The Influences of Poultry Litter Biochar and Water Source on Radish Growth and Nutrition

An Undergraduate Honors Thesis in the Department of Crop, Soil, and Environmental Sciences

Submitted in partial fulfillment of the requirements for the University of Arkansas Dale Bumpers College of Agricultural, Food and Life Sciences Honors Program

by

Julia Allen
November 2014

________________________________________
David E. Longer, Ph.D., Chair

________________________________________
Trenton L. Roberts, Ph.D.

________________________________________
J. Thad Scott, Ph.D.

________________________________________
Lisa S. Wood, Ph.D.
Table of Contents

Abstract..........................................................................................................................3

Introduction..............................................................................................................5

Research Objectives and Hypothesis.................................................................................13

Materials and Methods....................................................................................................13

Results..........................................................................................................................18

Discussion.....................................................................................................................18

Conclusion.....................................................................................................................21

Acknowledgements.......................................................................................................22

Literature Cited..............................................................................................................23

Tables and Figures..........................................................................................................27
I. Abstract

Many row-crop fields today have declined in soil fertility due to poor management practices and overuse of pesticides. Under these conditions, plant nutrient uptake can be sub-optimal. There are several soil amendments that can be used to improve soil quality and plant growth such as traditional fertilizers and manure applications. This study focused on the addition of biochar to the soil and the use of structured water to enhance plant growth. Biochar is a nutrient rich product that is produced by pyrolysis of organic feedstocks and contains high rates of carbon. Previous studies which focused on biochar have shown an increase in plant yield, nutrient availability in the soil, and soil water holding capacity. Structured water is the liquid crystalline state of water which has unique characteristics due to the ordering of the hydrogen bonds in the water molecules. Numerous claims have been made in the natural and organic health literature about the benefits of structured water in human and animal health, but little has been reported in the scientific literature concerning plant growth response. This study was conducted to evaluate the effect of biochar and structured water on the growth and nutrient content of radishes (*Raphanus sativa* (L.)). This study was conducted in October 2013 in the Rosen Alternative Pest Control Greenhouse at the University of Arkansas. A complete randomized design was used consisting of a total of six treatments including: equivalent rates of 0, 5,000, and 10,000 kg ha$^{-1}$ of biochar, each watered with either tap water or structured water. The plants grew for 22 days in the greenhouse and were then harvested and analyzed for leaf area, total fresh weight, root fresh weight, and mineral content using a Brix meter. Data showed that the water type had the most significant response. Tap water and biochar used together had a significant and positive interaction. Radishes grown in soil containing biochar and watered with tap water resulted in larger leaf area, total fresh weight, root fresh weight, and mineral contents as the rate of biochar
increased. Biochar alone had a negative effect on root fresh weight. Radish growth showed a negative response to structured water in almost every circumstance. This study concluded that tap water and 0 kg ha\(^{-1}\) of biochar produced the largest radish yield overall.
II. Introduction

In many agricultural settings, soil fertility has declined due to improper management (Laird et al., 2010a). In order to support the projected world population growth, more agriculture output is required (Schult and Glaser, 2012). However, soil degradation is currently becoming a common problem worldwide due over use from a rapidly expanding population which demands an increase in food supply. This increase in agricultural production increases greenhouse gas emission, contributing to climate change (Schult and Glaser, 2012). Plant uptake, leaching, runoff, and volatilization cause nutrient depletion from soil over time. Yearly or periodic fertilizer applications are used to compensate for the loss, though this is usually a temporary and often costly solution (Laird et al., 2010a). The application of biochar (BC) to agricultural fields may contribute to a long-term solution for increasing and maintaining minerals in the soil (Laird et al., 2010a).

Biochar is carbonized biomass created from organic feedstocks which have undergone pyrolysis (Revel et al., 2012b). Pyrolysis is defined as heating at extremely high temperatures in the absence of oxygen (Chan et al., 2007). Studies have indicated that BC is composed primarily of carbon (C) and can resist decomposition for hundreds or more years (Doydora et al., 2011). This implies that BC may factor into a long-term solution for increasing and sustaining soil fertility due to the potential for this carbonaceous material to persist in the soil and provide benefits for extended periods of time. (Laird et al., 2010a).

