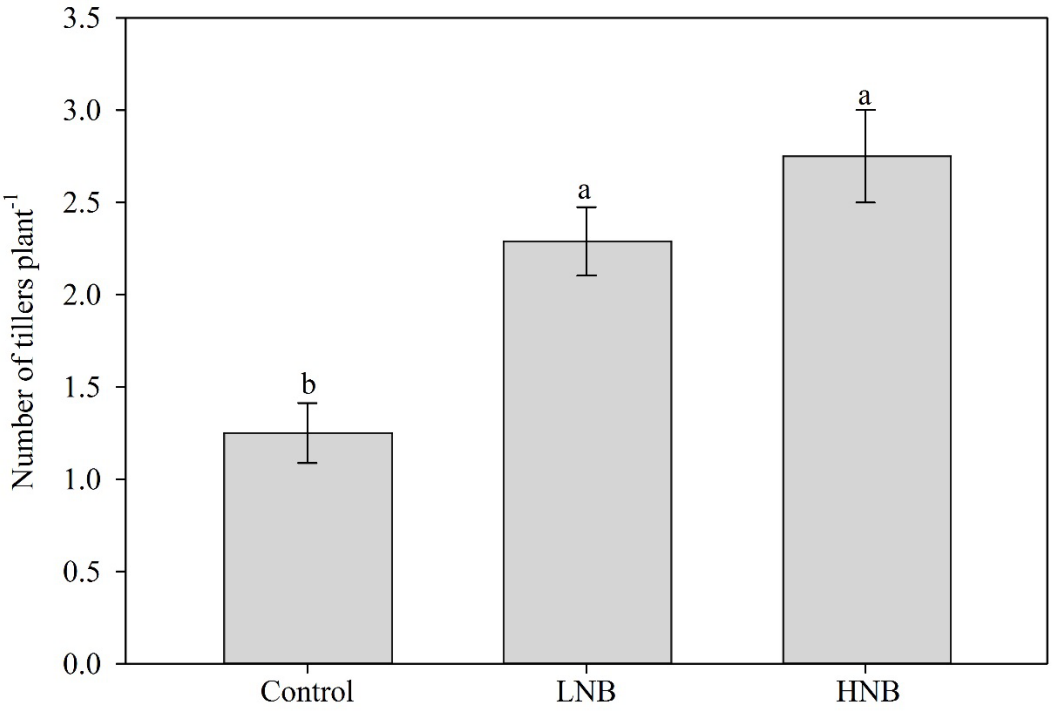

Figure 3.3. Number of tillers of large crabgrass grown in solid-pattern rhizobox with unamended field soil (Control), field soil with 2% low-nutrient biochar (LNB), or field soil with 2% high-nutrient biochar (HNB). Columns represent means (n=7 to 8); error bars represent standard error of the mean. Means with different letters indicate significant statistical difference (P<0.05).

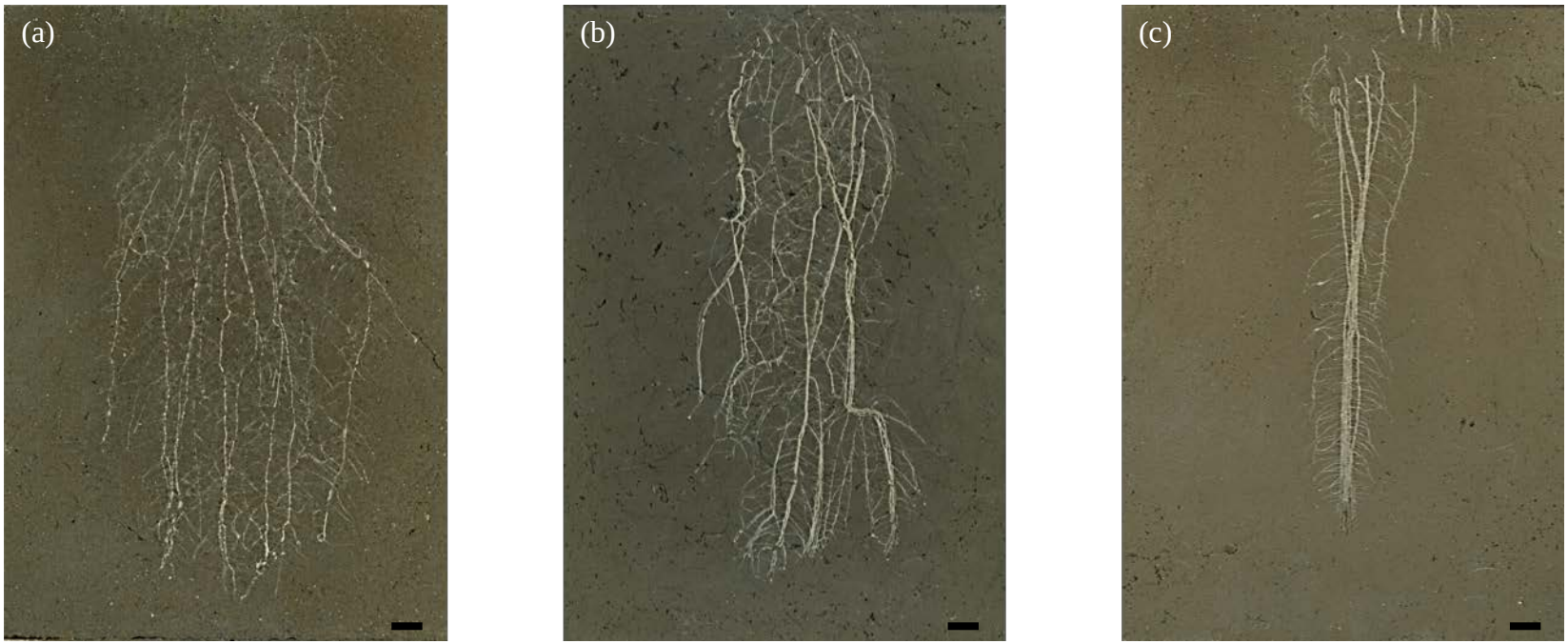

Figure 3.4. Root images of the solid-pattern rhizobox treatments were captured using a flatbed scanner. The control (a) rhizobox is uniformly filled with unamended field soil. The low-nutrient biochar (b) and the high-nutrient biochar (c) rhizoboxes are uniformly filled with field soil amended with 2% biochar (wt wt^{-1}). The black line at the bottom of each rhizobox is a 1 cm scale.

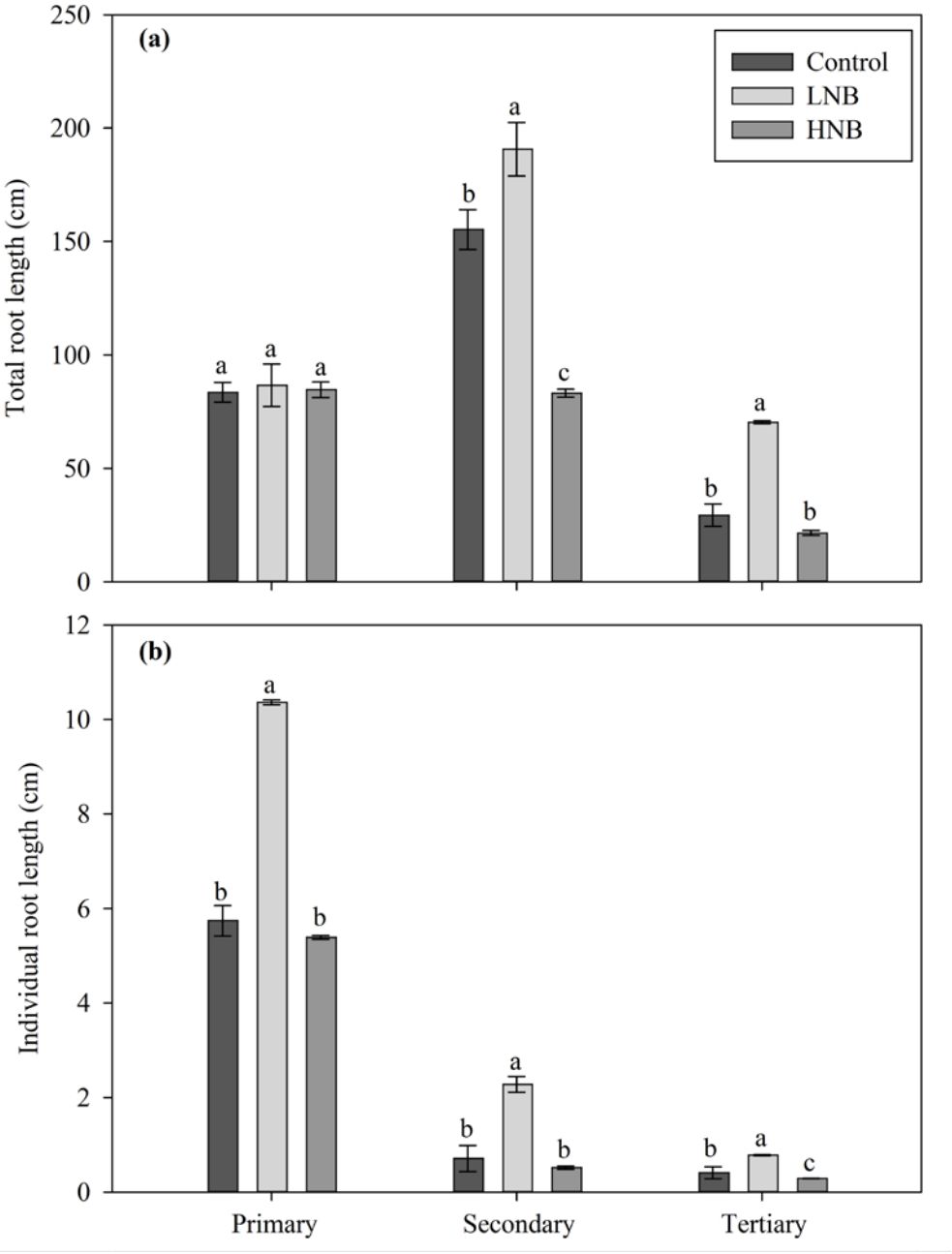

Figure 3.5. Root length of primary, secondary, and tertiary roots of large crabgrass grown in solid-pattern rhizobox with unamended field soil (Control), field soil with 2% low-nutrient biochar (LNB), or field soil with 2% high-nutrient biochar (HNB). Total root length (a) represents the sum of all roots within a root order (primary, secondary, or tertiary). Individual root length (b) represents the average length of a single root within a root order (primary, secondary, or tertiary). Columns represent means (n=7 to 8); error bars represent standard error of the mean. Within a root order, means with different letters indicate significant statistical difference (P<0.05).

