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The Effect of Biochar Source on Cotton Seedling Growth and Development and Association with Conventional Fertilizers

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Authors' contributions

This work was carried out in collaboration between all authors. Authors JMB, DEL, DMO and EMK designed the study and wrote the protocol. Author JMB wrote the first draft of the manuscript, managed the literature searches and performed analyses of the study. Authors JMB, EMK and DAL managed the experimental process and statistical analysis. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Growth chamber experiments were performed in order to determine the influence that biochars originating from two separate sources (mixed-hardwoods and poultry litter) have on the growth and development of cotton (*Gossypium hirsutum* L.) seedlings used alone or in combination with conventional fertilizers.
Study Design: The treatments consisted of a 3 by 3 full-factorial arrangement of biochar and fertilizer rates organized in a complete randomized design.
Place and Duration of Study: The trials were conducted in the fall of 2010 and 2012 at the Altheimer Laboratory located at the University of Arkansas Research and Extension Station, Fayetteville, Arkansas (USA).
Methodology: In both experiments, 54 1.5 liter pots were each filled with 1.8 kilograms of a Captina silt loam soil (Typic fragiudult). Both biochar types were added to pots at three equivalent rates: (1) no biochar (control); (2) 5,000 kg/ha; and (3) 10,000 kg/ha while

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fertilizer was also added to pots at three equivalent rates: (1) no fertilizer (control); (2) 31-23-49 kg/ha (N-P-K); and (3) 62-46-98 kg/ha (N-P-K). The plants were grown for eight weeks and then harvested. Data collected at harvest included plant height, chlorophyll content, leaf area, number of main-stem nodes and number of fruits along with plant dry matter.

Results: Both biochar types contributed to increases in numerous plant developmental characteristics. Statistical analysis showed that the hardwood chip based biochar had a more pronounced influence on most plant developmental measurements compared to the poultry litter based biochar. The main effect of biochar in 2010 demonstrated significant increases regarding response variables such as leaf area; control (654.41 cm^2), highest rate of biochar (748.63 cm^2) ($P < .0001$), stem dry matter; control (5.08 g), highest rate of biochar (6.08 g) ($P < .0001$) and fruit dry matter; control (0.20 g), highest rate of biochar (0.52 g) ($P < .0001$). The main effect of biochar in 2010 also significantly decreased chlorophyll content; control (53.80 SPAD units), highest rate of biochar (50.03 SPAD units) ($P < .0001$) and average node length; control (4.08 cm), highest rate of biochar (3.89 cm) ($P = .001$). The main effect of biochar in 2012 resulted in significant increases in the response variables of stem dry matter; control (2.87 g), highest rate of biochar (3.25 g) ($P = .040$), leaf dry matter; control (3.66 g), highest rate of biochar (4.21 g) ($P = .010$) and total plant dry matter; control (6.71 g), highest rate of biochar (7.55 g) ($P = .032$). Statistical analysis showed that the main effect of biochar in 2010 slightly surpassed the effects of the fertilizer main effect while generating more significant responses than the interaction of biochar and fertilizer. Further statistical analysis demonstrated that the main effect of fertilizer in 2012 exceeded the biochar main effect and an interaction between biochar and fertilizer was not observed.

Conclusion: Analyses of individual biochar rates for both experiments, along with the interaction of biochar and fertilizer treatments in 2010, demonstrated that the mixed-hardwoods based biochar had a more positive effect on cotton plant development than the poultry litter based biochar. Additional research is needed concerning the nature and ability of biochars of different origins to slowly release plant available nutrients over time that can contribute to cotton production.

Keywords: Biochar; cotton; pyrolysis; fertilizer; soil.

1. INTRODUCTION

In recent years, a process for waste management has evolved, wherein biological wastes are consumed by high temperature combustion, giving the products of excessive heat and biochar. Biochar analysis has revealed it to be a stable, carbon (C) and nutrient rich charcoal [1]. Biochar has been proposed as a soil amendment to increase soil efficiency [2] as well as having the capacity to significantly increase plant yields [3] while curtailing the demand for fertilizer [4]. Although conventional fertilization has been instrumental in not only maintaining but improving crop yields, there are drawbacks that accompany their use. Detrimental factors associated with the usage of conventional fertilizers include prolific nutrient groundwater leaching and surface runoff, substantial amounts of fossil fuel consumption used to create these fertilizers and the ever increasing costs associated with these fertilizers [2].