Extensive research efforts on BC resulted from the discovery of extremely fertile soil in the Amazon. This fertile soil is named ‘Terra Preta’ (Portuguese for ‘dark earth’). The Terra Preta soils are enriched with black carbon which has sparked interest in researching BC to evaluate if the addition of BC to soil will enhance soil properties similar to Terra Preta (Adams
et al., 2013). Terra Preta soils are known for containing stable soil organic matter and charcoal (Glaser et al., 2001). While many nutrients such as nitrogen (N), phosphorous (P), potassium (K), and calcium (Ca) are abundant in the Terra Preta soils, up to 70 times as much black carbon is found in these soils compared to the surrounding soil (Glaser et al., 2001). Zech et al. (1990) compared the Terra Preta soils with the surrounding Oxisols and found organic C content in the surface horizon of Terra Preta soil was 92 g kg\textsuperscript{-1} as compared to only 30 g kg\textsuperscript{-1} in the Oxisols. Similar trends were found for other nutrients: Terra Preta soils contained 6.8 g kg\textsuperscript{-1} total N and 2 g kg\textsuperscript{-1} total P and the Oxisols contained 2.7 g kg\textsuperscript{-1} total N and 0.22 g kg\textsuperscript{-1} total P (Zech et al., 1990). The charcoal found in these soils is from the incomplete combustion of organic material, mainly derive from cooking fires, and is the main cause for the persistence of organic matter in the Terra Preta soil (Glaser et al., 2001).

Biochar can be made from essentially any type of organic matter. Two common sources for BC production are plant waste and animal waste (Chan et al., 2007). Biochar has been made from green waste products like peanut shells (Yin et al., 2012), plant waste such as grass and plant clippings (Chan et al., 2007), olive (Olea europaea) husks, corncobs (Maize), and tea waste (Demirbas, 2004), woody material (Artiola et al., 2012), industrial waste (van Zwieten et al., 2010), and animal waste such as poultry litter (Chan, et al., 2008) and quail litter (Suppadit et al., 2012). Biochar from plant material has high C content while other macronutrients and micronutrients occur in smaller quantities. Biochar derived from wood has a high carbon content, around 70\%, and has been associated with increasing leaf area, leaf dry weight, and fruit yield when used as a soil amendment (Lehmann et al., 2003). Biochar derived from plant waste has shown to increase total N in the soil up to 7\% and soil organic C up to 69\% (Laird et al., 2010b). Biochar derived from animal waste has different properties than plant waste BC. Poultry litter
based BC contains less carbon than plant waste BC, anywhere between 27% and 42%, but does contain more nitrogen (N), phosphorus (P), and potassium (K). Poultry litter BC also contains soluble salts at a concentration of 24.2 dS m⁻¹ compared to BC made from plant matter which has smaller concentrations (Revell et al., 2012a & Revell et al., 2012b).

Poultry litter based BC is becoming a favorable soil amendment option due to the vast amounts of feedstock being produced and the growing need for environmentally friendly ways of disposal. Poultry litter is high in P and is commonly applied to land to increase plant available nutrients. In areas with numerous poultry operations, excessive land applications of litter has led to a buildup of soil-test P and is a major contributor to surface water eutrophication (Moore & Miller, 1994). Converting poultry litter into BC is a viable option that can reduce costs for poultry farmers and contribute to sustainable agriculture. (Revell et al., 2012). Since BC often increases soil pH (Laird et al., 2010a), traditional liming costs may also be reduced.

Most BC research has identified numerous benefits from its use as a soil amendment to increase soil fertility and promote plant growth. Biochar is known to often increase soil pH, water holding capacity, and the availability of nutrients – particularly N, P, K, and C – as well as improving cation exchange capacity (CEC) and promoting soil microbial activity and biomass (Adams et al., 2013 and Biederman & Harpole, 2012).

Most BC is alkaline by nature, though the pH varies depending on the source of the feedstock. A greenhouse study conducted by Revel et al. (2012b) showed that the addition of poultry litter BC positively influenced chemical and physical soil properties including an increase in soil pH. The sandy loam used by Revel et al. (2012b) started with a pH of 7.14 and increased to 9.20 with the addition of 100% BC by weight. Alkaline BC is more effective at increasing plant productivity and raising pH than an acidic BC (Biederman and Harpole, 2013).
The liming ability of BC may help stabilize soil pH when fertilizers, such as urea, are added to the soil. During nitrification, urea releases hydrogen ions which acidify the soil. The liming value of BC is roughly 30% CaCO$_3$ depending on the origin of the BC (van Zwieten et al., 2010). The liming ability of poultry litter BC can be beneficial in many agronomic soils since crop production, nutrient leaching, and fertilizer applications often acidify soils to produce potentially unfavorable growing condition (Revel et al., 2012b).