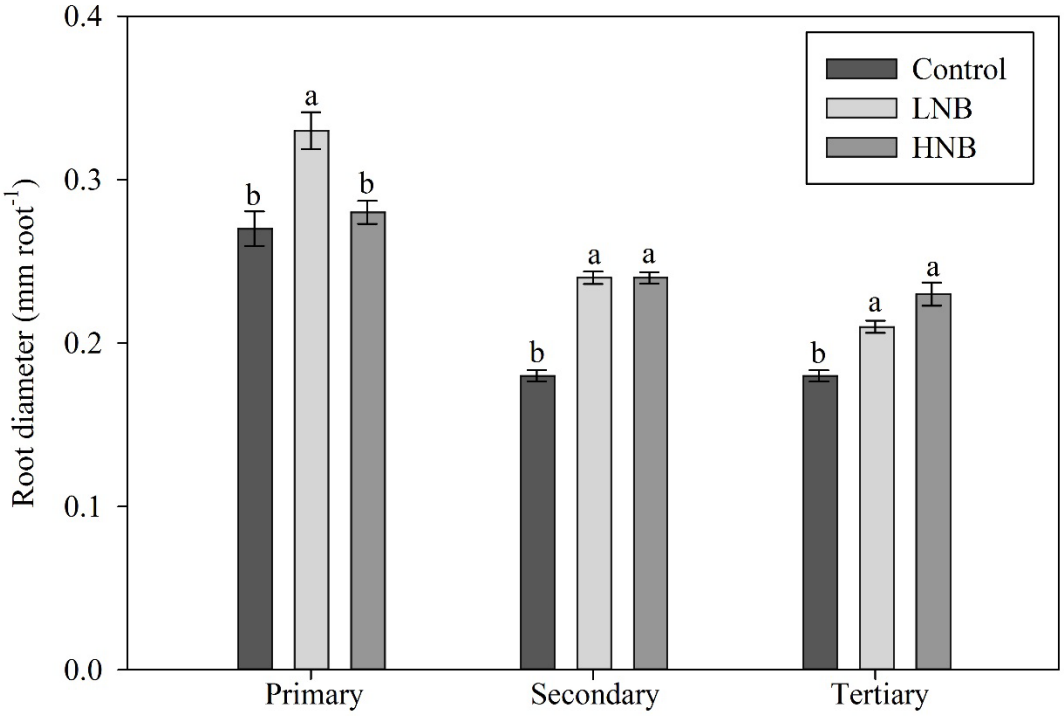

Figure 3.6. Root diameter of primary, secondary, and tertiary roots of large crabgrass grown in solid-pattern rhizobox with unamended field soil (Control), field soil with 2% low-nutrient biochar (LNB), or field soil with 2% high-nutrient biochar (HNB). Columns represent means (n=7 to 8); error bars represent standard error of the mean. Within a root order, means with different letters indicate significant statistical difference (P<0.05).

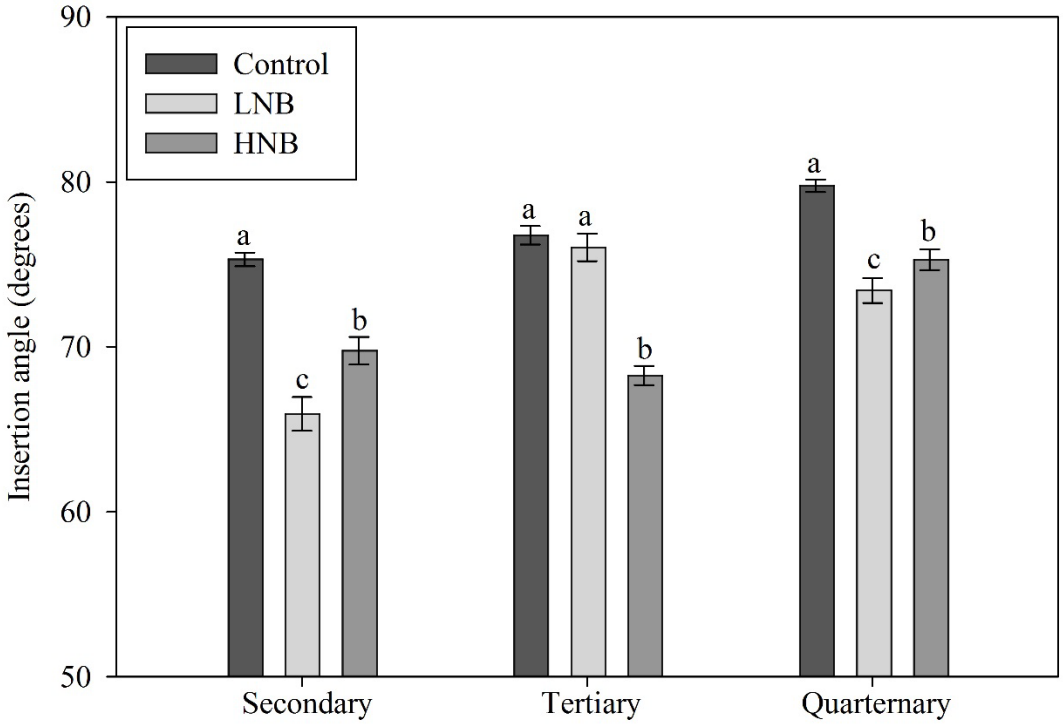

Figure 3.7. Branch insertion angle of secondary, tertiary, and quaternary roots of large crabgrass grown in solid-pattern rhizobox with unamended field soil (Control), field soil with 2% low-nutrient biochar (LNB), or field soil with 2% high-nutrient biochar (HNB). Columns represent means (n=7 to 8); error bars represent standard error of the mean. Within a root order, means with different letters indicate significant statistical difference (P<0.05).

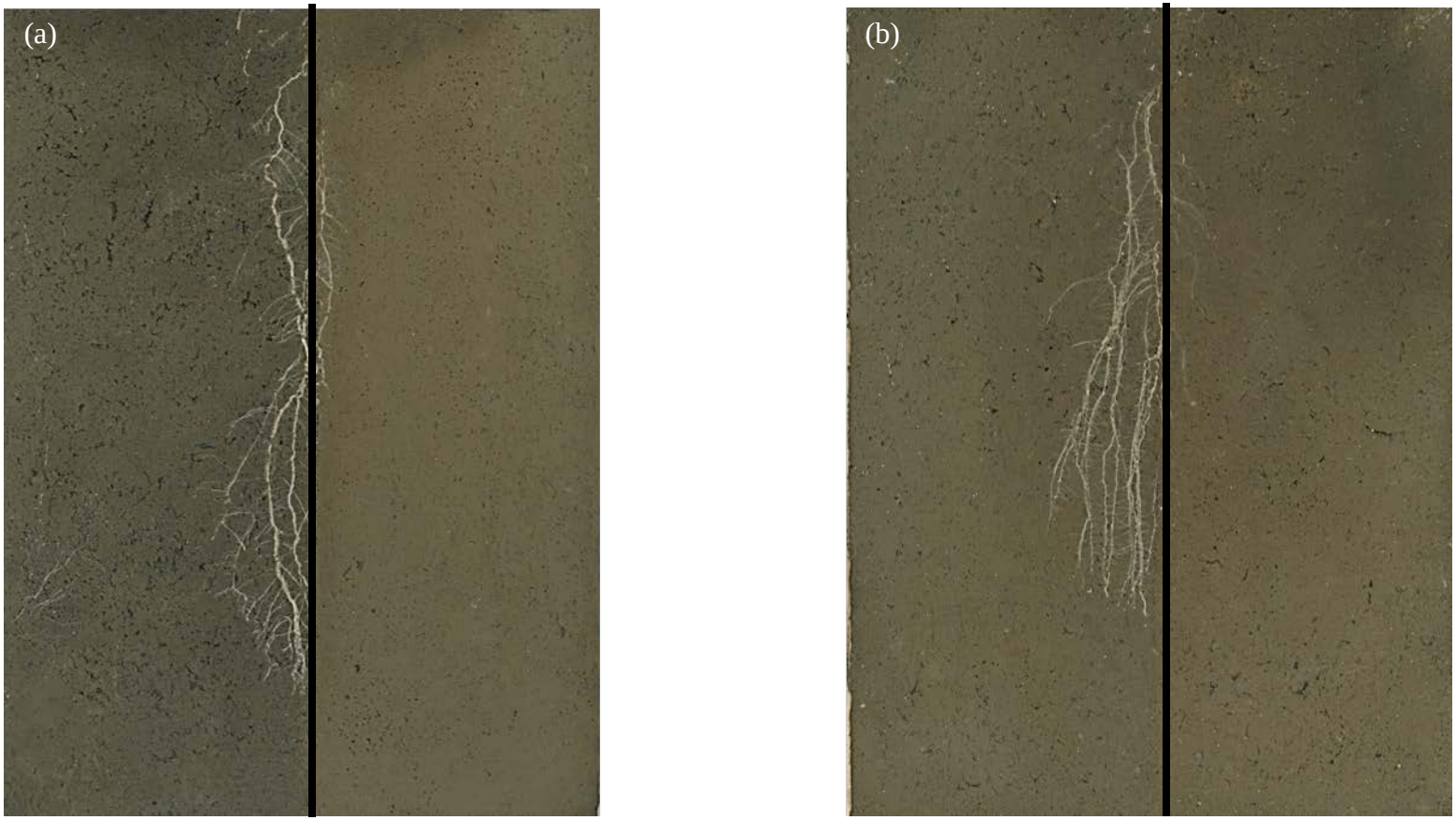

Figure 3.8. Root images of the split-pattern rhizobox treatments were captured using a flatbed scanner. The low-nutrient biochar (a) and the high-nutrient biochar (b) split rhizoboxes are filled with biochar-amended field soil to the left of the black line and unamended field soil on the right.

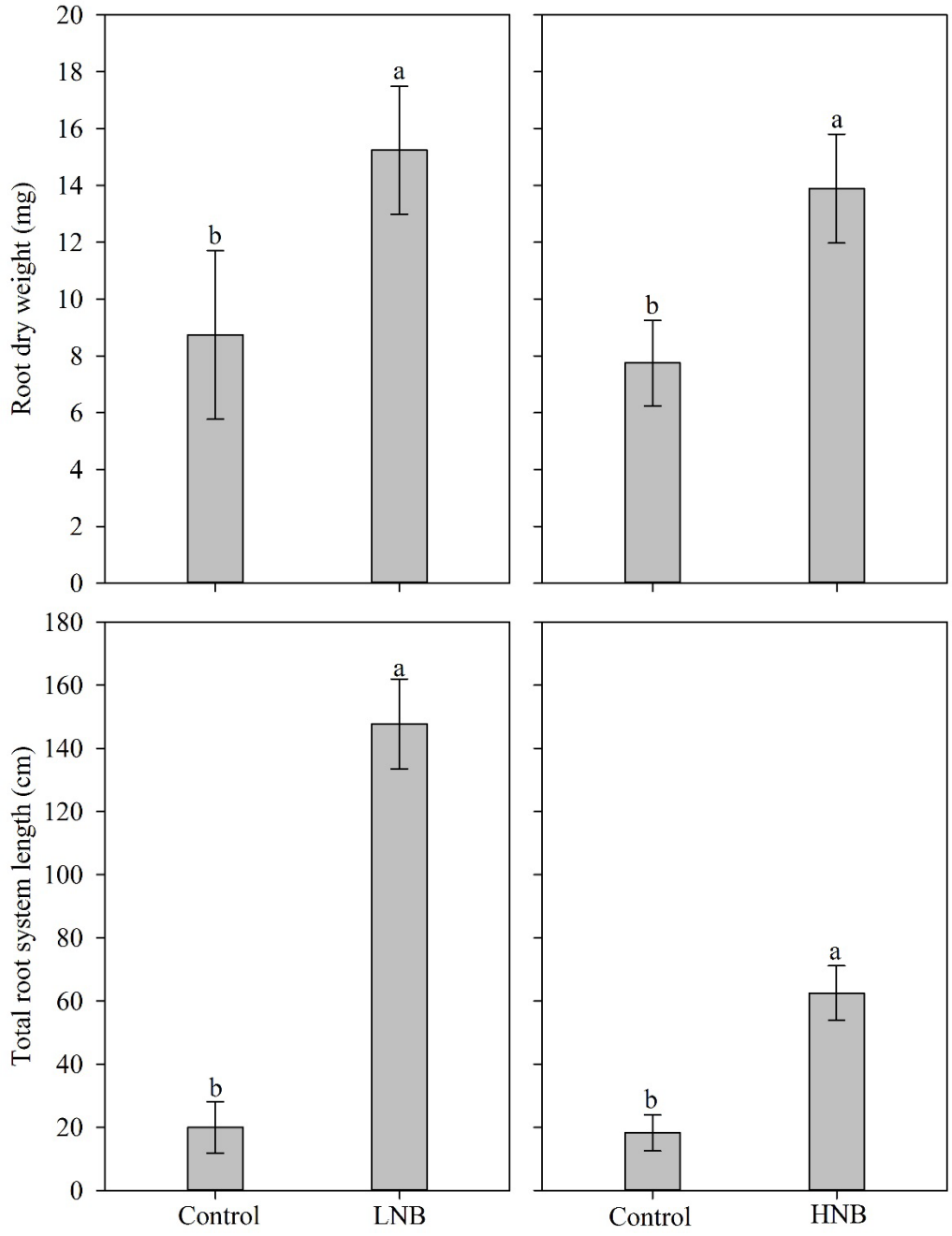

Figure 3.9. Root dry weight (DW) and total root system length of large crabgrass grown in split-pattern rhizobox. Large crabgrass seedlings were placed in the center of the rhizobox with unamended field soil (Control) and field soil amended with either 2% low-nutrient biochar (LNB) or high-nutrient biochar (HNB), each occupying half of the rhizobox vertically. The root system was cut down the center and each half was dried and weighed separately. Columns represent means (n=8); error bars represent standard error of the mean. Columns with different letters indicate significant statistical difference (P<0.05).

