12-1-2019

Evaluating effects of southern yellow pine biochar and wood vinegar on poultry litter

Maryam Mohammadi-Aragh

Evaluating effects of southern yellow pine biochar and wood vinegar on poultry litter

By

Maryam K. Mohammadi-Aragh

A Dissertation
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Doctor of Philosophy
in the College of Forest Resources
in the Department of Sustainable Bioproducts

Mississippi State, Mississippi

December 2019

Evaluating effects of southern yellow pine biochar and wood vinegar on poultry litter

By

Maryam K. Mohammadi-Aragh

Approved:

_____

C. Elizabeth Stokes
(Major Professor)

_____

Jason Tyler Street
(Committee Member)

_____

John Linhoss
(Committee Member)

_____

El Barbary M. Hassan
(Committee Member)

_____

Rubin Shmulsky
(Graduate Coordinator)

_____

George M. Hopper
Dean
College of Forest Resources

Name: Maryam K. Mohammadi-Aragh

Date of Degree: December 13, 2019

Institution: Mississippi State University

Major Field: the College of Forest Resources

Major Professor: C. Elizabeth Stokes

Title of Study: Evaluating effects of southern yellow pine biochar and wood vinegar on poultry litter

Pages in Study: 116

Candidate for Degree of Doctor of Philosophy

The objectives of this study were to investigate nutrient retention, *intI1* prevalence, and compost maturity rates for poultry litter co-composted with 5, 10, and 20% southern yellow pine biochar and with or without 2% wood vinegar (WV). Samples were collected at 0, 57, and 112 days to measure nitrogen, phosphorus, and potassium (N, P, K) concentrations, microbial counts, pH, moisture content, carbon to nitrogen (C:N) ratio, and *intI1* abundance. Composts were aerated once a week and the temperature was also recorded once a week. There was sufficient rainfall so no additional water was added. The results showed that N and P concentrations significantly increased over time in all treatments except 20% biochar and 20% biochar + wood vinegar, while K concentrations significantly decreased. In general, composting with wood vinegar significantly decreased nutrient concentrations; however, all nutrient concentrations were much higher than typical animal manure fertilizers. Increases in biochar level resulted in significantly lower bacteria counts and significantly higher fungi counts. Compost treatments containing wood vinegar had significantly lower bacteria and fungi counts, indicating that southern yellow pine wood vinegar had a biocide effect on microorganisms, and may be not suitable for composting at that application rate. *intI1* prevalence was not significantly different

among treatments, which may be due to insufficient thermophilic composting. Because thermophilic temperatures were not achieved, the compost was not mature by the end of the study; therefore, compost maturity rates could not be determined.

DEDICATION

This work is dedicated to the passionate scientists whose curiosity, ingenuity, and stubbornness continue to illuminate the unknowns left in this world. I also dedicate this work to my husband and best friend, David Leal. Without his patience, support, and love, I could not have completed my degree. Furthermore, I would like to thank Dr. Beth Stokes for her mentorship, and will pass on her kindness and dedication in my future endeavors. I would also like to thank my fellow graduate students: Julianna, Gabi, Shanice, Jazmine, Cody, Johnathan, Lakshmi, Telmah, and Dercilio for their unwavering assistance throughout my project. I also halfheartedly apologize to anyone whose nose never fully recovered from smelling poultry litter.

ACKNOWLEDGEMENTS

I would like to thank Dr. Beth Stokes and the Mississippi State Department of Sustainable Bioproducts for supporting my research. I would also like to thank Dr. Jason Street, Dr. John Linhoss, and Dr. El Barbary Hassan for their guidance and knowledge. Lastly, I would like to thank Dr. Heather Jordan, Dr. Donna Gordon, and Dr. Tom Tabler for their assistance.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

LITERATURE REVIEW

1.1 Compost

1.1.1 Background

Composting is defined as "the biological degradation of organic matter under controlled, aerobic conditions into a humus-like stable product" (Epstein, 1997). Composting is the best method for waste disposal because it recycles wastes to create a usable product that adds organic matter back into the soil (Epstein, 1997). Any organic matter can be composted under the proper conditions, including yard trimmings, food, and lignocellulosic residues. The benefits of composting are improving soil health and plant growth, reducing soil erosion and nutrient runoff, attracting earthworms, and reducing non-source pollution (Epstein, 1997). Therefore, composting organic wastes has positive impacts on agriculture as well as the environment.

There are advantages and disadvantages of using composting as a waste management strategy. The advantage is that composting can be applied to many community wastes, so one composting facility can manage municipal and industrial organic biosolids, yard wastes, food wastes, and agricultural wastes (Epstein, 1997; University of Arkansas Cooperative Extension Service, 2019). In addition, composting reduces the disposal of wastes in landfills. Ultimately, composting produces a usable material with high agricultural value. The disadvantages of using composting as a waste management method include (1) the production of odor and bio-aerosols

during the composting process, (2) composting facilities require more space than other waste management facilities, (3) there must be market demand (Epstein, 1997).

1.1.2 Composting Process

The most critical factors affecting the microbial decomposition of organic matter are oxygen and moisture (Epstein, 1997). Temperature is also an important factor; however, the temperature of a compost is the result of microbiological activity (Epstein, 1997). Therefore, microbiological activity is the driver of the composting process, and needs a specific range of oxygen and moisture to undergo aerobic cellular respiration (Figure 2.1). In addition to oxygen and moisture, pH and nutrients also affect microorganism growth. Composting can occur at a pH range of 5.5 to 9.0, with 6.5 to 8.0 being optimal (Rynk et al., 1992). The primary nutrients required by microorganisms are carbon and nitrogen. Carbon serves as the primary energy source and nitrogen provides molecules for cellular reproduction (Epstein, 1997). Carbon to nitrogen ratio (C:N) is used to provide a profile of the relative percentage of carbon to nitrogen in organic materials (University of Arkansas Cooperative Extension Service, 2019). Table 1.1 shows the ideal conditions at which rapid composting occurs. Microbes are most active at a C:N of 30:1; however, a good quality compost will be produced with a C:N range of 20:1 to 40:1 (Rynk et al., 1992; University of Arkansas Cooperative Extension Service, 2019). When the C:N ratio is too low, meaning there is an abundance of nitrogen, the nitrogen will volatilize as ammonia gas. If the C:N ratio is too high (>40:1), then there is not enough nitrogen for microorganisms to grow, and the composting process will be very slow (University of Arkansas Cooperative Extension Service, 2019). Commonly used carbon sources include hay, wood wastes, paper, and leaves. Compost nitrogen sources include animal manure, grass clippings, coffee grounds, and plant food scraps (University of Arkansas Cooperative Extension Service, 2019). During the

composting process, the C:N ratio gradually decreases due to loss of CO_2; however, if the initial C:N ratio is less than 15:1, the nitrogen lost as ammonia gas will be substantial enough to result in a similar final C:N ratio (Rynk et al., 1992). In addition, microorganisms require micronutrients such as Cu, Mg, Zn, P, S, and Na; however, little known about the function of micronutrients in the composting process (Epstein, 1997).

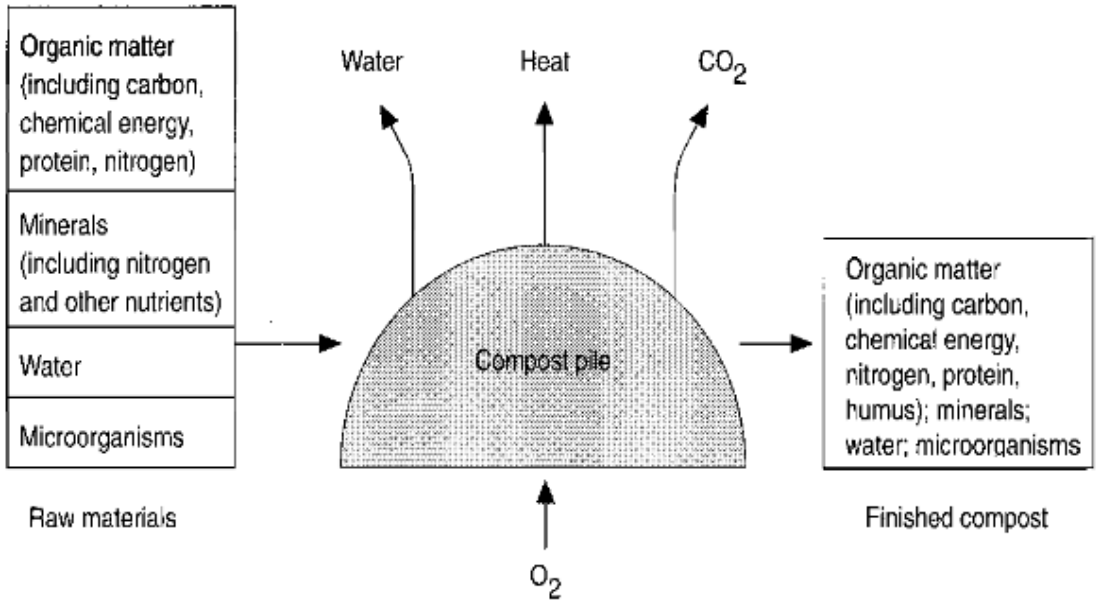


Figure 1.1 The composting process (Rynk et al., 1992)

Table 1.1 Conditions for rapid composting (Rynk et al., 1992)

Condition	Reasonable range [a]	Preferred range
Carbon to nitrogen (C:N) ratio	20:1–40:1	25:1–30:1
Moisture content	40–65% [b]	50–60%
Oxygen concentrations	Greater than 5%	Much greater than 5%
Particle size (diameter in inches)	1/8–1/2	Varies [b]
pH	5.5–9.0	6.5–8.0
Temperature (°F)	110–150	130–140

Note: As stated by the Rynk et al., these conditions are for rapid composting and are not required to yield successful results (1992).

Most of the heat produced in a compost is the result of microbial activity; therefore, composts are generally self-heated. This heat accumulates due to the organic mass acting as an insulator, trapping the heat inside. The temperature affects the microbial populations in the compost. Mesophilic bacteria are the primary drivers of initial decomposition and as the heat increases due to self-insulation, the mesophiles are inhibited and thermophilic bacteria are activated. Thermophilic bacteria are critical for reaching temperatures high enough to accelerate decomposition and inactivate pathogens and weed seeds. During aerobic respiration, microorganisms consume oxygen and release carbon dioxide (CO_2), water (H_2O), ammonia (NH_3), and other volatile compounds (Epstein, 1997).

In general, the composting process is not sensitive to pH; however, pH influences some factors depending on the raw material (Rynk et al., 1992). The pH is more critical in raw materials that are high in nitrogen, because at a pH above 8.5 nitrogen compounds are converted to ammonia, which further increases the pH. Lowering the pH to below 8.0 reduces ammonia emissions; therefore, adding alkalizing agents to composts is discouraged to prevent ammonia

volatilization (Rynk et al., 1992). The pH will fluctuate during the composting process due to the release of organic acids and ammonia production; however, a mature compost has a stable, neutral pH (Rynk et al., 1992).

Temperature is a crucial factor for composting duration and safety and is dependent on the activity of microorganisms. Composting undergoes three temperature phases named after the type of prevalent microorganisms: mesophilic (50 – 105°F), thermophilic (> 105°F), and a cooling and maturation phase that is also mesophilic. The most effective composting temperature range is between 110 - 150°F because it kills weed seeds and pathogens; however, composting will still occur at lower temperatures (Rynk et al., 1992). The critical temperatures for destroying human pathogens and weed seeds are 131 and 145°F, respectively (Rynk et al., 1992).

The types of microorganisms present in compost include bacteria, actinomycetes, fungi, protozoa, and rotifers. Bacterial activities control the temperature of the compost. Actinomycetes are filamentous, fungi-like bacteria that are more active in the maturation phase as they are responsible for breaking down the remaining resistant compounds such as lignin, cellulose, chitin, and proteins (Trautmann and Olynciw, 1996). Fungi are the primary organisms that break down complex plant-based carbohydrates such as lignin, cellulose, and hemicellulose.

1.2 Biochar

1.2.1 Biochar production, properties, and uses

Subjecting biomass to high temperatures with little oxygen, known as pyrolysis, creates biochar. Biochar is similar to charcoal; however, it is used primarily as a soil amendment to improve soil quality, sequester carbon, and remediate soil pollution (Kavitha et al., 2018)

In the forestry and forest products industries, wood wastes may be pyrolized to generate energy, leaving biochar as a residue (Steiner et al., 2010). Wood chips, wood shavings, bark, and

sawdust are sources for biochar production in the forestry and forest products industries (Dhungana, 2019). Around 97 million dry tons of wood residues are generated annually from residues produced at forest products processing mills, fuel-wood harvested from forestlands, and from forest fire risk reduction initiatives (Steiner et al., 2010). Carbon is the primary element found in biochar. Nitrogen, hydrogen, potassium, and magnesium, all of which are important source of plant nutrients, are also present in biochar (Kavitha et al., 2018).

Although studies have shown that biochar enhances soil qualities, several factors affect its efficacy. These factors include pyrolysis temperature, feed stock source, soil type, and biotic interactions (Kavitha et al., 2018). Biochar improves soil physiochemical and biological properties by adding organic matter to soils. However, studies have shown that biochar can have a stimulatory or inhibitory effect on soil microbiome environments (Kavitha et al., 2018).

The physiochemical and structural properties of biochar are primarily dependent on pyrolysis temperature, which range from 300 to 1000°C (Liu et al., 2010; Kavitha et al., 2018). The biomass sources for biochar are mostly comprised of cellulose, hemicellulose, and lignin, with lignin being the most resistant to degradation (Fahmi et al., 2008). During pyrolysis, volatile organic compounds and water evaporate from the biomass, which increases the aromatic content of the biochar. Pyrolysis at high temperatures increases the alkalinity of the resultant biochar due to the partial detachment of functional groups resulting in the formation of unpaired negative charges such as carboxyl (COO-) and hydroxyl (OH-) groups (Singh et al., 2010; Al-Wabel et al., 2017). These negative charges attract positive charges, which is crucial for the adsorption of cations such as potassium, calcium, sodium, and heavy metals. Increasing the attraction of positive charges to the negative functional groups increases the cation exchange capacity (CEC). Pyrolysis temperature not only affects the chemical properties of biochar, but also determines the

porosity, pore size, and surface area of biochar particles, with higher pyrolysis temperatures increasing these physical properties (Kavitha et al., 2018). This is due to the higher temperatures volatilizing organic compounds (Brewer et al., 2014). Feedstock is another important factor in biochar properties. Lei and Zhang reported that wood chip-derived biochars had higher porosity and surface area than dairy cow manure-derived biochars (2013). Higher surface area and porosity facilitates better absorption and adsorption, increasing the value of the biochar. Biochar properties can be optimized by manipulating production factors to meet specific needs.

1.2.2 Biochar as a soil amendment

Several studies have reported that biochar acts as an excellent soil amendment and soil conditioner (Kavitha et al., 2018). Biochar has shown to enhance low fertility soils through improving nutrient availability, soil physical properties, and crop productions (Sanchez-Monedero et al., 2018). Biochar increases soil aggregation and stability while reducing compaction. In addition, the porosity improves water-holding capacity. Biochar also improves soil fertility because it promotes nitrogen and phosphorous biochemical cycles (Gul and Whalen, 2016). Biochar is a highly recalcitrant and stable compound that resists decomposition in the soil. The average time required for biochar to degrade is 3000 years (Kavitha et al., 2018).

Biochar application has shown to improve plant growth and reduce plant stress by enhancing root development and promoting the growth of beneficial bacteria in the rhizosphere. In addition, studies show that biochar addition to soils stimulates plant defense systems to resist fungal pathogen infection (Kavitha et al., 2018).

Soil microbial populations are important for plant health because microorganisms facilitate nitrogen fixation, nutrient cycling, defense mechanisms, and decomposition of soil matter (Kavitha et al., 2018). Biochar amendments have shown to improve the soil microbiome

and several studies report that biochar significantly reduced arbuscular mycorrhiza fungi colonization, *Fusarium* chlamydospores infection, Fusarium crown rot, and root rot on tomato plants (Akhter et al., 2015; Han and Douds, 2016; Jaiswal et al., 2017).

There are some limitations for using biochar as a soil amendment. Kim et al. report that biochar's ability to adsorb nitrogen and other essential micronutrients could be counterproductive to plant growth (2015). Therefore, biochar's adsorbent properties may compete with plant nutrients, hindering plant growth. In addition, biochar may have an inhibitory effect on fungal growth, as Zheng et al. found in a study where biochar amendments reduced the abundance of Ascomycota and Basiomycota species by 11 and 66%, respectively (2016). The reduction of fungal populations may inhibit the decomposition of highly stable organic compounds, thereby increasing the decomposition time for organic matter. Yamato et al. (2006) reported, "a significantly increased peanut yield following biochar amendment on an infertile soil in Sumatra, with no significant change in yield for fertile soil, along with general increases in soil pH, N, available P, and CEC."

Finally, the benefits of biochar amendment to soils have been conducted under specific conditions that limit the scope of biochar's effects in soils. Therefore, more research is needed to thoroughly and properly describe the effects of biochar amendments and characterize any negative effects on agricultural soils.

1.2.3 Biochar in composting

When compost, manure, or chemical fertilizer is applied to agricultural fields, many of the vital nutrients such as nitrogen (N), phosphorous (P), and potassium (K) are lost through leaching and volatilization, resulting in financial strife for farmers and environmental pollution (Barrow, 2012). Biochar's physiochemical properties that confer improved water holding

capacity, cation exchange capacity, porosity, and surface area would help prevent these issues. Although there is a plethora of information on biochar's effects as a soil amendment, there is less information on how biochar influences the composting process of difference organic wastes (Sanchez-Monedero et al., 2018). Sanchez-Monedero et al. (2018) state, "The physicochemical, chemical, and microbiological properties of a composting pile are favorable for the interaction with biochar and, consequently, it is expected that a synergy would be established between the pool of organic matter, nutrients and microbial biomass of the composting material and the physicochemical properties of biochar." The composting process oxidizes biochar. This oxidation causes changes to biochar's surface chemistry, resulting in the activation of functional groups (Prost et al., 2013).

The addition of biochar has shown to stimulate microbial activity; therefore, the thermophilic stage is reached faster, and the maximum temperature is higher (Chen et al., 2010; Steiner et al., 2010). This increase in microbial activity results in several composting benefits, such as reducing composting time and faster stabilization (Sanchez-Garcia et al., 2015; Vandecasteele et al., 2016).

Application rate, feedstock, pyrolysis temperature, and particle size are biochar factors that impact the composting process (Sanchez-Monedero et al., 2018). Various biochar application rates have been studied in composting. A 10% application of biochar (dry weight) is recommended for optimum composting performance; however, small doses of 3-5% provides beneficial effects as well (Hua et al., 2009; Lopez-Cano et al., 2016). Steiner et al. (2010) and Dias et al., (2010) have reported successful composting with biochar application rates of 20 and 50%, respectively. However, studies have shown that using higher than 20% biochar in

composting can negatively affect microbial activity (Ishizaki and Okazaki, 2004; Liu et al., 2017).

Various particle sizes have been studied for optimizing biochar's physical properties such as porosity and water holding capacity. For composting, Sanchez-Monedero et al. (2018) report that there is no recommendation for biochar particle size in composting; however, particle size does significantly influence porosity and water holding capacity (Linhoss et al., 2019). A study conducted by Linhoss et al. (2019) reported that water holding capacity for broiler litter included with 20-50% biochar was not significantly different from each other but was significantly higher than inclusion rates of 0-10%. The study reported that adding 20% biochar to the broiler house bedding increased the overall water holding capacity of the litter by 49,210 L. In addition, the authors found that coarse biochar particles could hold more water than fine biochar particles because fine particles have lower porosity (Linhoss et al., 2019).

Agegnehu et al. (2015) found that although compost treated peanut fields had higher total soil P, the available P was lower than in peanut field soils treated with biochar. Because legumes can fix their own N, P is the most limiting nutrient. In addition, the authors found that organic amendments (biochar, compost, and biochar-compost blend) increased peanut plant growth and crop yield compared to application of solely inorganic fertilizer. The study also found that applying organic soil amendments supplemented with inorganic fertilizers yielded the highest plant growth and peanut yield. Biochar, compost, and biochar co-compost improved soil water content, CEC, and nutrient availability more than the inorganic fertilizer (Agegnehu et al., 2015). Legume crops experience a better response to biochar application, followed by vegetables and grasses (Liu et al., 2013). Steiner et al (2010) found that composting poultry litter with 20% biochar reduced total N losses by 52%, mostly through ammonia adsorption.

1.3 Wood Vinegar

Wood vinegar (WV), also known as pyroligneous acid, is another byproduct of biomass pyrolysis (Kishimoto and Tsuyoshi, 2019). Wood vinegar can be isolated during pyrolysis by capturing the smoke, allowing it to cool to its liquid form, and collecting the middle fraction from the distillate. This smoke distillate separates into three distinct fractions. The top fraction consists of a light oily substance, the middle layer consists of a transparent, yellowish-brown liquid (pyroligneous acid), and the lowest fraction contains a heavy wood tar substance (Kishimoto and Tsuyoshi, 2019). Figure 2.1 shows the basics of distilling WV from birch (*Betula* spp.) wood.

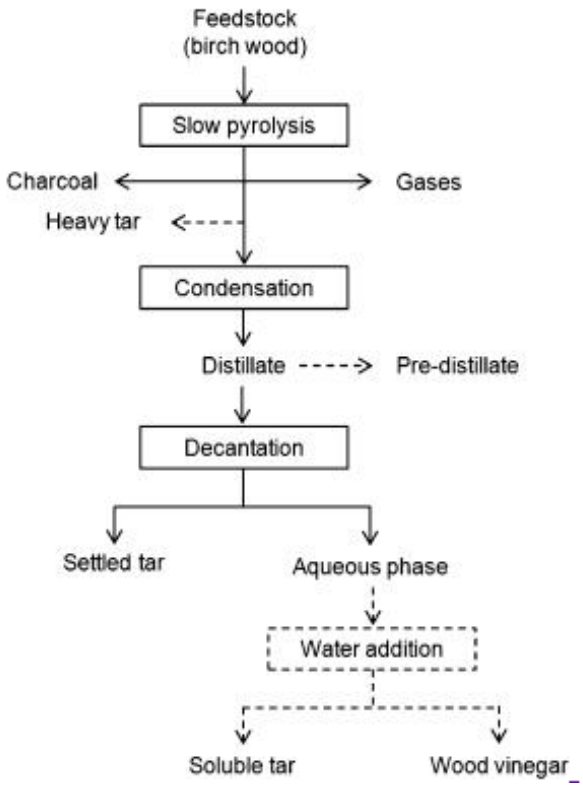


Figure 1.2 Diagram of the wood vinegar distillation process from birch wood (*Betula* spp.) feedstock (Fagernäs et al., 2012).

Wood vinegar contains mostly organic acid compounds along with phenolic substances, alcohols, and other carbon compounds, and it has been used as an insect repellent, wood preservative, odor-remover, soil fertilizer, and animal-feed additive (Yan et al., 2011; Wu et al., 2015). In addition, wood vinegar exhibits antimicrobial and antioxidant properties, which are associated with the presence of organic acids and phenolic compounds, respectively (Li et al., 2018). The phenolic and acetic acid groups also give WV termiticidal properties (Yatagai et al., 2002). Wood vinegar is dose dependent on microbial activity, as concentrated doses have a biocidal effect, while dilute doses stimulate microbial activity (Steiner et al., 2008; Baimark et al., 2009; Velmurugan et al., 2009).

Feedstock chemical composition determines the chemical make-up of pyroligneous acid. Cellulose, hemicellulose, lignin, and ash contents impart different qualities to pyroligneous acids; therefore, the effects of pyroligneous acid are highly specific depending on plant source material (Fujita et al., 2012; Mathew et al., 2015). For example, wood vinegars collected from hardwood and softwood trees are considerably different due to variations in carbohydrate composition (Fagernäs et al., 2012). Fagernäs et al. (2012) state "hardwoods contain on average 8 wt % less lignin and 3 wt % more hemicelluloses than softwoods. In contrast to softwood lignin composed of guaiacyl-type lignin, hardwood lignin consists of syringyl and guaiacyl units."

A greenhouse experiment conducted by Hagner et al. (2013) found that fine sandy soils mixed with birch biochar (3.3% w/v) had no effect on Ca, K, Mg, and P, and wood vinegar (0.26% w/v) showed no effect on concentrations of total N, Ca, K, Mg, and P in soils after 80 days. The wood vinegar used in this study had a pH of 2.04. Furthermore, the authors reported that biochar exhibited no effects on soil microbial activity at Day 4. However, there was a

significant increase in microbial activity in the biochar-amended soils at Day 46 and 80 (Hagner et al., 2013. The study also measured the effects of wood vinegar and biochar on the chemical composition of leachates. Hagner et al. (2013) found that (1) "the effects of wood vinegar and biochar on pH, conductivity, and total organic carbon (TOC) of leachates were slight and mostly transient," and that none of the 14 most abundant wood vinegar chemical compounds were detected in the leachates. The authors also found that wood vinegar did not significantly affect microbial activity. In addition, they reported that total N increased by almost 20% with addition of biochar, and biochar-amended soils lost 3% of total N, compared to the control losing 12% of total N. Biochar may affect N cycling through influencing nitrification rates, ammonia adsorption, and increasing ammonia (NH_4) storage by increasing the CEC in soils (Clough et al., 2010). The authors postulate that biochar decreased total N loss due to the strong N sorption capacity imposed by the surface area of the biochar (Hagner et al., 2013). Birch wood vinegar has a low environmental impact in fine sandy soils, and it rapidly degraded by soil microorganisms (Hagner et al., 2013).