Due to its physical properties, BC helps increase water holding capacity and reduces nutrient leaching. Biochar is very porous which increases adsorption properties allowing a greater retention of water and nutrients in the soil solution (Adams et al., 2013). One greenhouse study found that the water holding capacity nearly doubled when 15% poultry litter BC by weight was added to 9 kg of sandy loam soil (Revell et al., 2012b). The ability to retain a relatively large quantity of water aids plant growth when under water stress. In another greenhouse study, Artiola et al. (2012) found that soil amended with 2% and 4% BC by weight (15 kg of loamy sand used) had higher yields than control plants after undergoing water stress. Another study showed that BC reduced nutrient leaching significantly and concentrations of P and K were much higher in soils amended with BC (Biederman and Harpole, 2013).

Artiola et al. (2012) found that the addition of BC aided in decreasing bulk density of a loamy sand (from 1.59 g cm$^{-3}$ to 1.26 g cm$^{-3}$). It is important to keep bulk density low, usually around 1.33 g cm$^{-3}$, because it changes the physical properties of the soil. Another aspect of how the physical properties of BC changes soil properties is due to the color of BC. The dark color of the BC alters thermal dynamics in the soil which promotes faster seed germination (Biederman & Harpole, 2013).
In addition to affecting soil chemistry and physical soil properties, BC interacts with microorganisms in the soil. In order for BC to mineralize at a rapid rate, microorganisms must play a significant role in the rate of mineralization through metabolizing BC (Lehmann, 2003). A change in physical and chemical properties of the soil, such as increased pH, CEC, and aeration, induced by BC encourages microbial growth (Muhammad et al., 2014). The physical structure of BC also benefits microbes by providing a niche habitat for aerobic microorganisms and a sustained source of C. Bacteria and fungi can live on the surface and within the pores of BC (Lehmann, 2003). Not all types of BC are beneficial to microbes, though. Feedstocks that have undergone pyrolysis with low temperatures produce BC with smaller pore volume compared to BC created under high temperatures. Biochars created under low temperatures do not have enough aeration and porosity to sustain microorganism life (Muhammad et al., 2014).

Numerous studies have shown that BC can increase crop productivity and crop yield (Nogurea et al., 2012, Biederman and Harpole, 2013, and Artiola et al., 2012). An extensive literature review performed by Biederman et al. (2013) concluded that, overall, crop production and yield increased when soils were treated with BC. Another study found that shoot emergence and shoot length of ryegrass (*Lolium perenne L. var Cadix*) were significantly higher with soils treated with BC (Beesley et al., 2010). Another component of growth that has been reported to increase when BC is added to the soil is leaf area. One study on soybeans using quail litter derived BC showed that the leaf area of soybeans was the greatest with BC application rates of 1.6 kg m\(^{-2}\) (16,000 kg ha\(^{-1}\)) while control plants had the smallest leaf area (Suppadit et al., 2012).

Positive yield results have been found when a combination of BC and N fertilizer are used on crops. Chan et al. (2007) conducted research to evaluate the effects of BC and N fertilizer interactions in the growth of radishes. Two rates of BC were used in the study: 50,000
kg ha\(^{-1}\) and 100,000 kg ha\(^{-1}\). The results of the study showed a 320\% increase in radish dry matter when N fertilizer was combined with BC that was applied at 50,000 kg ha\(^{-1}\). Some studies suggest that N is tightly bound to BC which causes a limited amount of N to reach plants unless the soil is supplemented with N fertilizer (Adams et al., 2013 and Lehman et al., 2003). Plant uptake of nutrients is enhanced by a mixture of BC and fertilizer resulting in larger concentrations of nutrients, such as P, in plant tissue (Biederman and Harpole, 2013).

The use of BC in agricultural fields also affects the environment in many direct and indirect ways. There are several environmental factors that BC has been shown to impact such as reduction in greenhouse gas emissions and soil contamination remediation (Camps Arbestain et al., 2014). Due to the fact that pyrolysis of biomass increases the stability of the carbon in BC, the BC remains sequestered longer in the soil than raw biomass which can help mitigate greenhouse gas emissions from the soil (Lehmann et al., 2003). Biochar sequesters carbon and aids in alleviating anthropogenic CO\(_2\) emissions (Biederman & Harpole, 2013). Application of biochar in the soil could lessen the amount of atmospheric CO\(_2\) while also resulting in agricultural benefits. Fertilizers and liming agents used in crop production have been linked to contributing greenhouse gases to the atmosphere. Since studies have shown that BC increases fertilizer use efficiency (Chan et al., 2007) and has liming properties, the use of BC may reduce the amount of greenhouse gases emitted (Lehmann et al., 2003). New methods to increase C storage while lessening atmospheric CO\(_2\) are important because atmospheric CO\(_2\) is estimated to be increasing by 4.1 billion tons per year (Steinbeiss et al., 2009). The use of BC may also help remediate contaminated soils by binding metals so that they are not water extractable (Beesley et al., 2010). In a field study examining the effects of BC on metals in the soil, Beesley et al. (2010) found that BC significantly (p < 0.05) reduced the amount of cadmium (Cd) and zinc (Zn) in the
soil water. A spike in other elements, such as arsenic (As) and copper (Cu), in the soil water was higher than the control soil but decreased with time.