Many of the benefits of biochar as a soil amendment can be attributed to biochar's large surface area and elaborate pore structure [5], which is favorable for the bacteria and fungi that some plants require in order to take in nutrients from the soil [6,7]. This permeable quality enhances the soils capacity for retaining water and agricultural compounds [8] along with inhibiting the contamination of water systems [1]. Biochar can also improve soil nutrient holding capacity and replenishment [9]. Soil incorporation of biochar reinstates C, nitrogen (N) and a variety of plant nutrients that are extracted from the soil upon the removal of biomass [8,10]. However, knowledge of the type and composition of the biomass used in the production of biochar is crucial in determining what it is to be used for, i.e. soil amendment or as a nutrient source for crop and plant growth [11].

Biochars can be derived from a myriad of organic feed stocks which can culminate in materials that possess numerous characteristics along with beneficial plant and soil assets [2]. Biomass materials such as wood, leaves, paper waste and poultry litter can all be utilized as sources of biochar. Biochar derived from poultry litter has garnered much interest in several poultry producing regions across the United States, namely Northwest Arkansas, where the poultry industry is a driving force of the area's economy. Biochar originating from poultry litter has been discovered to contain higher concentrations of N and P than biochar derived from plant resources [12]. Consequently, the determination of the availability and total plant nutrient uptake provided by biochar and its association with the soil depend on several factors. Factors such as crop variety, regional soil types/climates and pre-existing soil nutrient conditions all contribute to the possibility of biochars originating from different sources supplying nutrients to plants. When combined, these compositional and nutritional aspects of multiple source biochars are vital when assessing their respective contributions to agronomic productivity and sustainability.

Even though various forms of biochar remediation have been practiced in some parts of the world for many years, it is still a relatively new concept for much of the modernized world. Studies and experiments have been undertaken to observe the environmental, agricultural and economical benefits that biochar can have on these systems. However, many of these trials were either localized or produced on a small-scale, giving biochar scarce recognition. The objective of this experiment was to test the hypothesis that an enhancement of early stage cotton development could be realized by sole applications of multiple source biochar or in concert with varying rates of conventional fertilizers.

2. MATERIALS AND METHODS

2.1 Experimental Location and Materials

Growth chamber experiments were conducted in Fayetteville, AR in the fall of 2010 and 2012. Cotton (*Gossypium hirsutum* L.) cultivar ST 4288 B2F was planted in a complete randomized design with 9 treatments and 6 replicates in a 3 by 3 full-factorial arrangement. A Fine-silty, siliceous, active, mesic Typic Fragiudult Captina silt loam soil was selected from the University of Arkansas Research and Extension Station in Fayetteville, AR. A fine mixed-hardwood based biochar (EE) and a pelletized poultry litter based biochar (BES) were used as biochar sources in 2010 and 2012 respectively. Biochar samples were tested for Mehlich-3-extractable, pH and electrical conductivity (EC) and total nitrogen (N) and carbon (C) at the University of Arkansas soil testing facility.

2.2 Treatments and Methodology

In both experiments, 54 1.5 liter pots were each filled with 1.8 kilograms (kg) of soil. Biochar application rates were calculated based on kg/ha using a 15 cm furrow slice as a reference.

The 3 biochar rates included an untreated (0B) control (no biochar), (1B) 5,000 kg/ha (4.92 g/pot) and (2B) 10,000 kg/ha (9.84 g/pot). Fertilizer rates were also calculated using a 15 cm furrow slice and based on University of Arkansas Division of Agriculture data [13] recommending a pre-plant N-P-K application rate of 62-46-98 (kg/ha) for Arkansas cotton production. The 3 fertilizer rates included a (0F) control (no fertilizer), (1F) 31-23-49 kg/ha (0.06 g urea/pot, 0.036 g $NH_4H_2PO_4$/pot, 0.076 g KCl/pot) and (2F) 62-46-98 kg/ha (0.12 g urea/pot, 0.072 g $NH_4H_2PO_4$/pot, 0.152 g KCl/pot). Treatments were mixed thoroughly with the soil prior to their placement in the pots according to the treatment levels.

