FEEDSTOCK AND AMENDMENT EFFECTS ON COMPOST CHARACTERISTICS AND USE IN VEGETABLE PRODUCTION

By

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ABSTRACT

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The research objectives were the production of compost for transplant root media and managing fertility and disease in vegetable cropping systems. The priorities were on using local organic materials to capture carbon and nutrients, proliferation of beneficial microorganisms and reducing the need for off farm inputs, particularly in an organic certification system.

Composts were made and the soluble and total nutrients, electrical conductivity, pH, percent organic matter, the final C:N ratio and bulk density are reported and correlated to the growth of transplants in greenhouse bioassays. In the first experiments (Chapter I), small-scale (1yd^3) thermophilic compost piles were constructed with variations in feedstocks to produce ten treatments focused primarily on starting carbon to nitrogen ratios, and the feedstock contributions observed in the physiochemical characteristics of the finished composts. Fall-collected leaves and on-farm fresh cut grass (1:1 v:v) as a base recipe and variations thereof by adjusting volume ratios of those feedstocks, or adding (1:1:1) dairy/horse manure, coffee grounds, shredded office paper, softwood shavings, or sphagnum peat, wrapping the base mix in plastic, and a standard mixture developed and used in previous research. Three species of plants: cucumber (*Cucumis sativus*), kale (*Brassica oleracea*), and tomato (*Solanum lycopersicum*) were grown and evaluated for dry and wet weights, plant heights, root ratings and number of leaves. Growth response in compost substrates were not consistent between species. Composts containing peat as an ingredient had lower pH and generally resulted in better growth of transplants while growth was minimal in wood shavings compost. Cucumber transplants were

grown in the composts after storage for 2, 3 or 4 years which identified differences in growth as the compost characteristics had changed over time. A second group of experiments (Chapter II) had the same base feedstocks with the addition of biochar (BC) and anaerobic digester effluent (ADE), on their own and combined. For cucumbers grown in these compost mixes, addition of biochar improved all metrics of transplant growth; in contrast, addition of ADE reduced several metrics of transplant health including shoot dry weight and root ratings. Most of the compost media produced acceptable transplants of varying quality without additional fertility added over the 3 to 5-week greenhouse production period. A laboratory vermicomposting bioassay (Chapter III) was designed to evaluate the effects of pineapple, melon, onion, carrot, spent coffee grounds (SCG) and a mixture of all five kitchen preparation residues from campus food service as vermicomposting feedstocks. Data were collected for the impact on worms, finished compost chemical characteristics and biota by community level physiological profiling using Biolog EcoPlates. SCG as a feedstock had elevated total N but nearly undetectable soluble NO_3-N, and greater microbial community functional diversity. Compost teas from three composts (dairy-manure based and leaf/grass/coffee based thermophilic composts, and food waste based vermicompost) were assessed for impacts on tomato leaf mold (*Fulvia fulva)* and winter squash powdery mildew *(Podosphaera xanthii)* management in a farm setting (Chapter 4). Aerated compost teas (CT) were produced for weekly foliar application on two tomato (*Solanum lycopersicum)* varieties and three winter squash varieties (*Cucurbita moschata*, *C. pepo,* and *C. maxima*) (five separate experiments). While there was some efficacy for management of disease in some of these plant/pathogen systems, this varied by species/variety; and as disease pressure increased over the season CT efficacy ceased. Use of a spreader-sticker appeared to increase disease management in some trials.

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INTRODUCTION

While composting has been used by farmers as a means of increasing soil fertility for centuries, in recent times/decades, compost has also been used as a component of root media/substrates for container grown plants and as a method of increasing plant resistance to insects and diseases through foliar application of compost tea. The quality and characteristics of the composts used for these specific added-value purposes can be managed and enhanced through a greater understanding of the effects of specific feedstocks and/or amendments and through the use of alternative composting methods such as vermicomposting.

The objectives of this research project were the production of compost, with a focus on locally available materials that might otherwise end up in the waste stream and the use of those composts for growing high value crops. Much of my work was specifically focused on "closing the food cycle loop" with thermophilic composting and vermicomposting of food residues from campus dining halls, so nutrients and organic matter could be returned back to the land. One of our responsibilities as researchers / academics is anticipating possible future sources of on-farm fertility as those currently mined from the earth and oceans or produced with fossil fuels are depleted or increase in cost. Intensive systems such as year-round high tunnels or greenhouses that are becoming increasingly popular on small scale diversified specialty crop farms and urban agriculture settings, especially in cold climates such as Michigan, are particularly benefited by such compost research.

A primary motivating factor of this research was to increase effective fertility and pest management options in organic production for which nutrient and pesticide inputs are limited. This research project required a foundation of information from a range of topics including production of both thermophilic compost and vermicompost, biochar, anaerobic digestion, compost teas and vegetable transplant production.

Transplant production can be an important part of a farm plan, especially for organic growers. Organic transplants can be harder to find commercially available for purchase, and even harder to make sure that the timing of transplants being ready for the field is in line with a farm's production plan, particularly for diversified vegetable operations. Growers/farmers using certified organic methods have a more limited set of options for suitable container root media and fertility compared to conventional growers.

Compost use for growing transplants has been dominated by the question of what percentage can be added to a media as a replacement for peat, while very few studies have assessed compost as the sole media component. Additionally, target/acceptable ranges for nutrients and other physiochemical characteristics of root media are generally geared towards peat-based media that would have soluble nutrients routinely added. Routine addition of soluble nutrients is contrary to the principles of organic agriculture. Such recommendations that stem from inert or low CEC media do not take into account the slow release of nutrients from compost in a biologically active system and other nutrient cycling dynamics. Nutrient assessment and management recommendations specific for compost-based root media are needed as they can be based on the different physical, chemical and biological characteristics.

Chapters I and II both have two parts: first compost production and then use of compost for growing transplants. The overall objective of this work was not to produce the "best" compost and test it, but rather to characterize feedstock benefits to a thermophilic compost system intended for use as a plant growing medium. Proportional effects of these feedstocks could then be calculated/estimated to develop a compost root media with the desired characteristics. Development of affordable compost-based transplant media using feedstocks that are readily available and would be generally consistent throughout the United States could increase the adoption of compost based media as an alternative for peat based media and use of fertilizers. Both chapters provide more evidence to the body of knowledge about the effect of specific feedstocks/amendments to a composting system and the resultant compost chemical and physical characteristics. Feedstock selection for the first experiment of ten treatments focused primarily on starting carbon to nitrogen ratios, while the second experiment had the same base recipe for compost as the first but was testing the impacts of biochar (BC) and anaerobic digester effluent (ADE), on their own and in tandem. Chapter III is similarly focused on the effect of feedstocks on physical and chemical properties, and additionally biological properties of compost made in a laboratory setting using vermicomposting as the composting method to test specific food residue feedstocks. This relates back to the bulk of my farm-scale experimentation with vermicomposting systems for a cold climate using food residue for vermicomposting, the primary constituents of which were selected as the treatment feedstocks.