Another relatively new area idea and with potential for study is the effect of “structured water” on plant growth. Structured water (SW) is the liquid crystalline form of water that has several unique characteristics when compared to “normal” water that has not undergone “structuring” (Pangman, 2011). Another term that is interchangeable for SW is hexagonal water. This is because when the water becomes structured, it forms in a hexagonal shape with each water molecule sharing one hydrogen atom compared to tap water (TW) which are constantly moving and creating short-lived bonds (Aqua Technology, 2014). In order to structure water, TW is run through a mechanical vortex. Many SW units are sold that attach onto a faucet or can be installed into pipe work to create the vortex. Vortex structuring units alter the molecular structure of the water which removes the suspended solids and contaminants and keeps the beneficial minerals (Betterton, 2012). In contrast to distilled water which removes impurities through distillation, differences in SW have been detected in molecular stability, a negative electrical charge, greater viscosity, molecular alignment, and an improved ability to absorb a certain spectra of light (Pangman, 2011).

Structured water has to do with how water interfaces within cells. According to Pangman (2011), the water molecules line up and become ordered. In most water, hydrogen bonds are random. In SW, the hydrogen bonds gain some molecular stability while in motion. This is what happens when water molecules lose their randomness and become ordered. These ordered water molecules can create a few million molecular layers when a hydrophilic interface is present. Most constituents within cells are hydrophilic interfaces. Water molecules also contain a charge. Each molecule in the lineup has the opposite charge of the molecule beside it. This chain of
charges acts like a battery and gives the SW energy (Mercola and Pollack, 2011). Since structured water molecules have a slightly different angle between bonds, the oxygen atoms are more readily available to be used, and this promotes aerobic bacterial activity. This increases the amount of aerobic bacteria and purges anaerobic bacteria out of the human body (Aqua Technology, 2014).

Structured water is not a new concept but it is not well known. Among those in the scientific community who know about SW, according to Mercola (2011) there is much controversy surrounding the concept and it has even been described as a hoax due to lack of scientific evidence. Aqua Technology claims that credentialed professionals in chemistry have voiced strong and nearly uniform skepticism with regard to the benefits of structure (hexagonal) water, or SW, is not physically possible. Aqua Technology has countered that argument by stating that hexagonal water is water physics, not chemistry. Structured water was included as a factor in this research because it has grown in popularity with organic farmers and commercial greenhouse growers and has provided yield and nutritional increases in numerous undocumented testimonies. One user of SW, Dr. Calvin Bey – retired from the USDA Forestry Service and now an organic farmer – uses SW in his personal gardens and has seen impressive growth and production without using fertilizer additives. The average tomato plant in his garden produced approximately 100 pounds of fruit in a growing season (C. Bey, personal communication, October 11, 2013). The majority of studies on SW focus on how SW interacts with human cells while little research has been conducted on how SW interacts with plant cells. The objectives of this study were to assess the effects of different rates of BC alone and in combination with SW on radish growth and plant nutrient development.
III. Research objectives and hypothesis

Previous research has shown that the incorporation of biochar in soil is beneficial to plant growth at certain rates. Throughout literature, the addition of BC to soil increases plant growth. Little research has been conducted to evaluate the effects of structured water on plant growth. The objectives of this study were to evaluate the effects of biochar and water type on the total fresh weight, root fresh weight, leaf area, and mineral content of radishes. It was hypothesized that radish leaf area, total fresh weight, and root fresh weight would be greatest for radishes grown with 5000 kg ha\(^{-1}\) application rate of BC and watered with SW and that they would also have the highest plant mineral content.

IV. Materials and Methods

*Greenhouse Experiment*

This experiment was conducted in the Rosen Alternative Pest Control Center Greenhouse located on the University of Arkansas campus, in Fayetteville, AR. The experiment was initiated on 30 October 2013 and ended on 5 December 2013. The study was established as a completely randomized design. There were 6 treatments: three rates of poultry litter biochar and two water types. Each treatment was replicated 12 times.