Once the treatments and soil had been placed into the pots, 5 cotton seeds were planted at 2.5 cm depth. The pots were placed in a large walk-in growth chamber (Model PGW36, Conviron, Winnipeg, Canada) set for 30/20C (day/night) temperatures, 14 hr photoperiods and 60% humidity. Watering of the plants occurred daily using 150 mL of deionized water per pot. Approximately 2 weeks after planting, plants were thinned to 2 uniform plants per pot and a week later, thinned to 1 based on the most vigorous of the remaining seedlings.

2.3 Cotton Growth and Development Measurements

In 2010, the first measurements at week six included chlorophyll content from 2 leaves on each plant; one from the first node and one from the fifth node. In 2012, chlorophyll content was measured at harvest from fifth node leaves. In both years, chlorophyll content was measured by using a digital SPAD meter (Konica Minolta Sensing Inc. Osaka, Japan). Measurements taken during harvest (eight weeks after planting) included plant height, measured from the plant/soil surface to the apical meristem, number of main-stem nodes and fruits, leaf area and plant dry weight. In the 2010 experiment, Verticillium wilt (*Verticillium dahliae*) was noticed on the plants on harvest day. The infestation appeared to be uniform and not severe. A disease rating system was devised, based on the number of symptomatic leaves per plant. Disease occurrence was not present in the 2012 trial. Leaf area was calculated by removing every leaf of an individual plant and processing with a leaf area analyzer (Licor-3100, Licor Inc., Lincoln, NE) and expressed as total leaf area per plant. For plant dry weight data, the leaves, fruits and stems were collected from each plant and dried in an oven at 55ºC for one week prior to weighing.

2.4 Statistical Analysis

A statistical analysis of two factors (biochar and fertilizer rate) with 6 replicates was used to evaluate the results with a JMP software versions 9.1 and 9.3 (SAS Institute Cary, NC). Analysis of variance and conventional LSD (α=0.05) post hoc analysis were used to compare statistical significance between means. If a two way interaction effect was not observed, the main effects of biochar and fertilizer rate were investigated by averaging data across biochar and fertilizer respectively. Statistical outliers were excluded from response variable analyses if their respective values were greater than 2 standard deviations from the overall mean.

3. RESULTS

Results from both growth chamber experiments will be examined individually by denoting the 2010 experiment as “Trial 1” and the 2012 experiment as “Trial 2”. Individual biochar analyses (EE and BES) are presented in Tables 1 and 2.

Table 1. Compositional analysis of Enginuity (EE) Biochar

pH	EC	P	K	Ca	Mg	S	Na	Fe	Mn[a]
	µmhos/cm				mg/kg				
9.54	48000	11730	53732	11679	5021	14680	12120	88	245
P	**K**	**Ca**	**Mg**	**S**	**Na**	**Fe**	**Mn**	**Zn**	**Cu[b]**
				mg/kg					
52530	92605	100470	22343	19146	26025	4594	2244	1973	2826
				%TN	**%TC[c]**				
				1.57	16.62				

[a]pH (1:2 soil ratio), Mehlich 3 extractable (1:10 ratio) Analysis by SPECTRO ARCOS ICP
[b]Total Recoverable Metals, EPA method 3050, measured on Spectro Arcos ICP
[c]Total N and total C by combustion, Elementar VarioMax

Table 2. Compositional analysis of BioEnergy Systems LLC (BES) Biochar

pH	EC	P	K	Ca	Mg	S	Na	Fe	Mn[a]
	µmhos/cm				mg/kg				
10.2	16680	7076	26412	3271	3071	3525	6880	32	190
P	**K**	**Ca**	**Mg**	**S**	**Na**	**Fe**	**Mn**	**Zn**	**Cu[b]**
			mg/kg						
46915	72298	67904	15298	10486	19919	2453	1397	1261	801
			%TN	**%TC[c]**					
			3.00	32.02					

[a]pH (1:2 soil ratio), Mehlich 3 extractable (1:10 ratio) Analysis by SPECTRO ARCOS ICP
[b]Total Recoverable Metals, EPA method 3050, measured on Spectro Arcos ICP
[c]Total N and total C by combustion, Elementar VarioMax

3.1 Trial 1 (EE 2010)

3.1.1 Effect of EE (Mixed Hardwoods) biochar and fertilizer rates on chlorophyll content, plant height, leaf area and average node length