A composting study conducted by Wang et al. (2018) observed nitrogen conservation and greenhouse gas emission in pig manure combined with tobacco stalk biochar (B), wheat straw, zeolite (Z), and wood vinegar (WV). The composting duration was 50 days and took place in 130L polyvinyl chloride (PVC) reactors. Different concentrations of wood vinegar were tested, and compost blends treated with 2% wood vinegar reached the thermophilic phase faster than other blends, and reached the highest temperature (Wang et al., 2018). These results were similar to a study conducted by Chen et al. (2010), where bamboo biochar and bamboo vinegar were used as additives in pig manure composting. Wang et al. (2018) reported a significant increase in pH ($p<.05$) after adding biochar to pig manure compost. In this study, the initial pH for pig

manure composts mixed with biochar and 0.5% (B + Z + 0.5% WV), 1.0% (B + Z + 1.0% WV), and 2.0% (B + Z + 2.0% WV) wood vinegar was 8.02, 7.84, and 7.8, respectively. At the end of the composting period, the pH values were 7.96, 8.5, 8.55, 8.64, and 8.56 for the control, B, B + Z + 0.5% WV, B + Z + 1.0% WV, B + Z + 2.0% WV were not significant ($p > .05$). Therefore, the authors concluded that the addition of WV had no significant effect on the pH when applied up to 2.0% w/v.

1.4 Poultry litter

Major improvements in the efficiency of livestock systems is needed to be able to meet the rising demands for meat and egg products while reducing environmental impacts (FAO, 2019). According to the FAO (2019), there are three ways to accomplish this goal with existing technology: "reduce the level of pollution generated from waste and greenhouse gases; reduce the input of water and grain needed for each output of livestock protein and recycle agro-industrial by-products through livestock populations." Since 1967, the global production of poultry meat and eggs has increased by almost 700% and 350%, respectively, which is much higher than demands for porcine and ruminant meats (FAO, 2019). Bolan et al. (2010) states, "the poultry industry is one of the largest and fastest growing agro-based industries in the world." The most significant problem facing the poultry industry is the wastes disposal issues caused by the large accumulation of poultry manure and litter (Bolan et al., 2010). In 2008, an estimated 44 million tons of poultry manure was produced in the United States (McDonald et al., 2009). Poultry litter is composed of bedding material, which can be wood shavings, cereal straw, husk, or paper clippings, along with feathers, manure, and spilt feed (Kelley et al., 1996; Swain and Sundaram, 2000, Tasistro et al., 2004). Poultry litter is rich in nutrients such as N, P, and K, as well as trace elements such as copper (Cu), zinc (Zn), and arsenic (As), calcium (Ca),

magnesium (Mg), and sulfur (S) (Bolan et al., 2010). Other, less desirable, constituents may include pesticide residues, coccidiostats, and endocrine disruptors (Bolan et al., 2010). Currently, most poultry litter is applied to agricultural land as a fertilizer (Bolan et al., 2010). Other uses of poultry litter include feeding it to livestock and generating fuel (Bolan et al., 2010).

Manure can be recycled by applying it to agricultural lands, which may benefit soil fertility by increasing plant nutrient availability, altering soil pH, increasing organic matter content, increasing cation exchange capacity, water holding capacity, and soil tilth (Bolan et al., 2010). However, there are some significant environmental and public health concerns over applying poultry litter to arable lands (Bolan et al., 2010). These include N leaching into groundwater, P contamination of surface water, increasing air pollution through greenhouse gas and volatile organic compounds emissions, and increased metal deposits (Williams et al., 1999, Ribaudo et al., 2003, Harmel et al., 2004, Casey et al., 2006). Arsenic (As), copper (Cu), and zinc (Zn) are trace elements that can be disseminated into the environment at toxic levels through poultry litter application (Bolan et al., 2010). In addition, poultry litter reduces air quality by releasing dust, foul odors, and bio-aerosols (Millner, 2009). The noxious gases released from poultry litter, which include amines, amides, mercaptans, sulphides, and disulphides, can cause respiratory diseases in humans and animals (Schiffman and Williams, 2005). Furthermore, ammonia gas, carbon dioxide, methane, and nitrous oxide are released from poultry litter while in use, during storage, and after application to agricultural fields (Walker et al., 2000a; Walker et al., 2000b; Bolan et al., 2010). Many of these gases are implicated in ozone depletion, acid rain, and foul odors; therefore, it is imperative to improve poultry litter handling and storage practices to reduce these emissions (Aneja et al., 2006).

There are three common practices for managing poultry litter in broiler houses: single use, partial re-use, and multi-use litter (Bernhart et al., 2010). Single-use litter involves removing all of the litter after removing one flock and adding new bedding material. Partial re-use litter involves removing litter from the brooding area and spreading it on the grower section and adding new bedding material to the brooding area (Bolan et al., 2010). Partial re-use litter may be composted in house for a few days to kill pathogens. Some of the partially spent litter is removed, and so a poultry house may remove all of the old litter after two to five flocks by rotating out some spent litter and adding new bedding material. (Bolan et al., 2010). Multi-use litter involves only removing caked material after each flock and adding 25-50 cm of new bedding material to the surface (Sistani et al., 2003). This practice yields a spent litter that has a higher nutrient concentration, but also may increase the occurrence of pathogens and parasites (Kelley et al., 1996).

Trace element supplements (Cu, As, iron (Fe), Mn, cobalt (Co), selenium (Se) and Zn) are commonly used in the poultry industry to prevent deficiencies and diseases, promote growth and feed conversion, and increase egg production (Miller et al., 1991; Tufft and Nockels, 1991; Sims and Wolf, 1994; Moore et al., 1995; Powers and Angel, 2008; Burel and Valat, 2009). Growth promoters help improve nutrient absorption efficiency to reduce N and P excretion. However, some growth promoters contain heavy metals, which result in manures having high levels of these metals (Nahm, 2002). Trace elements and heavy metal concentrations in the excreta have a linear relationship to the ingested elemental concentrations; therefore, decreasing the concentration of trace elements in feed and metals in growth promoters results in lower concentrations of those elements in the excreta (Krishnamachari, 1987; Miller et al., 1991; Van Ryssen, 2008).

Poultry manure carries a high microbial load that consists of viruses, bacteria, fungi, and protozoa (Bolan et al., 2010). Gram-positive bacteria, such as *Actinomycetes*, *Clostridia*, *Bacilli*, *Lactobacilli*, and *Eubacteria*, consist of almost 90% of bacterial species in poultry manure (Lu et al., 2003; Enticknap et al., 2006; Lovanh et al., 2007). The two most important groups of microorganisms in the poultry litter environment are nitrogen mineralizing microorganisms and pathogens (Bolan et al., 2010).

Nitrogen mineralizing microorganisms transform organic nitrogen compounds, primarily urea and uric acid, into inorganic nitrogen (Brinson et al., 1994; Moore et al., 1996). The major inorganic nitrogen compounds are nitrate (NO_3^-) and ammonium (NH_4^+), which are converted to more stable molecules. More than half of inorganic nitrogen in poultry litter is lost through ammonia volatilization as a result of microbial activity (Brinson et al., 1994; Moore et al., 1996). Nitrogen mineralization and reducing ammonia volatilization is critical for fertilizer potential because plants uptake inorganic N. The dominant uric acid mineralizing bacteria in poultry litter are *Bacillus* spp., and *Arthrobacter* spp. (Schefferle, 1965; Kim and Patterson, 2003). In addition, *Aspergillus* spp. can mineralize organic nitrogen species found in poultry litter (Cook et al., 2008).

Pathogens are the second important group of microorganisms residing in poultry litter. Pathogens in poultry litter have been extensively studied because poultry litter is a major reservoir for several zoonotic pathogens, including *Escherichia coli*, *Salmonella* spp., *Campylobacter jejuni*, *Listeria monocytogenes*, and *Clostridium perfringens* (Williams et al., 1999; Terzich et al., 2000; Line, 2002; Bull et al., 2006; Line and Bailey, 2006; Rothrock et al., 2008). Therefore, judicious application of poultry litter to agricultural fields is imperative for protecting public health.

Although poultry litter is mostly used as an organic nutrient source in forage, cereal, and fiber crop production, it is also used as an animal feed, fuel source, and more recently, as a bioremediation method to restore the biological fertility of mine tailings (Franzluebbers and Doraiswamy, 2007; Bolan et al., 2010). Poultry litter serves as nutrient rich food for cattle and fish (Bolan et al., 2010). This practice has been implemented in the United States for almost 40 years; however, using poultry litter as an animal feed is becoming less common due to negative public perception (Bolan et al., 2010). Therefore, recycling poultry litter as an animal feed is of limited use.

Poultry litter may be burned as a fuel source to generate heat (Bolan et al., 2010). There are some complications that prevent this practice from becoming an optimal way to dispose of spent poultry litter. One of the problems is that poultry litter generally has a high moisture content. In addition, combustion of poultry litter has the potential to release air pollutants such as particulates, nitrogen oxides, carbon monoxide, and sulphur dioxide (Turnell et al., 2007). Rather than burning, anaerobic digestion of poultry litter has been used to produce biogas, which can be burned to generate heat energy (Bolan et al., 2010). However, few poultry producers utilize this disposal method because of low biogas yield and technical operational difficulties (Williams et al., 1999).

Poultry waste has several storage, handling, and disposal problems. These issues include foul odor, N loss due to ammonia volatilization, N loss due nitrate leaching, and potential injury to seedlings and crops due to excessive application of poultry litter (Bolan et al., 2010). Gaseous loss of N compounds is of great concern, because not only does this reduce the fertilizer value, these gases also contribute to atmospheric pollution (Bolan et al., 2010). Furthermore, N leaching can contaminate ground and surface waters (Bolan et al., 2010). Therefore, it is crucial

to develop and implement technologies that are environmentally sustainable, economically feasible, and effective for managing poultry waste.

1.5 Class 1 Integrons

1.5.1 Background

Although the thermophilic phase of composting kills most pathogenic bacteria, it is sometimes insufficient for effectively degrading antibiotic-resistant genes (ARGs) and mobile genetic elements (MGEs) (Xie et al., 2016). In recent years, the increasing emergence of antibiotic-resistant bacteria has become a major global public health issue (WHO, 2014). One of the factors contributing to the rise in antibiotic resistance is the regular use of sub-therapeutic antibiotics in animal feed (The National Academies, 1999). Therefore, animal manure is a major reservoir of antibiotic residues and ARGs that can spread to humans via manure applications to farmlands as fertilizer (Heuer et al., 2011, Zhu et al., 2013). Rahube et al. (2014) detected ARGs in soils and vegetables, and ARGs may spread to previously nonpathogenic bacteria through horizontal gene transfer via MGEs. Therefore, it is paramount that ARGs and MGEs are destroyed or immobilized in manures before land application (Li et al., 2017).

Administering sub-therapeutic doses of antibiotics has several benefits in animal husbandry, such as increasing the feed-to-mass ratio, increasing animal density, and decreasing animal fatality rates (The National Academies, 1999). Over time, this practice has led to a rising emergence of antibiotic-resistant bacteria as they evolve to survive. In addition, antibiotic-resistant bacteria can spread genes through horizontal gene transfer, where bacteria can exchange genetic material such as plasmids and transposons to adjacent bacteria (Gillings, 2015). This can be an important issue when a highly adapted, yet benign, bacterium shares resistance genes with potential pathogens.

Class 1 integrons are genetic elements located in some plasmids and transposons that have a unique ability to capture genes and integrate them into the genome (Gillings et al., 2008). The selective pressure caused by the overuse of antibiotics has resulted in class 1 integrons accumulating a diverse array of ARGs, which has contributed to the rise in multi-drug resistant bacteria outbreaks (Gillings et al., 2015).

1.5.2 Effect of composting on antibiotics, antibiotic resistance genes, and class 1 integrons

Although the composting process kills most pathogenic bacteria, it is not always sufficient for degrading antibiotic-resistant residues or destroying ARGs and MGEs (Xie et al., 2016, Li et al., 2017). Heavy metals such as Cu and Zn are widely found in animal manures and play an important role in determining the abundance of ARGs (Ji et al., 2012; Zhang et al., 2012). Because heavy metals effect ARG abundance, biochar may help remove or immobilize ARGs in manures through heavy metal sorption (Mitchell et al., 2015). Cui et al. (2016) state that different types of biochars have various effects on ARGs; therefore, it is important to study how different biochars, and biochar proportions, influence ARG prevalence. For example, Jeong et al. (2012) found that hardwood biochar had a much higher sorption rate than softwood biochar for macrolide sorption.

Antibiotic sorption depends on the antibiotic's molecular mass, water solubility, hydrophobicity, and acid dissociation constant (Zhang et al., 2013). Biochar properties that affect antibiotic sorption include surface area, surface charge, and porosity. Environmental determinants of antibiotic sorption to biochar include pH and solution ionic strength. Biochar formed at high pyrolysis temperatures (>500°C) is more hydrophobic, has higher amounts of positive charges, and greater surface area (Mitchell et al., 2015). Therefore, higher temperature

biochars may not bind to cations as easily due to lower abundances of negative surface charges and would decrease CEC. On the other hand, biochars created at moderate pyrolysis temperatures (<500°C) have more negative surface charges, which would improve CEC, and tend to be hydrophilic (Mitchell et al., 2015). These properties are critical for determining a biochar's efficacy as an antibiotic adsorbent. Even though biochar sequesters some antibiotics, it should undergo thermophilic composting to degrade the antibiotic residues. Furthermore, not all antibiotics have the same properties, so it is critical to choose a biochar with the most appropriate properties to sequester the target antibiotic residues.

The diverse interplay between antibiotic sorption, ARG abundance, and biochar properties can complicate monitoring ARG dissemination in any environment. Focusing on a single gene or small set of genes that are indicative of not only ARG abundance, but also the potential for spreading ARGs would be ideal. Antibiotics are diverse in their mechanisms and properties can exhibit a significant biological impact at extremely low levels; therefore, it would be useful to use a biomarker that exhibits rapid, consistent responses to various environmental pressures (Gillings et al., 2015). Class 1 integrons harbor resistance genes for a multitude of ARGs, so the abundance of class 1 integrons may help identify the extent of ARG contamination and the risk of disseminating ARGs to surrounding bacteria (Zhu et al., 2013; Johnson et al., 2016; Xie et al., 2016). Because class 1 integrons, identified by the *intI1* gene, are significantly correlated with total ARG abundance, they can be used as a biomarker to determine a rapid assessment of overall ARG contamination (Xie et al., 2016).

Current studies show conflicting results on ARG dynamics during manure composting (Selvam et al., 2012; Zhu et al., 2013; Wang et al., 2015). Xie et al. (2016) found that there was less change in ARG abundance during composting for poultry manure than cattle manure. The

authors reported that ARGs such as *aadA*, *aada2*, *qacEΔ1*, *tetL*, *cintI-1*, *intI1*, and *tnpA-04* were detected at high concentrations in poultry, swine, and cattle manure and after undergoing thermophilic composting for weeks. This result is significant, because composting did not significantly reduce ARGs from disparate antibiotic classes or MGEs such as *cintI-1*, *intI1*, and *tnpA-04* in manure composts sourced from different animal species. In addition, there was no correlation found between the presence of free antibiotic residues and their associated resistance genes (Xie et al., 2016).

A composting study conducted by Li et al. (2017) found that class 1 integrase (*intI1*) abundance decreased during the thermophilic stage of composting and higher biochar proportions resulted in significantly lower ($p<.05$) *intI1* abundance. The authors postulate that biochar's highly porous structure may help create distance between bacteria, reducing their connectivity; therefore, reducing the probability that horizontal gene transfer will occur. The article reports "Pearson's correlation coefficients indicated that there were significant positive correlations between the relative abundances of *intI1* and most ARGs (except *sul1* and *drfA1*) during the composting process, which suggests that *intI1* plays and important role in the propagation of ARGs" (Li et al., 2017).

1.6 Objectives

The objectives of this dissertation are to evaluate how co-composting poultry litter with biochar and wood vinegar affects nutrient profiles, class 1 integron abundance, and compost maturity rates over time.

Composting in this dissertation refers to the composting process. Because of complications during the composting process that hindered the degradation of organic materials,

the final product was not a true compost. However, for clarity and brevity, the term “compost” will refer to the poultry litter, biochar, wood vinegar organic materials.

CHAPTER II

MATERIALS AND METHODS

2.1 Preliminary Study to Detect Class 1 Integrase (*intI1)*

The preliminary study was conducted to determine if class 1 integrons were present in Mississippi State University's poultry broiler houses. To detect class 1 integrons, a method was developed to isolate and amplify the class 1 integrase (*intI1*) gene, because this gene is strongly associated with class 1 integrons and is commonly used to identify class 1 integrons in a variety of sample mediums (Nandi et al., 2004). Dr. Heather Jordan graciously donated a series of bacteria to test for a positive control from Mississippi State University's department of Biological Sciences. The bacterial strain identified as a positive control for *intI1* was *Mycobacterium marinum* strain 1218. All donated bacteria strains were heat killed before transportation to Laboratory 3203 at the Department of Sustainable Bioproducts. A local forest products manufacturer provided southern yellow pine (SYP) biochar.

Poultry litter was collected from Mississippi State University Poultry Science broiler chicken house 2 after the flock had been removed. The litter was transported in plastic one-gallon zip top bags and stored at -20°C until ready to undergo analysis. To identify *intI1*, the bacteria were isolated from poultry litter and the DNA was extracted. Polymerase chain reaction (PCR) was performed on the DNA extracts to amplify *intI1* if it was present. After amplification, the PCR products were visualized using gel electrophoresis. In addition, poultry litter blended

with 10% biochar was tested to determine if the presence of biochar interfered with *intI1* identification.

The bacteria were collected using a method adapted from Lu et al. (2002). After multiple trials the following procedure was established and used to isolate bacteria from poultry litter and poultry litter containing 10% biochar. First, 20 g of poultry litter was added to an Erlenmeyer flask containing 100 mL of sterile phosphate buffer saline (PBS) (Fisher Scientific ™). The flask was loosely covered with aluminum foil and the mixture was shaken at room temperature overnight. Next, the poultry litter buffer mixture was sieved through sterilized cheesecloth that was fastened over a glass beaker to remove large particulates. (Figure 2.1) One milliliter of the filtrate was aliquoted into 1.5 mL Eppendorf tubes to undergo bacteria isolation. Up to 25 mL of the remaining filtrate was transferred to screw-cap tubes. Both tubes were stored at -20°C.

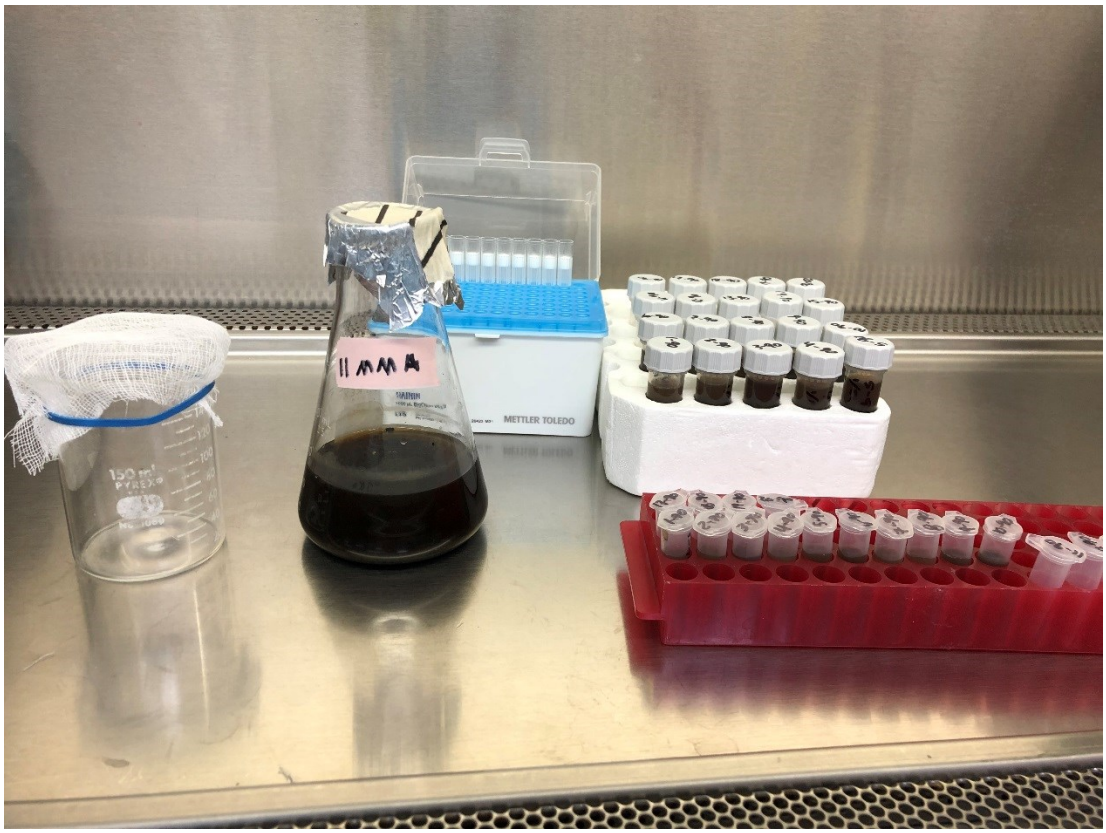


Figure 2.1 Filtration of poultry litter/biochar blends to remove large particulate matter prior to bacterial isolation.

For bacterial isolation, the 1.5 mL aliquot was thawed, and the bacteria cells were collected through a series of centrifugation steps. First, the 1.5 mL tubes were centrifuged at 4°C for 10 min at 5,000 x g to separate heavy particulate matter from bacterial cells that were suspended in the supernatant. The supernatant was removed and transferred to a sterile 1.5 mL tube. Next, the tubes were centrifuged for 30 min at 5000 x g at 4°C and the supernatant was discarded. The bacterial pellet was resuspended with 1 mL of PBS, vortexed, and centrifuged again for 15 min at 13,000 rpm at 4°C to wash out impurities. The supernatant was discarded, and the bacterial pellet was resuspended with 500 uL of PBS and 500 uL of 30% glycerol. The bacterial suspensions were then stored at 20°C until ready for DNA extraction.

QIAamp® DNA Mini Kit (Qiagen) was used to extract DNA using the DNA isolation from gram-positive bacteria protocol. This protocol was chosen because gram-positive bacteria are the predominant (>85%) species endemic in poultry litter (Nandi et al., 2002). DNA extracts were either stored at 4°C or -20°C, depending on when the PCR would be performed. DNA extracts were not stored longer than two weeks at 4°C to prevent excessive DNA degradation. After PCR, all DNA extracts were stored at -20°C for future use.

PCR amplification was performed on DNA extracts using the procedure described by Koeleman et al. (2001). Forward and reverse primers targeting *intI1* (160 base pairs) were purchased from Integrated DNA Technologies and were diluted to 10 uM working solutions. The forward and reverse primers, designated intI1F and intI1R, encoded the following nucleotide sequence, respectively: 5' CAG TGG ACA TAA GCC TGT TC 3' and 5' CCC GAG GCA TAG ACT 3'. Both primers have a melting temperature (Tm) of 53.2°C. PCR reactions were assembled in 0.2 mL PCR tubes (Thermo Scientific) on an ice block. For each reaction tube, 5 uL of DNA extract, 4 uL of colorless 5x GoTaq® Buffer (Promega), 2 uL of 2uM dNTPs (Sigma-Aldrich), 2.5 uL of 10uM of intI1F and intI1R, 3.8 uL of nuclease free water (Fisher Scientific), and 0.2 uL of GoTaq® DNA polymerase (Promega) were combined in the PCR reaction tube. The tubes were then centrifuged for five sec in a microcentrifuge (USA Scientific) and placed into the thermocycler (Eppendorf Mastercycler) for amplification. The denaturation phase was set to 94°C for 30 sec, the annealing phase was set to 55°C for 30 sec, and the elongation phase was set to 72°C for 30 sec. These temperature cycles were repeated for 35 cycles and then held at 4°C until the samples were removed and stored at 4°C to undergo gel electrophoresis.

Gel electrophoresis was performed on PCR products to visualize *intI1* using a 1.5% agarose gel (Sigma-Aldrich) run through Tris-acetate-EDTA (TAE) buffer. After dissolving the agarose in TAE buffer, it was cooled briefly and the nucleic acid gel stain dye (Lonza GelStar®) was added per instruction. After gentle mixing to evenly distribute the stain, it was poured into a gel electrophoresis cassette securely fixed into the electrophoresis system (Fisher Biotech). The liquid gel was allowed to cool for at least one hr to ensure the gel had solidified. Next, TAE buffer was poured into the system and the gel was loaded with 2 uL of 100 base pair (bp) DNA ladder (Fisher Scientific) and each following well was loaded with 2 uL of PCR product mixed with 1 uL loading dye. The loading dye imparted color to the PCR products to monitor the migration across the gel and ensured the PCR products sank to the bottom of the well. The last two wells were reserved for the positive control and negative control, respectively. Negative controls contained all PCR reagents and no DNA template. The negative control is used to eliminate false positives due to reagent contamination or primer mishybridization. After loading the gel with the ladder and PCR products, the power supply (Thermo Scientific EC 1000 XL) was set at 100 volts and ran for approximately 90 min. Then, the power supply was turned off and the gel was transferred to the Gel Doc XR^+ Imaging System (BioRad) to visualize *intI1* amplification using ultraviolet light (Figure 2.2).