Seventy-two 1- L plastic non-reactive pots were used. They were washed and sterilized prior to planting. A single coffee filter was placed in the bottom of each pot to prevent soil leaking from the base. Each pot was filled with approximately 1 L of the growing media which was a blend of 45% soil, 45% perlite, and 10% compost. The compost, made by Dr. Calvin Bey, was uniform in appearance and texture and was produced from lawn and plant waste. BC was
then ground to a fine powder and weighed to the appropriate values and added to their respective pots. The BC was then incorporated into the top few cm of growing media in each pot. These rates were equivalent to BC applications to each pot at the following rates: 0 kg ha\(^{-1}\), 5000 kg ha\(^{-1}\), and 10,000 kg ha\(^{-1}\).

The filled pots were transported from the preparation lab to the greenhouse and flushed with either SW or TW and allowed to drain overnight. When the soil settled, 3 radish seeds were planted per pot. The radishes were watered daily with 50 mL of their respective water type. Half of the radishes were watered with TW and the other half were watered with SW. Upon germination, each pot was thinned to one uniformly sized radish per pot. The radish pots were randomized and rotated weekly from one end of the bench to the other to avoid any biases from sunlight and air flow differences.

After the radishes completed their growth cycle, they were harvested and analyzed for total fresh weight, root fresh weight, leaf area, and root mineral content. Total plant and root fresh weights were determined by weighing at harvest on a Mettler analytical balance at the 0.00 g level of precision. Leaf area was analyzed using a LI-COR leaf area meter, LI-3100C Area Meter, (LI-COR Environmental and Biotechnology Research Systems, Lincoln, Neb.). Radish mineral content was determined with a Digital Hand-Held “Pocket” Refractometer PAL, which measures in units of percent Brix (ATAGO U.S.A., Inc.) which measures the amount of light refracted through a liquid, which can be used to detect sugar and mineral content of a liquid or slurry. The roots of the radish were pressed until liquid emerged. This liquid was used for the analysis.
Soil

The soil used in this study was obtained from a landowner in western Washington County from a small field adjacent to his commercial organic vegetable garden. This soil had experienced a minimum of 10 years of chemical-free operation prior to sampling. The soil was mapped as a Captina silt loam (fine-silty, siliceous, mesic, Typic Fragiudult) and described as prime farmland by the Natural Resource Conservation Service (USDA, 2013). The soil was combined with compost. A soil test on the mixture of soil and compost was performed by the University of Arkansas Division of Agriculture Soil Testing and Research Laboratory located in Marianna, AR. Soil was analyzed using the Mehlich 3 method and reported that 520 ppm NO$_3$-N, 189 ppm P, and 1009 ppm K were plant available. Soil test levels of P and K were “above optimum”. They recommended that only N needed to be applied at 2 lb/1000 ft$^2$.

Biochar and Structured Water

The two main factors under study in this project were BC and SW. The BC used in this study was derived from pyrolysed poultry litter as previously described. The BC used in this study was obtained from BioEnergy Systems, an Arkansas based LLC. The BC was analyzed for nutrient content and chemical properties results of which are depicted in Table 1. The SW was donated by Dr. Calvin Bey for use in this project. Dr. Bey structures water using a Dynamically Enhanced Portable Structured Water Unit available through Natural Action Technologies. This unit simply connects to any water faucet. When the tap is turned on, the TW flows through the unit and becomes structured through a series of vortexes.
Radish Seed

Radishes, *Raphanus sativa*, members of the Brassica family and grown worldwide, were used for this study due to their quick maturation time and being well suited for greenhouse culture. Radishes were grown from seed. Seeds were planted directly into the treated pots.

Measurements

After the growth cycle was complete, determined by the appearance of root swelling above the soil surface, the radishes were harvested and measurements were taken. Immediately after harvesting, each of the 72 radishes was weighed individually. Then, the roots were removed from the plant and a root fresh weight was measured. The leaves were then cut from the plant and run through a LI-COR leaf area meter (LI-3100C Area Meter, LI-COR, Lincoln, NE) to measure leaf area for each plant. Once the weights and leaf area were measured, the tap root of each radish was pressed using a garlic press to produce liquid and mineral concentration was measured using a Digital Hand-Held “Pocket” Refractometer PAL (ATAGO U.S.A., Inc.), commonly known as a Brix meter. Brix measures the amount of dissolved solids, but does not differentiate mineral type. Higher values indicate better quality, flavor, and mineral content.