The main effects of biochar and fertilizer rates on chlorophyll content, plant height, leaf area and average node length are presented in Table 3. Chlorophyll content was significantly higher in the main effects for biochar (P < .0001) and fertilizer (P = .002) rates in 2010. Biochar treatment 0B experienced the highest level of chlorophyll content and was significantly different from biochar treatments 1B and 2B which had no significant difference between them. Fertilizer treatment 2F had the highest chlorophyll content and was significantly different from treatment 0F. Treatment 1F registered no significant difference with both 0F and 2F. Plant height was also affected by the biochar main effect (P = .001) and fertilizer main effect (P = .0002) rates. Biochar treatment 0B had a significantly higher plant height than treatment 1B while treatment 2B was not significantly different than the two other biochar treatments. Fertilizer treatment 2F produced the highest value when compared to treatments 0F and 1F which in turn were not significantly different from each other. Leaf area in 2010 was affected by the biochar main effect (P < .0001) and the fertilizer main effect (P < .0001). Biochar treatment 2B had the highest leaf area level when compared to treatments 0B and 1B with treatment 1B being significantly higher than 0B. Fertilizer treatment 2F produced the highest leaf area values and was significantly different from treatments 0F and 1F with fertilizer treatment 1F being significantly higher than treatment 0F. Average node length, calculated by dividing the number of nodes per plant by plant height, was only affected by the main effect of biochar (*P* = .001). Biochar treatment 0B experienced the highest height to node ratio. This level was significantly different from biochar treatments 1B and 2B which were not significantly different from each other.

Table 3. Effect of EE (Mixed Hardwoods) biochar and fertilizer rates on chlorophyll content (Chl.), plant height, leaf area and average node length (ANL.) means for Trial 1

Treatment	Chl. (SPAD units)	Plant height (cm)	Leaf area (cm^2)	ANL. (cm)
Biochar 0B	53.80a[§]	43.89a[§]	654.41c[§]	4.08a[§]
Biochar 1B	51.21b	41.27b	695.92b	3.78b
Biochar 2B	50.03b	42.59ab	748.63a	3.89b
Fertilizer 0F	50.63b[§]	40.74b[§]	601.73c[§]	3.95a[§]
Fertilizer 1F	51.56ab	42.28b	687.23b	3.90a
Fertilizer 2F	53.28a	44.35a	815.39a	3.92a

[§]Columns for biochar and for fertilizer treatments not sharing a common letter are significantly different (P = .05)

<u>3.1.2 Effect of EE (Mixed Hardwoods) biochar and fertilizer rates on stem and fruit dry matter and plant disease rating</u>

The main effects of biochar and fertilizer rates on stem and fruit dry matter along with plant disease rating are shown in Table 4. In 2010, stem dry matter analysis was affected by the main effects of biochar (*P* < .0001) and fertilizer (*P* < .0001) rates. Biochar treatment 2B allowed the highest level of stem dry matter compared to treatments 0B and 1B with treatment 1B being significantly higher than treatment 0B. Fertilizer treatment 2F expressed the highest level of stem dry matter when compared to treatments 0F and 1F with treatment 1F being significantly higher than treatment 0F. Analysis of fruit dry matter in 2010 was also affected by the main effects of biochar (*P* < .0001) and fertilizer (*P* < .0001) rates. Biochar treatments 1B and 2B were not significantly different from each other in terms of fruit dry matter and were at a higher level than biochar treatment 0B. Fertilizer treatment 2F produced the highest level of fruit dry matter compared to treatments 0F and 1F which were not significantly different. Verticillium wilt ratings were only influenced by the biochar rate (*P* < .0001) main effect in 2010. The highest disease rating belonged to treatment 0B. Treatments 1B and 2B were significantly different and lower than treatment 0B. However, there were no significant differences between treatments 1B and 2B. These data indicate that biochar inclusion may have suppressed Verticillium wilt.

<u>3.1.3 Effect of EE (Mixed Hardwoods) biochar and fertilizer rates on node and fruit numbers, leaf and total plant dry matter</u>

The interactive effects between biochar and fertilizer rates on node and fruit numbers along with leaf and total plant dry matter are shown in Tables 5 and 6 respectively. The number of nodes per plant showed a significant interaction effect between biochar and fertilizer rates (*P* = .004). Table 5 shows that treatment 1B/2F generated the highest node level compared to other treatments except for treatment 2B/2F which had no significant difference with 1B/1F.