The second parts of Chapters I and II are growing transplants with the composts as the root media, often testing the boundaries of what would typically be expected as an acceptable range of certain physio-chemical properties such as electrical conductivity. Using greenhouse studies the effect of compost treatment on growth parameters of cucumber (*Cucumis sativus*), kale (*Brassica oleracea*), and tomato (*Solanum lycopersicum*) were related this back to the compost physio-chemical characteristics. Research reported in Chapter I used all three plant species while only cucumbers were grown in compost made in research reported in Chapter II. Most of the compost media used at 100% were able to produce acceptable transplants without additional fertility added, but of varying quality.

Still the benefits afforded transplants grown in microbially active compost-based media may not be enough to ward off disease once planted out in the field, and then compost designed for use as foliar sprays via compost tea could be used. Compost tea trials both in controlled greenhouse settings and in the field have produced varied results that are dependent on the host / pathogen system being studied. This area of compost research needs more experimentation, especially into specific diseases that have not yet been studied. Research reported in Chapter IV evaluates three compost teas and a spreader-sticker for their efficacy in management of *Fulvia fulva* on two tomato (*Solanum lycopersicum)* varieties, and *Podosphaera xanthii* on three winter squash varieties butternut (*Cucurbita moschata),* delicata (*C. pepo)*, and hubbard (*C. maxima)*.

Although composting is widely adopted, it is still often considered more of a waste management strategy for many organic waste streams generated by society. Changing that paradigm to a resource management strategy requires additional scientific backing of how to best valorize the materials that we have. Marketing of compost to increase its adoption for its intended uses at a price that generates profits associated with collection and production is another key step in furthering the use of compost. Knowledge gained from this dissertation contributes to a base of information for what compost characteristics a farmer might want, and from which locally sourced feedstocks can be calculated and combined to achieve desired outcomes of the finished compost product. Cultivating a circular ecology and economy in which waste streams are turned into resource streams that can increase the sustainability of a farming system has been a large driver of this work. This work will hopefully increase adoption of compost for growing organic vegetable transplants and compost teas for disease management by providing more information to compost producers and growers. An increased value for the compost product can stimulate increased interest in and motivation for the composting process.

CHAPTER I

FEEDSTOCK EFFECTS ON FINISHED COMPOST FOR GROWING ORGANIC VEGETABLE TRANSPLANTS

ABSTRACT

Thermophilic compost was made in static piles with alterations in feedstocks to adjust carbon to nitrogen ratios and other nutrients, with the objective to quantify feedstock effects on compost physiochemical characteristics, and on transplant growth in the composts as root media. The base recipe for all piles was fresh cut grass and fall collected municipal leaves with variations in additional feedstocks primarily designed to alter the starting carbon to nitrogen (C:N) ratio. The ten recipes were made with replication over a period of three consecutive years with a similar schedule for start date, turning, and maintenance. After a period of six months from the time of starting the piles samples were taken for nutrient analysis of total and water soluble nutrients, and physical characteristics. Cucumber, kale, and tomato vegetable transplants were grown in media comprised of 100% compost from the different feedstocks and a commercial transplant medium for comparison. Transplants were also grown in 100% compost from all 3 years simultaneously when the different recipes for feedstocks were of varying ages (nearly 2, 3 or 4 years old), and basil was grown in the compost to full maturity for harvest. There were distinct differences between composts for many of the physiochemical characteristics analyzed, including N, P, K, Ca, Mg, S, Na, Zn, Mn, Cu and B (soluble and total nutrients), EC, pH, bulk density, organic matter content and final C:N. There were differences in transplant growth based on compost feedstocks, and also compost age for certain compost recipes but not others. Compared as a proportion of the peat-based control medium, the cucumber, kale and tomato transplants had higher above ground biomass and other transplant metrics in all but one treatment (which was high in starting and final C:N), displaying similar treatment responses regardless of species. Basil plants were chlorotic in many treatments, though grew in all despite alkaline pH, above 8 for most treatments. While the compost recipes used in this study produced

a compost media that could be used for transplant and containerized production, blending of different composts or other medium components to optimize characteristics and enhance growth of plants is seen as a next step in development of compost media.

INTRODUCTION

Composting is a managed aerobic *process* of the biological conversion of organic matter into compost, the biodegradation and conversion being mediated by the resident microbial community (Stoffella & Kahn, 2001). Compost is the final *product* of the composting process; it is a humus-rich stable substrate that is free of pathogens (plant and human) and viable seeds. Composting has been employed by farmers for centuries, with compost being used to increase soil organic matter and nutrients, soil water holding capacity and nutrient retention. With an increasing emphasis on organics recycling in the United States, it is important that composting make the shift from a waste management strategy to a resource management strategy necessary for the sustainability of farming systems, creating a circular economy in which nutrients flow back to the land (Peng & Pivato, 2019). Refinement of the process can result in higher value compost products for specific end uses.

Compost use in root media cultivation of transplants or containerized plant production, either at 100% or as a component, provides a regenerative/sustainable system. Development of affordable compost-based transplant media using feedstocks that are readily available and would be generally consistent throughout the United States could increase the adoption of compost based media and reduce use of fertilizers. The high water holding capacity and low bulk density of compost are generally suitable for such use (Walker et al., 2006). These are some of the positive attributes of peat moss that have made it the prominent media component in the horticultural industry (Raviv, 2011). Specific pH, soluble salt, and nutrient balance recommendations are needed to improve the probability of success when using compost for transplants or as a substrate (Sterrett, 2001; Sullivan & Miller, 2001). Substrate components for growing plants in containers must be standardized, reproducible, available, and economical (Pelaez-Samaniego et al., 2017), and the inability to necessarily check all of these boxes has hampered the adoption of compost as a substrate. Transplant/container culture involves a restricted root zone, in which sufficient water, nutrients and aeration for the growing transplant must be provided (Raviv, 2011).