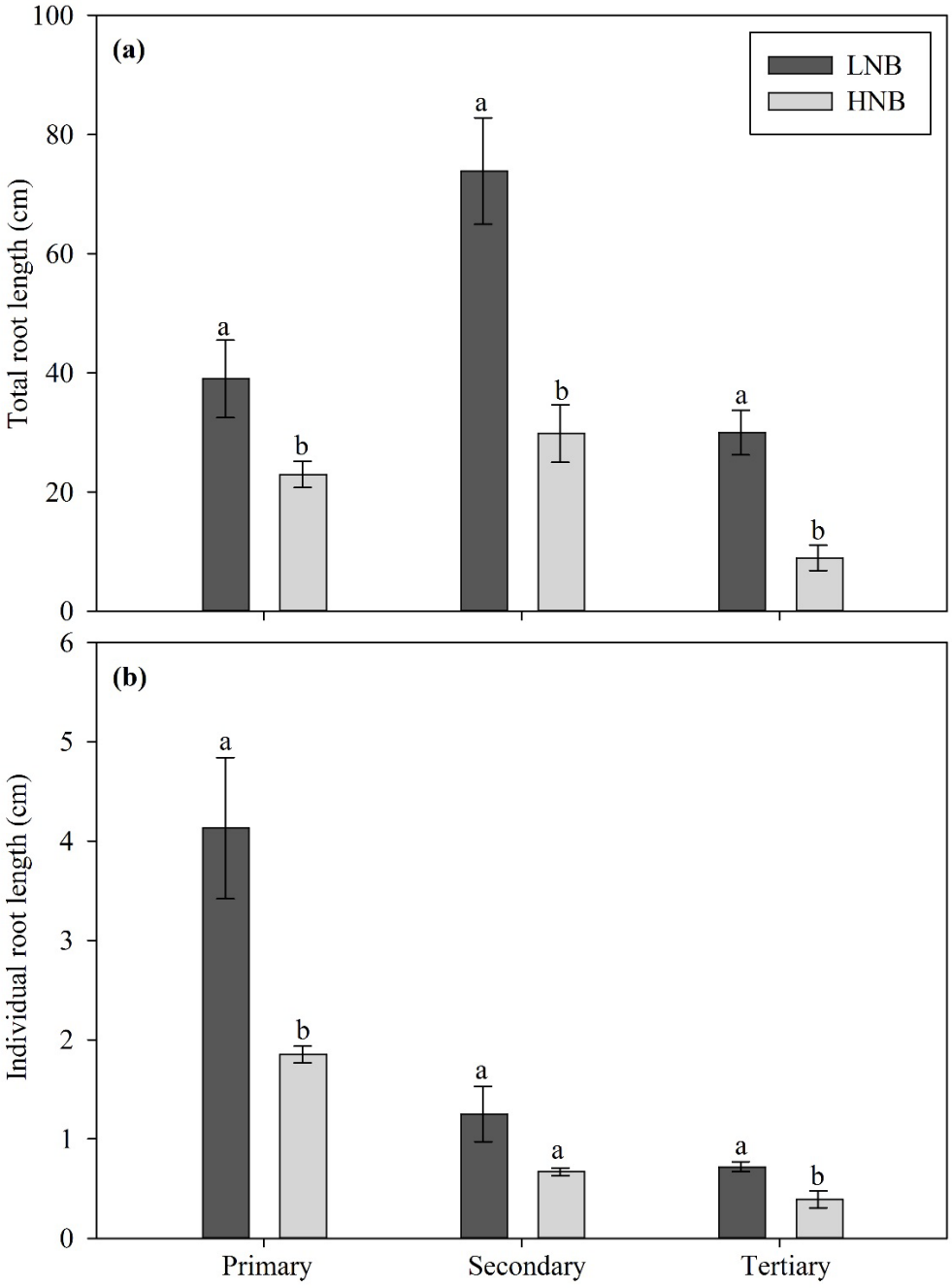

Figure 3.10. Root length of primary, secondary, and tertiary roots of large crabgrass grown in split-pattern rhizobox with field soil amended with either 2% low-nutrient biochar (LNB) or 2% high-nutrient biochar (HNB). Total root length **(a)** represents the sum of all roots within a root order (primary, secondary, or tertiary). Individual root length **(b)** represents the average length of a single root within a root order (primary, secondary, or tertiary). Columns represent means (n=8); error bars represent standard error of the mean. Within a root order, means with different letters indicate significant statistical difference (P<0.05).

3.5. Discussion

Large crabgrass growth was affected by biochar feedstock. Plants grown in HNB produced greater shoot DW, greater secondary and tertiary root diameters, lower insertion angles, more tillers and partitioned more biomass to shoots than plants grown in the unamended soil. Cumulatively, this suggests a fertilizer effect in which the addition of HNB to the soil reduced the need for large crabgrass to invest in roots in order to take up nutrients. Plants grown in the LNB soil had greater root DW, root mass and root length densities, and total and individual root lengths for secondary and tertiary roots than plants grown in the unamended soils. However, no differences were detected in biomass partitioning between the LNB and control treatments. Increased root length and root proliferation can reduce the distance nutrients travel by diffusion and mass flow while also improving uptake by increasing root surface area (White et al. 2013). Root proliferation can occur in response to the presence of nutrients (Drew 1975). If nutrient availability was relatively high in the HNB treatment, intermediate in the LNB treatment, and low in the control (HNB>LNB>Control) then one might expect biomass partitioning to favor shoots in HNB, a proliferation of roots in the LNB treatment in response to nutrients, and more limited growth in the nutrient-poor control soil. This explanation is consistent with root growth in the split rhizoboxes where large crabgrass roots preferentially foraged in soil containing both types of biochar.

Large crabgrass preferentially foraged in biochar-amended soil in the split design rhizoboxes. The effect of biochar on crop root growth was not addressed in this study but it is reasonable to expect that cereal crops might also preferentially forage in soil

amended with biochar. Conventional fertilizers and irrigation are commonly applied in bands to supply resources and limit weed competition in crop rows (Anderson 2000). Herbicides can also be applied in bands to control weeds either in or between rows (Bates et al. 2012). Biochar could also be applied in bands to limit positive effects to crops. This could be accomplished by banding biochar within but not between crop rows or by banding biochar vertically in the soil profile. The latter approach might be particularly useful for discriminating between small-seeded species that germinate on or near the soil surface and larger-seeded species that can emerge from greater depths.

Davis et al. (1967) compared the root profiles and shoot dry weights of seven weed species grown in field conditions and found that mature large crabgrass had a relatively large root profile of approximately 5-m wide and 2-m deep, but had the smallest shoot DW of all seven species. Similarly, large crabgrass plants grown in the control and LNB-amended soils had a root : shoot ratio greater than one thereby exhibiting a root-dominated plant allometry. In a review of maize root systems, Lynch (2013) suggested that maize will develop longer primary roots to capture mobile nutrients such as N and greater lateral insertion angles to capture immobile nutrients such as P and K. Lateral insertion angles were greater in the control treatment than in the HNB biochar treatment, suggesting that the roots in the control soils were foraging for immobile nutrients, which is consistent with the soil analysis results where HNB-amended soil had significantly greater P and K than the control (Table 2.1.). The lateral insertion angles for the biochar treatments varied by root order (Figure 2.7.). However, the relatively small insertion angles of large crabgrass laterals in biochar soils suggest foraging for N, which is a mobile nutrient and generally found deeper in the soil profile (Lynch 2013). This is

supported by the root length and diameter of plants grown in the biochar soils. Plants grown in the LNB soil had fewer but significantly longer and thicker primary roots than plants grown in the control soil (Figure 2.5.(b)). A longer, narrower root system may also be a characteristic of drought tolerance (Rogers and Benfey 2015). However, since biochar generally increases water holding capacity of soils (Novak et al. 2012) and the LNB-amended soils had significantly less N than both the control and the HNB-amended soils (Table 2.1.), it is more likely that the responses seen in the root system architecture are related to nutrient availability in the current study. Plant tissue analyses for nutrient content are necessary to confirm these findings.

This is the first detailed characterization of large crabgrass root growth and RSA. More research, combining phenotyping with genotyping and plant tissue analyses for nutrient content, is necessary to fully understand the complex root system of large crabgrass and how it interacts with the soil environment. However, this study suggests that the roots of large crabgrass, a globally important weed, will preferentially spread into soil enriched with biochar. Furthermore, the addition of biochar to soils has the potential to increase large crabgrass tiller production and to increase the ability of large crabgrass to produce longer primary roots to forage for nutrients.

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CHAPTER 4. THE EFFECT OF TWO TYPES OF BIOCHAR ON THE GROWTH OF FOUR SPECIES

4.1. Abstract

Amending agricultural soils with biochar, a carbon-rich product similar to charcoal, has been suggested as a way to increase crop yields while sequestering carbon. However, biochar may also increase weed growth, which could reduce crop yields. The objective of this study was to determine the effects of two types of biochar on the growth of two crops (sweet corn, red clover) and two weed species (large crabgrass, redroot pigweed). Plants were grown under greenhouse conditions with or without biochar using a randomized complete block design. Two types of biochar produced at 450 C were used to amend a sandy loam field soil at a rate of 2% of the soil dry weight: a low-nutrient biochar produced from a mixture of softwoods and a high-nutrient biochar produced from loblolly pine and switchgrass. Unamended field soil was used as the control. Plants were grown to maturity and plant biomass was dried and weighed. Biochar, regardless of type did not affect height or total plant dry weight of redroot pigweed but the high-nutrient biochar increased inflorescence dry weight. Biochar, regardless of type, increased redroot pigweed partitioning of biomass to shoots at the expense of roots. High-nutrient and low-nutrient biochar increased stem, root, and total dry weight of large crabgrass. Large crabgrass plants grown in the high-nutrient biochar produced more tillers than

plants grown in the control. However, biochar did not affect biomass partitioning or tiller length of large crabgrass. Red clover plants grown with the high-nutrient biochar were taller and had greater stem, petiole, and inflorescence dry weight than control plants or plants grown in the low-nutrient biochar. However, differences in red clover height or dry weight were not detected between the low-nutrient and control treatments. The low-nutrient biochar increased nodule fresh weight of red clover 20% over the control and 36% over the high-nutrient biochar. Sweet corn produced more ear and total dry weight in the high-nutrient treatment than in the control treatment. Biochar did not affect sweet corn height or root dry weight. However, both types of biochar accelerated sweet corn phenology relative to the control. Biochar increased the growth of both crop species suggesting that the incorporation of biochar, especially high-nutrient biochar, into temperate agricultural soils may increase crop yields. However, biochar also increased weed growth, which may complicate current weed management practices.