Statistical Analysis

The data were analyzed using SAS® PROC MIXED. A completely randomized analysis of variance was performed to find the main effects and the interaction effect on the measurements. Estimates of predicted outcome were calculated for each variable within each treatment. Significant differences in total fresh weight, root fresh weight, leaf area, and nutrient density were based on P < 0.10.
V. Results

Radishes were used in this study due to their quick maturation time of approximately 30 days. Radishes are a vigorous, easy-to-grow, cool-season vegetable with potential for multiple crops per year. They are valued because of their ease of planting, their low management, and they can be eaten directly from the garden.

Fresh weight was significantly higher for radishes watered with TW than the radishes watered with SW (P < 0.05) (Figure 1). The mean fresh weight for plants watered with TW was 15.34 g. Biochar application were not statistically significant.

The main effects of water type and BC were evaluated for their independent influences on root fresh weight. Water type alone had a statistically significant response (P < 0.05). Plants watered with TW had a larger mean root fresh weight (7.61 g) than those watered with SW (4.82 g) (Figure 2). However, BC alone had a significant negative effect on the radish root fresh weight (P < 0.05) (Figure 3). Mean root fresh weight decreased significantly when 10,000 kg ha⁻¹ of BC was applied compared to the 0.00 kg ha⁻¹. There was no significant difference in root fresh weight when 5,000 kg ha⁻¹ of BC was applied compared to 10,000 kg ha⁻¹, but root fresh weight was significantly greater when 5,000 kg ha⁻¹ of BC was applied compared to the control of 0.00 kg ha⁻¹.

Leaf area was affected by water type (P < 0.05) (Figure 4) but not by BC application rate (data not shown). The average leaf area for radishes watered with TW was 300.80 cm² whereas plants watered with SW only averaged 257.83 cm² (Figure 4).

The Brix measurements showed a statistically significant interaction between water type and BC application (P < 0.10) (Figure 5). Tap water alone gave a mean Brix reading of 3%. The
Brix measurement increased to 3.79% for radishes treated with 10,000 kg/ha of BC and watered with TW. However, the treatments combining BC and SW had a very different reaction. When plants were only influenced by SW, the mean Brix reading was 3.4%. When 5,000 kg/ha of BC was added, Brix measurements dropped to 2.5%. With 10,000 kg/ha of BC added and watered with SW, the Brix reading went back up slightly to 3.08%, which is still less than the plants with no BC added.

VI. Discussion

When examining the effect of water type on radish growth, TW outperformed SW in total fresh weight, root weight, and leaf area. There was no interaction between the water type and the BC rate except in the Brix measurements. Biochar did not independently affect radish growth on nutrition at a statistical level.

Biochar had adverse affects on plant growth when compared to much of the previous research. When looking at the affects of BC alone, there was a significant decrease in root fresh weight as the BC rate increased (p = 0.0431). BC did not affect total fresh weight or leaf area. This is contradictory to what the majority of previous studies have found. The experiment conducted by Chan et al. (2008) showed an increase in radish dry matter with the lowest application of BC (10,000 kg ha$^{-1}$). While 10,000 kg ha$^{-1}$ was the highest application rate used in this experiment, we saw a significant reduction in total fresh weight. Biochar may need to be applied at rates greater than 10,000 kg ha$^{-1}$. Though we were analyzing the radishes for fresh weight and Chan et al. (2008) analyzed for dry weight, both measurements are valid for discussion. The dry weight of the radishes measures the weight of above ground dry matter. The
fresh weight includes the weight of the plant matter as well as nutrients, minerals, and the water retained in the plant. Leaf area has also been reported to increase when soils are treated with BC (Suppadit et al., 2012). We did not see a significant increase in leaf area in soils treated with BC. This may have been the result of lower application rates. Our highest rate was 10,000 kg ha\(^{-1}\) and Suppadit et al. (2012) used 16,000 kg ha\(^{-1}\) and found positive leaf area increase at that rate.

Other studies have found a similar trend. In our study, the control plants out yielded the biochar treated plants. Adams et al. (2013) found inconsistent results with the affects of BC on big bluestem. Big bluestem grasses grown in sandy soil and treated with BC were positively affected by BC but big bluestem grown in the silt soil had less consistent results with no response to 2% and 4% BC additions to the soil. Adams et al. (2013) concluded that the response of big bluestems to BC depended on soil type. Another study showed a significant reduction in yields of macadamia nut when BC was added to the soil compared to the control soil with no BC added (Revell et al., 2012a). In another study examining the effects of poultry litter BC, germination of telluuce decreased significantly when applied at rates of 2.5% by weight and greater (Revell et al., 2012b). This was likely salt toxicity since poultry litter contains inorganic salts.