Organic agriculture is expanding and with it the demand for root media that are approved for use in an organic system. Fertilization regimen in organic agriculture is limited in options from natural sources such as fish emulsion, feather, bone or blood meal, and producers are often weighing the benefits against the high costs and environmental footprint of these choices (Clark & Cavigelli, 2005; Kuepper & Everett, 2004). For organic vegetable farmers the general recommendation has been to invest in a uniform, high quality transplant growing medium, which are most often peat-based, and may or may not include compost, as well as using these limited fertilizer options.

Peat-based root media may be the most commonly used in horticulture, however, there is growing demand for an alternative. The positive physical characteristics of peat that make it an ideal media component have been difficult to match. However, peat harvest may result in environmental degradation, the regeneration time is long, and increasing production and transportation costs make it increasingly expensive (Clark & Cavigelli, 2005; Lazcano et al., 2009; Pelaez-Samaniego et al., 2017; Ridout & Tripepi, 2011; Treadwell et al., 2007). Still, most container substrates on the market have peat in the range of 80% and upwards (Ceglie et al., 2015).

Compost, whether thermophilic or vermicompost, provides soluble and long term nutrients, water holding capacity, and carbon sources for microbes. Composts with high total porosity and air-filled porosity are more likely to be used as a transplant media (Raviv, 2005). However, compost variability can be a concern (Beozzi et al., 2017; Michael Raviv, 2011), making more careful management of transplants necessary (Burnett et al., 2016). Concerns with compost have been salinity levels, bad physical characteristics such as high of bulk density, phytotoxicity, high pH and heavy metals (Beozzi et al., 2017; Ceglie et al., 2015; Rogers, 2017). For widespread adoption on a larger scale, growers would need assurances that a compost can reliably provide a uniform, stable, and readily available product (Susan B. Sterrett, 2001). There have been numerous studies with compost used in root media. Many have used compost as a component and not at 100%, often using volume percentages to arrive at the "optimal" or cut-off at which the negatives outweigh the positives of its inclusion in media. The goal herein is to remove these negatives by investigating compost component characteristics and how those compost characteristics affect transplants. Understanding these shifts by compost component, ingredients can then be combined in such a way as to make compost that is based on necessary root media characteristics.

Transplant use provides a number of benefits for vegetable farmers. By avoiding emergence problems encountered with direct seeding, transplants ensure uniform plant establishment and dense plant populations (Burnett et al., 2016; Sterrett, 2001). Keeping young plants in one easy to monitor and manage protected location helps with protection from diseases and pests until field planting, at which time they should be better able to withstand such pressures. This in turn helps with improved ground cover (weed) management. Use of transplants can better ensure predictable harvest times as well. They shorten the production cycle, providing more time for cover crops to improve soil quality. Nearly every organic vegetable farm, regardless of size, uses transplants, but because organic transplants are difficult to source, farmers often produce their own, with inherent difficulties. The qualities of the root media generally have long-lasting ramifications on yield (Dufault, 1998; Jack et al., 2011; Raviv et al., 1998).

There are many factors in the production of compost to make it suitable for use as a transplant media that can be managed to affect the process and the end product. For example, variations in feedstock (Campitelli & Ceppi, 2008), mixing frequency (Tiquia et al., 2002) and storage time (Fracchia et al., 2006) can impact the chemical, biological and physical characteristics. EC increases as composts mature, while conversely pH and carbon to nitrogen ratios decrease (Campitelli & Ceppi, 2008; Tognetti et al., 2005). The relationship between nitrate-Nitrogen (NO_3-N) and ammoniacal-N (NH_4-N) also shifts as NO_3 increases and NH_4 decreases with compost maturity (Goyal et al., 2005). These parameters have cascading ramification for the end use of the compost as a microbial inoculant, a source of stable organic matter, a supplemental source of fertility, or a sole or substantial component of planting media (Waqas et al., 2018). Additional management factors that influence the process and finished product range from the initial feedstocks, feedstock relative ratios and starting particle size, temperature, moisture, turning or physical mixing, maturation and curing phases, etc. Storage time and conditions are yet other factors of compost management process quality. It is important that compost be stored under conditions that will maintain both the nutrients present and the biota (Fracchia et al., 2006). Moisture content is of particular concern; if compost is not protected from rainfall, leaching of nutrients may occur, and/or compost may become waterlogged and anaerobic, while excessive drying is also a concern more in relation to the active biota present.

A target carbon to nitrogen ratio (C:N) of approximately 25:1 to 35:1 for mixed feedstock materials is desirable (Day & Shaw, 2001). Low starting C:N (<20:1) can result in N losses through ammonia volatilization (Tiquia et al., 2002) which is detected by human smell and deleterious to human health at low concentrations (<5ppm), and high starting C:N (>60:1) will impede decomposition. Calculations of mixtures can be made with the %C and %N of each feedstock and their relative proportions/weight of the compost recipe. Examples of carbon sources (C:N>30:1) for feedstocks include straw, paper, woodchips, sawdust and bark. Nitrogen sources (C:N<30:1) may include animal manures, sewage sludge, or municipal solid waste (MSW) (Neher et al., 2013). Materials with lower C:N are often higher in other nutrients as well. As the C:N affects the composting process and nutrient content going into a compost pile, the resultant compost should display distinct characteristics.

A primary consideration in treatment selection was alteration of carbon to nitrogen ratios (C:N) and the effect this would have on the finished compost characteristics, though additional considerations of alteration of pH, better retention of nutrients and moisture, and addition of other nutrients from the specific feedstocks were also considered. Composts can be highly variable, but a better understanding of how feedstocks influence compost parameters could improve their use in transplant production. An emphasis was also on local resources that are often considered wastes, which would be used in a composting system, such as spent coffee grounds (SCG), manure (combination of dairy cow and horse), pine wood shavings, sphagnum peat moss, and shredded office paper. The higher C:N feedstocks and SCG in particular are more recalcitrant and the effect on the finished C:N ratio is not well understood (Stylianou et al., 2018). A motivating question is what makes a good compost for transplants, and if these characteristics will be the same for various plant species. Ultimately, different composts could be made and blended depending on the desired characteristics for the specific end use.

Electrical conductivity is commonly used as a quick test for nutrient content or soluble salts of peat and bark-based root media for containers (Nelson, 2011). A recommended range of test values for acceptable plant growth is 1.0 to 3.0 dS/m for root media saturated media extract analysis for peat-based media (Warncke & Krauskopf, 1983). Recommended EC ranges for compost-based media are based on peat-based media values and not actual research trials measuring plant growth in compost. Another factor complicating the reliability of EC readings for organic fertilizers is the presence of non-fertilizer salts (Na, Cl, etc.) that increase EC without increasing nutrient concentration and fertility. Furthermore, nutrients bound in organic material do not affect EC. Organic anions may also contribute to the measured electrical conductivity values. Research is needed to differentiate compost from other root media for such diagnostic tests as EC may be a key in compost use as media for growing transplants.