Nomenclature: Large crabgrass, *Digitaria sanguinalis* L. Scop.; red clover, *Trifolium pretense* L.; redroot pigweed, *Amaranthus retroflexus* L.; sweet corn, *Zea mays* L. var. Fisher's Earliest.

Keywords: Biochar, biomass partitioning, black carbon, char, crop productivity, legume, nodule fresh weight, weeds.

4.2. Introduction

Biochar is a carbon-rich soil additive similar to charcoal that is produced by heating biomass in a low oxygen chamber at temperatures typically between 300 and 1000 C (Verheijen et al. 2010). The incorporation of biochar into soils has been proposed as a means to sequester carbon and to improve soil health and crop yields. Two recent meta-analyses have suggested that, on average, adding biochar to agricultural soils can increase crop productivity by 10% (Jeffery et al. 2011) or 11% (Liu et al. 2013). A third meta-analysis (Biederman and Harpole 2013) also reported increased crop yields across a wide range of soils and climates. Biochar has multiple and complex effects on soils and soil organisms including greater water retention (Novak et al. 2012), enhanced microbial activity (Quilliam et al. 2012), and increased nutrient retention and availability (Cornelissen et al. 2013). Thus the effect of biochar on crop yields can go beyond a simple fertilizer effect. Positive relationships have been observed between crop productivity and a wide range of biochar feedstocks, i.e. the biomass used during production (Jeffery et al. 2011). However, Liu et al. (2013) found that the magnitude and direction of the crop response varied with feedstock type and pyrolysis temperature.

It is well known that weeds reduce crop yields; however, the majority of published research on biochar focuses on its effect on crop species (Jeffery et al. 2011; Biederman and Harpole 2013) and there is little published research on the effect of biochar on weed species (Major et al. 2005; Quilliam et al. 2012). In a study conducted on the low fertility, highly acidic soils of the Central Brazilian Amazon (Major et al. 2005), biochar incorporated into the soil at a rate of 11 t ha^{-1} did not increase weed cover.

However, biochar plus an inorganic fertilizer increased weed cover more than the inorganic fertilizer alone. In a similar study conducted on temperate agricultural soils (Quilliam et al. 2012), biochar incorporated into a sandy clay loam soil at a rate of 25 or 50 t ha^{-1} produced no long-term effect on weed seedling emergence. When biochar was reapplied after three years weed seedling emergence was reduced at both the 25 and 50 t ha^{-1} rates. Quilliam et al. (2012) suggested that soil microbial activity or residue from a pre-emergent herbicide may have played a role in the reduction of weed emergence.

The objective of this work was to determine the effects of two types of biochar on two common agricultural weed species and two crop species grown under greenhouse conditions. Large crabgrass, a C_4 monocot, (Turner et al. 2013) and redroot pigweed, a C_4 dicot, (Weaver and McWilliams 1980) are summer annual weed species. Both are considered problematic in agriculture due to their competitive nature and are found throughout most temperate regions worldwide (Mitich 1988; Weaver and McWilliams 1980). Sweet corn and red clover are commonly grown crops in temperate regions. Sweet corn, a C_4 monocot, is a warm season annual while red clover, a C_3 dicot, is a leguminous short-lived perennial.

4.3. Materials and Methods

A randomized complete block design with two main treatments (biochar type and plant species) in four blocks was used. Two weed species, large crabgrass and redroot pigweed (Azlin Seed Service, 112 Lilac Drive, Leland, MS, 38756), and two crop species, red clover and sweet corn (High Mowing Organic Seeds, 76 Quarry Road,

Wolcott, VT, 05680), were grown in 2.5 L soil-filled pots with four replicates of each treatment in two greenhouse trials initiated in Fall of 2013.

4.3.1. Soil and biochar properties

A sandy loam field soil, Desker series (coarse-loamy, mixed, superactive, mesic Mollic Hapludalfs), was collected in June 2013 from the top 10 cm of a conventional agriculture field located at Throckmorton Purdue Agricultural Center (8343 South US 231, Lafayette, IN 47909, US; 40°17’42.0”N 86°54’33.8”W). The field soil was pulverized using a Model 112 Royer Shredder-Mixer (Royer, Ind., 6856 Howlett Road, Oshkosh, WI 54902, US). Two types of biochar were used: a high-nutrient biochar (HNB) and a low-nutrient biochar (LNB). Both types of biochar were produced at the same temperature (450 C) but from two different feedstocks. The HNB was produced from a mixture of loblolly pine (*Pinus taeda*) and switchgrass (*Panicum virgatum*) by a commercial producer (Eprida, Inc., 3020 Canton Road Suite 105, Marietta, GA 30066, US). The LNB was produced from a mixture of fir, pine, and spruce by a commercial producer (Diacarbon Energy, Inc., 2250 Boundary Road 120, Burnaby, BC V5M 3Z3, Canada). Both types of biochar and the field soil were passed through a 4-mm mesh sieve separately to achieve uniform particle size. The field soil was amended with one of the two types of biochar at a rate of 2% of the soil dry weight (DW) and thoroughly mixed together in a 50 L electric concrete mixer for 2 h. Unamended field soil was also mixed for 2 h and used as the control.

Total carbon and nitrogen were determined in quintuplicate for all treatments by element analyzer (Table 1) (Thermo Scientific FlashEA 1112 series). Four 500g samples of each type of biochar, each type of amended soil, and the unamended soil were sent to a commercial laboratory for analysis of organic matter (OM), pH, cation exchange capacity (CEC), and extractable Bray 2-phosphorus, potassium (K), magnesium (Mg), and calcium (Ca) (Table 1) (A&L Great Lakes Laboratories, 3505 Conestoga Drive, Fort Wayne, Indiana 46808, US). Loss-on-ignition of the dry mass at 360 °C was used to measure percent OM content (Nelson and Sommers 1996). Plant-available nutrients (K, Mg, and Ca) were extracted using the Mehlich III method and analyzed by inductively coupled plasma-atomic emission spectroscopy (Mehlich 1984). The CEC was measured using a modified ammonium-acetate compulsory displacement and pH was determined by a 1:1 ratio of soil:water (Sumner and Miller 1996).

4.3.2. Growth conditions

The greenhouse trial was initiated on 2 December 2013 and repeated on 16 December 2013. Red clover seeds were inoculated with *Rhizobium leguminosarum* biovar trifolii. Seeds were germinated on moist filter paper and three seedlings of a consistent height were transplanted into each 2.5 L pot filled with field soil (+/- biochar). Pots were thinned to a single plant within two weeks of transplanting. Every two weeks, pots within each block were re-randomized to limit micro-climate effects. Minimum and maximum air temperatures and humidity were recorded daily. Average minimum and maximum temperatures were 9.6 C (±0.4 SE) and 26.1 C (±0.4 SE) in the first trial and

8.4 C (±0.4 SE) and 27.3 C (±0.3 SE) in the second. Average minimum and maximum humidity were 21.3% (±0.3 SE) and 42.2% (±0.6 SE) in the first trial and 23.4% (±0.4 SE) and 40.5% (±1.0 SE) in the second. Supplemental lighting was used to simulate the 14.5 h photoperiod of an average day in May in Indiana.

4.3.3. Harvest and data collection

Sweet corn was grown for 112 days after transplanting (DAT). Large crabgrass and redroot pigweed were grown for 120 DAT. Red clover was grown for 140 DAT. Daily observations on plant phenology were recorded for all species. Plant height was recorded before harvest. Large crabgrass has a prostrate growth habit and the length of the longest tiller was measured rather than height. The number of large crabgrass tillers was also recorded. Plants were harvested by cutting the stem at the soil surface and roots were carefully washed over a fine mesh to remove soil. Immediately after harvest, the red clover root systems were divided into four sections. One section of root was selected at random, nodules were counted, and the root section was dried at 60 C to a constant weight. Number of nodules per gram of root DW was calculated (nodule count divided by root section DW). A minimum of 100 nodules were excised from a separate root section selected at random, nodules were counted, and fresh weight was measured. The average nodule fresh weight was calculated (nodule fresh weight divided by number of nodules). Plant organs, i.e. leaves, stems, roots, and inflorescences, were placed into separate paper bags and dried at 60 C to a constant weight. Plant biomass partitioning ratios were calculated. Root shoot ratio (RSR) is root DW divided by above-ground DW.

Root weight ratio (RWR) is root DW divided by total plant DW. Shoot weight ratio (SWR) is above-ground DW divided by total plant DW.

4.3.4. Statistical analysis

Data were checked for normality and no transformations were required. Error variances between greenhouse trials were tested to determine if trials could be combined. Variances were found to be homogeneous and data from greenhouse trials were combined. Analyses of variance (ANOVA) was conducted using SAS 9.4 (SAS Institute Inc., Cary, NC, USA). Least significant difference (LSD) was used to compare means.

4.4. Results

4.4.1. Biochar and soil analyses

The HNB had greater total C and N, higher CEC, and more P, K, Mg, and Ca than the LNB (Table 3.1.). However, pH and C:N ratio were greater in the LNB than in the HNB. Differences in percent OM were not detected between the biochar types. The pH and C:N ratio were greater for the LNB-amended soil than for the control and the HNB-amended soils. The pH did not differ between the control soil and the HNB-amended soil but C:N ratios were greater in the HNB-amended soil than in the control soil. Percent C was greater in both biochar-amended soils than in the control soil. Percent N was greater in the control and the HNB-amended soils than in the LNB-amended soil. The OM was greatest in the HNB-amended soil and lowest in the control soil. No differences were

detected among soils for CEC or Ca. Soil test K and Bray phosphorus were greater in the HNB-amended soil than in the control soil or LNB-amended soil. Available Mg was greater in the control and HNB-amended soils than in the LNB-amended soil (Table 3.1.).

4.4.2. Redroot pigweed

Biochar did not affect the total or component DW of redroot pigweed, with the exception of inflorescence DW (Figure 3.1.(a)). Inflorescence DW was greater for plants grown with the HNB than for plants in the control treatment. Although redroot pigweed total DW was not affected by biochar, plants grown with biochar increased biomass partitioning to shoots at the expense of roots (Figure 3.1.(b)). There were no differences detected in biomass partitioning between the biochar types. Biochar did not affect redroot pigweed height; averaged across treatments redroot pigweed plants were 64.7 cm SE±2.8.

4.4.3. Large crabgrass

Large crabgrass total and component DW were greater for plants grown in the HNB treatment than in the control treatment (Figure 3.2.(a)). Large crabgrass grown with HNB produced nearly twice as much total DW as the control plants. Panicle DW was also nearly twice as high for plants grown with HNB than for control plants. Stem, root, and total DW were also greater in the LNB treatment than in the control. However, plants grown in the LNB soil produced less stem, root, and total DW than plants grown in the HNB soil. Biochar did not affect large crabgrass biomass partitioning ratios (Figure

3.2.(b)) or length of the longest tiller. Large crabgrass tiller length, averaged across treatments, was 108.7 cm SE±4.4. Plants grown with HNB produced more tillers than plants grown in the control or LNB treatments (Figure 3.3.). The number of tillers did not differ between the control and LNB treatment.

4.4.4. Red clover

Stem, petiole, and inflorescence DW were greater for plants grown with HNB than for plants grown in the control treatment (Figure 3.4.(a)). HNB more than tripled inflorescence DW compared to the control. Differences between the HNB and LNB treatments were only detected for stem DW. Total DW was not affected by either biochar treatment (Figure 3.4.(a)). Biochar increased the partitioning of biomass to shoots at the expense of roots (Figure 3.4.(b)). The RWR in the control treatment was greater than in the LNB or HNB treatments and RWR was greater for plants grown with LNB than for plants grown with HNB (Figure 3.4.(b)). Both types of biochar increased SWR relative to the control; SWR was greater for the HNB treatment than for the LNB treatment. Red clover height was increased by the HNB treatment relative to the control (Figure 3.5.). No differences were detected in height between the control and LNB treatments (Figure 3.5.). Nodule fresh weight increased with the LNB treatment but did not differ between the control and HNB treatments (Figure 3.6.(a)). Biochar did not affect the number of nodules per gram of root DW (Figure 3.6.(b)).