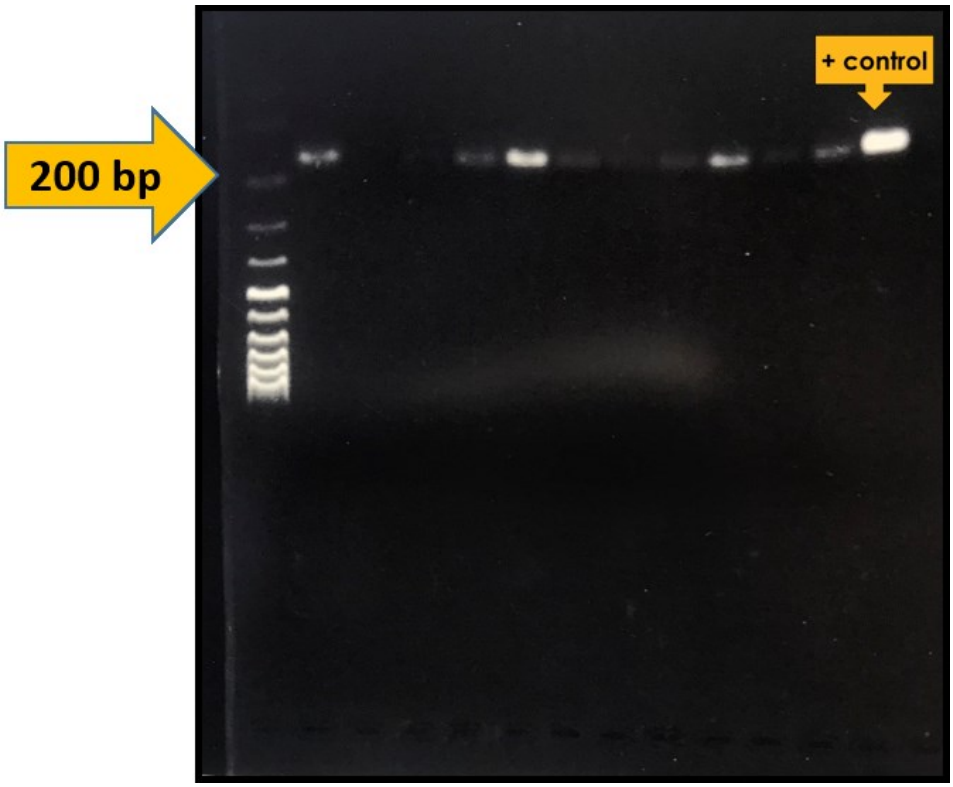


Figure 2.2 White bands indicated *intI1* was present in the poultry litter/biochar blend, and the positive control verified that the bands were not false positives. Lane 1 = 100 bp ladder, Quad 2 PBS 2, Quad 3 Water 2, Quad 3 Water 3, Quad 3 PBS 1, Quad 3 PBS 1, Quad 3 PBS 2, Quad 3 PBS 3, Quad 4 Water 2, Quad 4 Water 3, Quad 4 PBS 1, Quad 4 PBS 2, Quad 4 PBS 3, positive control (*M. marinum*), negative control.

After performing gel electrophoresis on PCR products obtained from the compost study, the beginning and end date PCR products were sent to Eurofins MWG Operon Inc. for DNA sequencing. Positive controls were sent for DNA sequencing to verify sequence similarities between *M. marinum* 1218 and other bacterial strains containing *intI1*. A sequence similarity of >95% indicated a good match, and >97% indicated a strong similarity between *intI1* genes detected in the PCR samples and *intI1* sequences stored in the National Institute of Health's GenBank® BLAST database. In addition to >97% sequence similarity, *intI1* matches needed to

have three or fewer nucleotide gaps in order to be considered a high similarity. Some PCR product (*intI1*) sequences were trimmed of 20-30 nucleotides in order to increase the quality of the match in BLAST.

2.2 Compost Experiment

2.2.1 Compost Set up

A local forest products company donated SYP biochar that was pyrolyzed at 500°C. Wood vinegar was produced from destructive distillation of loblolly pine (*Pinus taeda*) from a farm scale retort located in North Carolina. Dry poultry litter was collected from Mississippi State University Poultry Science broiler house two and was air dried for 48 hr (Figure 2.3). A tarp was placed over the poultry litter during transport and was removed afterwards to facilitate drying.

Figure 2.3 Poultry litter collection from Mississippi State University Poultry Science broiler house.

After air drying, compost treatments were assembled and then the initial moisture content was determined. The study was conducted outdoors adjacent to Building 6 at Sustainable Bioproducts in 35-gallon heavy duty plastic bins that were purchased from a local hardware store. Five 3 cm holes were drilled into the bottom to facilitate water drainage. A single layer of water permeable gardeners mesh was placed at the bottom of the bin to prevent solids from falling out. The bins were color-coded with forestry ribbon to designate the treatment, and the sample name was written on the ribbon and the plastic bin. The experimental set up for the

containers were based on two previous studies conducted by Bakhshizadeh (2012) and Bahsi Kaya (2018).

This experiment consisted of eight treatments with five replicates. A total of 11 kg of material was used for each replicate. Varying proportions of poultry litter (PL), biochar (BC), and wood vinegar (WV) were prepared as follows:

1. Control containing 11 kg of poultry litter (PL)
2. 5% BC (0.55 kg) added to 10.45 kg PL
3. 10% BC (1.1 kg) added to 10.9 kg PL
4. 20% BC (2.2 kg) added to 8.8 kg PL
5. Control containing 11 kg of PL and WV (w/w)
6. 5% biochar (0.55 kg) added to 10.45 kg PL and WV (w/w)
7. 10% biochar (1.1 kg) added to 10.9 kg PL and WV (w/w)
8. 20% biochar (2.2 kg) added to 8.8 kg PL and WV (w/w)

Poultry litter, biochar, and wood vinegar were weighed out individually for each compost replicate. The dry materials were then placed in a cement mixer and mixed for 30 sec to ensure homogenization. Then, the compost blends were transferred to the designated bin (Figure 2.4).

Figure 2.4 Compost set-up using individually weighed poultry litter (blue buckets) and biochar (clear containers) as described above.

A sample from each replicate was collected in a plastic zip top bag and the plastic bin (black trash can) was weighed again to record a total final weight. The sample collected was designated “Day 0.” Because the scale (ULINE LP7510A) was only sensitive to 0.5 kg, five 0.1 kg bags were used to improve the accuracy of the scale by subtracting the number of 0.1 kg bags needed to reach the next 0.5 kg mark (Figure 2.5).

Figure 2.5 Bags containing 0.1 kg of sand were used to improve the accuracy of the scale

The weight of the bin was subtracted from the total final weight to give the final compost weight (F_w). To determine the volume of wood vinegar to add, the moisture content of each sample was measured. The weight of an aluminum weighing dish was recorded, then approximately five g of sample was added to the dish. The weight of the sample was recorded. The weight of the aluminum weighing dish (A_w) was subtracted from the total weight (T_w) to yield the wet weight (W_w) of the sample. The samples were dried in a drying oven set to 100°C for 18 hrs and then the weight was recorded. A_w was subtracted from the total sample dry weight to yield the final dry weight (W_d). Then the moisture content (M_c) was calculated as follows:

$$Mc = \frac{Ww - Wd}{Wd} \; x \; 100 \qquad (2.1)$$

The compost moisture content was adjusted to 50% moisture by adding deionized water for treatments without WV, or a combination of deionized water and wood vinegar. To achieve 2% wood vinegar, the final compost weight (F_w) was multiplied by 0.02 to calculate the amount of wood vinegar needed to reach 2% w/w. Then, F_w was divided by two to determine the weight of 50% of the compost. Once the moisture content had been calculated, the amount of water needed to reach 50% was determined by subtracting M_c (in liters, 0.1 x M_c) from 50% of F_w (kg). In samples that included wood vinegar, the amount of wood vinegar needed to reach 2% w/w was included in the subtraction as follows:

$$((0.5 \times Fw - (Mc \times 0.1) - Fw \times 0.02) \quad (2.2)$$

Figure 2.6 Wood vinegar and deionized water were measured to bring the total moisture content of the composts up to 50%.

All compost samples received at least 1 L of deionized water to reach an appropriate moisture content required for composting to occur (50-60%). The bins were then transported to the experiment site and randomly placed on stone blocks to facilitate drainage (Figure 2.7). The composting study was initially designed to run for 90 days with sampling periods at Day 0, 45, and 90. However, due to the weather the composting study was extended to 112 days with the midpoint sample collection at Day 57.

Figure 2.7 Compost bins were placed outdoors in a random arrangement.

2.2.2 Aeration, Irrigation, and Temperature

All containers were aerated by mixing with a flathead shovel. The containers were mixed thoroughly twice during the first week to ensure aerobic conditions, and then at least once a

week for the remainder of the study depending on the weather. Mixing the containers helped to stabilize moisture content and prevent anaerobic conditions from developing. Care was taken to not disturb the mesh on the bottom during mixing. One treatment was mixed at a time, and the shovel was disinfected using 70% ethanol between treatments.

Because there was ample rainfall during the composting period, no additional water was added. After a period of prolonged heavy rainfall, some containers were observed to not have adequate drainage. Henceforth, lids were loosely placed on all compost containers during rainfall to prevent excess water from accumulating. Lids were removed after the precipitation ended.

The temperature was recorded once a week, as the weather permitted. A thermometer probe (REOTEMP) was inserted approximately in the same position in the center of the compost pile each time. The thermometer was held until the temperature stabilized, approximately 30 sec. The temperature was recorded, and the thermometer probe was disinfected with 70% ethanol between each container.

2.2.3 Sample collection

Samples were collected on day 0, 57, and 112 to analyze moisture content, pH, total fungi counts, total bacteria counts, *intI1*, and nutrient analysis. In addition, Day 0 and 112 would be used in a compost maturity test at the end of the composting experiment.

A trowel sanitized with 70% ethanol was used to mix the compost bins immediately prior to collection to ensure homogeneity. One treatment was done at a time to reduce contamination between treatments. The trowel was sanitized with 70% ethanol between treatments, and residual compost was wiped off with a paper towel between replicates. First, compost bins were weighed to the nearest 0.1 kg, the sample was taken and stored in one-gallon zip top bags, and then the final weight was recorded. This final weight would be used to calculate weight loss at the end of

the study. At day 57, rainwater had not sufficiently evaporated or drained from samples that contained less than 10% biochar. The same procedure was performed for day 57; however, it was difficult to determine the amount of compost sample taken because of the excess rainwater. Therefore, a true estimate of moisture content and weight loss could not be determined for water-logged samples. By day 112, composts did not have any standing water, but some composts retained excess moisture and had a sludge-like appearance and texture. These sludge-like composts included Control 2 and 5% BC 3.

After samples were collected, they were stored at 4°C for up to 72 hr in order to take samples for microbiological enumerations tests. Then, compost samples were placed at -20°C. There were two temperature fluctuation periods where composts were not held at -20°C due to equipment malfunction. The first incident occurred after adding Day 57 samples to the -20°C freezer. The freezer door gaskets did not seal properly, so ice accumulated around the samples due to fluctuating temperatures. The ice pushed the gaskets further apart, allowing ambient air into the freezer. It was noticed after approximately one month after Day 57 samples were collected that some composts bags were not frozen. The samples were retrieved to take out 20 g for *intI1* analysis, and then stored in the cold room, which was held between 32-36°F. However, the cooling system in the cold room malfunctioned, leaving compost samples at 45-60°F for several days. The samples were then transferred to multiple -20°C freezers and some had to be stored in the freezer with poor gaskets. Excess ice was removed from the freezer by hand to allow the gaskets to connect, and the door was taped tightly shut using heavy-duty tape fastened at three equidistant points on the freezer door.

2.2.4 Total fungi and bacteria enumeration

Total fungi and bacteria enumerations were performed on compost samples taken at day 0, 57, and 112. Three replicates were performed on each sample. First, serial dilutions were made by suspending 1 g of compost in 9 mL of phosphate buffer saline (PBS), vortexing for 5 sec, and transferring 1 mL of solution to 9 mL of fresh PBS. These steps were repeated until the desired dilutions were achieved. One milliliter was transferred from the PBS compost dilution to the growth media (Figure 2.8).

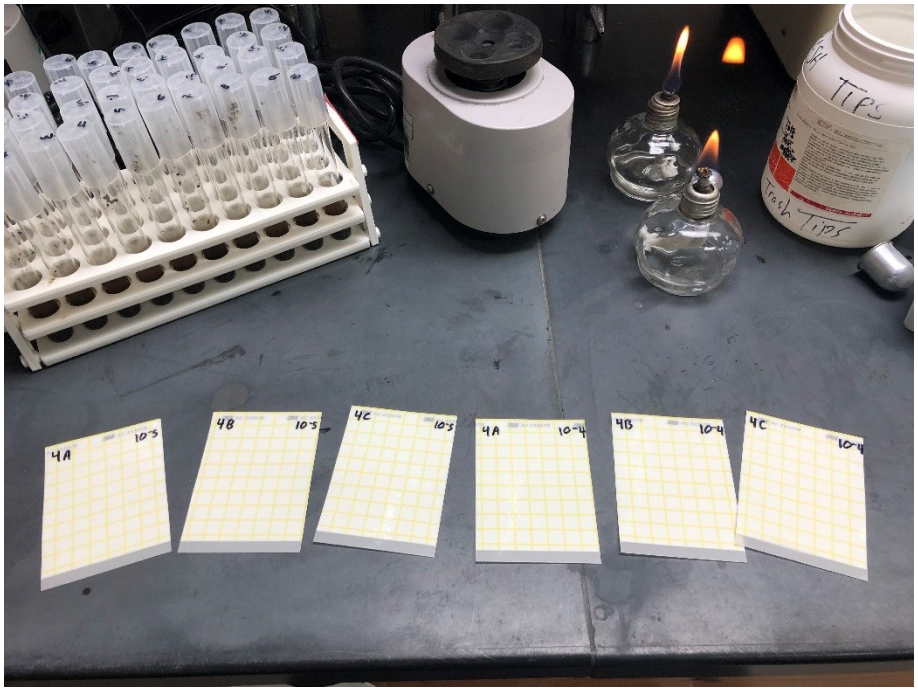


Figure 2.8 APC and YM petri films were prepared with serial dilutions of compost in PBS as described above.

Day 0 fungi were enumerated on potato dextrose agar with antibiotics (PDAA). PDAA was weighed into deionized water per the manufacturer's instruction. The mixture was stirred to break up large clumps and then sterilized in the autoclave. The media was cooled in a water bath

until it reached 45-55°C. While the media was cooling, the following antibiotic solution was assembled for 1.5 L of PDAA: 0.045 g of chlortetracycline and 0.18 g of streptomycin sulfate (ICN Bio chemicals, Inc.). The antibiotics were dissolved in 5 mL of deionized water and injected into the media using a sterile syringe. It was mixed thoroughly and poured into petri dishes (Fisher Scientific). The media in the petri dishes was cooled and then flipped over to prevent condensation on the agar surface. The media was stored at 4°C in plastic bags until ready for use. One milliliter of dilution was transferred to the center of the petri dish and spread evenly around the dish using a sterile spreader (Fisher Scientific). The spreader was discarded after each use. Bacteria were cultivated on Aerobic Plate Count (APC) petri films (3M™ Petrifilm™).

Day 0 and 57 dilutions were made up to 1/10,000 (10^{-4}) and day 112 dilutions were made up to 1/100,000 (10^{-5}). Dilution was necessary for counting bacterial and fungal colonies because poultry litter carries a high microbial load (Figure 2.9). Day 0 total bacteria were taken from 10^{-3} and 10^{-4} dilutions. Day 0 fungi were taken from 10^{-2}, 10-^{3} and 10^{-4} dilutions. Initial fungi enumerations were evaluated from a wider range of dilutions because it was unclear which dilutions would provide countable colonies.

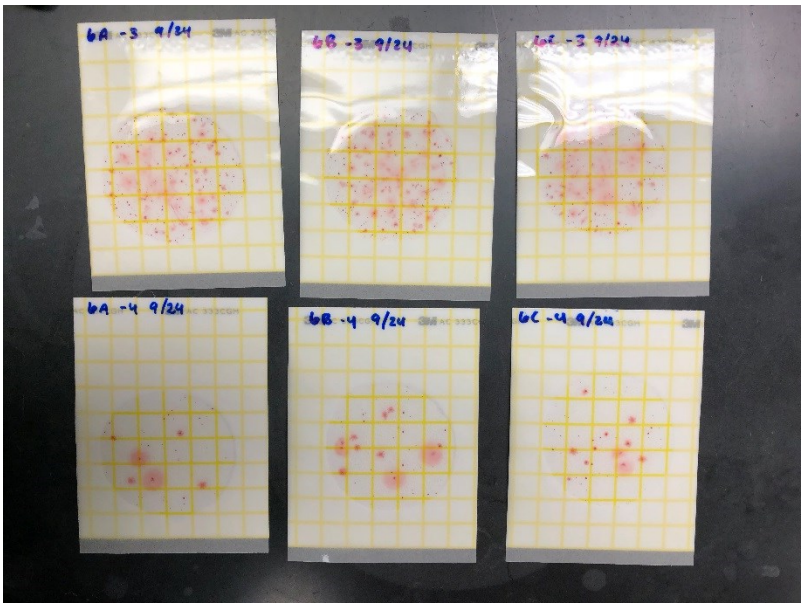


Figure 2.9 The dilution chosen for enumeration showed easily distinguishable colonies Note: The 10^{-4} dilution (bottom) was chosen for bacterial (APC) enumeration because the colonies were more distinguishable than 10^{-3} (top).

Because of the high bacteria abundance at Day 0, only Day 57 bacteria petri films were plated only from the 10^{-4} dilution, and fungi were enumerated from the 10^{-3} and 10^{-4} dilutions. For Day 112, fungi petri films were inoculated from the 10^{-3} and 10^{-4} dilutions while bacteria petri films were inoculated from the 10^{-4} and 10^{-5} dilutions. An additional dilution was added for bacterial enumeration because of the high abundance of the previous sampling period (Day 57).

Bacteria petri films were incubated for 24 hr at 30°C and then colonies were counted. Day 0 fungi were grown on PDAA plates at 30°C for one week. Because of the prolonged incubation time, total yeast and mold petri films (YM) were used to enumerate fungi on Day 57 and 112 (Figure 2.10). Yeast and mold petri films (3M™ Petrifilm™) were also incubated at 25°C, but the incubation period was decreased to 72 hr as per the manufacturer's instruction. Day 112 YM petri films were repeated because no growth occurred.

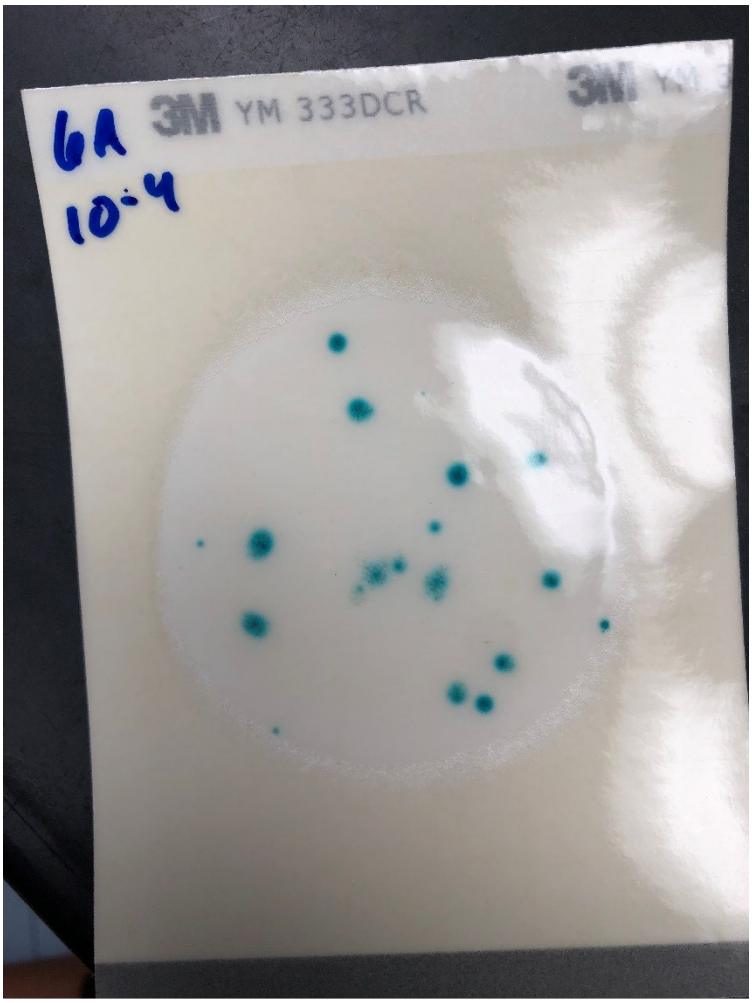


Figure 2.10 Fungal colonies were indicated by blue or yellow colonies on YM petri films

To calculate the number of colonies in the sample, the number of colonies counted on the films/plates were multiplied by their dilution factor. For example, if 30 colonies were counted from a 10^{-2} dilution, then there were approximately 30*100 or 3,000 colonies in the compost sample.

2.2.5 Class 1 integrase (*intI1*) identification

Class 1 integrase (*intI1*) was detected using the protocol described in Section 2.1.

2.2.6 Nutrient Analysis, pH, and moisture content

The Mississippi State University Soil Testing Laboratory performed nutrient, pH, and moisture content analysis on compost samples. Initial moisture content was determined at the Sustainable Bioproducts Department to calculate how much liquid to add to reach a 50% moisture content as previously described. The same moisture content procedure was performed on Day 0, Day 57 and 112 at the Soil Testing Laboratory. Approximately 45 g of compost sample was weighed into 50 mL Falcon tubes and transported to the Soil Testing Laboratory after all sampling was completed. The Mississippi Soil Testing Procedure, a standard method used in the state, was performed to determine P (inorganic), K, Ca, Mg, Na, and Zn, and pH. C and N content was determined using an Elementar VarioMax C:N analyzer (Elementar Americas, Inc.). N levels reported are for total N.

2.2.7 Compost Maturity Test

Compost maturity tests, also known as plant germination tests, were performed using Day 0 and Day 112 compost samples to compare maturity between treatments and over time. The maturity tests were performed according to the University of Florida's Composting Center protocol using radish seeds (2019). Radish a commonly used seed in maturity tests because they germinate quickly. The germination time is approximately seven days and they mature in 21 days. In compost maturity tests, the germination rate is how many seeds sprout out of the total number of seeds planted after seven days. A control sample containing commercial potting soil is used to compare the germination rate of the compost to commercial potting soil.

Compost maturity tests were assembled by pooling equal amounts of treatment replicates and distributing them into six plastic containers with perforated bottoms (Figure 2.11). The containers were labeled with the treatment and replicate number. The control soil used was

Miracle-Gro® Garden Soil for in-ground use. Because this project aims to provide information on how to incorporate compost into soil for agricultural land, garden soil would represent agricultural land better than potting soil. Furthermore, garden soil is designed to grow vegetables, while many potting soils/mixes are designed to grow flowers, shrubs, and other plants.

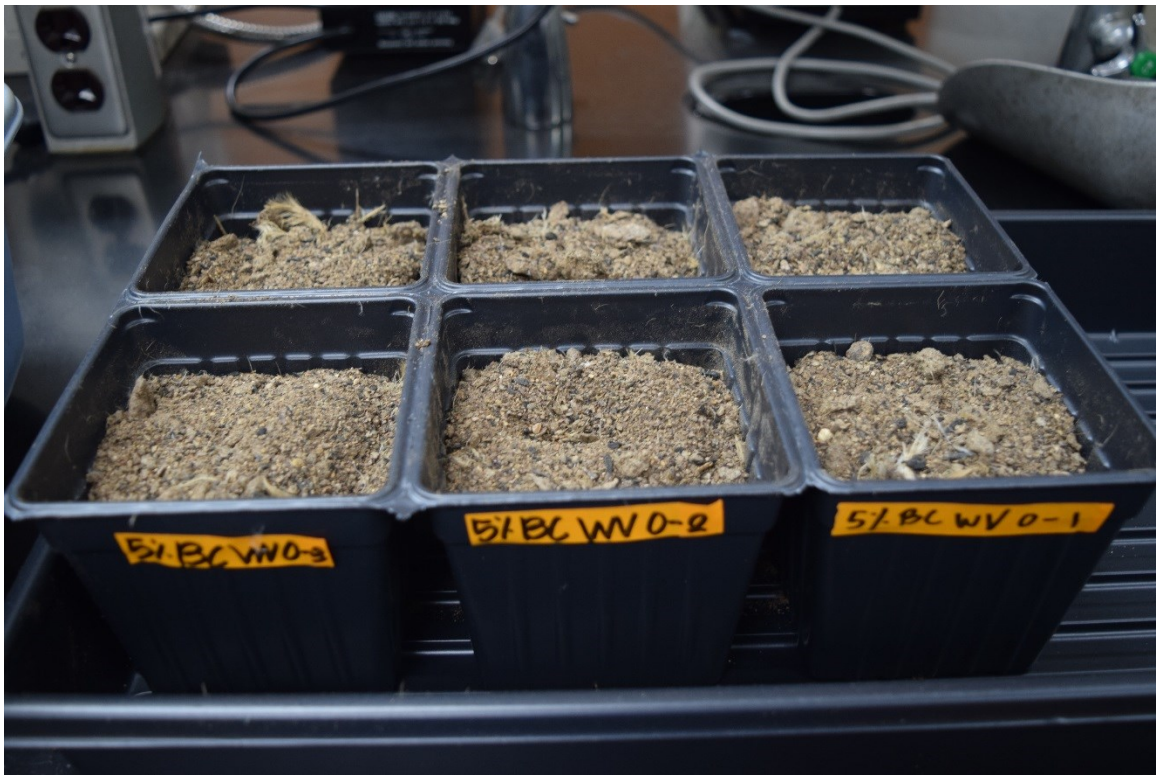


Figure 2.11 Compost maturity test containers filled with equal parts of pooled compost treatment replicates.

Two compost treatment replicates from the Day 112 samples (Control 2 and 5% BC 3) were not included in the maturity tests because they contained an abundance of liquid. Six radish seeds (Radish Rover Hybrid Seeds, Garden David's Seeds®) were planted in each container. The

containers were taken to the greenhouse at Sustainable Bioproducts and watered with 80 mL of deionized water. The greenhouse fan was turned on and the door was propped open to facilitate better air circulation and to lower the temperature, as radishes prefer cooler temperatures. Radish seed germination was observed after seven days and were kept in the greenhouse for an additional 21 days for a plant growth test. At the end of the 21 days, any plants would be removed and dried to compare dry biomass weights between compost treatments and the control.