The soluble fraction of nutrients is what will be most readily available to plants as a nutrient source (Sullivan & Miller, 2001). Nitrogen content of compost can vary greatly and is particularly dependent on the parent feedstocks. Ranges are typically between 0.5-3% total N. Saturated media extract (SME) quantifies inorganic N, which is either in the nitrate (NO_3-N) form or ammonium (NH_4-N) form, which in part can help in the evaluation of compost maturity as NH_4-N values are generally low in mature compost; if values are high it is an indication a compost is not yet mature. The immediate or first-year nitrogen availability effect of an application of compost is generally estimated at less than 15% of the total N; the remainder will become more slowly available over subsequent years, at a rate of about 2-8% per year (Amlinger et al., 2003). Thus there is a lingering, cumulative slow-release N availability effect of a compost application to a field or in container culture.

The first objective of this study was to characterize the effects of locally available or on-farm feedstocks with a range of starting C:N ratios. We hypothesized that the nutrient levels (soluble and total) and balance of nutrients (ratios) of the finished compost would be different between treatments. We further hypothesized that there would also be treatment effects on finished compost for the pH, EC, bulk density and final C:N. The second objective was to contribute towards the development of affordable compost-based root media for production of vegetable transplants or growing plants to full maturity. We hypothesized that the compost media could grow acceptable transplants without modification, and that feedstock treatments would result in differences in the transplant morphological features assessed for three species of transplants grown (cucumber, kale, and tomato). Additionally, we hypothesized that compost age would affect transplants as compost characteristics change over time, assessed in a separate experiment growing cucumber seedlings only. A third plant growth experiment consisted of growing basil to full maturity, with the hypothesis being that there would be differences in harvest weights dependent on feedstock treatment.

The overall objective of this work was not to produce the "best" compost and test it, but rather to characterize feedstock benefits to a thermophilic compost system intended for use as a plant growing medium. Proportional effects of these feedstocks could then be calculated/estimated to develop a compost root media with the desired characteristics. Development of affordable compost-based transplant media using feedstocks that are readily available and would be generally consistent throughout the United States could increase the adoption of compost based media as an alternative for peat based media and use of fertilizers.

MATERIALS AND METHODS

Compost Treatments and Experimental Design

Compost was made at the Michigan State University (MSU) Horticulture Teaching and Research Center (HTRC) in Holt, MI (42°40” N, 84°28” W). Experimental treatments consisted of ten feedstock combinations (Table 1.1). The base mix for nine of the ten compost recipes was grass freshly cut from organically managed/certified property at the HTRC and municipal leaves from the city of East Lansing, MI, at equal parts by volume. The senesced whole leaves, from various tree species, had been collected in the previous fall in large piles that sat over winter, covered with plastic tarping most of that time, until use for composting (approximately seven months) and were moist from rain. Volume was measured with 20-gallon black plastic pots, compressed by hand to fill space, with grass weighing approximately half as much as leaves of equal volume. Additional feedstocks were added at equal proportions to the grass and leaves of the base mix in a 1:1:1 ratio of grass to leaves to other by volume. The recipe treatments were as follows: grass and leaves at 1:1 (‘Base Mix’); base mix plus more leaves (‘More Leaves’); base mix plus more grass (‘More Grass’); the base mix wrapped in plastic (‘Wrapped’); base mix plus fresh manure, which was comprised of high moisture content dairy manure and low moisture content horse manure at 1:1 by volume, both without bedding and mixed thoroughly prior to addition to compost piles (‘Manure’); base mix plus used moist SCG (‘SCG); base mix plus shredded office paper (‘Paper’); pine wood shavings plus base mix (‘Shavings’); and base mix plus sphagnum peat moss (‘Peat’). A tenth recipe referred to as the ‘Control’ is a recipe comprised of sphagnum peat moss (3ft^3 compressed bale, 53lbs), pine wood shavings (3ft^3 compressed bale, 43lbs), straw (1 standard bale, 30lbs) and hay (2 standard bales, 100lbs) which has been a standard compost recipe used for growing organic transplants for over a decade at MSU (Jost, 2008). An additional treatment of the base mix plus food residue ('Food') was made in year two only and not included for most of the analyses due to the lack of replication, with the exception of growing basil to full maturity for harvest.

Each experimental unit was a static compost pile of approximately a cubic yard at the start of composting. Compost piles were all contained under an open-ended high tunnel for protection from rain and wind. Placement of each treatment was randomized. Each treatment was replicated over time (with approximately the same start date of making the compost piles) with one replication in 2013, 2014 and 2015.

Compost Management and Data Collection

Composts were made using standard (40"x48") wooden pallets lined with cardboard creating three-sided bays for static piles to allow for approximate size of a cubic yard per piles. The feedstock components were layered on the pile by 20 gallon increments (approximately 4" layers) and roughly combined by pitchfork to ensure sufficient interaction of all components to gain proper heating and create a relatively homogeneous mixture; there were nine total 20-gallon layers per pile. The size of 'Control Recipe' treatment was larger at approximately 1.5yd^3 with five feedstocks divided into four layers for twenty total layers. The feedstocks of peat, paper, shavings, and both hay and straw from the 'Control' that were very low in moisture were all soaked in water and gravitational water drained, then added to their respective compost piles. Other feedstocks were considered to be of adequate moisture, and additional water was added to piles by a hose with a breaker, approximately one gallon per 12" height of pile. This was intended to achieve desired initial moisture content of the pile of approximately 40-60%.

The 10 compost piles were maintained under a moveable (sled) open-ended high tunnel for rain exclusion. The experiment was arranged in a randomized complete block design (RCBD) with the location of treatments within the tunnel being randomized every year. Piles were turned manually with a pitchfork when internal temperatures fell below 130°F (four times). Piles were removed out of the bay to combine and then returned by stacking while adding moisture by a hose breaker as needed to adjust moisture (approximately 3 gallons per compost pile) and ensuring all material that was on the outside was put into the middle. Piles were turned simultaneously, a total of two times, when average internal temperatures fell below 130°F. The first turn was around day nine, and the second turn 13 days later (approximate, turning schedule varied by year depending on compost temperatures).