4.4.5. Sweet corn

The HNB increased ear DW by 198% and total DW by 52% over the control (Figure 3.7.(a)). The LNB increased ear DW but not total DW relative to the control. Biochar did not affect stem, leaf, tassel, or root DW. The HNB increased the partitioning of biomass to shoots at the expense of roots (Figure 3.7.(b)). The RWR was greater for the control plants than for HNB plants while the SWR was greater in the HNB treatment than in the control. Biochar did not affect height of sweet corn; averaged across treatments sweet corn plants were 105.5 cm SE±7.0. Both types of biochar accelerated sweet corn phenology relative to the control (Figure 3.8.).

Table 4.1. Characteristics of unamended field soil (Control)[a], low-nutrient biochar (LNB)[b], high-nutrient biochar (HNB)[c], and the soils amended with 2% biochar (LNB- and HNB-amended soil) prior to conducting experiment. Values are means of four samples; parentheses enclose standard error of the mean. Values with different letters indicate significant differences were detected (P<0.05).[d]

	pH	C : N	Total C	Total N	OM	CEC	K	Mg	Ca	P
			%	%	%	meq 100 g^{-1}	ppm			
LNB	9.52 **a**	259.0 **a**	55.2 **b**	0.21 **b**	65.4 **a**	0.8 **b**	135 **b**	14 **b**	60 **b**	1 **b**
	(0.14)	(14.2)	(1.0)	(0.01)	(0.3)	(0.1)	(14.1)	(2.9)	(10.0)	(0.2)
HNB	7.08 **b**	45.0 **b**	61.4 **a**	1.36 **a**	64.4 **a**	15.5 **a**	3,742 **a**	361 **a**	588 **a**	296 **a**
	(0.03)	(1.3)	(1.2)	(0.05)	(0.8)	(0.2)	(66.5)	(2.4)	(12.5)	(6.0)
Control	7.03 **b**	11.7 **c**	2.1 **b**	0.18 **a**	2.7 **c**	8.9 **a**	175 **b**	236 **a**	1,300 **a**	55 **b**
	(0.03)	(0.1)	(0.1)	(<0.01)	(0.04)	(0.2)	(3.4)	(3.2)	(20.4)	(0.6)
LNB-amended soil	7.25 **a**	21.0 **a**	3.2 **a**	0.15 **b**	2.9 **b**	8.7 **a**	191 **b**	223 **b**	1,263 **a**	54 **b**
	(0.03)	(0.5)	(0.1)	(<0.01)	(0.1)	(0.2)	(2.8)	(4.3)	(31.5)	(0.9)
HNB-amended soil	7.08 **b**	15.5 **b**	3.0 **a**	0.19 **a**	3.7 **a**	9.3 **a**	365 **a**	244 **a**	1,275 **a**	67 **a**
	(0.03)	(0.4)	(0.1)	(<0.01)	(0.03)	(0.2)	(7.6)	(5.2)	(32.3)	(1.1)

[a]Desker series (coarse-loamy, mixed, superactive, mesic Mollic Hapludalfs) consisting of approximately 68% sand, 22% silt, and 10% clay.

[b]Low-nutrient biochar (LNB) was produced by slow-pyrolysis at 450°C from a mixture of fir, pine, and spruce.

[c]High-nutrient biochar (HNB) was produced by slow-pyrolysis at 450°C from loblolly pine and switchgrass.

[d]Abbreviations: C : N, carbon : nitrogen ratio; OM, organic matter; CEC, cation exchange capacity; meq 100 g^{-1}, milliequivalent per 100 grams of dry soil; ppm, parts per million.

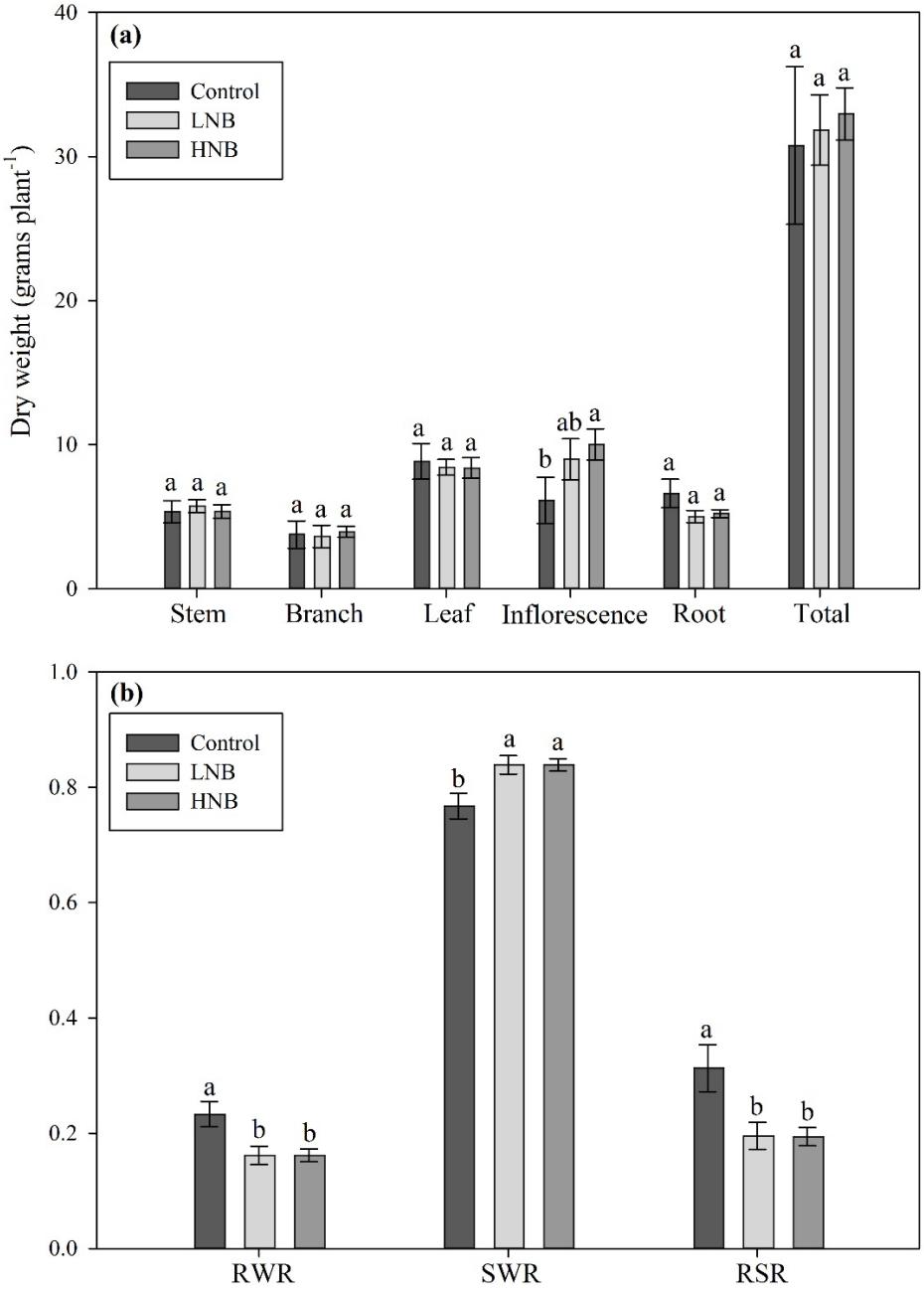

Figure 4.1. (a) Plant component and total dry weight (DW) and (b) plant biomass partitioning ratios of redroot pigweed grown in unamended field soil (Control) and field soil amended with 2% low-nutrient biochar (LNB) or 2% high-nutrient biochar (HNB). Root weight ratio (RWR) is the root DW divided by the total plant DW. Shoot weight ratio (SWR) is the above-ground DW divided by the total plant DW. Root : shoot ratio (RSR) is the root DW divided by the above-ground DW. Columns represent means (n=7 to 8); error bars represent standard error of the mean. Means with different letters indicate significant statistical difference (P<0.05).

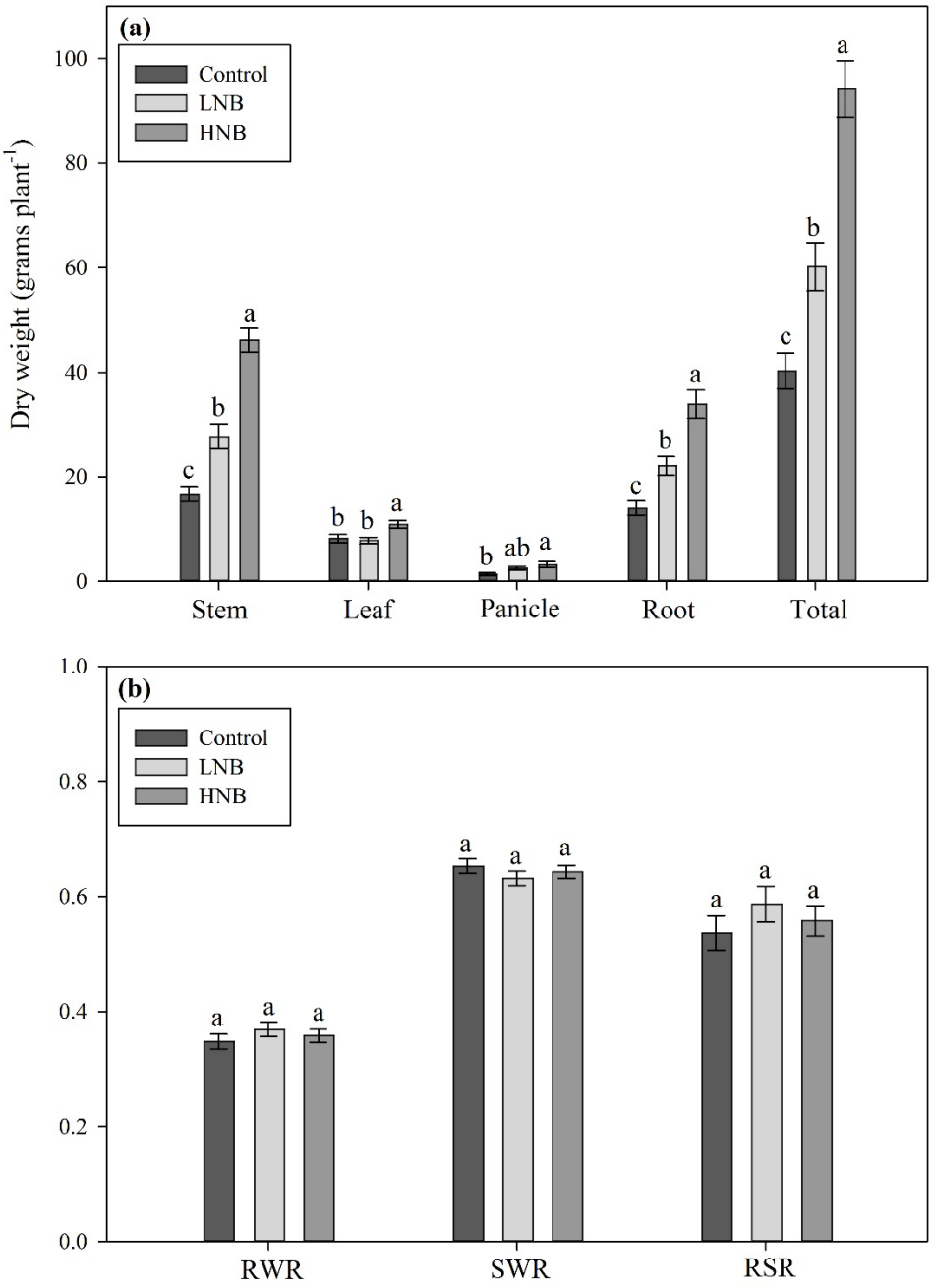

Figure 4.2. (a) Plant component and total dry weight (DW) and (b) plant biomass partitioning ratios of large crabgrass grown in unamended field soil (Control) and field soil amended with 2% low-nutrient biochar (LNB) or 2% high-nutrient biochar (HNB). Root weight ratio (RWR) is the root DW divided by the total plant DW. Shoot weight ratio (SWR) is the above-ground DW divided by the total plant DW. Root : shoot ratio (RSR) is the root DW divided by the above-ground DW. Columns represent means (n=8); error bars represent standard error of the mean. Means with different letters indicate significant statistical difference (P<0.05).