Throughout the selected literature, the effects that BC has on plant growth have been very inconsistent. In the literature review by Biederman and Harpole (2013), eight of the 20 papers reviewed showed negative responses on plant growth, ten resulted in positive growth responses, and two had no effect. This variation in results is likely due to the wide array of feedstocks that can be used to create BC which results in different chemical properties. Variation could also be the result of by different soil types used.
One potential reason for lack of positive and significant results from the BC could be due to the fairly intensive irrigation (50 mL of water per day). Schulz and Glaser (2012) saw similar trends in their greenhouse experiment with BC and fertilizer. They found that the second growth stage of oats (*Avena sativa L.*) was substantially smaller than the first growth and speculated that it was due to the leaching of nutrients from daily irrigation.

Similar to the results found in the study by Chan et al. (2007), total weight and root weight were not affected by BC alone. Chan et al. (2007) saw a positive effect when BC was combined with N fertilizer. Van Zwieten et al. (2009) also concluded that BC and N fertilizer had a significant interaction. As the rate of BC and N fertilizer increased, there was a significant increase in radish total fresh weight compared to the control and to BC without N fertilizer. The results from our study indicate that BC did not significantly affect plant growth by itself but did have a positive effect on radish nutrient content when BC was combined with TW and a negative effect when in combination with SW as shown in Figure 5. This interaction was significant (P < 0.10). When the interaction between TW and BC is examined for nutrient content, as the BC rate increases, Brix measurement estimate increased.

The one parameter measured that showed a significant (P < 0.10) increase was the mineral content of the radish root which was measured using a Brix meter. The Brix meter measures the amount of light that is refracted in a liquid. The amount of total suspended solids (TSS) in the liquid is responsible for the amount of refracted light. In fruits and vegetables, this value, given in percent Brix, indicates the density of dissolved minerals, amino acids, and carbohydrates in the juice, but does not quantify the levels of each. Higher Brix measurements signify that the juice contains more TSS and have higher quality (Bionutrient Food Association, 2008). The results of our study showed a significant change in nutrient density due to an
interaction between BC and water type. When watered with TW and as the BC rate increased from 0 kg ha\textsuperscript{-1} to 10,000 kg ha\textsuperscript{-1}, the reading increased. The SW and BC interaction had a different affect on Brix measurements. When watered with SW, Brix measurements decreased at the medium rate (5,000 kg ha\textsuperscript{-1}) of BC then increased slightly at the maximum rate but was still lower than the Brix measurements of the control plants. In a study examining the effects of bagasse BC on sugarcane, researchers found that the Brix estimate was higher for sugarcane that was grown in soil treated with BC (Chen et al., 2010). Another study examined the effects of BC on sweet potatoes found that BC had a positive effect on Brix measurements resulting in a 20\% Brix increase (Dou et al., 2012). Our results were similar to these findings when TW was used.

Unlike the testimonials for the benefits of SW, our study showed that SW had a statistically significant (P < 0.05) negative impact on the growth parameters we examined in the radishes. Mercola and Pollack (2011) claim that drinking SW can be beneficial to cellular health in humans with the inference that all cellular health, including cellular health in plants, might benefit from SW. In our study we saw that SW seemed to have the opposite effect of what we expected and hypothesized. We predicted that plants watered with SW would grow more vigorously and be larger, and healthier than radishes watered with TW. Total fresh weight, root weight, and leaf area were all significantly smaller for radishes watered with SW. Our results contradict the results found by Bey in his organic garden. The significant growth and plant health in Bey’s garden could be attributed to other factors such as residual nutrients in the soil or the added nutrients from compost. Further research on SW should be done to evaluate if SW has an effect on plant growth and nutrition or if other variable are causing changes. There is a lack of scientific evidence on the existence of SW and its effects on plant and animal health. Scientists
should be skeptical and more research should be done to examine how the structuring unit alters water.

VII. Conclusion

In summary, the data analyses indicated that the type of water used for irrigation had the most pronounced influence on radish growth and development. Plants watered with TW had higher total fresh weights, root weights, and larger leaf areas (P < 0.05). There was also an interaction between the water type and BC (P < 0.10). When TW and BC were combined, the nutrient content increased as the BC rate increased. When SW and BC were combined, nutrient content was lower for plants that had BC added to the soil. Based on the results of this study, further trials to examine the effects of SW and the interaction between BC and water type would be appropriate. One area in particular that needs further research is examining SW in different conditions and with different plants.