During the composting process daily temperatures of all compost piles were recorded. Piles were allowed to mature and when composts were approximately six months old composite samples of one-gallon volume from ten areas within the freshly turned compost pile were taken for laboratory analysis of chemical and physical properties. Key responses measured from mature compost samples were for nutrients including water soluble nutrients using saturated media extract (ppm nitrate-N, ammonium-N, P, K, Ca, Mg, Na, Cl), and total nutrients (% N, P, K, Ca, Mg, S, Na; ppm Zn, Mn, Fe, Cu, B, Al) and chemical characteristics of electrical conductivity (EC), pH, organic matter (%) and physical characteristics of bulk density and moisture (%). Calculations for the percent carbon and the carbon to nitrogen ratio were obtained from the data and the percent of each nutrient contribution to the electrical conductivity was also calculated using the equation

[1]	% Element = ((ppm element) / (EC mg/L * 700)) * 100 (Warncke & Krauskopf, 1983).

At the time compost analysis was done (December of each respective year) the remainder of the compost was put into lined crates and moved to a heated head house at MSU HTRC maintained at approximately 65°F. Moisture was checked and water added by mister nozzle to surface to maintain a minimum moisture content of approximately 40%. Compost media were maintained this way until being used for transplant production the summer following when it was made. After this time each compost was stored in multiple 5-gallon buckets in an unheated barn with lids fastened to all buckets with holes drilled for maintenance of aerobic conditions but decrease moisture loss during storage. At the start of 2017 these were moved again to the same heated head house prior to being used to grow cucumber transplants in the compost treatments of different ages. Compost buckets were thoroughly mixed and samples from all three replications of each treatment (30 in total) were tested again by saturated media extract for soluble nutrients, with each replication being of a different age at the time of analysis. Ages of composts at this time were approximately 2, 3 and 4 years old.

Plant Experimental Design, Management and Data Collection

Experiment 1 Effect of compost type on tomatoes, kale and cucumbers. Finished composts (Table 1.1) were used as planting media for organic vegetable transplant production. An additional treatment of Sunshine Potting Mix (Sun Gro Horticulture) was used as a control substrate, which is approved in organic production systems. Transplants were grown in July/August of 2014, 2015 and 2016 at the MSU Research Greenhouses, with each compost-based media treatment having been made the previous year (compost aged 1 year). Three species of transplants were grown: tomato cv. Amish Paste (*Solanum lycopersicum*), kale cv. Red Russian (*Brassica oleracea* var. *sabellica*), and cucumber cv. Marketmore 76 (*Cucumis* *sativus*). An experimental unit comprised one 4-cell pack from a 48-cell flat, with four replications of each treatment for each of the three plant species. Design was a randomized complete block design (RCBD), each block being arranged on a single greenhouse bench.

Composts were filled into cells with settling and seeded, planting 2 seeds per cell and obtaining emergence data after approximately 1 week (number of seeds germinated out of eight possible). Cells were then thinned to one plant per cell by shoot removal. Supplemental lighting was supplied with three overhead 400W HPS lamps suspended 4’ above a 3’x18’ bench for a 16-hour photoperiod. Air temperature was maintained at a minimum heating setpoint of 65°F with ventilation at 75°F. Transplants were watered daily by overhead watering and no supplemental fertilizer was added.

Transplant data was taken at the stage at which each transplant would have been put into the field at a transplantable size. The data collected to assess quality of transplants were above ground biomass (both wet and dry weights), root rating (categorical variable 0-5 of qualitative rating), average plant height (cm), and number of true leaves. Cucumber transplants were assessed at three weeks, kale at four weeks, and tomatoes at five weeks. The observational unit was all four plants from each experimental unit (4-cell pack). Plant height was a visual average of all plants, while for the other measurements the total number of plants was taken into account if less than four and the data was divided by the total number of plants present. For analysis the per plant average were the data used.

Experiment 2. Effect of compost type and age on cucumber transplants. Transplants were also grown in composts from all 3 years simultaneously when the different treatments for feedstocks were of varying ages (nearly 2, 3 or 4 years old) in March of 2017. In this trial cucumber cv. Marketmore 76 (*Cucumis sativus*) was the only species grown. The number of plants in each experimental unit was increased, using one quarter of a 48 cell flat for a total of 12. This was again a RCBD arranged in the same manner as the other transplants, but with only three replications per treatment and 30 treatments total (3 different ages of the compost treatments with the exception of 'SCG' at only 2 and 3 years old, and a peat-based control media).

Transplant management and environmental conditions were the same as in Experiment 1. Data collection for emergence at one week and plant growth characteristics at 3 weeks was also consistent with procedures for Experiment 1, with the exception that data was obtained for eight plants instead of four since the experimental unit size had been increased. Data reflect per plant averages within each replication.

Experiment 3. Effects of compost type on basil. Additionally, composts that were made in 2014 were used in the summer of 2015 to grow basil cv. Genovese (*Ocimum basilicum*). One experimental unit was a bulb crate (dimensions 12"x24" x 6" deep), lined with landscape fabric and filled with a compost treatment. In each bulb crate four basil plants were transplanted, having been started in the same commercial media three weeks prior. There were six replications of each compost media treatment arranged in a RCBD, blocks being situated on individual growing benches. These were in an unheated hoophouse with no supplemental lighting, grown from July through September.

Basil was grown to full maturity and multiple harvests of top growth collected. Fresh weight of each harvest was recorded on a total of four dates from July to September 2015. The new pH of media samples collected from the root zone within a week after basil were planted into bulb crates was measured using a 1:2 dilution with deionized water and pH probe (IQ 150pH Meter with ISFET Probe). Recently matured leaf tissue was analyzed for 4 replicates of two of the treatments: the ‘Control Compost’ in which the basil was particularly green and healthy looking, and the ‘Food’ waste compost in which the basil was chlorotic.

Statistical Analysis

Initial chemical and physical properties of the media were analyzed for treatment significance by analysis of variance (ANOVA). Significant differences between means of treatments were determined by pairwise comparison using Tukey’s Honestly Significant Difference (HSD) test ($P \leq 0.05$). For additional testing of specific hypotheses, Welch’s two sample t-test was used to evaluate the differences between specific groupings of treatments which were as follows: High Starting C:N which included treatments ‘Paper,’ ‘Shavings,’ and ‘Peat,’ and Low Starting C:N which included treatments ‘More Grass,’ ‘SCG,’ and ‘Manure.’ Differences between the two groups was performed to analyze starting C:N on the dependent variable of final C:N, total %N, and soluble NO_3-N (ppm) of finished compost.