Table 4. Effect of EE (Mixed Hardwoods) biochar and fertilizer rates on stem dry matter (DM), fruit dry matter (DM), and disease rating means for Trial 1

Treatment	Stem DM (g)	Fruit DM (g)	Disease rating
Biochar 0B	5.08c[§]	0.20b[§]	4.33a[§]
Biochar 1B	5.60b	0.51a	2.84b
Biochar 2B	6.08a	0.52a	2.33b
Fertilizer 0F	5.02c[§]	0.28b[§]	3.11a[§]
Fertilizer 1F	5.71b	0.38b	3.76a
Fertilizer 2F	6.13a	0.57a	2.66a

[§]Columns for biochar and for fertilizer treatments not sharing a common letter are significantly different (P = .05)

Treatment level 0B/2F, which is a typical rate for Arkansas cotton production, showed lower levels of nodes but was not significantly different than treatment 1B/1F. Fruit numbers were also affected by the interaction between biochar and fertilizer rates (P < .0001). Table 5 shows that treatment 1B/2F brought about the highest fruit level over all other treatments except for treatments 2B/2F, 0B/2F and 1B/1F. Treatment level 0B/2F showed no significant differences between treatment levels 1B/2F, 2B/2F, 1B/1F and 2B/1F. Leaf dry matter analysis was also affected by the interaction between biochar and fertilizer rates (P = .001).

Table 5. Interaction of EE (Mixed Hardwoods) biochar and fertilizer rates on the number of nodes and fruits per plant for Trial 1

Biochar/Fertilizer treatment	Node number	Fruit number
0B/0F	10.66c[§]	0.50e[§]
0B/1F	10.50c	2.66cd
0B/2F	10.83c	3.50ab
1B/0F	9.83d	2.50d
1B/1F	11.00bc	3.20abc
1B/2F	11.66a	3.66a
2B/0F	10.50c	2.83cd
2B/1F	10.83c	3.00bcd
2B/2F	11.50ab	3.50ab

[§]Columns not sharing a common letter are significantly different (P = .05)

Table 6 shows that treatment 2B/2F exhibited the highest leaf dry matter rate over all other treatments except for 2B/0F and 1B/1F. Treatment 0B/2F showed a substantial decrease in leaf dry matter when compared to 2B/2F but was not significantly different from treatments 2B/1F, 1B/2F, 1B/0F and 0B/1F. Analysis of total plant dry matter also was affected by the interaction between biochar and fertilizer rates (P = .009). Table 6 shows that treatment 2B/2F expressed the highest level of total plant dry matter sharing significant differences with the other treatments. Treatment 0B/2F came in at a lower level then 2B/2F but had no significant differences with treatments 2B/1F, 2B/0F, 1B/2F, 1B/1F and 1B/0F.

Table 6. Interaction of EE (Mixed Hardwoods) biochar and fertilizer rates on leaf dry matter (DM) and total plant dry matter (DM) for Trial 1

Biochar/Fertilizer treatment	Leaf DM (g)	Total plant DM (g)
0B/0F	3.79f§	8.20e§
0B/1F	4.66e	9.96d
0B/2F	5.02de	11.18bc
1B/0F	5.43bcd	10.79cd
1B/1F	5.58abc	11.94b
1B/2F	5.26cd	11.85b
2B/0F	5.79ab	11.87b
2B/1F	5.47bcd	11.85b
2B/2F	5.99a	13.37a

§Columns not sharing a common letter are significantly different (P = .05)

3.2 Trial 2 (BES 2012)

3.2.1 Effect of BES (Poultry Litter) biochar and fertilizer rates on chlorophyll content, plant height, leaf area and average node length

The main effects of biochar and fertilizer rates on chlorophyll content, plant height, leaf area and average node length are shown in Table 7. In 2012, only the main effect of fertilizer was significant ($P < .0001$) concerning chlorophyll content. Table 7 shows that fertilizer treatment 2F was significantly higher than fertilizer treatments 1F and 0F. Additionally, fertilizer treatment 1F was significantly different and higher than treatment 0F. With regards to plant height, neither biochar nor fertilizer nor their interaction was determined significant. The main effect of fertilizer was significant ($P = .012$) on leaf area where fertilizer treatments 1F and 2F were significantly higher than treatment 0F. There were no significant differences between treatments 1F and 2F. Average node length was not a significant factor in 2012 for biochar, fertilizer or their interaction.