Acknowledgements

Thank you to BioEnergy Systems LLC for producing the biochar used in this experiment. Thank you to Dr. Calvin Bey for supplying the structured water, soil, and compost as well as much guidance. Dr. Edward Gbur, Agricultural Statistics Laboratory, Univ. of Arkansas and his M.S. student Lichen Hao completed the statistical analysis and provided guidance on graphics.
Literature Cited


Aqua Technology. Understanding hexagonal water.


## Tables and Figures

### Table 1. Compositional analysis of BioEnergy Systems, LLC (BES) Biochar

<table>
<thead>
<tr>
<th>Measured Property (unit)</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (pH units)</td>
<td>10.20</td>
</tr>
<tr>
<td>Electrical Conductivity (μmhos cm⁻¹)</td>
<td>16680.00</td>
</tr>
<tr>
<td>P (mg kg⁻¹)</td>
<td>7076.00</td>
</tr>
<tr>
<td>K (mg kg⁻¹)</td>
<td>26412.00</td>
</tr>
<tr>
<td>Ca (mg kg⁻¹)</td>
<td>3217.00</td>
</tr>
<tr>
<td>Mg (mg kg⁻¹)</td>
<td>3071.00</td>
</tr>
<tr>
<td>S (mg kg⁻¹)</td>
<td>3525.00</td>
</tr>
<tr>
<td>Na (mg kg⁻¹)</td>
<td>6880.00</td>
</tr>
<tr>
<td>Fe (mg kg⁻¹)</td>
<td>32.00</td>
</tr>
<tr>
<td>Mn (mg kg⁻¹)</td>
<td>190.00</td>
</tr>
<tr>
<td>P (mg kg⁻¹)</td>
<td>46915.00</td>
</tr>
<tr>
<td>K (mg kg⁻¹)</td>
<td>72298.00</td>
</tr>
<tr>
<td>Ca (mg kg⁻¹)</td>
<td>67904.00</td>
</tr>
<tr>
<td>Mg (mg kg⁻¹)</td>
<td>15298.00</td>
</tr>
<tr>
<td>S (mg kg⁻¹)</td>
<td>10486.00</td>
</tr>
<tr>
<td>Na (mg kg⁻¹)</td>
<td>19919.00</td>
</tr>
<tr>
<td>Fe (mg kg⁻¹)</td>
<td>2453.00</td>
</tr>
<tr>
<td>Mn (mg kg⁻¹)</td>
<td>1397.00</td>
</tr>
<tr>
<td>Zn (mg kg⁻¹)</td>
<td>1261.00</td>
</tr>
<tr>
<td>Cu (mg kg⁻¹)</td>
<td>801.00</td>
</tr>
<tr>
<td>%Total N</td>
<td>3.00</td>
</tr>
<tr>
<td>%Total C</td>
<td>32.03</td>
</tr>
</tbody>
</table>

* pH (1:2 soil ratio), Mehlich 3 extractable (1:10 ratio) Analysis by SPECTRO ARCOS ICP
* Total Recoverable Metals, EPA method 3050, measured on Spectro Arcos ICP
* Total N and C by combustion, Elementar Variomax

### Table 2. ANOVA table for main effects and interaction effects

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>BRIX</th>
<th>RFW¹</th>
<th>FW²</th>
<th>AREA³</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC</td>
<td>2</td>
<td>0.1793</td>
<td>0.0413</td>
<td>0.6572</td>
<td>0.1279</td>
</tr>
<tr>
<td>WATER</td>
<td>1</td>
<td>0.1190</td>
<td>0.0049</td>
<td>0.0001</td>
<td>0.0092</td>
</tr>
<tr>
<td>BC*WATER</td>
<td>2</td>
<td>0.0595</td>
<td>0.6948</td>
<td>0.5336</td>
<td>0.3698</td>
</tr>
</tbody>
</table>

¹Root fresh weight
²Fresh weight (total)
³Leaf Area
Fig 1: Estimated total radish plant fresh weight for structured water (SW) and tap water (TW) treatments. Error bars indicate standard error. Different letters indicate significant differences.

Fig 2: Estimated root fresh weight for structured water (SW) and tap water (TW). Error bars indicate standard error. Different letters indicate significant differences.
Fig 3: Estimated root fresh weight for each biochar application rate. Error bars indicate standard error. Different letters indicate significant differences.

Fig 4: Estimated leaf area for each structured water (SW) and tap water (TW) treatment. Error bars indicate standard error. Different letters indicate significant differences.
Fig 5: Changes in Brix estimate for each biochar treatment with structured water (SW) and tap water (TW) treatments.