Principal component analysis (PCA) was conducted on the standardized compost physical and chemical characteristics data. PCA is a standard tool for data compression and used to capture the main features and extract information from multivariate, high-dimensional data sets. With the large number of dependent variables, PCA is descriptive technique used to explore and visualize the data and differences between treatments. The PCA was run for 27 different variables- the soluble and total nutrients, EC, pH, percent organic matter, carbon to nitrogen ratio (C:N), bulk density and moisture. While % soluble salts were calculated from the ppm of each nutrient and the EC (Equation 1), these numbers were not included in the PCA analysis because this data was already captured by the ppm of each nutrient. Visual representation of the PCA was made by creating bi-plots of the first and second PCs, looking at treatments individually and by the groupings described above for starting C:N. Loading contribution of compost characteristics to PC1 and PC2 were used for interpretation of the PCA.

For transplant performance (Experiments 1 and 2) the plant metric data of dry and wet weights, plant height, number of leaves, and root rating were analyzed for treatment significance by ANOVA. Species growth data for cucumber, kale and tomato grown in the one-year-old composts was also combined, using a 2-factor ANOVA with plant species (3 levels) and compost treatment (10 levels) as factors. Significant differences between treatment means were determined by pairwise comparison and separated according to Tukey's HSD test ($P \leq 0.05$).

An ANOVA was performed for the basil harvest data grown in the year two compost only (Experiment 3). Significant differences between treatment means were determined by pairwise comparison and separated according to Tukey's HSD test ($P \leq 0.05$). The pH data collected from the media was used to complete linear regression analysis establishing if there was a relationship between basil harvest data and pH. For the leaf tissue analysis from the two treatments 'Control' and 'Food,' nutrient values were also subject to ANOVA.

Data analysis was carried out using R statistical software package, and additional packages for R including multcomp (glht function) for pairwise comparisons and ggbiplot for visualization of PCA (R Core Team, 2019). Unless otherwise noted, statistical significance is determined at $a<0.05$. Data were assessed to ensure that ANOVA assumptions were not violated and were log transformed when needed prior to analysis. Sattherthwaite's degrees of freedom was employed to account for potential nonequal variance in the data. All data presented in figures and tables are non-transformed.

RESULTS

Composting Temperature

Temperatures curves for the compost pile were not consistent year to year, and timing of turning the piles varied slightly so it was not possible to combine the temperature data for statistical analysis (data not shown). The piles in 2013 did not heat as much or for as long as piles in the other two years. Various environmental factors may have come into play, as well as maintenance of moisture, etc. There were, however, some trends that were consistent regarding temperature of piles by treatment. Peat in particular had the lowest temperatures, and only in 2015 did it go above 130°F at all which was only for a period of four days.

Compost Physio-chemical Characteristics

Physical data: Bulk density of ‘Manure’ at 0.36 g/cc was higher than all other treatments except ‘SCG’ and ‘Paper,’ while ‘Shavings’ and ‘Control Compost’ had values at 0.15 and 0.14 g/cc, respectively, equivalent only to ‘Peat’ (Table 1.2). Percent moisture ranged from 60% (‘More Leaves’) to 73% (‘Wrapped’) with no significant differences between treatments (data not shown).

EC and pH: ‘Manure’ had the highest EC values at 10.6 ds/m, while the range of values for the remaining treatments was from 6.7 (‘More Grass’) to 1.3 (‘Peat’). The range of pH values for all composts that did not contain peat as a feedstock was between 7.7 and 8.8 (Table 1.3). The ‘Control’ compost in contrast had a mean pH of 6.3 (~20% peat) and ‘Peat’ compost 5.7 (~33% peat).

Soluble nutrients: The results of the saturated media extract (SME) for soluble nutrients displayed differences between treatments for NO_3-N (range of 1 - 734ppm), NH_4-N (0.6 - 47ppm), P (6 - 118ppm), K (218 - 2241ppm), Ca (115 - 880ppm), Mg (42 - 199ppm) and Na (25

- 377ppm, Table 1.3). Soluble Cl (103 - 1210 ppm) was the only one of the soluble nutrients with no differences between treatments.

The 'Base Mix' compost NO_3-N concentration was within a mid-range of all the composts at 250ppm and only significantly lower than 'Manure' compost with a mean value of 734ppm, higher than all the other treatments. 'SCG' had the second lowest value for NO_3-N of 4.5ppm ('Shavings' with 1ppm), while also having the highest NH_4-N concentration of 47ppm, over 700% higher than the treatment with the next highest mean NH_4 value of 5.6 ('Shavings'). P levels for 'SCG' were higher than all treatments other than 'Control' compost with a mean value of 118ppm.

'Manure' compost also had highest values K (2241ppm), and Na (337ppm) when compared to the other treatments. All nutrient values for 'Manure' have a many fold increase as compared to the published acceptable ranges for these nutrients in peat-based media (given in Table 1.3), with the exception of Ca and Mg for which the recommendation is a minimum concentration only.

For the nutrient balance of soluble nutrients (the degree of contribution to the electrical conductivity) no differences between treatments for Na, Cl, Ca and Mg were observed (data not shown; calculated by dividing elemental ppm by (EC * 700).

Total nutrients: For total nutrients analyzed, of the 13 nutrients 11 displayed significant differences between treatments and are presented in Table 1.4. The 'SCG' compost at 3.69% total N was greater than all other composts except 'More Grass' at 2.55%. 'Peat' compost N concentration of 1.34% was lower or equivalent to all other treatments. Phosphorus values ranged between 0.09% (Peat) to 0.52% for Manure which was higher than all other treatments. The K concentration ranged from 0.44 to 1.63%. The 'Manure' treatment had values higher or equivalent to other treatments for nearly all nutrients, of note Mg at 0.95%, Zn at 154%, Mn at 297%, Cu at 51%, and Na at 0.20%. 'Paper' treatment had the highest % Ca at 8.82%, the range of other treatments being from 1.25 to 4.05%. The higher total Ca did not result in higher SME/soluble Ca relative to the other treatments (Table 1.3). The nutrients with no differences between treatments were total Al (263 – 2621 ppm) and Fe (1725 - 3893 ppm, data not shown).

Nitrogen concentration displayed differences dependent on starting C:N of the compost feedstocks. Final C:N was nearly twice as high in the high starting C:N group with 23.6 versus C:N of 12.6 for the low C:N group. Total N (%) was 1.7 versus 2.8, and soluble NO_3-N (ppm) was 137 versus 444 for the high versus low C:N starting groups, respectively (significance at $p<0.001$, $p=0.001$, and $p=0.037$, respectively).