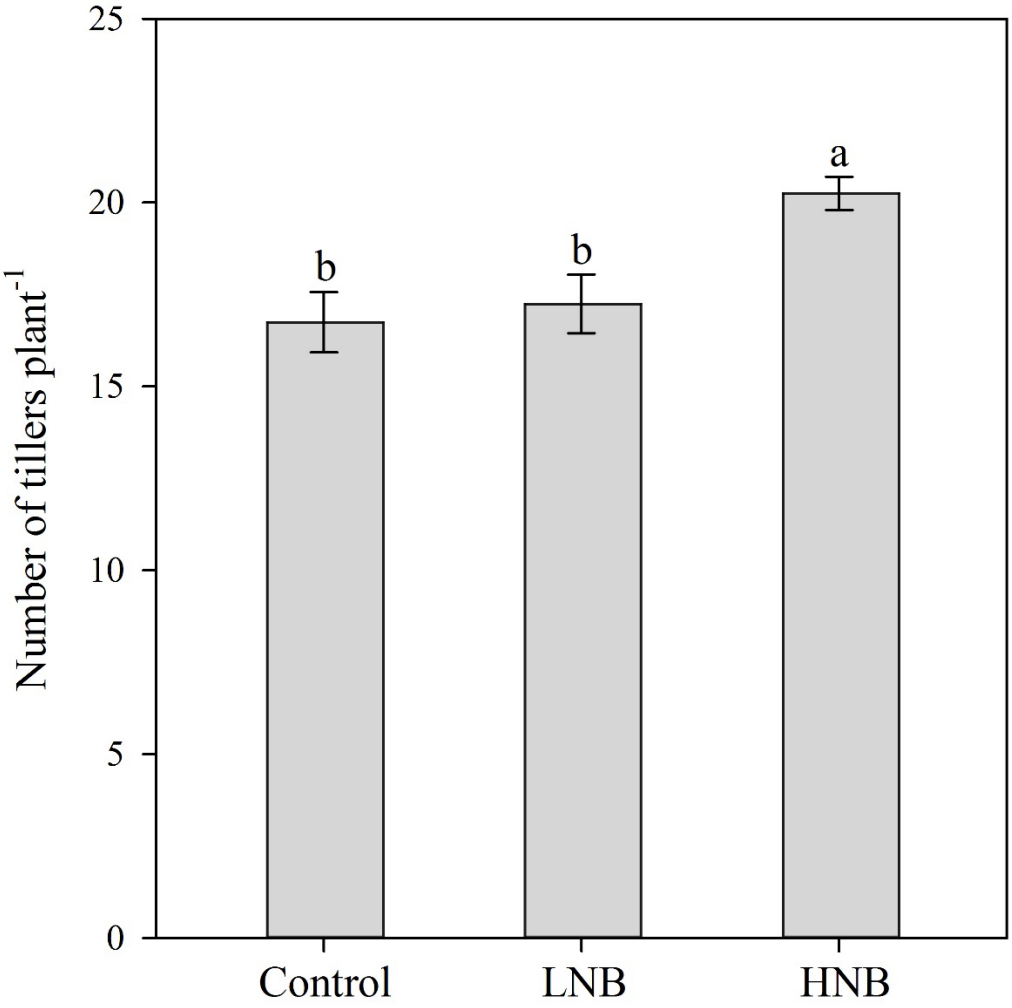

Figure 4.3. The number of tillers produced by large crabgrass grown in unamended field soil (Control) and field soil amended with 2% low-nutrient biochar (LNB) or 2% high-nutrient biochar (HNB). Columns represent means (n=8); error bars represent standard error of the mean. Means with different letters indicate significant statistical difference (P<0.05).

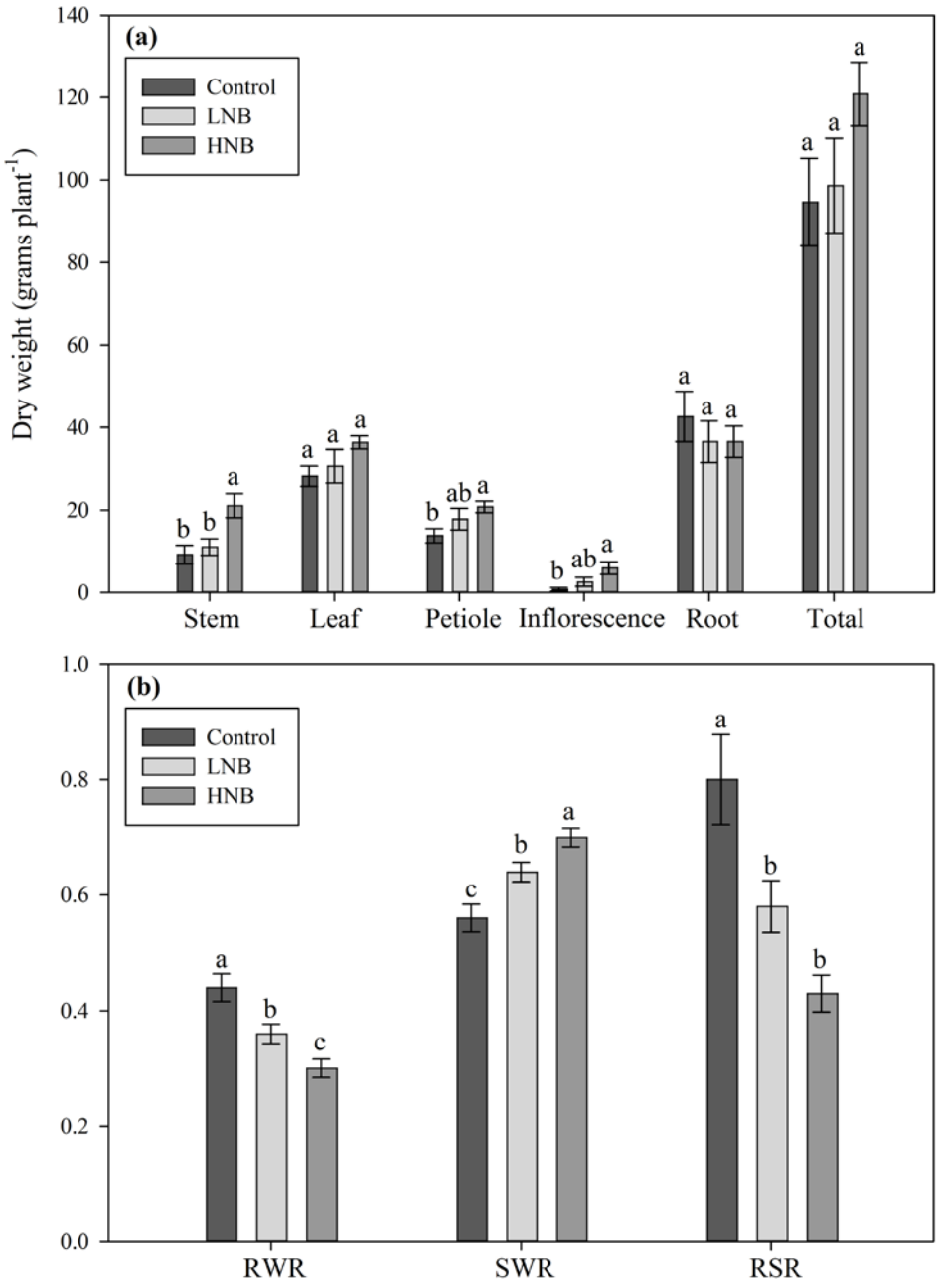

Figure 4.4. (a) Plant component and total dry weight (DW) and (b) plant biomass partitioning ratios of red clover grown in unamended field soil (Control) and field soil amended with 2% low-nutrient biochar (LNB) or 2% high-nutrient biochar (HNB). Root weight ratio (RWR) is the root DW divided by the total plant DW. Shoot weight ratio (SWR) is the above-ground DW divided by the total plant DW. Root : shoot ratio (RSR) is the root DW divided by the above-ground DW. Columns represent means (n=8); error bars represent standard error of the mean. Means with different letters indicate significant statistical difference (P<0.05).

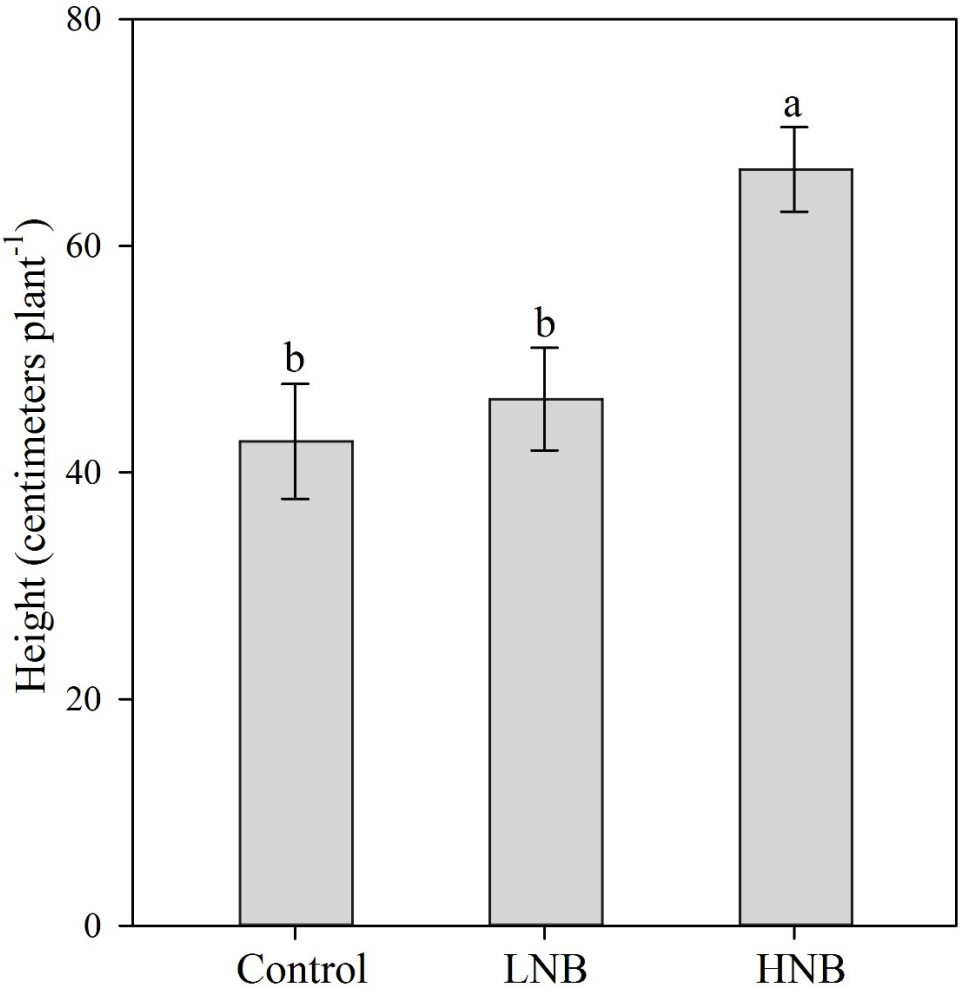

Figure 4.5. Height of red clover grown in unamended field soil (Control) and field soil amended with 2% low-nutrient biochar (LNB) or 2% high-nutrient biochar (HNB). Columns represent means (n=8); error bars represent standard error of the mean. Means with different letters indicate significant statistical difference ($P<0.05$).