Table 7. Effect of BES (Poultry Litter) biochar and fertilizer rates on chlorophyll content (Chl.), plant height, leaf area and average node length (ANL.) means for Trial 2

Treatment	Chl. (SPAD units)	Plant Height (cm)	Leaf Area (cm^2)	ANL. (cm)
Biochar 0B	45.10a§	32.47a§	434.58a§	3.65a§
Biochar 1B	44.06a	31.98a	454.21a	3.58a
Biochar 2B	45.45a	32.06a	482.25a	3.59a
Fertilizer 0F	40.17c§	31.46a§	418.60b§	3.62a§
Fertilizer 1F	45.66b	32.33a	464.98a	3.64a
Fertilizer 2F	48.78a	32.72a	487.75a	3.57a

§Columns for biochar and for fertilizer treatments not sharing a common letter are significantly different (P = .05)

3.2.2 Effect of BES (Poultry litter) biochar and fertilizer rates on node and fruit numbers

The main effects of biochar and fertilizer rates on node and fruit numbers are shown in Table 8. In 2012, the number of nodes per plant was only significant for the main effect of fertilizer rate ($P < .0001$). Table 8 shows that fertilizer treatments 1F and 2F had higher node totals per plant than fertilizer treatment 0F. There were no significant differences between fertilizer treatments 1F and 2F. Fruit totals were only affected by the fertilizer main effect (*P* < .0001). Fertilizer treatments 1F and 2F generated the highest fruit numbers and were significantly different than fertilizer treatment 0F. There were no significant differences between fertilizer treatments 1F and 2F.

Table 8. Effect of BES (Poultry Litter) biochar and fertilizer rates on the number of nodes and fruits per plant for Trial 2

Treatment	Node number	Fruit number
Biochar 0B	8.777a[§]	1.944a[§]
Biochar 1B	8.866a	2.111a
Biochar 2B	8.944a	2.333a
Fertilizer 0F	8.444b[§]	1.388b[§]
Fertilizer 1F	9.000a	2.388a
Fertilizer 2F	9.233a	2.611a

[§]Columns for biochar and for fertilizer treatments not sharing a common letter are significantly different (P = .05)

3.2.3 Effect of BES (Poultry litter) biochar and fertilizer rates on stem, fruit, leaf and total plant dry matter

The main effects of biochar and fertilizer rates on stem, fruit, leaf and total plant dry matter are shown in Table 9. In 2012, leaf dry matter was only significant for the main effect of biochar (*P* = .010). Biochar treatment 2B was significantly higher in leaf dry matter than biochar treatment 0B. Biochar treatment 1B was not significantly different than biochar treatments 0B and 2B. Additionally, total plant dry matter was only affected by the main effect of biochar (*P* = .032). Biochar treatment 2B was significantly higher in total plant dry matter than biochar treatment 0B. Biochar treatment 1B was not significantly different from treatments 0B and 2B. Stem dry matter was significant for the main effects of biochar (*P* = .040) and fertilizer rates (*P* = .021) in the 2012 trial.

Table 9. Effect of BES (Poultry Litter) biochar and fertilizer rates on stem, fruit, leaf and total plant dry matter (DM) means for Trial 2

Treatment	Stem DM (g)	Fruit DM (g)	Leaf DM (g)	Total Plant DM (g)
Biochar 0B	2.873b[§]	0.121a[§]	3.665b[§]	6.717b[§]
Biochar 1B	3.113ab	0.139a	3.894ab	7.147ab
Biochar 2B	3.253a	0.161a	4.216a	7.558a
Fertilizer 0F	2.864b[§]	0.050b[§]	3.807a[§]	6.738a[§]
Fertilizer 1F	3.143ab	0.157a	3.873a	7.174a
Fertilizer 2F	3.289a	0.198a	4.021a	7.510a

[§]Columns for biochar and for fertilizer treatments not sharing a common letter are significantly different (P = .05)

Table 9 shows that biochar treatment 2B was significantly higher in stem dry matter over treatment 0B while treatment 1B was not significantly different from treatments 0B or 2B. Fertilizer treatment 2F had a significant increase in stem dry matter over treatment 0F but was not significantly different than treatment 1F. Fertilizer treatment 1F was not significantly different than 0F or 2F. Analysis of fruit dry matter in 2012 was only significant for the main effect of fertilizer rate (*P* < .0001). Fertilizer treatments 1F and 2F were significantly higher in fruit dry matter than treatment 0F. Fertilizer treatments 1F and 2F were not significantly different from one another.