2.2.8 Statistical Design

The split-plot design model was used for the nutrient analysis, microbial analysis, pH, weight loss, and compost maturity tests. Biochar and wood vinegar level were the main plot factors and time was the subplot factor. The statistical data was generated using Statistical Analysis Software (SAS). All statistically significant interactions occurred at $P<.05$. First, PROC GLM was run on a single response variable, such as bacteria counts. Main variables (BC and WV) /time significant interactions ($P<.05$) were run on least square means under the PROC MIXED METHOD = TYPE3 to account for treatment factors for biochar, wood vinegar, and time. GPLOTs were then generated that compared responsible variable measurement on the y axis and time on the x axis with four biochar levels (0, 5, 10, 20) and were separated by WV (1 or 0). Response variables that were significant with biochar OR wood vinegar and time were run independently using the same procedure. The statistical model equation followed:

$$Y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta_{ij}) + \varepsilon_{l(ij)} + \tau_k + (\alpha\tau)_{ik} + (\beta\tau)_{jk} + (\alpha\beta\tau)_{ijk} + ejkl, \text{ where } i=1,2,3,4 \text{ (BC level);}$$
$$j=1, 2 \text{ (WV level); } k=1, 2, 3 \text{ (sampling time); } e=1, 2, 3, 4, 5 \text{ (rep)} \qquad (2.3)$$

An example of the SAS code used to generate the data is provided in the appendix. Because of the extraordinarily lengthy SAS output for each response variable, an example has

been provided in the appendix to demonstrate how significant relationships were determined. The complete SAS output for each variable is available upon request.

CHAPTER III

RESULTS AND DISCUSSION

3.1 Preliminary Experiment

The preliminary results showed that 95% (n=38) poultry litter samples tested under the developed *intI1* amplification protocol and 62% (n=16) of poultry litter with 10% biochar were positive for *intI1*. From these results, it was hypothesized that biochar may reduce the prevalence of *intI1* and needed further investigation.

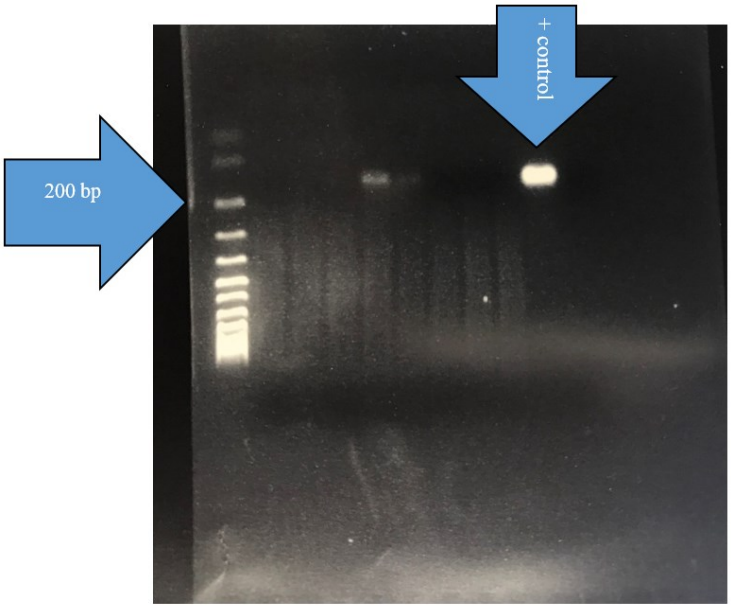


Figure 3.1 There were fewer and fainter *intI1* bands in poultry litter/10% biochar blends. Lane identification from the left are as follows: 100 bp ladder, 1A, 1B, 2A, 2B, 3A, 3B, 4A, 4B, positive control (*M. marinum*), negative control.

3.2 Observational physical characteristics of compost treatments

3.2.1 Insect activity

There was an abundance of insect larvae and pupae on the surface and within the composts approximately two weeks after setting up the experiment. The insect activity throughout the composts indicated that aerobic conditions were present in the center of the pile. At approximately three weeks, insect activity was higher in compost treatments that contained less biochar. Therefore, increasing the biochar percentage appeared to deter insects, which is a favorable quality in composts (Epstein, 1997). There were no observable differences in insect activity based on the presence of wood vinegar.

After approximately 6 weeks (October 25, 2018), a weather system brought a significant amount of rain that resulted in some compost containers accumulating standing water, which killed any insect larvae/pupae that was present. No insect activity was observed in the compost after this event. This may indicate that anaerobic conditions developed in compost treatments containing no biochar and 5% biochar. Although 10% and 20% biochar treatments were draining sufficiently, there was little initial insect activity; therefore, the lack of insect activity does not indicate anaerobic conditions had developed in 10 and 20% biochar treatments.

3.2.2 Biochar effects on compost drainage after heavy rainfall

After a period of heavy rainfall, some compost treatments were not draining sufficiently. This resulted in standing water accumulating in the containers, and as the level of biochar increased, the amount of standing water decreased (Figure 3.2).

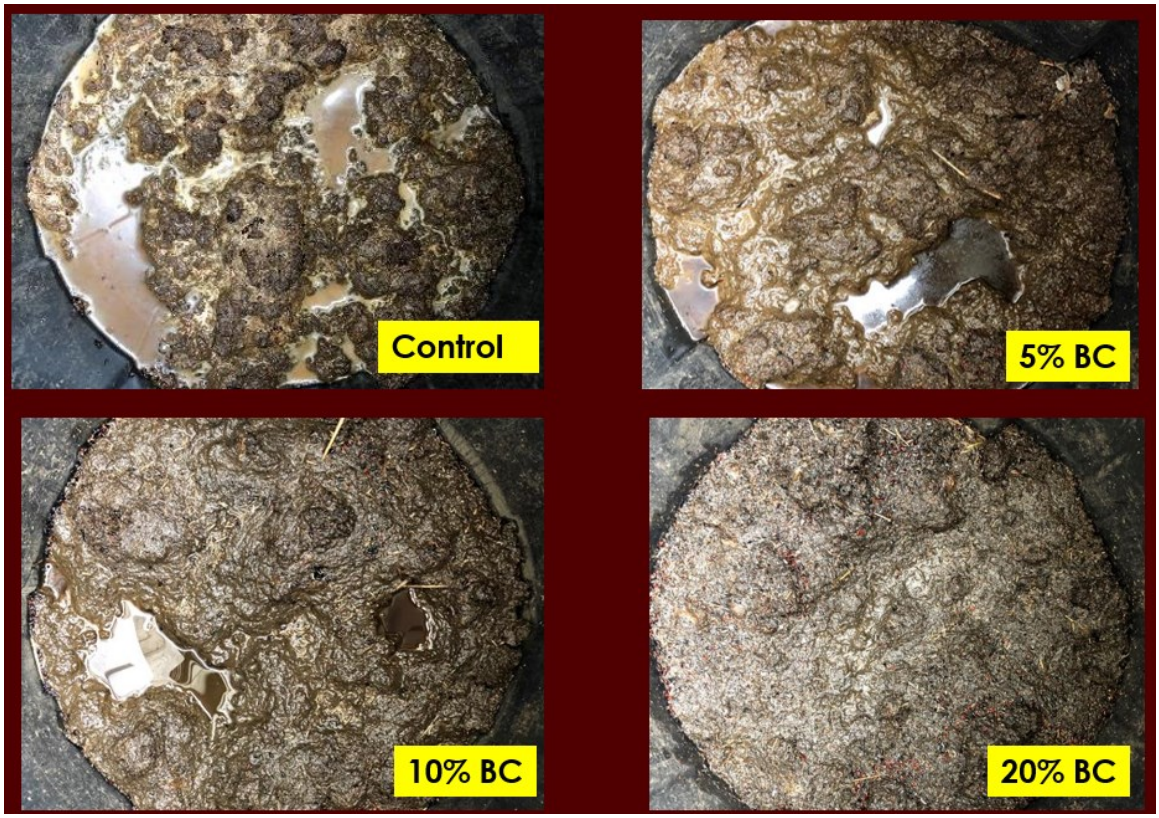


Figure 3.2 The amount of standing water decreased as biochar level increased.

Standing water is an issue in composting because it can result in anaerobic conditions, which drastically slows down the composting process. It was observed that water was not permeating through the mesh at the bottom of the container in Control, PL + WV, 5% BC, 5% BC + WV, and some 10% BC/WV treatments. Treatments containing no biochar had a sludge or slurry-like consistency, and it is possible that the solids were compacted at the bottom of the container, clogging the permeable mesh. Because biochar has a rigid, porous structure, it may have reduced compaction on the mesh and facilitated drainage. Furthermore, biochar's absorptive properties may have reduced the amount of free water in the compost. This observational result further demonstrates the high absorption capacity and porosity of biochar. In

addition, it illustrates how biochar improves compost's physical properties. From these results, 20% BC would be recommended for uncovered compost piles in regions that experience high rainfall. 20% BC may help prevent anaerobic conditions from developing; therefore, compost maturation would be achieved faster. In addition, 20% biochar compost blends may benefit soils with poor drainage or high compaction.

3.2.3 Final compost collection observations

As noted above, composting in this dissertation refers to the composting process. Because of complications during the composting process that hindered the degradation of organic materials, the final product was not a true compost. However, for clarity and brevity, the term "compost" refers to the blends of poultry litter, biochar, and wood vinegar.

At the end of the composting experiment, 20% BC and 20% BC + WV blends had a fine, light texture with little odor. Compost treatments with less biochar had a clumpy, sticky, and compacted texture, while composts with no biochar resembled sludge (Figure 3.3). Foul odors were more intense as the level of biochar decreased.

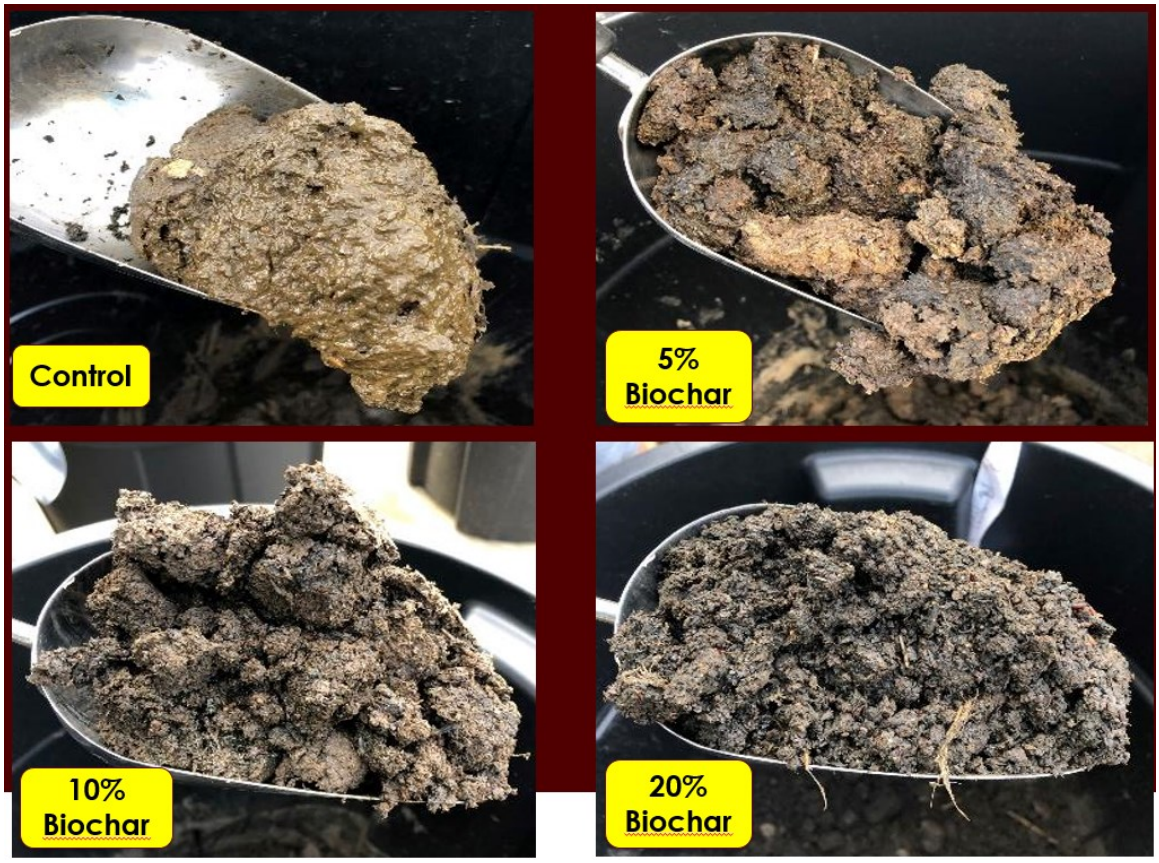


Figure 3.3 Biochar level had an observable impact on final compost texture and odor

3.3 Temperature, pH, and weight loss

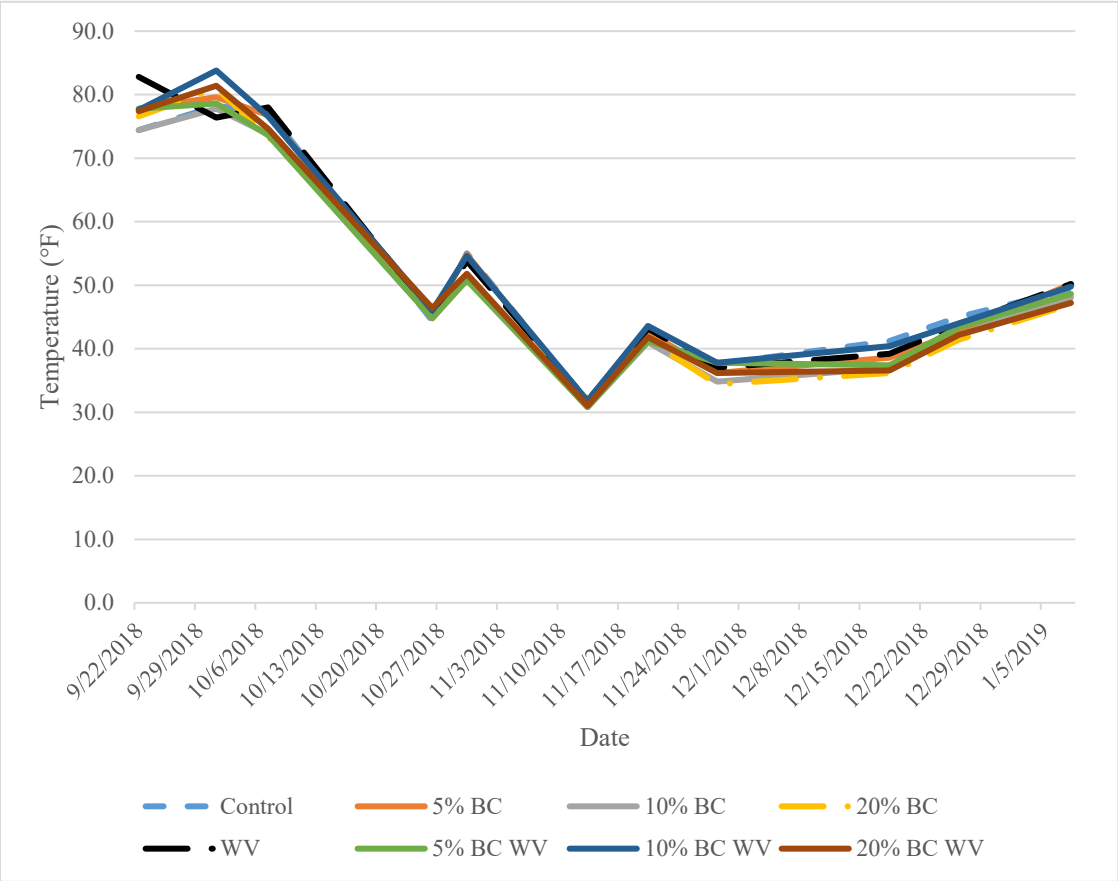


Figure 3.4 Compost temperatures (°F) recorded from 9/22/2018 to 1/08/2019

Compost temperatures did not undergo the typical composting temperature phases and did not achieve thermophilic temperatures (>105°F). Initially, all compost treatments excluding WV experienced a rise in temperature for approximately two weeks. During this time, mesophilic bacteria and fungi were most active and decomposing organic matter. However, the temperature did not rise enough to activate thermophilic bacteria to undergo the thermophilic phase. After two weeks, the temperature consistently decreased for all treatments. Compost temperature were recorded below mesophilic (50°F) temperatures on October 26th, which is also

soon after the excessive rainfall. The abundance of water and cooler temperatures most likely impeded aerobic microbiological growth, which reduced heat generation. Average compost temperatures did not reach above 50°F for the remainder of the study.

Several factors may have resulted in atypical compost temperature phases. These factors include moisture content, compost volume, and ambient air temperature. Water conducts heat, so too much moisture in the composts would hinder insulating the heat generated by microorganisms. As state previously, compost material acts as an insulator; therefore, sufficient material (volume) should be used in order to insulate heat. Although 11 kg of compost material has been successful in previous studies, the composts in this study contained a denser material (poultry litter) that had a lower volume for the same weight (Bakhshizadeh, 2012; Bahsi Kaya, 2018). Therefore, it is important to consider the density of the materials used in the compost to ensure proper heat insulation. The cooler temperatures of fall and winter in addition to the low compost volume and high moisture may have resulted in low compost temperatures. As composts began to dry, the temperature gradually increased. The increase in temperature is most likely due to increased microbial activity.

Weight loss could not be determined because Day 57 and 112 moisture contents exceeded 100%. This is due to first excessive rain event where containers were not covered because the mesh clogging was unexpected. Henceforth, containers were covered if it was raining to prevent further water accumulation. However, covering the composts, cool temperatures, and high humidity prevented water from evaporating.

There was a significant relationship found ($P = .0419$) between pH, biochar level, WV level, and time. pH values were significantly higher ($P<.05$) at the end of the compost study than the beginning for all treatments (Figure 3.5 and 3.6). 20% BC + WV had the most alkaline pH at

9.08, and increasing biochar levels increased the pH, except for 10% BC. In addition, the rate of pH increase was proportional to the level of biochar, with 20% having the fastest rate increase. Adding wood vinegar to the compost appeared to have a stabilizing effect for 20 and 10% biochar composts. Control and 5% biochar also remained stable until after the midpoint collection. Control and 5% BC were not significantly different at Day 112, and PL + WV, 5% BC + WV, and 10% BC + WV were not significantly different at Day 112. Biochar and wood vinegar have a significant interaction with pH, but it depends on time, as Day 0 pH values were not significantly different from each other, but all treatments Day 0 and Day 112 were significantly different.

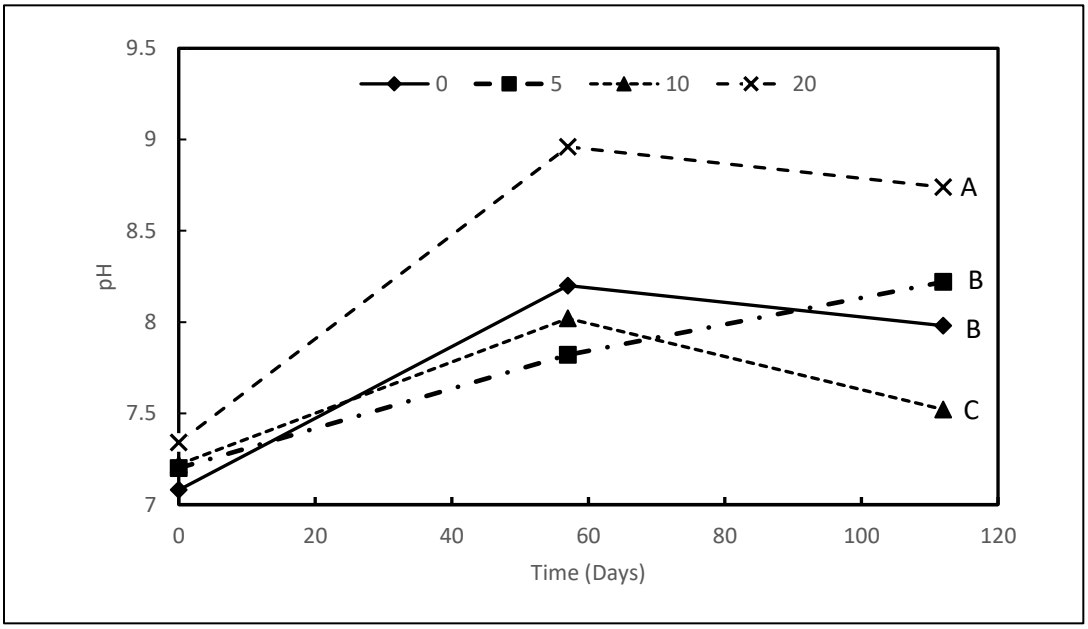


Figure 3.5 pH response to biochar (bc) levels over time without wood vinegar (WV=0).

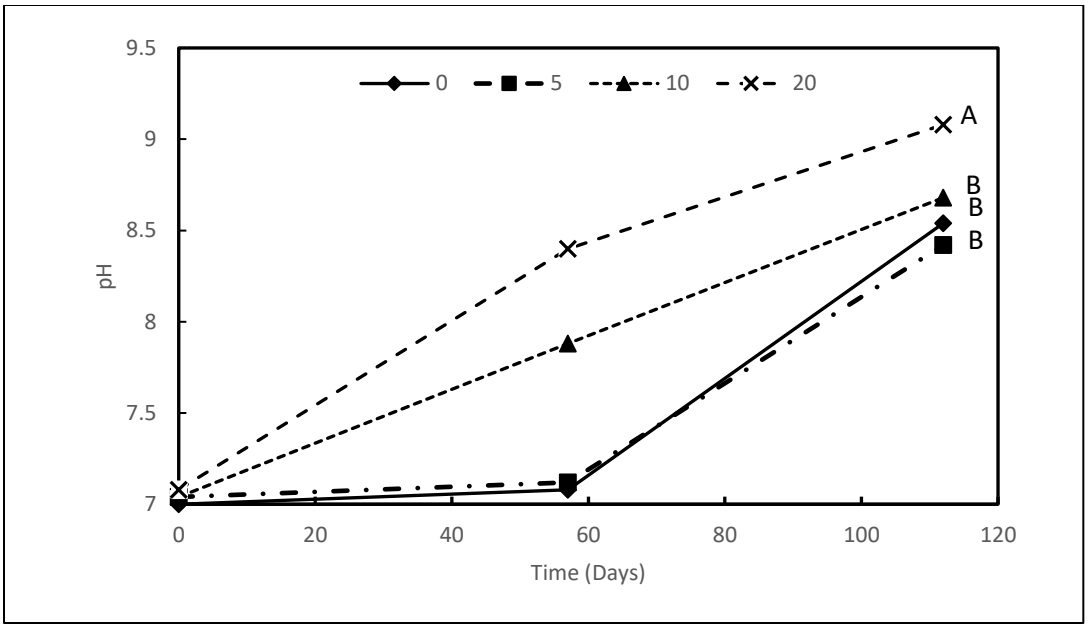


Figure 3.6 pH response to biochar (bc) levels over time with wood vinegar (WV=1).

Wang et al. (2018) support these results, as the authors reported a significant increase in pH with 10% biochar addition to pig manure compost. However, the authors found that 2% WV had no significant impact on compost pH at a 10% BC inclusion rate, while this study found that 10% BC and 10% BC +WV were significantly different (P = <.001) at Day 112. In addition, the control and PL + WV were significantly different at Day 112 (P = .0057). At Day 112, 5% and 20% biochar treatments were not significantly different regarding WV inclusion.

An increase in pH during composting may be related to ammonia production and accumulation, while a decrease in pH could be due to "ammonium volatilization, nitrification, or the production of low molecular organic acids" (Chen et al., 2010). In addition, excess moisture and cool temperatures may have hindered microbiological activity, so the production of organic acids through microbial decomposition decreased. WV showed inhibitory effects for bacteria and fungi, which may have resulted in lower amounts of organic acids being produced. This may

explain why treatments with WV generally had higher alkalinity at the end of the study. In treatments absent of WV, it is possible that microorganisms were able to increase cellular activity as the moisture content decreased after Day 57, and the acids produced resulted in a decrease in pH by the end of the study. As the moisture content decreased after Day 57 and more oxygen was available, the bacteria may have become more active and generated acidic metabolites. These acidic metabolites may have decreased the pH of the compost as shown on Day 112 for composts without WV.

3.4 Total Bacteria and Fungi Counts

3.4.1 Total Bacteria Counts

Bacteria counts were significantly affected by biochar level (P = <.0001), but it depended on time. Bacteria counts were also significantly affected by WV (P = .0322), but it depended on time. However, bacteria counts were not significantly affected by the combination of biochar and WV over time. Therefore, the variables (BC and WV) had independent fixed effects on bacteria counts.

For biochar, there were no significant differences in bacteria counts between Day 0 and 57 for all treatments (Figure 3.7).

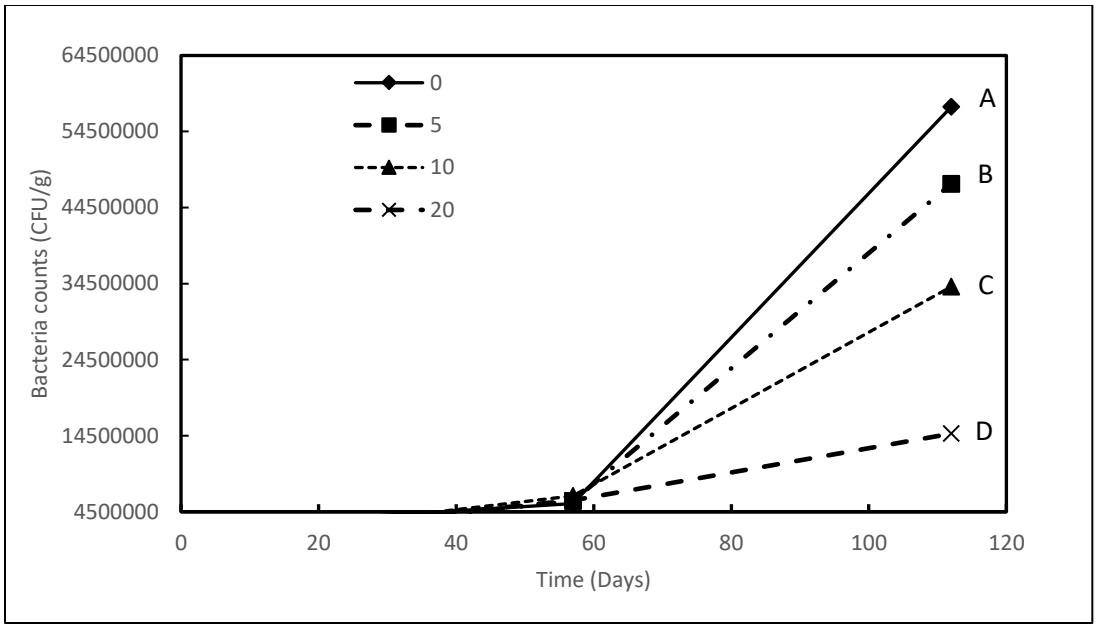


Figure 3.7 Compost bacteria counts (CFU/g) at different biochar application rates over time.