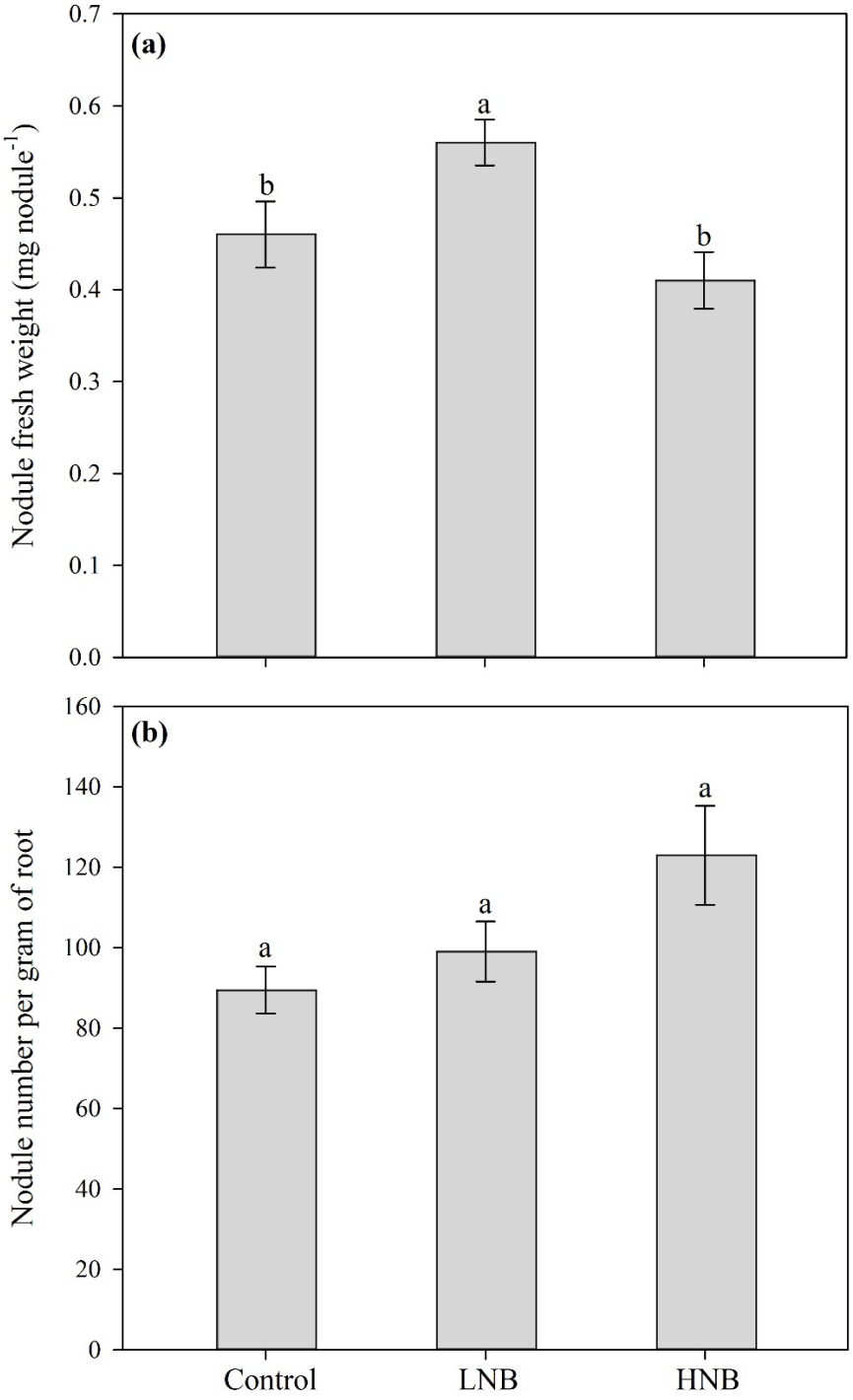

Figure 4.6. (a) Average nodule fresh weight and (b) the number of nodules produced per gram of root dry weight of red clover grown in unamended field soil (Control) and field soil amended with 2% low-nutrient biochar (LNB) or 2% high-nutrient biochar (HNB). Columns represent means (n=8); error bars represent standard error of the mean. Means with different letters indicate significant statistical difference (P<0.05).

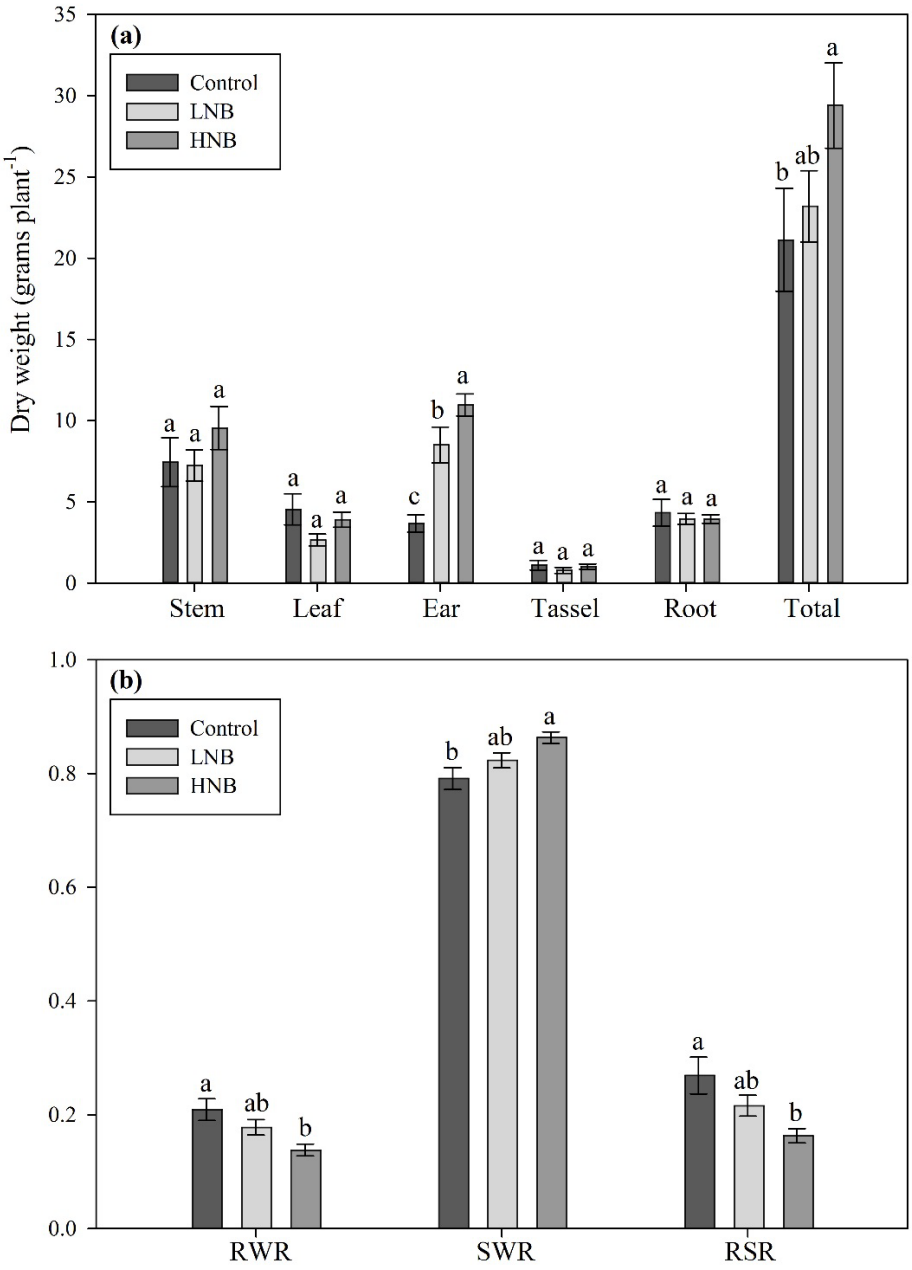

Figure 4.7. (a) Plant component and total dry weight (DW) and (b) plant biomass partitioning ratios of sweet corn grown in unamended field soil (Control) and field soil amended with 2% low-nutrient biochar (LNB) or 2% high-nutrient biochar (HNB). Root weight ratio (RWR) is the root DW divided by the total plant DW. Shoot weight ratio (SWR) is the above-ground DW divided by the total plant DW. Root : shoot ratio (RSR) is the root DW divided by the above-ground DW. Columns represent means (n=8); error bars represent standard error of the mean. Means with different letters indicate significant statistical difference (P<0.05).

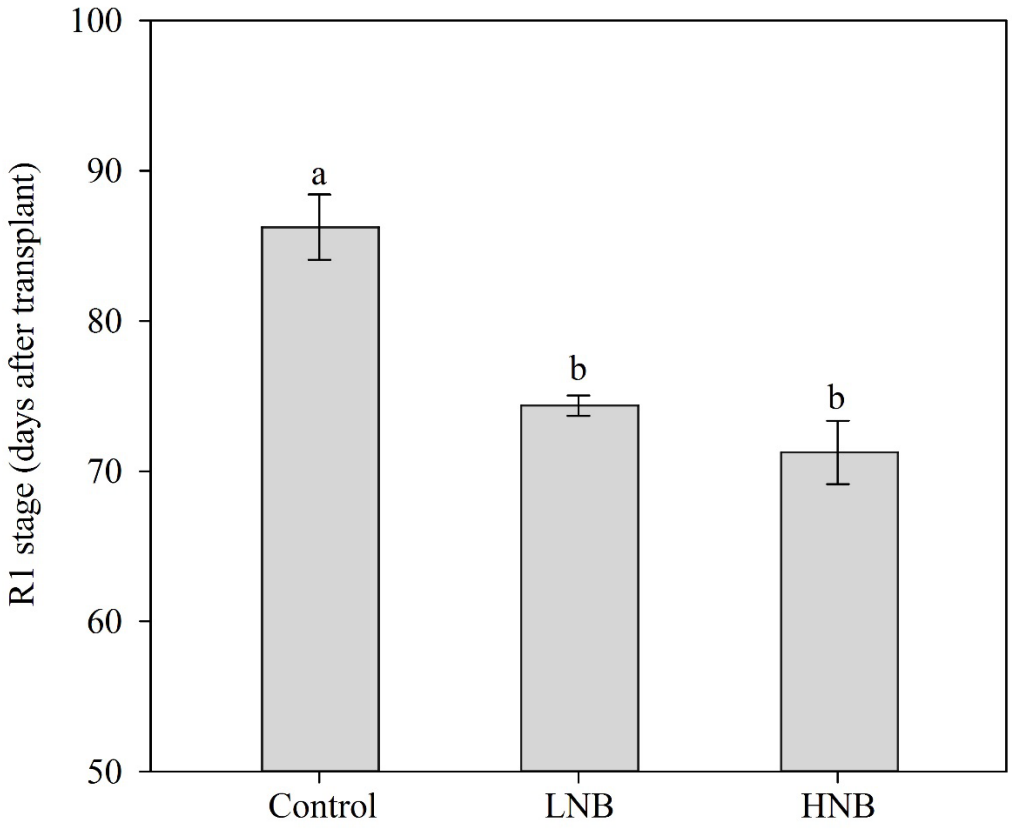

Figure 4.8. The days after transplant that sweet corn grown in unamended field soil (Control) and field soil amended with 2% low-nutrient biochar (LNB) or 2% high-nutrient biochar (HNB) reached the R1 or silking stage. Columns represent means (n=8); error bars represent standard error of the mean. Means with different letters indicate significant statistical difference ($P<0.05$).