4. DISCUSSION

The goal of this experiment was to test the hypothesis that increasing applications of biochar originating from mixed hardwoods or poultry litter could enhance numerous aspects of cotton seedling development and growth alone or in association with varying rates of conventional fertilizers. However, while some significant effects were observed through this interaction, there were many significant main effects resulting from separate biochar and fertilizer rate analyses. Nonetheless, the majority of plant development characteristics tested expressed increases when biochar was present. The following discussion will link several of these aspects and results to explain how biochar additions affected the development of cotton.

4.1 Trial 1 (EE 2010)

Regarding chlorophyll content, plant height and average node length, it appears that biochar may have depressed plant development in the 2010 trial. Biochars originating from plant materials have a high C/N ratio and depresses N availability in the soil [6,14], thereby limiting development of proteins, enzymes and chlorophyll molecules. However, the plant dry matter data in 2010 does not support this theory since increments of biochar resulted in higher plant dry matter production which is consistent with the meta-analysis conducted by [3] regarding biochar pot research. Chlorophyll content for individual biochar treatments decreased from 0B to 1B and 2B, this possibly could be due to a dilution effect since increase of biochar rates resulted in higher plant leaf area. The opposite was observed for individual fertilizer treatments.

Plant height with respect to the fertilizer treatments in 2010 only showed a significant difference between 2F, producing the highest height, and 0F and 1F which were lower than 2F but not significantly different from each other. Plant height results for biochar treatments however, were not as conclusive. Though there was a significant difference from 0B to 1B, 2B was not significantly different from either 0B or 1B. The reasoning for these results is not clear and supports the inconsistencies observed by [15] concerning biochar's effect on plant height. Average node length results showed that treatment 0B had the highest level while 1B and 2B registered lower levels and no significant difference. In cotton production, excessive values of node length are detrimental to lint yield and often controlled with applications of growth regulators (e.g. Mepiquat Chloride). Thus, the biochar affect in 2010 which saw a decrease in node length could be considered beneficial.

Biochar played a significant role in leaf area development as well. All of the biochar treatments showed significant differences with 2B producing the highest leaf area. The mixed hardwoods biochar used for this trial had an ample supply of P and K. Coupled with this amount of P and K were other macro and micro nutrients provided by this biochar that may have assisted with the provision of these nutrients as well as reducing competition for them; possibly as a result of this biochar possessing an alkaline pH of 9.54 [3].

Stem and fruit dry matter in 2010 were positively affected by applications of biochar and fertilizer. In both treatments, stem dry matter rose with increasing additions of both biochar and fertilizer inputs. For the mixed hardwoods based biochar, the supply of macro and micro nutrients must have been sufficient to influence stem development. Although both biochar and fertilizer treatments exhibited similar gains of stem dry matter in 2010, the fact remains that stem development is one of the first priorities in plant development and most nutrients taken up by the plant in this critical time will be used for this purpose. If an adequate nutrient reserve is not available, further plant growth, such as fruit and leaf development can be compromised. The fact that this did not occur in the biochar treatments in 2010 indicates that nutrients were released over time. The nature of that release is not well understood. Fruit dry matter likewise, was affected by separate biochar and fertilizer rates. However, unlike stem dry matter where there were gains and significant differences with each increasing fertilizer and biochar input, fruit dry matter experienced less fluctuation.