There were significantly higher bacteria counts at the end of the study for all treatments. Composting increases the abundance of microorganisms, so this result was not unexpected; however, it was expected that bacterial proliferation at the midpoint collection would be significantly higher than Day 0. Some potential reasons for the low bacteria counts during the midpoint collection may be related to high moisture content that created anaerobic conditions, cool temperatures, competition with an abundance of fungi, as well as high alkalinity in 20% BC and 20% BC+WV. At Day 112, treatments containing no biochar had significantly higher bacteria counts than other treatments. Each increasing level of biochar resulted in significantly lower bacteria counts. This is an unusual occurrence, as biochar has been shown to stimulate microbial activity up to 20% biochar application rates (Dias et al., 2010; Steiner et al., 2010).

For WV, bacteria counts were not significantly different at Day 57 from Day 0, but all were significantly higher at Day 112 (Figure 3.8).

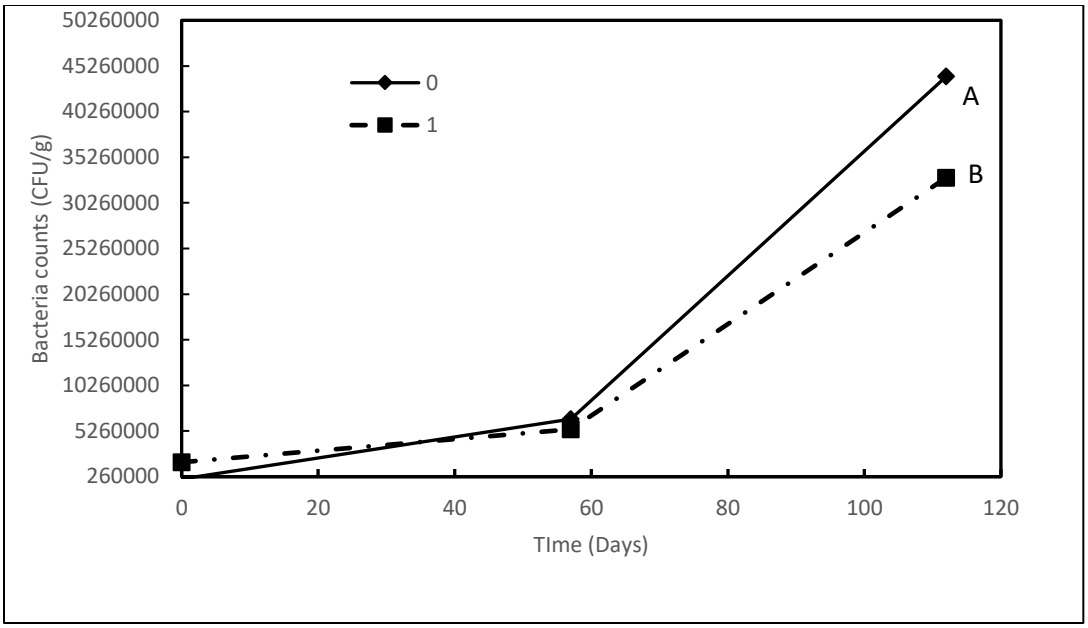


Figure 3.8 Effects of WV on compost bacteria counts (CFU/g) over time.

Compost treatments without WV exhibited significantly higher bacteria counts, indicating that WV may inhibit bacterial growth at a 2% application rate. WV's effects on microbial activity is dose dependent, as higher doses have biocidal properties while dilute doses stimulate activity (Steiner et al., 2008; Baimark et al., 2009; Velmurugan et al., 2009). Hagner et al. (2013) found that adding wood vinegar at 0.26% w/v (pH=2.04) did not significantly affect microbial activity. Wang et al. (2018) reported that pig manure composts treated with 2.0% wood vinegar, 10% biochar, and 10% zeolite achieved thermophilic temperatures faster than the control. Although the authors were not measuring microbial activity, it is well established that microbial activity is the primary driver of compost temperature (Epstein, 1997). The source of the wood vinegar was not disclosed, which is important because pyroligneous acid properties vary significantly depending on feedstock (Fujita et al., 2012; Mathew et al., 2015). Furthermore, Wang et al. (2018) did not test for the effects of WV as the single variable; all treatments with

WV were combined with biochar and zeolite. Therefore, it is unknown if WV alone would have had a significant impact on temperature rates, which could provide some information on the microbial activity within the compost.

3.4.2 Total Fungi Counts

Fungi counts were significantly affected by the presence of WV (P = 0.0020) depending on time. Biochar level had a significant effect on fungi counts (P = 0.0495), and biochar*time effects were close to the significance limit at P = 0.0596. Because of the inherent variation attributed to conducting outdoor experiments, the biochar*time effect was considered significant and included in this discussion. Fungi counts were not significantly affected by the combination of biochar and WV over time. Therefore, the variables (BC and WV) had independent fixed effects on fungi counts.

Fungal abundance was significantly higher at Day 57 for all treatments (Figure 3.9, Figure 3.10). As with bacteria, WV showed an inhibitory effect at 2% WV for fungi. The concentration of WV that serves as a microbiological stimulator or biocide are nuanced, as studies have used a wide range of concentrations up to 2% and had successful results. Fungi counts were also significantly higher as the level of biochar increased (Figure 3.10). Biochar appeared to facilitate fungal growth, while it inhibited bacterial proliferation.

It is possible that the high fungal activity at Day 57 could have resulted in unexpectedly low bacteria counts at Day 57 because of nutrient competition. Furthermore, while bacteria appeared to be inhibited by biochar, fungi appeared to thrive in higher biochar environments. As the composting time progressed, fungi counts significantly decreased and were not significantly different than Day 0 counts.

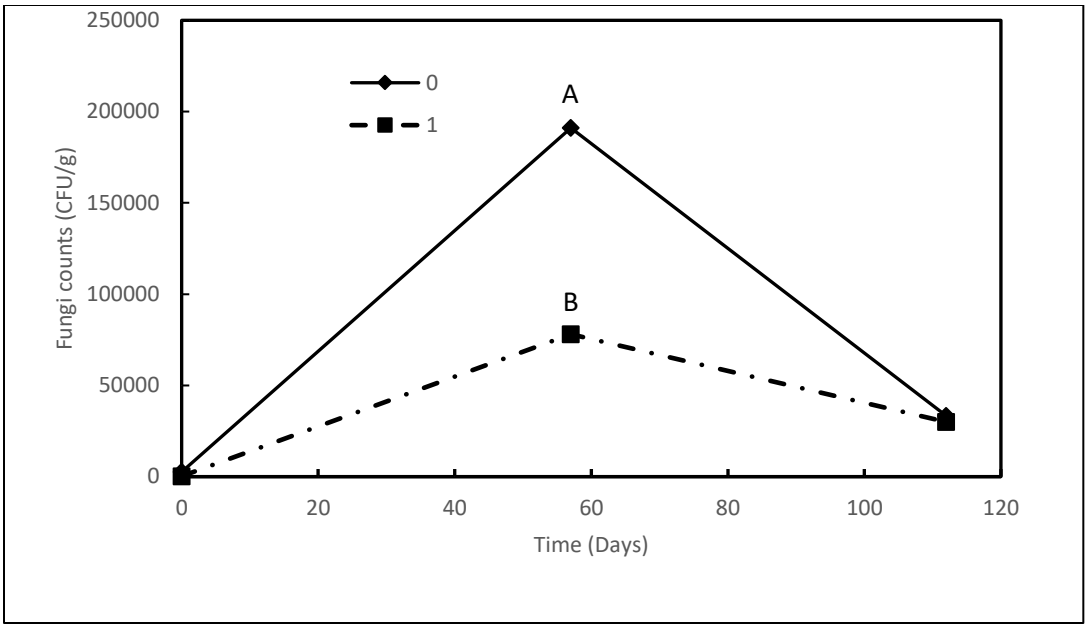


Figure 3.9 Compost total fungi counts (CFU/g) between WV treatments over time.

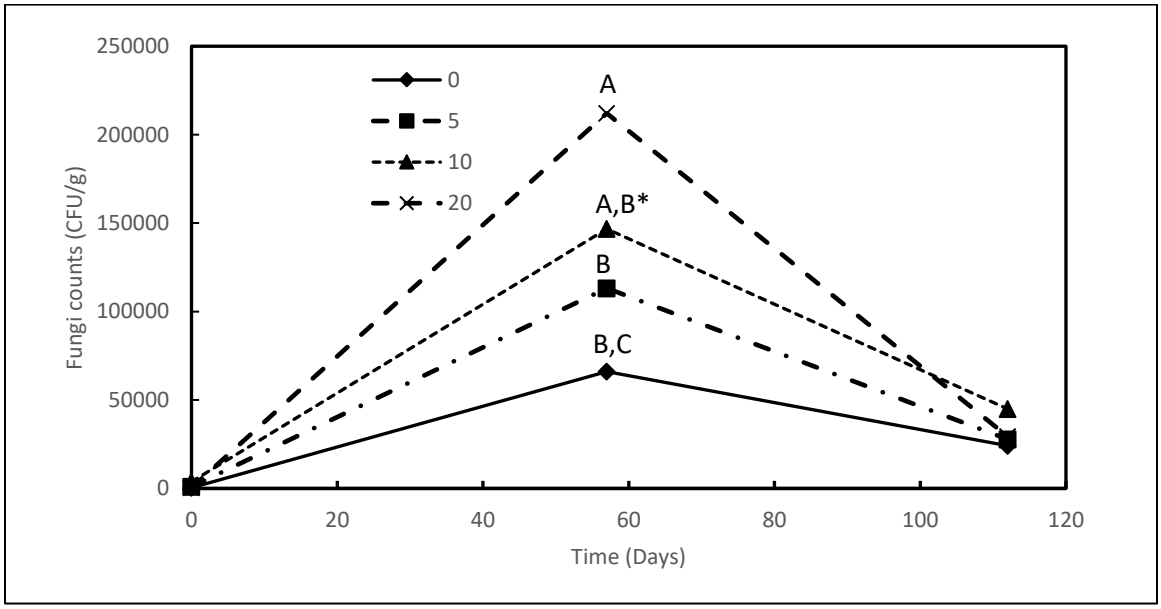


Figure 3.10 Compost total fungi counts (CFU/g) between biochar treatments over time.

3.5 Nutrient Analysis

3.5.1 Nitrogen

Total nitrogen concentration had a significant relationship with biochar level, presence of wood vinegar, and time (P =<.0001). Treatments without WV had significantly different initial N levels at Day 0, with the control being the highest, then 5%, 10%, and 20% BC being the lowest (Figure 3.11). It was expected for the control to begin with the highest nutrient concentrations because it has not been diluted by the addition of biochar.

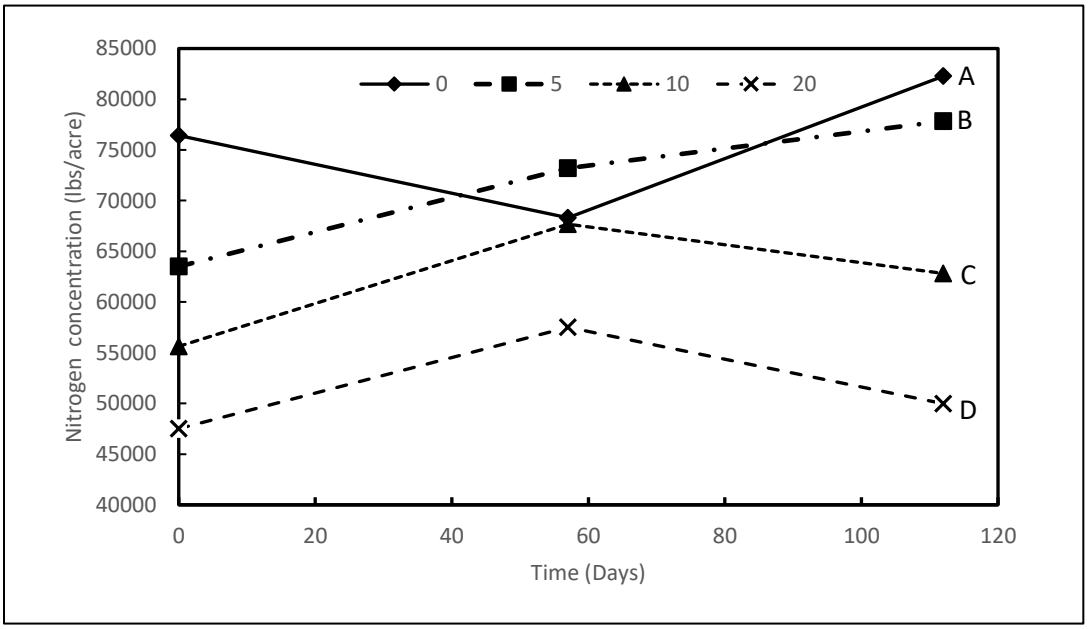


Figure 3.11 Compost nitrogen concentration (lbs/acre) for biochar levels without WV (WV=0) over time.

Treatments containing biochar trended upwards to Day 57, and 10% and 20% BC lost N from Day 57 to 112. The control experienced a decrease in N and then a subsequent increase after Day 57. The control, 5%, and 10% BC treatments had significantly higher N concentrations at Day 112 from Day 0. 20% BC was not significantly different from Day 0 to 112, so N was

retained even though it was significantly lower than other treatments. The high pH associated with increasing biochar levels could have favored ammonia volatilization, which would decrease N concentration. The trends in Figure 3.11 suggest that adding biochar to the compost changed the N fluctuation trend. In 10 and 20% biochar treatments, N concentrations increased from Day 0 to 57, and then decreased at varying degrees. 5% BC showed a less drastic increase in N from Day 57 to 112 compared to the control. For the control, N concentrations decreased significantly and then increased between the mid-point and end of the study. The decrease from the beginning could be a result of a low C:N ratio, which favors N volatilization (Epstein, 1997). In treatments with biochar, N could be adsorbed to the porous structure and less volatilization would occur between Day 0 and Day 57 because of a higher C:N ratio. Although the pH at Day 57 was high in biochar treatments, N volatilization could have been decreased due to adsorption to the biochar surface. Another factor to consider is the clogged mesh at the bottom at the container. Because 10% and 20% biochar did not experience clogging, leachate would have been able to permeate the mesh. Therefore, nutrients could leach out of the 10% and 20% biochar treatments, while control and 5% biochar would not leach nutrients or leaching would occur very slowly. This could have potentially skewed the results of N concentrations at Day 112, as the amount of N in leachates was not measured. The increased N concentrations for the control at Day 112 could be the result of high bacterial activity, which was discussed in the previous section.

Only the 0% and 5% biochar treatments were significantly different at Day 112 depending on the presence of WV, with WV treatments having lower final N concentrations (Figure 3.12). Although 0% and 5% biochar + WV began with significantly higher N concentrations than 10% and 20% BC + WV, the two treatments lost a significant amount of N by the end of the study (Figure 3.12). 10% and 20% BC +WV did not have significantly different

N concentrations between Day 0 and 112. This indicates that the higher biochar levels aided in retaining N. Furthermore, the addition of WV appears to have a stabilizing effect on N levels for the control (0% BC); however, the cause of this phenomena is unknown and warrants further investigation.

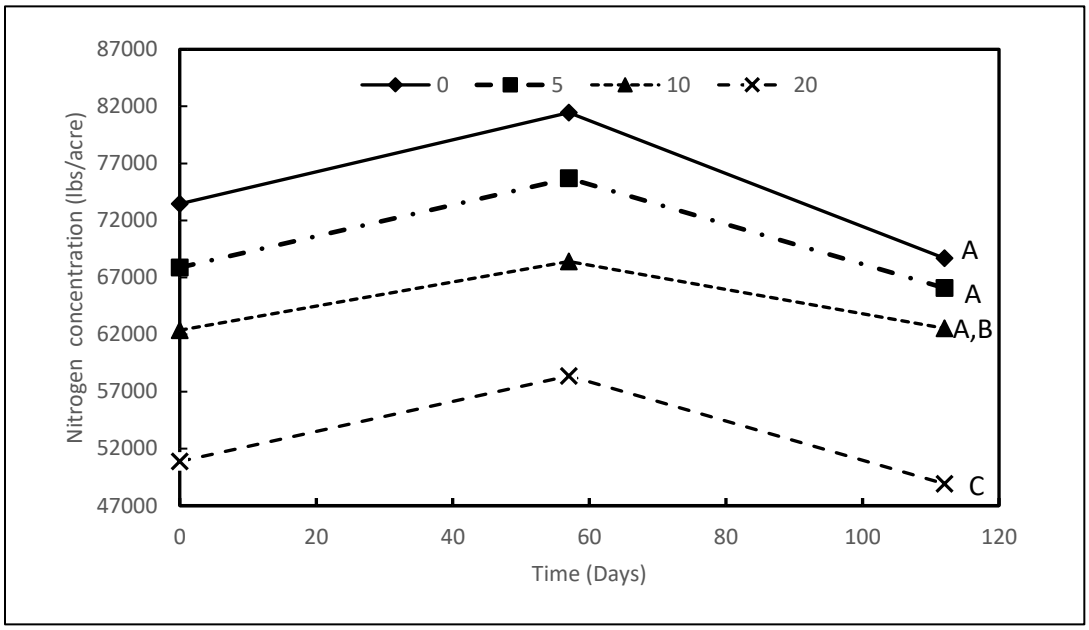


Figure 3.12 Compost nitrogen concentration (lbs/acre) for biochar levels with WV (WV=1) over time.

3.5.2 Phosphorous

Phosphorous (P) concentration had a significant relationship among biochar level, presence of wood vinegar, and time. All treatments showed a significant increase in P levels from Day 0 to Day 112 (Figure 3.13 and Figure 3.14). For treatments without WV, 10%, 5%, and 0% BC were not significantly different from each other at Day 112, and were significantly higher than 20% BC at Day 112 (Figure 3.13).

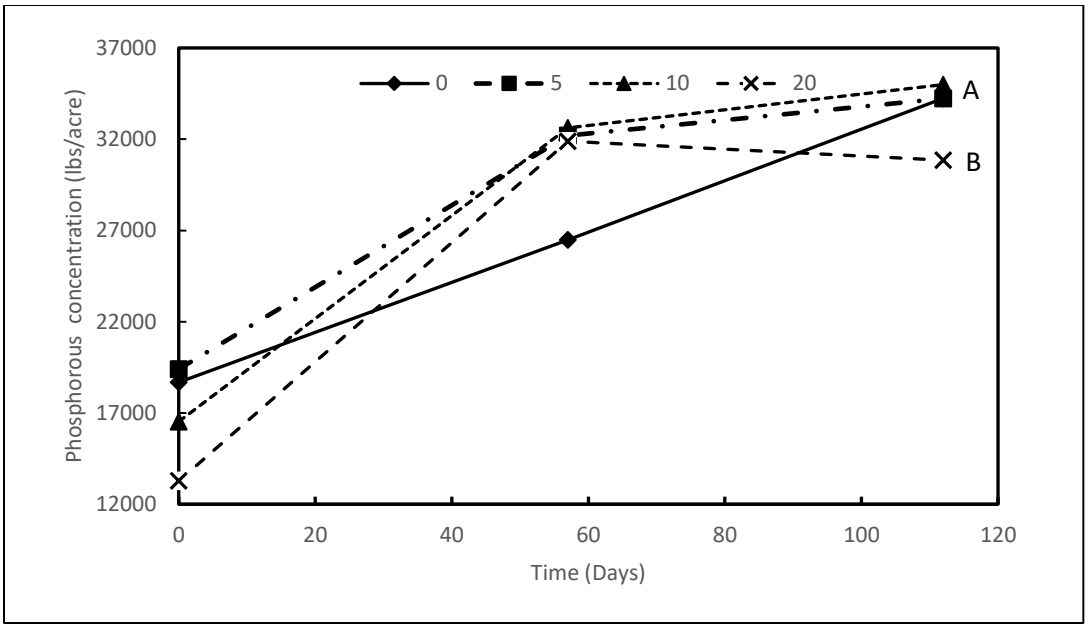


Figure 3.13 Compost phosphorus concentration (lbs/acre) for biochar levels without WV (WV=0) over time.

Although 20% BC decreased from Day 57 to 112, it was not significantly different. The control (0% BC) did not experience the same trend as treatments containing biochar. Treatments with biochar showed a slower rate of increase from Day 57 to 112, and 20% BC was the only treatment that showed decreased P concentrations. The control maintained a steady increase in P levels over time. One explanation for the decrease in P from 20% BC is through leaching, as P is at a higher risk of leaching in coarse textured soils (NRCS, 2006). P dynamics are similar to N, except that P does not enter a gaseous form so volatilization is not a cause of P loss (NRCS, 2006).

Compost P concentrations followed a similar trend as N when WV was added, because the 0% BC treatment exhibited similar patterns as biochar treatments. (Figure 3.14).

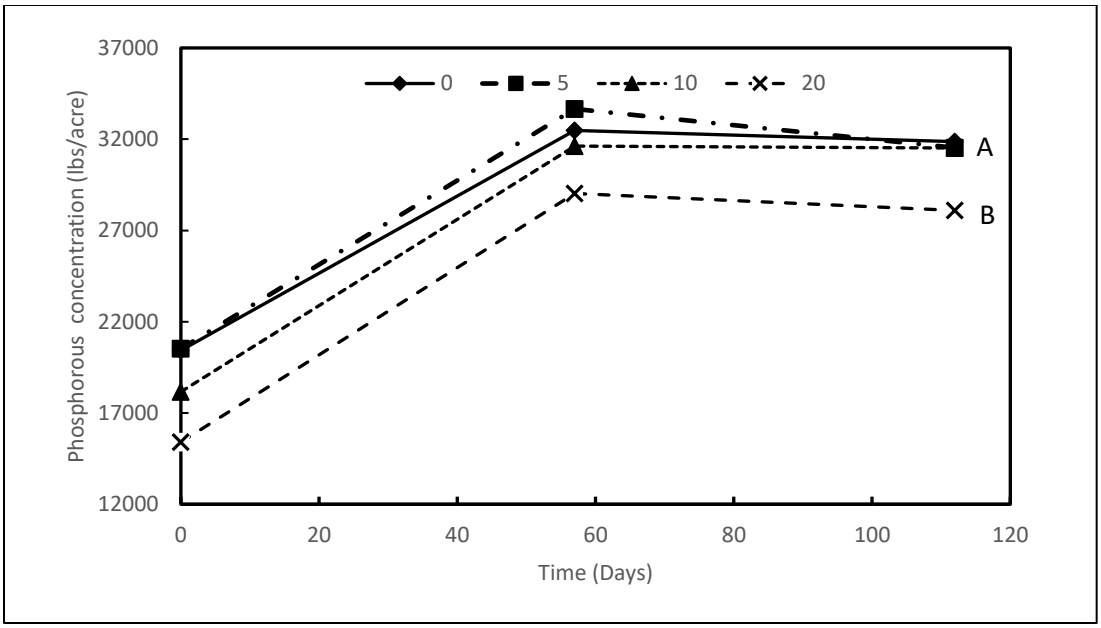


Figure 3.14 Compost phosphorus concentration (lbs/acre) for biochar levels with WV (WV=1) over time.

At Day 112, P concentrations in treatments containing WV were significantly lower than treatments containing no WV. Therefore, WV did not increase P concentrations. 5% BC + WV was the only WV compost treatment that significantly decreased from Day 57 to Day 112.

3.5.3 Potassium

There were no significant interactions between potassium (K) levels and biochar or WV. K is not a constituent of soil organic matter like N and P; however, it is a crucial plant nutrient. Manures contain plentiful amounts of potassium, so providing enough K to plants is generally not an issue when animal manures are applied (Parnes, 2013).

3.5.4 C:N ratio

All C:N ratios significantly decreased from Day 0 to Day 112. 20% BC and 20% BC + WV had the significantly highest C:N ratios at Day 112 (Figures 3.15 and 3.16); however, an

appropriate range for composting is 20:1 – 40:1 (Rynk et al., 1992; University of Arkansas Cooperative Extension Serve, 2019). C:N ratio is expected to decrease over time as microorganisms break down carbonaceous material (Jindo et al., 2012), so a significant decrease in C:N ratio between Day 0 and Day 112 was projected. At Day 112, WV treatments with 5% and 10% biochar had significantly lower C:N ratios than 5% and 10% biochar treatments.

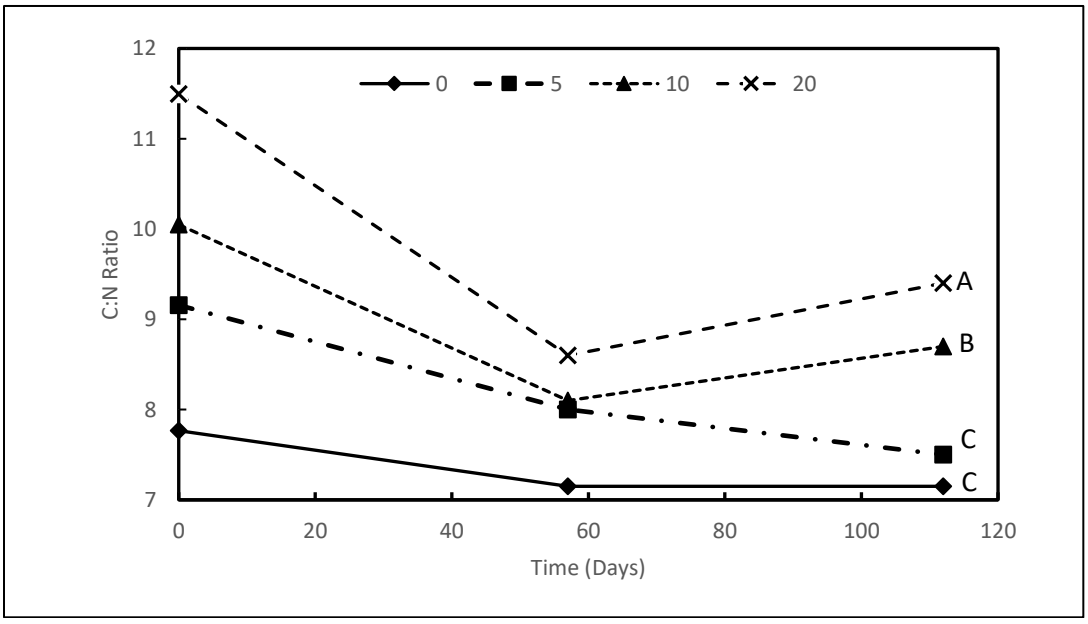


Figure 3.15 Compost C:N ratio within biochar levels without wood vinegar (WV=0) over time.