The wrapped Mix did not have any odors that would signify anaerobic or putrid conditions that would warrant concerns of composters. While the percent moisture of finished compost was not different, the wrapped compost moisture throughout the composting process maintained without additions while water had to be added to all other treatments when piles were turned (twice).

Principal Component Analysis: The distribution of the composts in the biplot defined by the two PCs resulted in the separation of some of the compost treatments, "SCG", "Peat", "Manure" and "Control" composts in particular while the other treatments displayed much more overlap (Figure 1.1A). When the treatments were grouped by starting C:N, the differences in distribution of these groups in the bi-dimensional space is even more apparent (Figure 1.1B). The first and second principal components accounted for 63% of the systems total variability of the physiochemical compost variables. The loadings or correlations between the PC axes and the original data are displayed in Figure 1.2. The first PC which captured 47.1% of the variance is dominated by EC and soluble nutrients (K, Mg, NO3-N, Na, Cl, Ca) and by C:N, and organic matter content in the opposite direction. This would indicate a potential inverse relationship between higher carbon content and soluble nutrient concentrations. The second PC is dominated by total N, P, K, S, moisture, organic matter, and in the opposite (positive) direction by C:N.

Comparison of Plant Performance

Experiment 1. Mean emergence across all species ranged from 71% to 98% depending on compost treatment (Table 1.5). By species, cucumber emergence ranged from 83% in 'More Leaves' to 98% in 'Wrapped' compost; kale exhibited the smallest range of emergence by treatment, from 81% in the 'SCG' compost to 91% in the 'Control' compost; tomato emergence rates ranged from 71% in the 'Manure' compost to 92% in the 'Control media'.

Data for all transplant characteristic is displayed in Table 1.6. There were significant differences between treatments for all species and all plant growth characteristics ($p<0.001$ for all but plant height for cucumber, for which $p=0.009$). For kale and tomato there was high correlation between these growth metrics (0.62 - 0.95); however, for cucumber all metrics were highly correlated (0.65 - 0.79) except for root rating (correlation of 0.11 - 0.29). A dominant trend in the data was "Shavings" compost consistently having the lowest values for all growth parameters, regardless of plant species. While there were no differences between "Shavings" and the control media for any of the metrics for cucumber, for root rating, height and leaves for kale and tomato "Shavings" and the control were significantly different.

The model predicting performance of transplants dry weight as a function of compost media treatment displayed differences between treatments (Figure 1.1, Cucumber: $F=12.5_{(10, 102)}$, $p=<0.001$, Kale: $F=18.8_{(10, 101)}$, $p=<0.001$, Tomato: $F=6.2_{(10, 111)}$, $p<0.001$). With the exception of the 'Shavings' treatment (and 'SCG' treatment for Kale), relative to the growth in the control medium plants had increased dry weight (127% to 246% more for kale, 40% to 89% more for cucumbers, 115% to 301% more for tomatoes). All compost except "Shavings" produced transplants with suitable growth characteristics for transplanting and handling.

Experiment 2. Transplants grown in the ten compost treatments at different ages demonstrated a distinct effect of compost age/maturity on transplant performance. There was a significant effect of compost that interacted with year ($F=12.8_{(8, 52)}$, $p<0.001$). For four of the compost treatments the interaction of compost and year was found to be highly significant ($p<0.05$). This was most pronounced for 'Shavings' and 'Peat' where the youngest compost (2yo) resulted in less growth ('Shavings') or more growth ('Peat') for wet/dry weight and plant height than the same compost at either 3 or 4 years old. For the 'Wrapped' treatment, the oldest compost at 4yo had dry/wet weight values of less than half the transplants from the two younger composts ($p<0.001$). In the case of the 'Control' compost, differences were observed in the 2yo versus 4yo compost for shoot dry weight ($p<0.05$), however neither of these were significantly different than the three -year-old compost, making it hard to establish any kind of a trend. The 'SCG' treatment increased plant growth with age (by 55% in dry weight, 22% for root rating) from two versus three years old.

Experiment 3. For basil grown to full maturity in bulb crates filled with one cubic foot 100% compost substrate, there was a large range in the average cumulative harvest with differences between treatments (Table 1.9, $F=66.0_{(9, 45)}$, $p<0.001$). Cumulative harvest weight was lowest for 'Shavings' (38g), while the treatment with the next cumulative basil weight was the 'Wrapped' compost at 134g, which was equivalent to five of other treatments. The 'Control' compost had the highest harvest weight (418g) followed by the 'Peat' compost at 351g.

Plant tissue analysis for total nutrient content in leaf tissue between the 'Control' and 'Food' composts revealed differences in N, P, K, S, B, Zn, Cu and Na (Table 1.10). The nutrients that were not different were Mg, Ca, Mn, Fe and Al. These two treatments were selected for nutrient analysis based upon their difference in appearance with the 'Food' treatment displaying signs of chlorosis and low harvest weights and the 'Control' looking particularly green with high harvest weights.

The relationship between the harvest weights and compost media pH was negative (Figure 1.4, $p<0.001$, $R^2=0.50$), further validating the hypothesis of the impact of compost media pH on plant growth**.**

DISCUSSION

The experiments completed and data collected provide a foundation to consider first the impacts of feedstocks on physiochemical properties, and second the impacts of resulting physiochemical properties on transplant growth.

Feedstock and Physio-chemical differences: 'SCG' and 'Manure' were hypothesized to have higher nutrient levels for multiple nutrients. This trend is observed in some nutrients but not all.

When compared against the same recipe that was not wrapped ('Base Mix') the mean values for all nutrients were not different in the 'Wrapped' treatment, contradicting our hypothesis that 'Wrapped' would have had greater nutrient retention. The hypothesis that 'Wrapped' would also have greater water holding capacity was not supported in the moisture content as there were no detectible differences between treatments.

The pH of composts generally is within the range of 6.0 to 8.0, dependent on feedstocks, the composting process and any additional amendments (Sullivan & Miller, 2001). The only two compost treatments to have an acidic pH after 6 mos. were the mix with peat (~33% by volume) and the control compost recipe that also included peat (~20% by volume). In general the pH values were higher than were expected for finished compost with many treatments remaining at alkaline pH ('Base Mix,' 'Wrapped,' 'More Leaves,' 'Paper' and 'Shavings' all above pH 8.0; Table 1.3) and indicate that the composts were not yet fully mature at the time of sampling. In contrast, ammonia levels had decreased to < 5 ppm while nitrate levels had increased, indicating compost maturity. Transplants were grown in the compost media nearly 10 months after these data were collected, and pH values would be likely to have dropped (SME of aged compost samples displayed a substantial drop in pH, Table 1.8). The pH values of media impact the solubility/availability of nutrients, and so while nutrients may be present in sufficient quantities, plants may still display deficiencies. Nutrient availability in relation to peat-based media pH is dependent on the nutrient, with P being particularly sensitive to pH. In the absence of mineral soil (clay, silt, sand) and therefore reduced Al and Fe, P availability increases in peat-based media more than in field soil with declining pH. This relationship needs to be characterized for compost-based media that may or may not contain mineral soil.