In 2010, the interaction of biochar and fertilizer had a major effect on cotton development in node number while fruit number was slightly affected by this interaction. Node production was at its highest when biochar treatments 1B and 2B were associated with full treatments of fertilizer. This could be attributed to the availability of other major and micro nutrients other than N already present in the mixed hardwoods biochar [16]. The biochar used in 2010 was alkaline in nature and contained sizeable amounts of K, Ca and Mg, elements essential for proper plant growth. If these nutrients could become available over time from the biochar in the soil, then the plant should receive a steady supply throughout its growth cycle [11]. Accompanying this increase of major and micro nutrient availability is the possible improvement of nutrient transport to the plant roots and up into the xylem tissue. Since biochar has been known to enhance pore spacing in soils [6], it seems likely that with an ample supply of water, adequate amounts of N, P, and K could find their way to the roots easier than in soils that had not been amended by biochar additions and may be more compacted, thus influencing nutrient uptake.

In the 2010 experiment, a sudden onset of Verticillium wilt was experienced on the final day of the experiment. After counting the number of diseased leaves on each plant, it was determined that only the biochar treatment had any effect on disease response. Biochar has already been known to provide a habitat for bacteria and fungal mycorrhizae [5]. Perhaps the biochar served as an alternate host for the pathogen or biochar could have increased the population of beneficial microorganisms in the soil that suppressed the Verticillium wilt pathogen [7,17]. This observation does encourage further research to better define the relationship between biochar and the occurrence of Verticillium wilt and perhaps other plant diseases.

4.2 Trial 2 (BES 2012)

Although biochar was not a significant factor in chlorophyll measurements in 2012, the increases in stem, leaf and total dry matter attributed to biochar may signify the temporal partitioning of specific nutrients throughout the plant. Chlorophyll content significantly rose with increasing rates of fertilizer, indicating either specified nutrient partitioning or that the nutrients contained within this pelletized form of biochar were not yet available for nutrient uptake and subsequent chlorophyll accumulation. Moreover, this may be the result of the high C content in this biochar, which in turn may have restricted the availability of N needed for chlorophyll production despite having a low C/N ratio [18]. Plant height and average node length were not significant factors for the main effects of biochar, fertilizer or their interaction.

The poultry litter based biochar was not a significant factor with regards to leaf area. However, the fertilizer treated plants significantly generated the highest leaf area totals over the non-fertilized control. Since only the nutrients N, P, and K are included in these treatments and no positive interaction with biochar was detected, it seems this response is due solely to increased fertilizer rates and high levels of availability. Since both experiments were somewhat similar concerning their respective responses to fertilizer in addition to only the mixed hardwood based biochar having a significant impact on leaf area, this may possibly suggest that the textural differences between both types of biochar may be a factor in nutrient uptake and assimilation [11].

In 2012, only the main effects of fertilizer and biochar increased node and fruit number as well as leaf and total plant dry matter respectively. However, both the biochar and fertilizer main effects generated significant increases in stem dry matter at their respective higher rates. There was a significant increase in both node and fruit number between both fertilizer rates and the control and the highest rate of biochar was significantly higher than the control for both leaf and total plant dry matter measurements. Additionally, only the main effect of fertilizer was observed to increase fruit dry matter. These observations further reinforce the fact that in 2012 the cotton plants obtained a significant portion of their nutrient supply from the applications of fertilizer and not the poultry litter based biochar.

5. CONCLUSION

Analyses of individual biochar rates, along with the interaction of biochar and fertilizer treatments in 2010 and 2012, demonstrated positive effects of cotton plant development concerning both types of biochars; EE (mixed hardwoods) and BES (poultry litter) with the EE biochar having more levels of interaction with the fertilizers used in this study. Through its addition and association with conventional fertilizers in this experiment, the EE biochar significantly impacted growth and development and had higher numerical values in more areas than the BES biochar. This could possibly be attributed to the fine-textured composition of the EE biochar which may have made the nutrients contained within more accessible to the developing root system. Consequently, the pelletized form of the BES biochar may have inhibited nutrient release and subsequent plant uptake resulting in lower numerical values for most measured variables. This is further illustrated by the fact that the main effect of fertilizer was observed more frequently than the main effect of biochar in the 2012 trial. Nonetheless, enhancements in areas such as leaf area and total plant dry matter weight indicate that physiological functions vital to cotton growth and development can be benefitted by plant/biochar interaction.

These experiments have pointed out the direction for the next series of biochar trials in cotton. Additional research is needed concerning the nature and ability of biochar to slowly release nutrients over time that can become made available for cotton production. Research should also include biochar's association with various pathogens and diseases and its responses in controlling them.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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