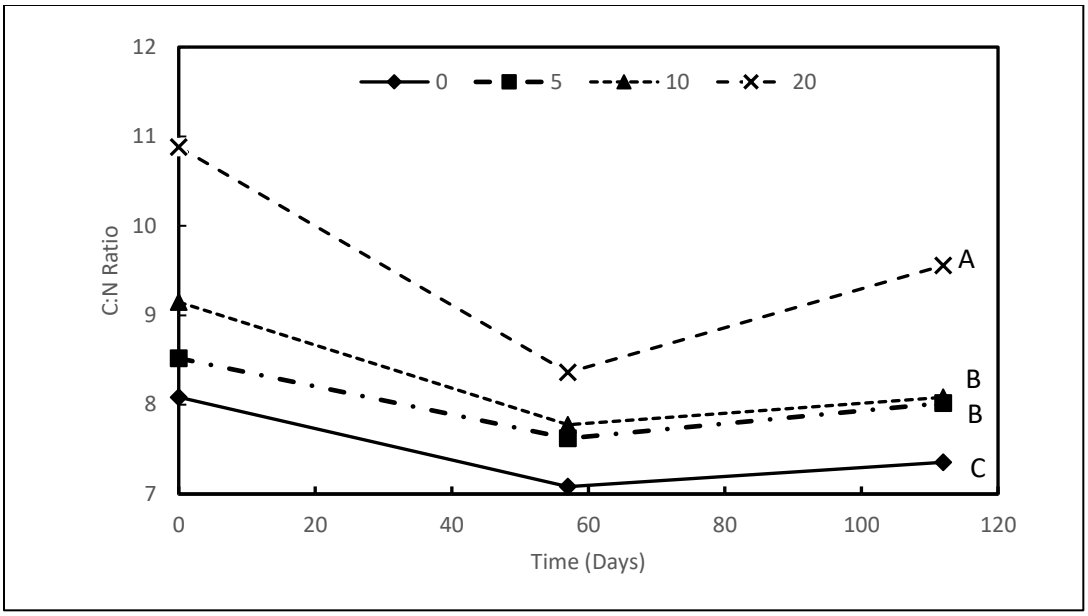


Figure 3.16 Compost C:N ratios within biochar levels with wood vinegar (WV=1) over time.

The low C:N ratios favored ammonia volatilization, which would have resulted in N losses. However, in this compost study treatments with higher C:N ratios resulted in lower total N at Day 112. This lower N could be the result of biochar increasing the pH, which also results in N volatilization. Furthermore, without knowing how much N leached during the heavy rainfall period, the true impact of higher C:N ratios on N retention cannot be determined.

Although biochar is predominantly comprised of C, it did not contribute substantially to the C:N ratio because of its resistance to microbial decomposition (Lehmann, 2007). Therefore, more C sources such as sawdust, wood chips, and wood shavings would be needed for future biochar compost studies.

3.6 *intI1* Prevalence

intI1 was found in 38/40 Day 0 compost bacteria PCR products after undergoing gel electrophoresis and in all samples thereafter. Therefore, *intI1* prevalence did not significantly

change due to biochar level, wood vinegar addition, or composting time. This result is in concurrence with the findings of other studies measuring *intI1* abundance after composting poultry litter and poultry litter with biochar (Qian et al., 2016; Xie et al., 2016). However, Li et al. (2017) found that *intI1* abundance was reduced during the thermophilic stage of composting and that higher biochar levels significantly reduced *intI1* abundance. Interestingly, Qian et al. (2016) and Xie et al. (2016) constructed compost piles at various scales, from small containers that were stored in an incubator to simulate composting or from large-scale industrial composting facilities, respectively. Qian et al. (2016) reported that *intI1* abundance did not change during normal thermophilic composting, significantly increased during insufficient thermophilic composting, and significantly decreased during continuous thermophilic composting. Therefore, it is likely the *intI1* abundances recorded in this dissertation compost study would not have decreased because thermophilic temperatures were not achieved. Although Qian et al. (2016) reported a significant decrease in *intI1* during continuous thermophilic composting, it is important to consider the experiment was conducted in a temperature-controlled incubator. Extending the thermophilic phase and increasing the maximum temperature in conventional compost piles may be highly valuable for decreasing *intI1* prevalence. Biochar has been shown to increase temperatures and prolong the thermophilic phase during composting (Li et al., 2017).

Because thermophilic temperatures were not achieved, it is unknown if *intI1* levels would have been effected by thermophilic temperatures. High temperatures may stress mesophilic bacteria and impede horizontal gene transfer, or kill mesophilic bacteria harboring the gene (Miller et al., 2016). As stated before, the effect of composting and biochar on *intI1* abundance is conflicting, and needs further investigation.

Control, PL + WV, 20% BC, and 20% BC + WV sample sequences were chosen to compare *intI1* sequence similarities because they represented the two most extreme biochar levels. DNA sequences were run in GenBank BLAST with the first 20 nucleotides removed to improve sequence quality (Figure 3.17). From PCR gel electrophoresis, all samples from Day 0 except 5% BC-2 and 20% BC-5 were positive for *intI1*. 5% BC-2 showed poor DNA quality and this was most likely the reason *intI1* was not amplified (Figure 3.18). 20% BC-5 was a false positive on agarose gel electrophoresis, as it was positive for *intI1* when run through BLAST. In addition, all samples from Day 112 were *intI1* positive on gel electrophoresis.

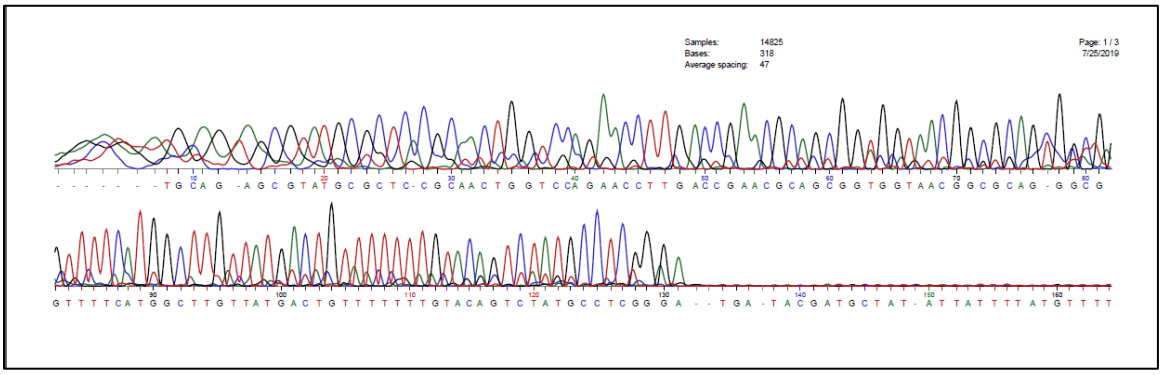


Figure 3.17 20% BC-5 Day 0 nucleotide sequence peaks. Discrete peaks indicate good DNA quality, and the first 20 nucleotides were trimmed.

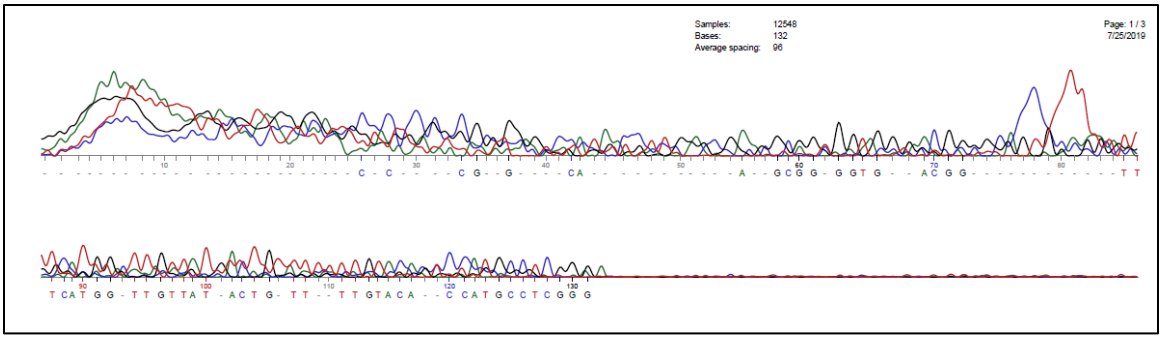
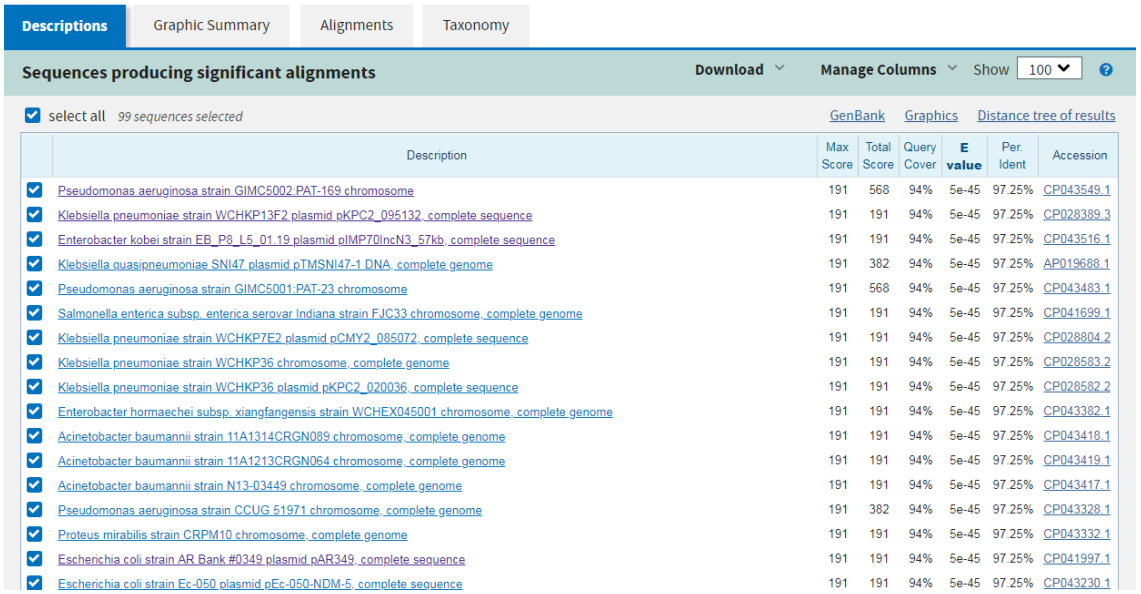


Figure 3.18 5% BC-2 showed poor sequence quality as indicated by overlapping, indistinct peaks.

The comparison of four treatments containing no biochar and 20% biochar yielded at least good sequence similarities to *intI1* (>95%), except 20% BC WV-1 Day 0 that exhibited 93.46% nucleotide homology to *intI1* with no nucleotide gaps. Furthermore, all samples except 20% BC WV-1 Day 0 showed the same bacteria strain matches for at least the first 16 strains. The top three bacteria strain similarities were for *Pseudomonas aeruginosa* strain GIMC5002-PAT-169 chromosome (Accession # CP043549.1), *Klebsiella pneumoniae* strain WCHKP13F2 plasmid pKPC2_095132 (Accession # CP028389.3), and *Enterobacter kobei* strain EB_PB_L5_01.19 plasmid pIMP70IncN3_57kb (Accession # CP043516.1), all of which showed the same sequence similarity to *intI1* isolated from compost samples (Figure 3.19).



Descriptions | Graphic Summary | Alignments | Taxonomy

Sequences producing significant alignments — Download — Manage Columns — Show 100

select all 99 sequences selected — GenBank | Graphics | Distance tree of results

Description	Max Score	Total Score	Query Cover	E value	Per. Ident	Accession
Pseudomonas aeruginosa strain GIMC5002:PAT-169 chromosome	191	568	94%	5e-45	97.25%	CP043549.1
Klebsiella pneumoniae strain WCHKP13F2 plasmid pKPC2_095132, complete sequence	191	191	94%	5e-45	97.25%	CP028389.3
Enterobacter kobei strain EB_P8_L5_01.19 plasmid pIMP70IncN3_57kb, complete sequence	191	191	94%	5e-45	97.25%	CP043516.1
Klebsiella quasipneumoniae SNI47 plasmid pTMSNI47-1 DNA, complete genome	191	382	94%	5e-45	97.25%	AP019688.1
Pseudomonas aeruginosa strain GIMC5001:PAT-23 chromosome	191	568	94%	5e-45	97.25%	CP043483.1
Salmonella enterica subsp. enterica serovar Indiana strain FJC33 chromosome, complete genome	191	191	94%	5e-45	97.25%	CP041699.1
Klebsiella pneumoniae strain WCHKP7E2 plasmid pCMY2_085072, complete sequence	191	191	94%	5e-45	97.25%	CP028804.2
Klebsiella pneumoniae strain WCHKP36 chromosome, complete genome	191	191	94%	5e-45	97.25%	CP028583.2
Klebsiella pneumoniae strain WCHKP36 plasmid pKPC2_020036, complete sequence	191	191	94%	5e-45	97.25%	CP028582.2
Enterobacter hormaechei subsp. xiangfangensis strain WCHEX045001 chromosome, complete genome	191	191	94%	5e-45	97.25%	CP043382.1
Acinetobacter baumannii strain 11A1314CRGN089 chromosome, complete genome	191	191	94%	5e-45	97.25%	CP043418.1
Acinetobacter baumannii strain 11A1213CRGN064 chromosome, complete genome	191	191	94%	5e-45	97.25%	CP043419.1
Acinetobacter baumannii strain N13-03449 chromosome, complete genome	191	191	94%	5e-45	97.25%	CP043417.1
Pseudomonas aeruginosa strain CCUG 51971 chromosome, complete genome	191	382	94%	5e-45	97.25%	CP043328.1
Proteus mirabilis strain CRPM10 chromosome, complete genome	191	191	94%	5e-45	97.25%	CP043332.1
Escherichia coli strain AR Bank #0349 plasmid pAR349, complete sequence	191	191	94%	5e-45	97.25%	CP041997.1
Escherichia coli strain Ec-050 plasmid pEc-050-NDM-5, complete sequence	191	191	94%	5e-45	97.25%	CP043230.1

Figure 3.19 GenBank BLAST sequence similarity results for *intI1* in 20% BC-2 Day 0 showing 97.25% sequence identity to various bacteria.

intI1 was found in the complete genetic sequence of *K. pneumonia* and *E. kobei,* but the *P. aeruginosa* 169 chromosome did not encode *intI1* (Figure 3.20). However, *Pseudomonas* spp. are known for being intrinsically resistant to antibiotics and for acquiring various antibiotic-resistance genes facilitated by class 1 integrons (Bonomo and Szabo, 2006). Therefore, it is probable that *intI1* would have been found if the complete sequence was provided. In addition, various strains of *Salmonella enterica*, *Enterobacter hormaechei*, *Acinetobacter baumannii*, *Proteus mirabilis*, and *Escherichia coli* were found to have at least 95% sequence similarities to the *intI1* positive compost samples (Figure 3.19).

Pseudomonas aeruginosa, *Acinetobacter baumannii*, and *Enterobacter* spp. are commonly found in soil and aquatic systems while *Klebsiella pneumoniae* is an enteric bacteria found in many animal species (Kim et al., 2005; Bonomo and Szabo, 2006; Mezzatesta et al., 2012). A study conducted by Kim et al. (2005) reported that multidrug-resistant *K.pneumoniae*

was prevalently isolated from turkey, cattle, and chicken farms as well as retail meat products in the state. Class 1 integrons were identified in 132 isolates and encoded the *aadA1* gene, which confers streptomycin and spectinomycin resistance. In addition, the study demonstrated the transfer of the multiple antibiotic-resistant genes through transconjugation to *Escherichia coli*, which is facilitated by mobile genetic elements such as class 1 integrons. The article states, "The bacterial genomic DNA restriction patterns by pulsed-field gel electrophoresis showed that the same clones of multidrug-resistant *K. pneumonia* remained in feathers, feed, feces, and drinking water in turkey environments, indicating the possible dissemination of antibiotic-resistance genes in the ecosystem and cross-contamination of antibiotic-resistant bacteria during processing and distribution of products." This further describes how multi-drug resistant bacteria can spread throughout multiple environments and enter the food chain.

```
gene            complement(154952..155965)
                /gene="intI1"
                /locus_tag="B7D34_00895"
CDS             complement(154952..155965)
                /gene="intI1"
                /locus_tag="B7D34_00895"
                /inference="COORDINATES: similar to AA
                sequence:RefSeq:NP_569372.1"
                /note="Derived by automated computational analysis using
                gene prediction method: Protein Homology."
                /codon_start=1
                /transl_table=11
                /product="class 1 integron integrase IntI1"
                /protein_id="AVW74135.1"
                /translation="MKTATAPLPPLRSVKVLDQLRERIRYLHYSLRTEQAYVHWVRAF
                IRFHGVRHPATLGSSEVEAFLSWLANERKVSVSTHRQALAALLFFYGKVLCTDLPWLQ
                EIGRPRPSRRLPVVLTPDEVVRILGFLEGEHRLFAQLLYGTGMRISEGLQLRVKDLDF
                DHGTIIVREGKGSKDRALMLPESLAPSLREQLSRARAWWLKDQAEGRSGVALPDALER
                KYPRAGHSWPWFWVFAQHTHSTDPRSGVVRRHHMYDQTFQRAFKRAVEQAGITKPATP
                HTLRHSFATALLRSGYDIRTVQDLLGHSDVSTTMIYTHVLKVGGAGVRSPLDALPPLT
```

Figure 3.20 The *intI1* gene was found in *K. pneumoniae* and is likely a source of the organism's drug resistance.

Although some poultry producing companies are trying to phase out the use of subtherapeutic antibiotics, that does not result in an immediate reduction in antibiotic resistance (Spratt, 1996). Bacteria populations can retain resistance genes for long periods of time even after the cessation of antibiotic administration (Luo et al., 2005). Therefore, it is imperative to continue research how to reduce or immobilize *intI1* in poultry environments. Even though the effect of biochar on *intI1* abundance is inconclusive, it may assist in preventing the spread of MGEs and ARGs through adsorption.

3.7 Compost Maturity Test

No radish seeds germinated in compost treatments after seven days. Radish seeds germinated in six out of seven Miracle Gro garden soil replicates; therefore, greenhouse conditions were favorable for seed germination (Figure 3.13).

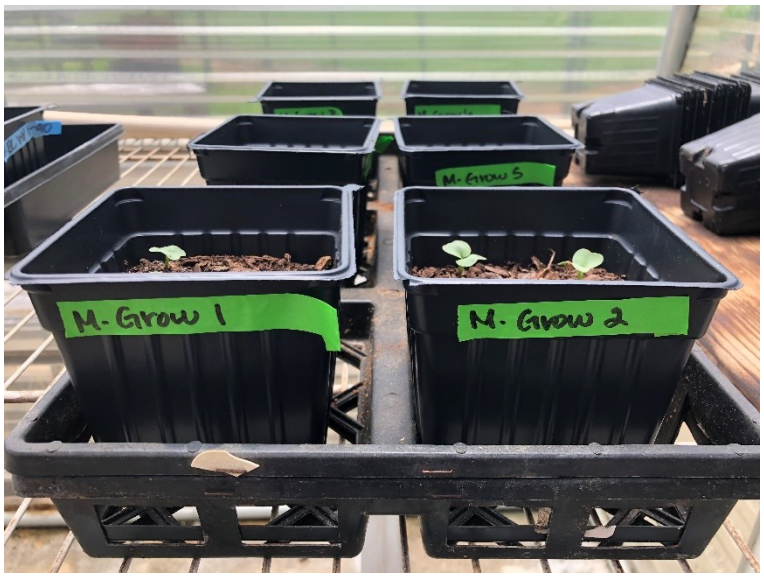


Figure 3.21 Radish seed germination after 7 days in commercial soil.

Compost maturation was not achieved after 112 days because composts did not reach thermophilic temperatures or undergo typical compost phases. These disruptions may have been due to the low material volume, low C:N ratio, cool ambient temperatures, and high moisture contents. Furthermore, the high nutrient concentrations may have inhibited radish seeds from germination. Diluting the compost with neutral soil may increase radish seed germination rates.

CHAPTER IV

CONCLUSIONS

Compost treatments containing 20% biochar facilitated drainage by imparting rigidity and porosity to the compost. In addition, biochar's absorptive properties may have reduced the amount of free water in the compost. 20% biochar would be recommended for composts being applied to agricultural lands with a history of drainage or soil compaction issues. Furthermore, higher amounts of biochar appeared to reduce insect activity and odor, which is valuable for composting facilities and landowners that apply poultry litter compost to agricultural fields because it would reduce odor complaints. Although composting does not have to be done under covered areas, it is recommended that future compost studies include covers to prevent issues caused by excessive precipitation.

Thermophilic temperatures were not achieved during composting. This is most likely a result of the low volume of the compost material, as heat generated by microbial activity could not be insulated. High moisture contents caused by heavy rainfall would have hindered microbial activity and conducted heat out of the compost pile, which further caused compost cooling. In future studies, the volume of the compost should be increased in order to reach proper temperatures.

Increasing biochar level did not significantly increase N, P, and K levels compared to the control at the end of the study. However, 20% biochar and 20% biochar + WV did not significantly decrease in N and P over time, so these nutrients were retained. Treatments

containing WV showed significantly lower N levels at the end of the study compared to treatments without WV. In addition, the type of analysis used measured total N, which does not provide information on the composition of N species (organic or inorganic). In future studies, this information would be valuable to further characterize how biochar and WV influence N cycles during composting.

Phosphorous concentrations were significantly lower in WV treatments at the end of the compost study; however, all treatments showed a significant increase in P concentration over time. It is possible that if the composting time was extended, the final N concentrations may have been higher in 10% and 20% biochar + WV for P concentrations. WV appeared to change the P and N dynamics for the control treatment; however, this mechanism remains unclear and should be investigated further.

Potassium concentration was not significantly affected by biochar or wood vinegar; however, K levels did significantly decrease from Day 0 to Day 112 for all treatments. However, animal manures are rich sources of K, and is generally not of great concern when applied as a fertilizer.

Because 0% and 5% biochar treatments had clogged meshes that limited water drainage, they could have retained more nutrients because leaching was impeded. On the other hand, 10% and 20% biochar treatments were draining properly; therefore, nutrients would have been able to leach out of the containers. This may have impacted the results, so the final concentrations of N, P, and K are not a true comparison between composts with different biochar levels. A laboratory-scale leaching study would be beneficial to quantify the leaching rate for N, P, and K in composts with varying biochar levels.

Although biochar significantly increased C:N ratio, it was not enough to reach an efficient range for composting. This is most likely due to the recalcitrant nature of biochar, so microorganisms cannot break it down. Poultry litter is partially comprised of carbon sources such as sawdust and wood shavings; however, the poultry litter collected for this study had a low C:N ratio, which favors ammonia volatilization. Since this is an issue in poultry houses, it may be beneficial for the industry to incorporate more carbon sources between flocks. Future composting studies should include additional carbon sources such as sawdust, wood shavings, and wood chips to increase C:N ratios to an appropriate level for efficient composting.

Class 1integrons (intI1) abundance was not significantly affected by biochar or wood vinegar. Although this result has been found in other studies, it is unknown if *intI1* would have been affected if thermophilic temperatures were achieved. However, these results do demonstrate the persistence of class 1 integrons in compost environments, which is of great concern for public and environmental health. In addition, it would be beneficial to measure *intI1* expression levels during composting to observe if biochar or wood vinegar affected gene expression. Furthermore, the protocol developed for isolating *intI1* in poultry litter and compost was successful with a false positive rate of 1.25%, and should be further explored as a rapid screening method to survey *intI1* abundance in poultry litter environments.

Composts were not mature at the end of the study. This is most likely due to low temperatures, which impeded the composting process.

Although there were several factors that hindered the composting process to result in a material that was not a true compost, 10% biochar would be recommended for composting poultry litter because it showed good physical properties, significantly higher N and P concentrations than 20% biochar, had a lower pH so less ammonia volatilization would occur,

had significantly more bacteria than 20% biochar, and showed significantly higher fungi counts than 5% BC and the control. However, more carbon sources should be added to increase the C:N ratio. Adding WV at 2% application rate is not recommended for composting because N and P concentrations were significantly lower than treatments without WV. In addition, WV treatments showed a significantly higher pH and significantly lower abundances for bacteria and fungi, which impeded the composting process.

REFERENCES

Agyarko-Mintah E, Cowie A, Singh BP, Joseph S, Van Zwieten L, Cowie A, Harden S, Smillie R (2017) Biochar increases nitrogen retention and lowers greenhouse gas emissions when added to composting poultry litter. Waste Manag. 61:138-149.

Akhter A, Hage-Ahmed K, Soja G, Steinkellner S (2015) Compost and biochar alter mycorrhization, tomato root exudation, and development of *Fusarium oxysporum* f. sp.*lycopersici*. Front. Plant Sci. 6:529.

Al-Wabel MI, Usman AR, Al-Farraj AS, Ok YS, Abduljabbar A, Al-Faraj AI, Sallam AS (2017) Date palm waste biochars alter a soil respiration, microbioal biomass carbon, and heavy metal mobility in contaminated mined soil. Environ. Geochem. Health 1-18.