4.5. Discussion

In this study, we investigated the effect of two biochars produced from different feedstocks on the growth of weed and crop species. The HNB-amended soil increased the reproductive DW (inflorescence, panicle, or ear) of both weed and crop species and the total DW of both large crabgrass and corn. The HNB also increased the partitioning of biomass to shoots at the expense of roots for all species except large crabgrass. The crop and weed species showed a similar but generally weaker response to the LNB. It is tempting to attribute the effect of biochar to a simple fertilization effect, particularly since the magnitude of plant responses was generally greater for the HNB. The HNB treatment did not increase soil pH, total N, Ca, or CEC relative to the control treatment but did increase percent OM, total C, K, and P. However, plant tissue analyses for nutrient content are necessary to confirm that the biochar had a fertilizer effect.

The LNB increased nodule fresh weight relative to the control and HNB treatments. Similarly, Ogawa and Okimori (2010) reported that soybean root nodule formation was increased by a wood charcoal. They suggested that the higher pH in the charcoal-amended soil was responsible or that biochar might serve as a habitat for root nodule bacteria. Soil pH was greater in LNB-amended soils than in the control or HNB-amended soils in our study as well. In contrast, Quilliam et al. (2013) grew white clover (*Trifolium repens*) with and without biochar derived from wood and found no effect of biochar on the total number of root nodules or the nodule dry weight at the end of three years. However, total nitrogenase activity was greater in biochar-amended soils than in the unamended soil. When biochar was reapplied after three years, the total number of

root nodules decreased while the nodule dry weight increased regardless of the application rate. In a similar study, Mia et al. (2014) reported that biochar increased the biological nitrogen fixation of red clover. Our results support previous research showing an effect of biochar on root nodulation but suggest that effects may vary substantially with feedstock. Further studies investigating nodulation and nitrogenase activity of legumes grown in biochar-amended soils, using biochars derived from different feedstocks, are warranted.

Our research supports the hypothesis that biochar amendments will increase both weed and crop growth. The effect of biochar on competition between weeds and crops remains unknown. However, several studies have demonstrated that biochar binds to herbicides, which may reduce the leaching or runoff of agrochemicals into the water but also result in a decrease in bioavailability and efficacy (Yu et al. 2006; Cao et al. 2009, Spokas et al. 2009; Zheng et al. 2010, Graber et al. 2012). Graber et al. (2012) tested the effect of two biochars, incorporated at rates of 0, 13, 26, and 52 Mg ha^{-1}, on the bioavailability of S-metolachlor and sulfentrazone at two dose rates. Herbicide efficacy was reduced even when the herbicides were applied at their maximum, or near maximum, recommended dose rates. The sorption of agrochemicals to biochar may prove useful in cases of environmental remediation; however, this same attribute could prove detrimental in an agricultural setting where farmers rely on the efficacy of these chemicals. With the reduction in efficacy, the unintentional underdosing of herbicides could lead to faster emergence of herbicide resistant weed species (Kookana et al. 2011). Therefore, further research is warranted on the effect of biochar on the growth of agricultural weed species.

4.6. References

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CHAPTER 5. CONCLUSION

Biochar is often referred to as a 'win-win' solution for increasing crop productivity while mitigating climate change (Biederman and Harpole 2013). Soil properties, and subsequently crop yields, may improve with the incorporation of biochar into agricultural fields. However, substantial challenges need to be addressed and overcome before the widespread application of biochar to agricultural fields is possible in the United States generally, and the Midwest in particular. First, the cost of biochar remains prohibitive at \$400 to \$600 ton^{-1}. With recommended application rates ranging from 9 to 22 tons $acre^{-1}$ (20 to 50 metric tons ha^{-1}) or more, a single application of biochar could cost anywhere from \$3,600 to \$13,200 $acre^{-1}$. Shipping and handling charges could potentially add hundreds to thousands of dollars to that cost. Second, the availability of biochar must be addressed when considering the application of biochar on a large-scale. Biochar is readily available to the homeowner or small gardener in small quantities of 10 to 100 lbs. However, farmers seeking to apply biochar to large-scale operations (hundreds to thousands of acres), would find it difficult to find a source capable of supplying the tons of biochar needed to apply to their fields. Third, if an increase in crop yield or soil pH balancing is desired, traditional fertilizers and lime are more readily available and cost effective than biochar for farmers in the United States. The current annual cost of fertilizer and lime for a continuous corn cropping system is

estimated to be \$154 acre^{-1} (Plastina 2015). Finally, although carbon sequestration is a potential benefit of biochar application, there is not a system in place to help offset the costs of biochar application by providing carbon credits or carbon sequestration payments to farmers in the United States.

In addition to the factors listed above, biochar may pose a particular challenge for conventional farmers by increasing their weed problems. Although considerable research on biochar has been conducted during the past decade, the effect of biochar on agricultural weeds has been neglected. It is well-known that weeds reduce crop yields, either by direct competition for nutrients and water or by harboring harmful insects and diseases. If the beneficial effect that biochar has on crop species extends to weed species, then potential gains in crop yields may be lost due to increased weed pressure. Most farmers rely heavily on herbicides for weed control but two factors call that reliance into question. First, no herbicides with new modes of action have been brought to market in nearly two decades (Green 2014). Second, glyphosate resistance as well as resistance to many other chemistries such as ALS and ACCase inhibitors have substantially reduced the efficacy of previous weed management practices. Increasingly, farmers are returning to the use of soil-applied pre-plant or pre-emergent herbicides in an effort to combat the resistance to glyphosate and ALS inhibitors. However, several studies found that biochar decreases the efficacy and bioavailability of soil-applied herbicides and other agrochemicals (Yu et al. 2006; Cao et al. 2009, Spokas et al. 2009; Zheng et al. 2010, Graber et al. 2012). The use of biochar may therefore result in lower herbicide efficacy and/or higher herbicide application rates being necessary for complete weed control, which would further increase the input costs for the farmer. If biochar also increases

weed growth, then applying it to agricultural soils may exacerbate weed problems. Three experiments were conducted to increase the understanding of how biochar affects both the above- and below-ground growth of common agricultural weed species.

In the first experiment, three common agricultural weed species, barnyardgrass, large crabgrass, and redroot pigweed, were grown to maturity under greenhouse conditions using a factorial design with biochar (0 and 2% of the soil dry weight) and nitrogen treatments (0 and 14 g N m^{-2}). It was hypothesized that biochar would increase the growth of all weed species and that the combination of biochar and nitrogen would have a synergistic effect, i.e. biochar plus a nitrogen fertilizer would increase weed growth more than biochar or nitrogen alone. However, each weed species had a different response to the biochar and there were no interactions detected between biochar and nitrogen. Biochar increased the total dry weight of barnyardgrass but did not affect root : shoot biomass partitioning. In contrast, biochar did not affect the total dry weight of large crabgrass but increased shoot dry weight by 34% and reduced root dry weight by 30%. Biochar increased both the height of barnyardgrass and tiller length of large crabgrass. Finally, biochar reduced the height of redroot pigweed by 30% but nearly doubled the branch dry weight. The unique, and sometimes unexpected, response of each species to the biochar-amendment suggests that, in this experiment, biochar did not have a simple fertilizer effect on all species.

The second experiment investigated the response of the large crabgrass root system in more detail. Large crabgrass was grown for 38 days after transplant in rhizobox mesocosms so the root growth and root system architecture could be analyzed *in situ*. The rhizoboxes were either filled uniformly with a field soil +/- biochar (solid) or

with a combination of amended and unamended soil (split) so that each type of soil occupied half of the rhizobox. Two types of biochar were used, one with a low-nutrient content and another with a high-nutrient content. Large crabgrass total dry weight did not vary among the treatments in the full treatment. However, root dry weight and root : shoot partitioning was greater for the low nutrient biochar than for the unamended soil. Shoot dry weight was greater and root : shoot partitioning was lower for the plants grown with the high nutrient biochar than for plants grown in the unamended soil. This suggests that the high nutrient biochar may have supplied nutrients in sufficient quantities that plants could reduce partitioning to roots. It also suggests that the low nutrient biochar did not supply nutrients at the same level as the high nutrient biochar. However, when given a choice between soil amended with either biochar type and unamended soil, plants produced more roots and root biomass in the amended soil. These responses suggest that large crabgrass responded to nutrients supplied by the biochar; however, plant tissue analyses for nutrient content would be necessary to confirm this conclusion.

In the third experiment, two crop (red clover and sweet corn) and two weed species (large crabgrass and redroot pigweed) were grown to maturity under greenhouse conditions with the same two types of biochar used in the second experiment. The high-nutrient biochar increased the stem, petiole, and inflorescence dry weight of red clover and the ear and total dry weight of sweet corn. The high-nutrient biochar reduced root : shoot ratio of both crop species. The response of the crop species indicates a fertilizer effect in which the crop was able to increase yield or above-ground biomass without investing resources into an extensive root system. Redroot pigweed also followed this pattern with greater inflorescence dry weight in the high nutrient biochar treatment and

reduced partitioning to roots in both biochar treatments compared to the control. Although redroot pigweed responses were similar to the crops in this experiment, they were drastically different than the results from the first experiment in which biochar reduced the height of redroot pigweed, had no effect on root : shoot biomass partitioning, and substantially increased branch dry weight. Both types of biochar increased large crabgrass total dry weight but neither type affect the biomass partitioning of large crabgrass. This conflicts with the results of both of the previous experiments where large crabgrass partitioning was affected by biochar.

Variability in results among the three experiments may be attributed to variability in soil type, differences between "aged" and fresh biochar, and to differences in the duration of the experiments. In all three experiments, a sandy loam soil was used; however, the soil used in the first experiment was collected from a 2-yr-old prairie restoration site while the soil used in the last two experiments was collected from a more than 10-yr-old conventional agricultural field. Although the biochar used in the first experiment was the same type as the high-nutrient biochar used in the last two experiments, the biochar in the first experiment had been aged for one growing season, i.e. mixed in with soil and used to grow big bluestem and sericea lespedeza during a previous experiment. Both types of biochar used in the last two experiments had not been used previously. Differences in large crabgrass growth between the second and third experiment may be a result of growing large crabgrass for 32 days in the second experiment and to maturity in the third. It is possible that we would have observed differences in biomass partitioning in the third experiment if we had harvested plants earlier. It should be noted that it is not uncommon for researchers to report variability in

results based on biochar type, soil type, plant species, and the duration of the experiment (Liu et al. 2013).

Previous studies attributed the beneficial effects of biochar to increasing the cation exchange capacity (Glaser 2002) or neutralizing the pH of the soil (Lehmann 2006) and this may be true when low-fertility, acidic soils are amended with biochar (Liu et al. 2013). However, the unamended soils used in the current experiments had a neutral pH and therefore, the 2% biochar-amendment had little to no effect on the soil pH. Also, biochar had no effect on the cation exchange capacity of the soils used. This suggests that the responses were due to factors other than an increase in cation exchange capacity or the neutralizing of the soil pH; possibilities include an increase in water retention of the soil (Novak et al. 2012), a reduction in soil bulk density (Laird et al. 2010), or an increase in the mycorrhizal fungi population (Warnock 2007). However, these soil attributes were not tested in the current experiments.

Cumulatively, current studies support the potential for biochar to improve plant growth generally and weed growth more specifically. To the extent that larger weeds could result in greater competition and yield losses, this research suggests that biochar may exacerbate weed problems. However, further research directly measuring the effect of biochar on weed: crop competition should be conducted. Ideally, that research would be conducted with and without soil-applied herbicides to better understand how biochar might affect both plant biology and weed management. The varying results from these experiments, in combination with other concerns (cost, availability, and potential effects on herbicides) suggest that biochar is unlikely to be adopted widely in the Midwest in the near future.

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