Aneja VP, Schlessinger WH, Niyogi D, Jennings G, Gilliam W, Nighton RE, Duke CS, Blunden J, Krishnan S (2006) Emerging national research needs for agricultural air quality. Eos, Transactions of the American Geophysical Union 87:25-36.

Bahsi Kaya, G (2018) Composting of cross-laminated timber (CLT) sawdust. MS thesis, Mississippi State University, Mississippi State, MS. 65 pp.

Baimark Y and Niasma N (2009) Study on wood vinegars for use as coagulating and antifungal agents on the production of natural rubber sheets. Biomass Bio-energy 33:994-998.

Bakhshizadeh H (2012) Accelerated composting of hardwood bark amended with organic and inorganic fertilizer. MS thesis, Mississippi State University, Mississippi State, MS. 70 pp.

Bernhart M, Fasina OO, Fulton J, Wood CW (2010) Compaction of poultry litter. Bioresource technology 101:234-238.

Blair JMA, Webber MA, Baylay AJ, Ogbolu DO, Piddock, LJV (2014) Molecular mechanism of antibiotic resistance. Nat. Rev. Microbiol. 13:42-51.

Bolan NS, Szogi AA, Chuasavathi T, Seshadri B, Rothrock Jr. MJ, Panneerselvam P (2010) Uses and management of poultry litter. World's Poultry Science Journal 66:673-698.

Bonomo RA and Szabo D (2006) Mechanisms of Multidurg Resistance in *Acinetobacter* Species and *Pseudomonas aeruginosa.* Clinical Infectious Diseases 43:49-56.

Brewer CE, Chuang VJ, Masiello CA, Gonnermann H, Gao X, Dugan B, Driver LE, Panzacchi P, Zygourakis K, Davies CA (2014) New approaches to measuring biochar density and porosity. Biomass Bioenergy 66:176-185.

Brinson Jr. SE, Cabrera ML, Tyson SC (1994) Ammonia volatilisation from surface-applied, fresh and composted poultry litter. Plant and Soil 167:213-218.

Bull SA, Allen VM, Domingue G, Jorgensen F, Frost JA, Ure R, Whyte R, Tinker D, Corry JEL, Gilliard-King J, Humphrey TJ (2006) Sources of Campylobacter spp. colonizing housed broiler flocks during rearing. Applied and Environmental Microbiology 72:645-652.

Burel C, Valat C (2009) The effect on the feed on the host-microflora interactions in poultry:an overview. Pages 365-383 *in* Proceedings in International Symposium on Sustainable animal production – the challenges and potential developments for professional farming, 13th Congress of the International Society for Animal Hygiene. Vechta, Germany.

Camps M and Tomlinson T (2015) The Use of Biochar in Composting. International Biochar Initiative. https://www.biochar-international.org/wp-content/uploads/2018/04/Compost_biochar_IBI_final.pdf. (2 June 2019).

Casey KD, Bicudo JR, Dschmidt DR, Singh A, Gay SW, Gates RS, Jacobsen LD, Hoff SJ (2006) Air quality and emissions from livestock and poultry production/waste management systems. Pages 1-40 *in* Rice JM, Caldwell DR, Humenik FJ, eds. Animal Agriculture and the Environment: National Center for Manure and Animal Waste Management White Papers, Publication No 913C0306. St. Joseph, MI, ASABE.

Chen B, Liang X, Nie X, Huang X, Zou S, Li X (2015) The role of class 1 integrons in the dissemination of sulfonamide resistance genes in the Pearl River and Pearl River Estuary, South China. J. Hazard. Mater. 282:61-67.

Chen YX, Huang XD, Han ZY, Huang X, Hu B, Shi DZ, Wu WX (2010) Effects of bamboo charcoal and bamboo vinegar on nitrogen conservation and heavy metals immobility during pig manure composting. Chemosphere 78:1177-1181.

Clough TJ, Clondron LM (2010) Biochar and the nitrogen cycle: introduction. J. Environ. Qual. 39:1218-1223.

Cook KL, Rothrock MJ, Warren JG, Sistani KR, Moore PA (2008) Effect of alum treatment on the concentration of total and ureolytic microorganisms in poultry litter. Journal of Environmental Quality 37:2360-2367.

Cui E, Ying W, Zuo Y, Hong C (2016) Effect of different biochars on antibiotic resistance genes and bacterial community during chicken manure composting. Bioresour. Technol. 203:11-17.

Enticknap JJ, Nonogaki H, Place AR, Hill RT (2006) Microbial diversity associated with odor modification for production of fertilizers from chicken litter. Applied and Environmental Microbiology 72:4105-4114.

Epstein E (1997) The Science of Composting. Technomic Publishing Company, Inc., Pennsylvania.

Fagernäs L, Kuoppala E, Tiilikkala K, Oasmaa A (2012) Chemical Composition of Birch Wood Slow Pyrolysis Products. Energy and Fuels. 26:1275-1283.

Fahmi R, Bridgewater AV, Donnison L, Yates N, Jones J (2008) The effect of lignin and inorganic species in biomass on pyrolysis oil yields, quality and stability. Fuel 87(7):1230-1240.

Franzluebbers AJ, Doraiswamy PC (2007) Carbon sequestration and land degradation. Pages 343-358 *in* International Workshop on Climate and Land Degradation. Arusha, Tanzania.

Gillings M, Boucher Y, Labbate M, Holmes A, Krishnan S, Holley M, Stokes H (2008) The evolution of class 1 integrons and the rise of antibiotic resistance. Journal of Bacteriology 190(14):5095-5100.

Gillings M, Gaze W, Pruden A, Smalla K, Tiedje J, Zhu Y (2015) Using the class 1 integron-integrase gene as a proxy for anthropogenic pollution. The ISME Journal 9(6):1269-1279.

Gul S, Whalen JK (2016) Biochemical cycling of nitrogen and phosphorus in biochar-amended soils. Soil Biol. Biochem. 103:1-15.

Hagner M, Penttinen O, Tiilikkala K, Setälä H (2013) The effects of biochar, wood vinegar and plants on glyphosate leaching and degradation. European Journal of Soil Biology 58:1-7.

Han Y, Douds Jr. DD, Boateng AA (2016) Effect of biochar soil-amendments on *Allium porrum* growth and arbuscular mycorrhizal fungus colonization. J. Plant. Nutr. 39(11):1654-1662.

Harmel RD, Torbert HA, Haggard BE, Haney R, Dozier M (2004) Water quality impacts of converting to a poultry litter fertilization strategy. Journal of Environmental Quality 33:2229-2242.

Ho YB, Zakaria MP, Latif PA, Saari N (2013) Degradation of beterinary antibiotics and hormone during broiler manure composting. Bioresour. Technol. 131:476-484.

Hua L, Wu W, Liu Y, McBride, MB. Chen Y (2009) Reduction of nitrogen loss and Cu and Zn mobility during sludge composting with bamboo charcoal amendment. Environ. Sci. Pollut. Res. 16:1-6.

Heuer H, Schmitt H, Smalla K (2011) Antibiotic resistance gene spread due to manure application on agricultural fields. Curr. Opin. Microbiol. 14(3):236-243

Ishizaki S, Okazaki Y (2004) Usage of charcoal made from dairy farming waste as bedding material of cattle, and composting and recycle use as fertilizer. Bull. Chiba Prefectural Livestock Res. Center 4:25-28.

Jaiswal AK, Elad Y, Paudel I, Graber ER, Gytryn E, Frenkel O (2017) Linking the belowground microbial composition, diversity and activity to soilborne disease suppression and growth promotion of tomato amended with biochar. Sci. Rep. 7:44382.

Jechalke S, Heuer H, Siemens J, Amelung W, Smalla K (2014) Fate and effects of veterinary antibiotics in soil. Trends Micrbiol. 22:536-545.

Jeong CY, Wang JJ, Dodla SK, Eberhardt TL, Groom L (2012) Effect of biochar amendment on tylosin adsorption-desorption and transport in two different soils. J. Environ. Qual. 41(4):1185-1192.

Ji X, Shen Q, Fang L, Jing M, Gang X, Wang Y, Wu M (2012) Antibiotic resistance gene abundances associated with antibiotics and heavy metals in animals manures and agricultural soils adjacent to feedlots in Shanghai; China. J. Hazard. Mater. 236-236(20):178-185.

Jindo K, Sanchez-Monedero MA, Hernandez T, Garcia C, Furukawa T, Matsumoto K, Sonoki T, Bastida F (2012) Biochar influences the microbial community structure during manure composting with agricultural wastes. Science of the Total Environment 416:476-481.

Johnson TA, Stedtfeld RD, Wang Q, Cole JR, Hashsham SA, Looft T, Zhu YG, Tiedje JM (2016) Clusters of antibiotic resitance genes enriched together stay together in swine agriculture. mBio 7:e02214-e02215.

Kavitha B, Reddy PVL, Kim B, Lee SS, Pandey SK, Kim K (2018) Benefits and limitations of biochar amendment in agricultural soils: A review. J. Environ. Manag. 227:146-154.

Kelley TR, Pancorbo OC, Merka WV, Thompson SA, Cabrera ML, Brnhat HM (1996) Elemental concentratons of stored whole and fractionated broiler litter. Journal of Applied Poultry Research 5:276-281.

Kim WK, Patterson PH (2003) Effect of minerals on activity of microbial uricase to reduce ammonia volatilisation in poultry manure. Poultry Science 82:223-231.

Kim S, Wei C, Tzou Y, An H (2005) Multidrug-resistant *Klebsiella pneumonia* isolated from farm environments and retail products in Oklahoma. Journal of Food Protection 68(10):2022-2029.

Kishimoto S, Tsuyoshi H (2019) How to improve crop quality while reducing dependence on agricultural chemicals.

Koeleman JGM, Stoof J, Van der Bijl MW, Vandenbrouche-Grauls CMJE, Savelkoul PHM (2001) Identificiation of epidemic strains of *Acinetobacter baumannii* by integrase gene PCR. Journal of Clinical Microbiology. 39(1):8-13.

Krishnamachari KAUR (1987) Fluorine. Vol 1, 5th Ed. Pages 265-416 *in* Mertz W eds. Trace Elements in Human and Animal Health. Academic Press, San Diego, CA.

Lehmann J (2007) A handful of carbon. Nature 447:143-144.

Lei O, Zhang R (2013) Effects of biochars derived from different feedstocks and pyrolysis temperatures on soil physical and hydraulic properties. J. Soils Sediments 13:1561-1572.

Li H, Duan M, Gu J, Zhang Y, Qian X, Ma J, Zhang R, Wang X (2017) Effects of bamboo charcoal on antibiotic resistance genes during chicken manure composting. Ecotoxicology and Environmental Safety. 140:1-6.

Li Z, Zhang L, Chen G, Wu L, Liu B, Sun S, Zhang H, Zhang Z, Wang Z (2018)A new method for comprehensive utilization of wood vinegar by distillation and liquid – liquid extraction. Process Biochemistry. 75:194-201.

Line JE, Bailey JS (2006) Effect of on-farm acidification treatments on Campylobacter and Salmonella populations in commercial broiler houses in northeast Georgia. Poultry Science 85:1529-1534.

Linhoss JE, Purswell JL, Street JT, Rowland MR (2019) Evaluation of biochar as a litter amendment for commercial broiler production. J. Appl. Poult. Res. 0:1-10.

Liu Z, Zhang F, Wu J (2010) Characterization and application of chars produced from pinewood pyrolysis and hydrothermal treatment. Fuel 89(2):510-514.

Liu N, Zhou J, Han L, Ma S, Sun X, Huang G (2017) Role and multi-scale characterization of bamboo biochar during poultry manure aerobic composting. Bioresour. Technol. 241:190-199.

López-Cano I, Roig A, Cayuela ML, Alburquerque JA, Sánchez-Monedero MA (2016) Biochar improves N cycling during composting of olive mill wastes and sheep manure. Waste Manage. 49:553-559.

Lovanh N, Cook KL, Rothrock Jr. MJ, Miles DM, Sistani K (2007) Spatial shifts in microbial population structure within poultry litter associated with physicochemcial properties. Poultry Science 86:1840-1849.

Lu J, Sanchez S, Hofrace C, Maurer JJ, Harmon B, Lee MD (2003) Evaluation of broiler litter with reference to the microbial composition as assesses by using 16S rRNA and functional gene markers. Applied and Environmental Microbiology 69: 901-908.

Luo N, Pereira S, Sahin O, Lin J, Huang S, Michel L, Zhang Q (2005) Enhanced *in vivo* fitness of fluoroquinolone-resistant *Campylobacter jejuni* in the absence of antibiotic selection pressure. Proceed Nat. Acad. Sci. 102:541-546.

Mcdonald JM, Ribaudo MO, Livingston MJ, Beckman J, Wang H (2009) Manure use for fertilizer and for energy. USDA Economic Research Service, Washington, D.C.

Mezzatesta ML, Gona F, Stefani S (2012) Enterobacter cloacae complex: clinical impact and emerging antibiotic resistance. Ruture Microbiol 7:887-902.

Miller RE, Lei X, Ullrey DE (1991) Trace elements in animal nutrition. Pages 593-662 *in* Mortvedt JJ, eds. Micronutrients in Agriculture, 2nd Ed. Soil Science Society of America, Madison WI.

Miller JH, Novak JT, Knocke WR, Pruden A (2016) Survival of antibiotic resistant bacteria and horizontal gene transfer control antibiotic resistance gene content in anaerobic digesters. Front. Microbiol. 7:263.

Millner PD (2009) Bioaerosols associated with animal production systems. Bioresource Technology 100:5379-5385.

Mississippi Department of Agriculture and Commerce (2017) Mississippi Agriculture Overview. https://www.mdac.ms.gov/agency-info/mississippi-agriculture-snapshot/. (9 June 2019).

Mitchell SM, Subbiah M, Ullman JL, Frear C, Call DR (2015) Evaluation of 27 different biochars for potential sequestration of antibiotic residues in food animal production environments. Journal of Environmental Chemical Engineering. 3:162-169.

Moore PA, Daniel TC, Edwards DR, Miller DM (1996) Evaluation of chemical amendments to reduce ammonia volatilisation from poultry litter. Poultry Science 75:315-320.

Moore PA, Daniel TC, Sharpley AN, Wood CW (1995) Poultry manure management – environmentally sound options. Journal of Soil and Water Conservation 50:321-327.

Nahm KH (2002) Efficient feed nutrient utilization to reduce pollutants in poultry and swine manure. Critical reviews in Environmental Science and Technology 32:1-6.

Nandi S, Maurer JJ, Hofacre C, Summers AO (2004) Gram-positive bacteria are a major reservoir of class 2 antibiotic resistance integrons in poultry litter. Proceedings of the National Academy of Sciences of the United States of America. 101(18):7118-7122.

National Research Council (US) Committee on Drug Use in Food Animals (1999) The use of Drugs in Food Animals: Benefits and Risks. Washington (DC): National Academies Press (US) https://www.ncbi.nlm.nih.gov/books/NBK232571/. (12 July 2019).

Natural Resources Conservation Service (NRCS) (2006) Model Simulation of Soil Loss, Nutrient Loss, and Change in Soil Organic Carbon Associated with Crop Production. https://www.nrcs.usda.gov/Internet/FSE_DOCUMENTS/nrcs143_012874.pdf. (16 September 2019).

Nguyen TTN, Xu C, Tahmasbian I, Che R, Xu Z, Zhou X, Wallace HM, Bai SH (2017) Effects of biochar on soil available inorganic nitrogen: A review and meta-analysis. Geoderma. 228:79-96.

Pedroso AA, Hurley-Bacon AL, Zedek AS, Kwan TW, Jordan APO, Avellaneda G, Hofacre CL, Oakley BB, Colett SR, Maurer JJ, Lee MD (2013) Can probiotics improve the environmental microbiome and resistome of commercial poultry production? Int. J. Environ. Res. Public Health 10:4534-4559.

Parnes R (2013) Soil Fertility: A Guide to Organic and Inorganic Soil Amendments. Northeast Organic Farming Association. https://www.nofa.org/soil/html/quality.php. (16 September 2019).

Powers W, Angel R (2008) A review of the capacity for nutritional strategies to address environmental challenges in poultry production. Poultry Science 87:1929-1938.

Prost K, Borchard N, Siemens J, Kautz T, Séquaris JM, Möller A, Amelung W (2013)Biochar affected by composting with farmyard manure. J. Environ. Qual. 42:164-172.

Qian K, Kumar A, Zhang H, Bellmer D, Huhnke R (2015) Recent advances in utilization of biochar. Renewable and Sustainable Energy Reviews. 42:1055-1064.

Rahube TO, Marti R, Scott A, Tien YC, Murray R, Sabourin L, Zhang Y, Duenk P, Lapon DR, Topp E (2014) Impact of fertilizing with raw or anaerobically digested sewage sludge on the abundance of antibiotic-resistant coliforms, antibiotic-resistance genes, and pathogenic bacteria in soil and on vegetables at harvest. Appl. Microbiol. Biotechnol. 80(22):6898-6907.

Ribaudo MO, Golleghon NR, Agapoff J (2003) Land application of manure by animal feeding operations: Is more land needed? Journal of Soil and Water Conservation. 58:30-38.

Rothrock Jr. MJ, Cook KL, Lovanh N, Warren JG, Sistani K (2008) Development of a quantitative real-time PCR assay to target a novel group of ammonia producing bacteria found in poultry litter. Poultry Science 87:1058-1067.

Rynk, R, van de Kamp M, Wilson GB, Singley ME, Richard TL, Kolega JJ, Gouin FR, Laliberty, Jr. L, Kay D, Murphy DW, Hoitink HAJ, Brinton WF (1992) On-farm composting handbook. Northeast Regional Agricultural Engineering Service (NRAES) Cooperative Extension.

Sánchez-García M, Alburquerque JA, Sánchez-Monedero MA, Roig A, Cayuela ML (2015) Biochar accelerates organic matter degradation and enhances N mineralization during composting of poultry manure without a relevant impact on gas emissions. Bioresour. Technol. 192:272-279.

Sánchez-Monedero MA, Cayuela ML, Roig A, Jindo K, Mondini C, Bolan N (2018) Role of biochar as an additive in organic waste composting. Bioresour. Technol. 247:155-1164.

Sarmah AK, Meyer MT, Boxall AB (2006) A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of beterinary antibiotics (VAs) in the environmental. Chemosphere. 65:725-759.

Schefferle HE (1965) The decomposition of uric acid in built up poultry litter. Journal of Applied Bacteriology 28:412.

Schiffman S, Williams M (2005) Science of odor as a potential health issue. Journal of Environmental Quality 34:129-138.

Selvam A, Xu D, Zhao Z, Wong JW (2012) Fate of tetracycline, sulfonamide, and fluoroquinolone resistance genes and the changes in bacterial diversity during composting of swine manure. Bioresour. Technol. 126:383:390.

Sims JT, Wolf DC (1994) Poultry Waste Management: Agricultural and Environmental Issues. Advances in Agronomy 52:1-83.

Singh B, Singh BP, Cowie AI (2010) Characterization and evaluation of biochars for their application as a soil amendment. Soil Res. 48(7):516-525.

Sistani KR, Brink GE, Mcgowen SL, Rowe DE, Oldham JL (2003) Characterization of broiler cake litter, the by-products of two management practices. Bioresource Technology 90:27-32.

Skog, K, Lebow P, Dykstra D, Miles P, Stokes B, Perlack R, Buford M, Barbour J, McKeever D (2011) Forest biomass and wood waste resources. In: U.S. billion-ton update: biomass supply for a bioenergy and bioproducts industry: Chapter 3. Oak Ridge, Tenn :Oak Ridge National Laboratory. 16-51.

Spratt BG (1996) Antibiotic resistance: counting the cost. Current Biol. 6:1219-1221.

Steiner C, Das KC, Garcia M, Förster B, Zech W (2008) Charcoal and smoke extract stimulate the soil microbial community in a highly weathered xanthic ferra-soil. Pedobiologia 51:359-366.

Steiner C, Das K, Melear N, Lakly D (2010) Reducing nitrogen loss during poultry litter composting using biochar. J. Environ. Qual. 39:1236-1242.

Swain BK, Sundaram RNS (2000) Effect of different types of litter material for rearing broilers. British Poultry Science 41:261-262.

Tasistro AS, Kissel DE, Bush PB (2004) Spatial variability of broiler litter composition in a chicken house. Journal of Applied Poultry Research 13:29-43.

Terzich M, Pope MJ, Cherr TE, Hollinger J (2000) Survey of pathogens in poultry litter in the United States. Journal of Applied Poultry Research 9:287-291.

Tilman D, Cassman K, Matson P, Naylor R, Polasky S (2002) Agricultural sustainability and intensive production practices. Nature. 418(6898): 671-677.

Trautmann N and Olynciw E (1996) Composting Microorganisms. Cornell Composting Science and Engineering. http://compost.css.cornell.edu/microorg.html. (21 June 2019).

Tufft LS, Nockels CF (1991) The effects of stress, *Escherichia coli*, dietary ethylene-diaminetetetraacetic acid, and their interaction on tissue trace elements in chicks. Poultry Science 70:2439-2449.

Turnell JR, Faulkner RD, Hinch GN (2007) Recent advances in Australian broiler litter utilization. World's Poultry Science Journal 63:223-231.

United States Department of Agriculture (USDA). https://www.usda.gov/our-agency/about-usda. (9 June 2019).

United States Department of Energy (2015) Waste to Wisdom: Utilizing forest residues for the production of bioenergy and biobased products. US Department of Energy, Bioenergy Technologies Office (BETO), Washington, D.C. https://www.energy.gov/sites/prod/files/2015/04/f22/demonstration_market_transformation_han_3414.pdf. (3 June 2019).

University of Arkansas Cooperative Extension Service. Compost and Composting Resources. https://www.uaex.edu/yard-garden/vegetables/compost.aspx (21 June 2019).

University of Florida IFAS Extension Service. Compost Maturity Tests. https://sfyl.ifas.ufl.edu/sarasota/natural-resources/waste-reduction/composting/what-is-composting/when-is-compost-ready/compost-maturity-test/. (19 September 2019).

University of Georgia Agricultural and Environmental Services Laboratories. Crop Code Sheets. http://aesl.ces.uga.edu/publications/soil/CropSheets.pdf. (19 September 2019).

Vandecasteele B, Sinicco T, D'Hose T, Vanden Nest T, Mondini C (2016) Biochar amendment before or after composting affects compost quality and N losses, but not P plant uptake. J. Environ. Manage. 168:200-209.

Van Ryssen JBJ (2008) Trace elements in poultry litter: prevalence and risks. Pages 101-113 *in* Durosy S, Jongbloed AW, eds. Trace Elements in Animal Production Systems. Wageningen Academic Publishers, Netherlands.

Velmurugan N, Chun SS, Han SS, Lee YS (2009) Characterization of chikusaku-eki and mokusaku-eki and its inhibitory effect on sapstaining fungal growth in laboratory scale. J. Environ. Sci. Technol. 6:13-22.

Walker JD, Aneja VP, Dickey DA (2000a) Atmospheric transport and wet deposition of ammonium in North Carolina. Atmospheric Environment 34:3407-3418.

Walker JD, Aneja VP, Dickey DA (2000b) Trends in ammonium concentration in precipitation and atmospheric ammonia emissions at a coastal plain site in North Carolina, U.S.A. Environmental Science and Technology 34:3527-3534.

Wang J, Ben W, Zhang Y, Yang M, Qiang Z (2015) Effects of thermophilic composting on oxytetracycline, sulfamethazine, and their corresponding resistance genes in swine manure. Environ. Sci. Technol. 17:1654-1660.

Wang Q, Awasthi MK, Ren X, Zhao J, Li R, Wang Z, Wang M, Chen H, Zhang Z (2018) Combining biochar, zeolite, and wood vinegar for composting of pig manure: The effect on greenhouse gas emission and nitrogen conservation. Waste Management. https://doi.org/10.1016/j.wasman.2018.01.015.

WHO (2014) Antimicrobial resistance: global report on surveillance. Aust. Med. J. 7(4):695-704.

Williams CM, Barker JC, Sims JT (1999) Management and utilization of poultry wastes. Reviews of Environmental Contamination and Toxicology 162:105-157.

Xie W, Yang X, Li Q, Wu L, Shen Q, Zhao F (2016) Changes in antibiotic concentrations and antibiotic resistome during commercial composting of animal manures. Environmental Pollution. 219:182-190.

Yan Y, Lu XD, Li L (2011) Components of pyroligneous solution from straw pyrolysis and its effect on growth and quality of pepper spice. J. Nanjing Agric. Univ. 34:58-32.

Zhang F, Li Y, Yang M, Li W (2012) Content of heavy metals in animal feeds and manures from farms of different scales in northeast China. Int. J. Environ. Res. Public Health 9(8)L2658-2668.

Zhang G, Liu X, Sun K, He Q, Qian T, Yan Y (2013) Interaction of simizine, metsulfuron-methyl, and tetracycline with biochars and soil as a function of molecular structure. J. Soils Sediments. 13(9):1600-1610.

Zheng J, Chen J, Pan G, Liu X, Zhang X, Li L, Bian R, Cheng K, Jinwei Z (2016) Biochar decreased microbial metabolic quotient and shifted community composition four years after a single incorporation in a slightly acid rice paddy from southwest China. Sci. Total Environ. 571:206-217.

Zhu YG, Johnson TA, Su JQ, Qiao M, Guo GX, Stedtfeld RD, Hashshsam SA, Tiedje JM (2013) Diverse and abundant antibiotic resistance genes in Chinese swine farms. Proc. Natl. Acad. Sci. USA 110(9):3435-3440

APPENDIX A

COMPOST RAW DATA

Table A.1 Compost Moisture Content

Treatment	Rep	% Biochar	WV	Day	Wet wt (g)	Dry wt (g)	Moisture Content	% MC
Control-0	1	0	0	0	10.03	7.62	0.316	31.6
	2	0	0	0	10.16	7.87	0.291	29.1
	3	0	0	0	10.29	7.93	0.298	29.8
	4	0	0	0	10.01	7.87	0.272	27.2
	5	0	0	0	10.06	7.38	0.363	36.3
5% BC-0	1	5	0	0	10.01	7.54	0.328	32.8
	2	5	0	0	10.00	7.78	0.285	28.5
	3	5	0	0	10.06	7.29	0.380	38.0
	4	5	0	0	10.04	7.64	0.314	31.4
	5	5	0	0	10.11	8.04	0.257	25.7
10% BC-0	1	10	0	0	10.01	8.14	0.230	23.0
	2	10	0	0	10.08	7.86	0.282	28.2
	3	10	0	0	10.08	7.78	0.296	29.6
	4	10	0	0	10.01	7.61	0.315	31.5

Table A.1 (continued)

	5	**10**	**0**	**0**	**10.01**	**7.24**	**0.383**	**38.3**
20% BC-0	1	20	0	0	10.00	8.05	0.242	24.2
	2	20	0	0	10.03	8.01	0.252	25.2
	3	20	0	0	10.00	7.44	0.344	34.4
	4	20	0	0	10.04	7.67	0.309	30.9
	5	20	0	0	10.08	7.53	0.339	33.9
WV-0	1	0	1	0	10.19	6.88	0.481	48.1
	2	0	1	0	10.08	7.27	0.387	38.7
	3	0	1	0	10.07	6.53	0.542	54.2
	4	0	1	0	10.05	7.08	0.419	41.9
	5	0	1	0	10.01	6.39	0.567	56.7
5% BC WV-0	1	5	1	0	10.00	6.66	0.502	50.2
	2	5	1	0	10.08	7.50	0.344	34.4
	3	5	1	0	10.00	6.82	0.466	46.6
	4	5	1	0	10.04	6.83	0.470	47.0

Table A.1 (continued)

	5	**5**	**1**	**0**	**10.03**	**6.36**	**0.577**	**57.7**
10% BC WV-0	1	10	1	0	10.04	6.62	0.517	51.7
	2	10	1	0	10.04	7.27	0.381	38.1
	3	10	1	0	10.08	6.73	0.498	49.8
	4	10	1	0	10.00	6.98	0.433	43.3
	5	10	1	0	10.01	6.81	0.470	47.0
20% BC WV-0	1	20	1	0	10.02	7.23	0.386	38.6
	2	20	1	0	10.05	6.87	0.463	46.3
	3	20	1	0	10.04	7.06	0.422	42.2
	4	20	1	0	10.01	6.94	0.442	44.2
	5	20	1	0	10.11	7.12	0.420	42.0
Control-57	1	0	0	57	10.04	3.34	2.006	200.6
	2	0	0	57	10.20	2.21	3.615	361.5
	3	0	0	57	10.38	2.63	2.947	294.7
	4	0	0	57	10.14	2.10	3.829	382.9

Table A.1 (continued)

	5	**0**	**0**	**57**	**10.08**	**2.11**	**3.777**	**377.7**
5% BC-57	1	5	0	57	10.31	2.35	3.387	338.7
	2	5	0	57	10.23	2.34	3.372	337.2
	3	5	0	57	10.08	2.15	3.688	368.8
	4	5	0	57	10.26	2.39	3.293	329.3
	5	5	0	57	10.36	2.45	3.229	322.9
10% BC-57	1	10	0	57	10.26	2.73	2.758	275.8
	2	10	0	57	10.30	2.51	3.104	310.4
	3	10	0	57	10.62	2.68	2.963	296.3
	4	10	0	57	10.12	2.26	3.478	347.8
	5	10	0	57	10.02	2.63	2.810	281.0
20% BC-57	1	20	0	57	10.02	3.14	2.191	219.1
	2	20	0	57	10.18	3.05	2.338	233.8
	3	20	0	57	10.18	2.73	2.729	272.9
	4	20	0	57	10.06	2.54	2.961	296.1

Table A.1 (continued)

	5	**20**	**0**	**57**	**10.24**	**2.58**	**2.969**	**296.9**
WV-57	1	0	1	57	10.15	2.27	3.471	347.1
	2	0	1	57	10.03	3.19	2.144	214.4
	3	0	1	57	10.03	2.57	2.903	290.3
	4	0	1	57	10.34	2.58	3.008	300.8
	5	0	1	57	10.05	2.34	3.295	329.5
5% BC WV-57	1	5	1	57	10.25	2.76	2.714	271.4
	2	5	1	57	10.29	2.60	2.958	295.8
	3	5	1	57	10.59	2.51	3.219	321.9
	4	5	1	57	10.34	3.55	1.913	191.3
	5	5	1	57	9.99	2.77	2.606	260.6
10% BC WV-57	1	10	1	57	10.43	2.57	3.058	305.8
	2	10	1	57	10.06	3.08	2.266	226.6
	3	10	1	57	10.08	2.61	2.862	286.2
	4	10	1	57	10.01	3.23	2.099	209.9

Table A.1 (continued)

	5	**10**	**1**	**57**	**9.99**	**2.41**	**3.145**	**314.5**
20% BC WV-57	1	20	1	57	10.19	3.12	2.266	226.6
	2	20	1	57	10.41	2.78	2.745	274.5
	3	20	1	57	10.04	3.01	2.336	233.6
	4	20	1	57	10.33	3.25	2.178	217.8
	5	20	1	57	10.21	2.99	2.415	241.5
Control-112	1	0	0	112	10.28	3.27	2.144	214.4
	2	0	0	112	10.04	3.57	1.812	181.2
	3	0	0	112	10.28	4.22	1.436	143.6
	4	0	0	112	10.05	3.22	2.121	212.1
	5	0	0	112	10.24	3.96	1.586	158.6
5% BC-112	1	5	0	112	10.30	3.74	1.754	175.4
	2	5	0	112	10.15	3.91	1.596	159.6
	3	5	0	112	10.09	2.94	2.432	243.2
	4	5	0	112	10.04	3.64	1.758	175.8

Table A.1 (continued)

	5	**5**	**0**	**112**	**10.08**	**3.76**	**1.681**	**168.1**
10% BC-112	1	10	0	112	10.10	3.66	1.760	176.0
	2	10	0	112	10.06	3.62	1.779	177.9
	3	10	0	112	10.23	3.98	1.570	157.0
	4	10	0	112	10.10	3.61	1.798	179.8
	5	10	0	112	10.03	3.68	1.726	172.6
20% BC-112	1	20	0	112	10.04	3.81	1.635	163.5
	2	20	0	112	10.41	3.17	2.284	228.4
	3	20	0	112	10.04	3.34	2.006	200.6
	4	20	0	112	10.12	3.75	1.699	169.9
	5	20	0	112	10.07	3.76	1.678	167.8
WV-112	1	0	1	112	10.22	3.81	1.682	168.2
	2	0	1	112	10.40	3.94	1.640	164.0
	3	0	1	112	10.16	3.52	1.886	188.6
	4	0	1	112	10.15	3.89	1.609	160.9

Table A.1 (continued)

	5	**0**	**1**	**112**	**10.00**	**3.96**	**1.525**	**152.5**
5% BC WV-112	1	5	1	112	10.17	3.71	1.741	174.1
	2	5	1	112	10.08	3.87	1.605	160.5
	3	5	1	112	10.14	3.82	1.654	165.4
	4	5	1	112	10.21	4.20	1.431	143.1
	5	5	1	112	10.05	3.86	1.604	160.4
10% BC WV-112	1	10	1	112	10.10	4.18	1.416	141.6
	2	10	1	112	10.06	3.97	1.534	153.4
	3	10	1	112	10.24	4.10	1.498	149.8
	4	10	1	112	10.06	4.10	1.454	145.4
	5	10	1	112	10.49	3.92	1.676	167.6
20% BC WV-112	1	20	1	112	10.18	3.95	1.577	157.7
	2	20	1	112	10.06	3.26	2.086	208.6
	3	20	1	112	10.20	3.76	1.713	171.3
	4	20	1	112	10.24	3.65	1.805	180.5

Table A.1 (continued)

	5	**20**	**1**	**112**	**10.21**	**3.42**	**1.985**	**198.5**

Table A.2 Raw Data for Compost N, P, and K concentrations (lbs/acre) and C:N ratios

Treatment	**Rep**	**BC**	**WV**	**Time**	**N**	**P**	**K**	**C/N ratio**
Control	1	0	0	0	76380	18992.76	61038.6	7.7126
	2	0	0	0	75940	15813.6	57985.56	7.6676
	3	0	0	0	78400	18941.1	59335.02	7.6493
	4	0	0	0	75080	18956.52	55935.42	7.9747
	5	0	0	0	76320	20710.32	60474	7.8261
5% BC	1	5	0	0	61560	20169.66	60718.2	9.2658
	2	5	0	0	62860	20102.64	56279.4	9.4699
	3	5	0	0	64000	17985.66	55173.72	8.8557
	4	5	0	0	63220	19506.72	60099.6	9.1107
	5	5	0	0	63620	17472	63240	9.0813

Table A.2 (continued)

10% BC	**1**	**10**	**0**	**0**	**52000**	**17686.98**	**58062.96**	**10.7864**
	2	10	0	0	56460	15972.06	61177.2	10.2071
	3	10	0	0	62180	17395.08	58332.96	8.9813
	4	10	0	0	55100	15496.8	67285.2	10.0522
	5	10	0	0	52400	16102.92	57780	10.2148
20% BC	1	20	0	0	45320	11424.12	45358.38	11.3156
	2	20	0	0	44060	12286.2	46741.26	12.5677
	3	20	0	0	46040	14572.68	50635.38	12.0471
	4	20	0	0	53060	14068.08	52697.88	10.6427
	5	20	0	0	49080	14039.34	49800.06	10.9064
WV	1	0	1	0	73580	19596.9	63427.2	8.0027
	2	0	1	0	70000	19835.52	61114.8	8.7166
	3	0	1	0	72880	21692.16	61371	8.0493
	4	0	1	0	76380	19841.34	64281.6	7.242

Table A.2 (continued)

	5	**0**	**1**	**0**	**74420**	**21181.02**	**66331.8**	**8.4069**
5% BC WV	1	5	1	0	71560	20768.28	62326.2	7.7852
	2	5	1	0	68100	19834.08	66822.6	8.4106
	3	5	1	0	65760	20770.62	71694	8.906
	4	5	1	0	65540	20842.5	69244.8	8.7816
	5	5	1	0	68460	20408.22	63452.4	8.7105
10% BC WV	1	10	1	0	63800	18733.68	57501.6	9.4037
	2	10	1	0	55740	18476.94	56568.6	10.2143
	3	10	1	0	66560	17672.4	59675.22	8.6764
	4	10	1	0	61680	17237.64	58418.94	9.1772
	5	10	1	0	64120	18689.28	57155.04	8.2651
20% BC WV	1	20	1	0	43960	16739.16	54461.1	11.4146
	2	20	1	0	48920	16757.76	48260.76	11.2949
	3	20	1	0	53060	14880.3	49112.4	10.6012

Table A.2 (continued)

	4	**20**	**1**	**0**	**53020**	**16635.9**	**51486.84**	**10.7173**
	5	20	1	0	55480	12020.64	37710.42	10.3858
Control	1	0	0	57	68700	23737.5	33214.92	6.361
	2	0	0	57	59380	22714.86	26559.18	7.3633
	3	0	0	57	74220	28546.56	36610.08	7.374
	4	0	0	57	64920	26270.64	23446.14	7.0444
	5	0	0	57	74340	31164.36	53897.88	7.1389
5% BC	1	5	0	57	73160	32011.02	42541.44	7.8901
	2	5	0	57	68500	28488.78	39167.58	8.0631
	3	5	0	57	73560	33027.12	444840.7	7.7643
	4	5	0	57	73360	34652.17	36824.55	7.7967
	5	5	0	57	77440	32800.57	37147.6	7.7939
10% BC	1	10	0	57	65980	33101.81	27089.44	7.861
	2	10	0	57	69400	32076.77	39201.4	8.0987

Table A.2 (continued)

	3	**10**	**0**	**57**	**69380**	**31479.63**	**29653.79**	**7.8339**
	4	10	0	57	70020	31385.31	29417.05	7.9289
	5	10	0	57	63600	35095.9	25698.93	8.028
20% BC	1	20	0	57	57300	33324.17	28723.59	8.3667
	2	20	0	57	49480	31896.69	21600.04	9.1835
	3	20	0	57	57720	30137.28	26751.45	8.5212
	4	20	0	57	62320	30610.16	23752.23	8.0887
	5	20	0	57	60720	33477.4	29615.6	8.231
WV	1	0	1	57	81780	33751.52	34410.88	6.996
	2	0	1	57	85120	28140.67	39569.95	6.7202
	3	0	1	57	77600	31877.37	30608.1	7.6603
	4	0	1	57	81100	33399.31	27669.01	7.3445
	5	0	1	57	81660	35239.75	35635.25	6.6923
5% BC WV	1	5	1	57	72700	33029.26	29488.45	7.6227

Table A.2 (continued)

	2	**5**	**1**	**57**	**73800**	**33011.83**	**28000.98**	**7.8359**
	3	5	1	57	76980	33249.27	31490.31	7.6089
	4	5	1	57	79700	34517.35	28755.51	7.3094
	5	5	1	57	75260	34485.64	30936.43	7.7374
10% BC WV	1	10	1	57	63280	32386.8	32376.3	8.6533
	2	10	1	57	69820	31078.53	26808.46	7.4789
	3	10	1	57	69660	30153.45	25146.59	7.777
	4	10	1	57	70260	32901.3	21263.2	7.2196
	5	10	1	57	69000	31600.07	29050.21	7.7489
20% BC WV	1	20	1	57	54440	29209.22	27829.9	8.5874
	2	20	1	57	54240	27313.65	12565.49	9.2213
	3	20	1	57	60540	28703.57	18127.13	8.1982
	4	20	1	57	60340	30408.11	19037.83	7.8921
	5	20	1	57	62300	29501.19	23598.75	7.9029

Table A.2 (continued)

Control	**1**	**0**	**0**	**112**	**88100**	**37241.05**	**23679.15**	**6.6174**
	2	0	0	112	80380	34247.68	32388.09	7.227
	3	0	0	112	80340	34480.78	31384.61	7.1201
	4	0	0	112	80800	32516.79	18064.76	7.163
	5	0	0	112	81760	32730.32	45236.45	7.2333
5% BC	1	5	0	112	76780	34634.25	34907.88	7.4688
	2	5	0	112	81360	33220.67	37770.25	7.3379
	3	5	0	112	74560	34125.42	26623.17	7.6782
	4	5	0	112	76360	34806.91	34182.37	7.5073
	5	5	0	112	80120	34358.38	32540.52	7.1888
10% BC	1	10	0	112	61800	34606.99	28839.51	8.924
	2	10	0	112	63620	35539	37898	8.754
	3	10	0	112	62580	34115.38	29295.46	8.9581
	4	10	0	112	66320	34432.02	28246.47	8.1973

Table A.2 (continued)

	5	**10**	**0**	**112**	**59760**	**36281**	**29696.84**	**8.9542**
20% BC	1	20	0	112	49300	33959.21	27642.9	9.7498
	2	20	0	112	42680	30308.36	16367.3	10.5944
	3	20	0	112	51060	29789.83	25350.85	9.2483
	4	20	0	112	53120	29829.35	24220.91	9.1255
	5	20	0	112	53740	30371.85	26072.94	9.4235
WV	1	0	1	112	65700	32857.86	28190.19	7.8026
	2	0	1	112	70480	32146.17	24116.51	6.8424
	3	0	1	112	62080	29674.3	23095.42	7.8838
	4	0	1	112	72220	32656.47	23743.51	6.9553
	5	0	1	112	72960	32028.96	29506.16	7.2898
5% BC WV	1	5	1	112	66640	32071.17	26201.81	8.1983
	2	5	1	112	63340	30063.57	21187.43	8.1054
	3	5	1	112	64660	30678.17	26353.64	7.7169

Table A.2 (continued)

	4	**5**	**1**	**112**	**68000**	**31723.86**	**26787.53**	**7.8921**
	5	5	1	112	67760	33172.79	27711.18	8.1685
10% BC WV	1	10	1	112	60020	32583.22	30941.05	8.6489
	2	10	1	112	63220	31731.42	21787.19	7.7703
	3	10	1	112	64380	31746.02	23497.64	7.9531
	4	10	1	112	62300	31574.66	20153.04	7.8789
	5	10	1	112	62740	29983.8	26782.63	8.1572
20% BC WV	1	20	1	112	47960	27870.47	25476.26	9.25
	2	20	1	112	43860	26143.99	11209	10.7514
	3	20	1	112	49400	28135.84	17751.93	9.4532
	4	20	1	112	52300	29657.95	17361.82	8.9142
	5	20	1	112	51080	28677.88	24023.79	9.4106

Table A.3 Comparison of Average Nitrogen, Phosphorous, and Potassium concentrations (lbs./acre) in Compost Treatments to the University of Georgia Established Fertilizer Recommendations

	N		**P**		**K**	
	Recommended	Recorded	Recommended	Recorded	Recommended	Recorded
Corn for grain (irrigated) code #002	180	65290	90	27059	90	41608
Cotton 1000 lbs yield goal code #501	75		60		60	

APPENDIX B

SAS CODE AND OUTPUT

B.1 Example of SAS code input for determining significant relationships (P<.05) between biochar, wood vinegar, and time with bacteria counts

```
proc glm data = test;
class bc wv time rep;
model bacteria_counts = bc wv bc*wv time bc*time wv*time bc*wv*time
rep(bc*wv);
test h = bc wv bc*wv e = rep(bc*wv);
run;
ods rtf close;

proc mixed method = type3;
class bc wv rep time;
model bacteria_counts = bc wv bc*wv time bc*time wv*time bc*wv*time;
random rep(bc*wv);
lsmeans bc*time / pdiff;
run;

proc means mean noprint;
var bacteria_counts;
class bc time;
ways 2;
output out = x mean = tc_mean;
run;

proc print data = x;
proc sort data = x out = y;
by wv;
run;

symbol1 interpol = join;
proc gplot data = x;
plot tc_mean*time = bc;
run;

proc mixed method = type3;
class bc wv rep time;
model bacteria_counts = bc wv bc*wv time bc*time wv*time bc*wv*time;
random rep(bc*wv);
lsmeans wv*time / pdiff;
run;
ods rtf close;
proc means mean noprint;
var bacteria_counts;
class wv time;
ways 2;
output out = x mean = tc_mean;
run;

proc means mean noprint;
var bacteria_counts;
class bc wv time;
ways 3;
output out = x mean = tc_mean;
```

```
run;

proc print data = x;
proc sort data = x out = y;
by wv;
run;

symbol1 interpol = join;
proc gplot data = y;
plot tc_mean*time = wv;
run;
```

B.2 Example of SAS output for bacteria counts (bc*time and WV*time)

Table B.1 Bacterial count SAS outputs for Proc GLM and Proc Mixed for biochar*time and WV*time

Type 3 Analysis of Variance		
Source	**VValue**	**Pr > F**
bc	9.96	<.0001
wv	3.16	0.0849
bc*wv	1.66	0.1958
time	135.37	<.0001
bc*time	9.26	<.0001
wv*time	3.63	0.0322
bc*wv*time	1.98	0.0812
rep(bc*wv)	0.97	0.5209
Residual	.	.

Table B.1 (continued)

Differences of Least Squares Means									
Effect	**bc**	**time**	**bc**	**time**	**Estimate**	**Standard Error**	**F**	**t Value**	**Pr > |t|**
bc*time	0	0	0	57	-3176667	4953525	64	0.64	0.5236
bc*time	0	0	0	112	-5.537E7	4953525	64	-11.18	<.0001
bc*time	0	0	10	0	1863667	4931767	64	0.38	0.7068
bc*time	0	0	10	57	-4211333	4931767	64	-0.85	0.3963
bc*time	0	0	10	112	-3.172E7	4931767	64	-6.43	<.0001
bc*time	0	0	20	0	1584000	4931767	64	0.32	0.7491
bc*time	0	0	20	57	-3614000	4931767	64	-0.73	0.4664
bc*time	0	0	20	112	-1.239E7	4931767	64	-2.5	0.0145
bc*time	0	0	5	0	1889667	4931767	64	0.38	0.7029
bc*time	0	0	5	57	-3521667	4931767	64	-0.71	0.4778
bc*time	0	0	5	112	-4.523E7	4931767	64	-9.17	<.0001
bc*time	0	57	0	112	-5.219E7	4953525	64	-10.54	<.0001
bc*time	0	57	10	0	5040333	4931767	64	1.02	0.3106
bc*time	0	57	10	57	-1034667	4931767	64	-0.21	0.8345
bc*time	0	57	10	112	-2.854E7	4931767	64	-5.79	<.0001
bc*time	0	57	20	0	4760667	4931767	64	0.97	0.3380
bc*time	0	57	20	57	-437333	4931767	64	-0.09	0.9296

Table B.1 (continued)

Differences of Least Squares Means									
Effect	**bc**	**time**	**bc**	**time**	**Estimate**	**Standard Error**	**F**	**t Value**	**Pr > |t|**
bc*time	0	57	20	112	-9214667	4931767	64	-1.87	0.0663
bc*time	0	57	5	0	5066333	4931767	64	1.03	0.3082
bc*time	0	57	5	57	-345000	4931767	64	-0.07	0.9444
bc*time	0	57	5	112	-4.205E7	4931767	64	-8.53	<.0001
bc*time	0	112	10	0	57231667	4931767	64	11.60	<.0001
bc*time	0	112	10	57	51156667	4931767	64	10.37	<.0001
bc*time	0	112	10	112	23646667	4931767	64	4.79	<.0001
bc*time	0	112	20	0	56952000	4931767	64	11.55	<.0001
bc*time	0	112	20	57	51754000	4931767	64	10.49	<.0001
bc*time	0	112	20	112	42976666	4931767	64	8.71	<.0001
bc*time	0	112	5	0	57257667	4931767	64	11.61	<.0001
bc*time	0	112	5	57	51846333	4931767	64	10.51	<.0001
bc*time	0	112	5	112	10136667	4931767	64	2.06	0.0439
bc*time	10	0	10	57	-6075000	4953525	64	-1.23	0.2245
bc*time	10	0	10	112	-3.359E7	4953525	64	-6.78	<.0001
bc*time	10	0	20	0	-279667	4931767	64	-0.06	0.9550
bc*time	10	0	20	57	-5477667	4931767	64	-1.11	0.2709

Table B.1 (continued)

Differences of Least Squares Means									
Effect	**bc**	**time**	**bc**	**time**	**Estimate**	**Standard Error**	**F**	**t Value**	**Pr > |t|**
bc*time	10	0	20	112	-1.426E7	4931767	64	-2.89	0.0052
bc*time	10	0	5	0	26000	4931767	64	0.01	0.9958
bc*time	10	0	5	57	-5385333	4931767	64	-1.09	0.2789
bc*time	10	0	5	112	-4.71E7	4931767	64	-9.55	<.0001
bc*time	10	57	10	112	-2.751E7	4953525	64	-5.55	<.0001
bc*time	10	57	20	0	5795333	4931767	64	1.18	0.2443
bc*time	10	57	20	57	597333	4931767	64	0.12	0.9040
bc*time	10	57	20	112	-8180000	4931767	64	-1.66	0.1021
bc*time	10	57	5	0	6101000	4931767	64	1.24	0.2206
bc*time	10	57	5	57	689667	4931767	64	0.14	0.8892
bc*time	10	57	5	112	-4.102E7	4931767	64	-8.32	<.0001
bc*time	10	112	20	0	33305333	4931767	64	6.75	<.0001
bc*time	10	112	20	57	28107333	4931767	64	5.70	<.0001
bc*time	10	112	20	112	19330000	4931767	64	3.92	0.0002
bc*time	10	112	5	0	33611000	4931767	64	6.82	<.0001
bc*time	10	112	5	57	28199667	4931767	64	5.72	<.0001
bc*time	10	112	5	112	-1.351E7	4931767	64	-2.74	0.0080

Table B.1 (continued)

Differences of Least Squares Means									
Effect	**bc**	**time**	**bc**	**time**	**Estimate**	**Standard Error**	**F**	**t Value**	**Pr > |t|**
bc*time	20	0	20	57	-5198000	4953525	64	-1.05	0.2980
bc*time	20	0	20	112	-1.398E7	4953525	64	-2.82	0.0064
bc*time	20	0	5	0	305667	4931767	64	0.06	0.9508
bc*time	20	0	5	57	-5105667	4931767	64	-1.04	0.3044
bc*time	20	0	5	112	-4.682E7	4931767	64	-9.49	<.0001
bc*time	20	57	20	112	-8777333	4953525	64	-1.77	0.0812
bc*time	20	57	5	0	5503667	4931767	64	1.12	0.2686
bc*time	20	57	5	57	92333	4931767	64	0.02	0.9851
bc*time	20	57	5	112	-4.162E7	4931767	64	-8.44	<.0001
bc*time	20	112	5	0	14281000	4931767	64	2.90	0.0052
bc*time	20	112	5	57	8869667	4931767	64	1.80	0.0768
bc*time	20	112	5	112	-3.284E7	4931767	64	-6.66	<.0001
bc*time	5	0	5	57	-5411333	4953525	64	-1.09	0.2787
bc*time	5	0	5	112	-4.712E7	4953525	64	-9.51	<.0001
bc*time	5	57	5	112	-4.171E7	4953525	64	-8.42	<.0001

Table B.1 (continued)

Effect	wv	time	wv	time	Estimate	Standard Error	F	tValue	Pr >|t|
wv*time	0	57	0	112	-3.753E7	3502671	64	-10.72	<.0001
wv*time	0	57	1	0	4740500	3487286	64	1.36	0.1788
wv*time	0	57	1	57	1124167	3487286	64	0.32	0.7482
wv*time	0	57	1	112	-2.644E7	3487286	64	-7.58	<.0001
wv*time	0	112	1	0	42272167	3487286	64	12.12	<.0001
wv*time	0	112	1	57	38655833	3487286	64	11.08	<.0001
wv*time	0	112	1	112	11093333	3487286	64	3.18	0.0023
wv*time	1	0	1	57	-3616333	3502671	64	-1.03	0.3057
wv*time	1	0	1	112	-3.118E7	3502671	64	-8.90	<.0001
wv*time	1	57	1	112	-2.756E7	3502671	64	-7.87	<.0001