Maturity of compost affects the pH, primarily through the process of mineralization and nitrification. Immature compost may test at 8.0 to 8.5, prior to the initiation of ammonification and bacterial nitrification. Decreasing pH with increasing NO_3 concentration is common during compost maturation as protons are released during the nitrification process (Cooperband et al., 2003). The compost samples being analyzed only at one point in this study did not allow us to assess our findings in relation to this trend, however. At early stages (6 mos.) 'SCG' compost maturation indices appear to be anomalous in regards to progression of nitrification with NO_3-N levels still near zero and NH_4-N levels nearly 50x higher than the other treatments (Table 1.3).

However, in years 2 and 3 of maturation pH and NH_4 levels appear to drop, while NO_3 increased (though this is unreplicated data, Table 1.8). This would suggest a possible inhibition in nitrification in less mature composts that changes with additional curing; there could be differences in the biota that are driving these observations, and future research that assesses biological differences in the compost treatments may help elucidate these processes.

Experiment 1: Transplant differences.

Although many of the composts proved to be adequate for growing transplants at 100%, 'Peat' and 'Control' had some of the highest values for the plant metrics, and it is likely that pH may have had one of the biggest roles in this. P is particularly impacted by alkaline versus acidic pH with increased availability at slightly acidic pH versus alkaline, and other nutrients to a lesser degree also. Additionally, the pH of the environment will likely impact the biota which drives mineralization.

Acceptable range numbers for soluble nutrient values, pH and EC of root media are listed in Table 1.3 (Warnke and Krauskopf, 1983), but these values are based on SME for peat-based media. Nearly all nutrient concentrations in the composts well exceeded the recommended ranges often by a factor of two to four but in more extreme cases by a factor of nine.

The nutrient reserve of compost-based root media is somewhat buffered, having higher total nutrient concentrations and biota for mineralization, may have a greater impact when production cycle is longer and no supplemental fertilizer is used. Cucumbers have a shorter production phase as compared with other vegetables such as brassicas or solanaceous transplants at four to six weeks, or alliums at 10-12 weeks. Kaya et al. (2016) reported differential responses in plant variables between treatments in which high salt content resulted in dry matter and chlorophyll reductions that were more pronounced in pepper transplants as compared with cucumber. Our results, however, do not appear to display differences in responses between plant species in regards to which media have higher or lower EC or salt content (Figure 1.3).

Transplants grown in peat-based media are typically fertilized with water soluble nutrients at each irrigation or weekly, where these acceptable numbers would provide adequate fertility. A goal with compost-based media could be that supplemental water soluble fertilization is not needed due to the higher reserve (CEC), and mineralization of nitrogen from the compost over time. It is important to note when making comparisons that the control peat-based medium in this study was low in fertility and would typically be fertilized, however, during this study it was not. Although differences between the control medium and compost treatments could be overcome by fertilization, there is a monetary cost associated with this, additional labor, and environmental cost both from the production or extraction of nutrients, and also the higher potential for nutrient pollution by highly soluble nutrients leaching from substrates.

Experiment 2: Effects of compost age.

Changes in soluble nutrients, pH and EC over time are displayed in Table 1.8. While there was no replication of the saturated media extracts for each compost by year, some trends can be observed, most notably for pH and EC. While pH values were quite high in the fresher compost tested at less than one year from the time of making, in all of the different ages of the aged compost only three samples were pH 8 or above (highest was 2yo Shavings at 8.4). There is a trend observed that pH decreased with age though not consistently. EC and soluble nitrate had the opposite trend, with older composts increasing in EC and NO_3-N. While ammonium trended downward over time most levels were quite low even in the fresher compost; 'SCG,' however, was notable in the original data for having high ammonium concentration (47ppm) and so had the most drastic decrease with values of the older compost averaging just 0.8 ppm. While there are other interesting potential trends, the lack of replication of the soluble nutrients from the compost of the different ages limits what is able to be deduced in regards to relationships between the interaction of compost and age of compost. There are also variables that in hindsight should have been accounted for, such as compost storage temperature, moisture, and aeration. While the trends of decreasing pH is well documented in compost research, the rate as a function of these and other variables is not as researched or well understood.

The hypothesis that compost age would affect transplants as the compost characteristics changed over time was supported for many of the compost treatments. How these changes in the compost as it aged affected transplant health is exemplified most in the treatments 'Shavings' and 'Peat' which had the starkest differences in regard to compost age. While 'Peat' had some of the highest plant metrics in Experiment 1 when composts were 1yo, the positives of this compost root media changed over time where there was a trend of a decrease in plant height with the older composts, and a decrease in shoot dry weight. The opposite was true for 'Shavings' which had the smallest transplants with the least developed root systems at 1yo in Experiment 1, in the 3 and 4yo composts of Experiment 2 the plant metrics increased significantly over the original 1yo compost and also the 2yo compost. Many of the other treatments, however, did not display an effect, or did not have as clear of a trend, of compost age.

Experiment 3: Basil to full maturity.

Our hypothesis of differences observed between treatments in basil grown to maturity was supported and is related to pH values. 'Control' and 'Peat' had the lowest pH values, both of them having acidic sphagnum peat moss as compost feedstocks, and the highest and second highest harvest weights. With the high pH values observed in the compost media overall, it was expected that many of the treatments at high pH may have displayed chlorosis and other nutrient deficiency symptoms, which was evident to varying degrees in 'Food,' 'Shavings,' 'More Leaves,' 'More Grass,' 'Paper,' and 'Wrapped;' however, the basil still grew in all treatments.

The selection of the 'Control' and the 'Food' compost treatments for further evaluation of the basil by leaf tissue analysis was based primarily on the visual difference of plant health, with the former being dark green and the latter the treatment with the most apparent chlorosis. The mean cumulative harvest weights were also considerably different at 418g and 197g, respectively. There are interactions between many different factors and this would not be due to pH alone but there is substantial evidence that pH is a particularly important factor which is observed in the inverse linear relationship between pH and harvest weight (Figure 1.4). Other interactions allowing for growth at high pH may be that the biota are able to influence mineral availability more than expected at high pH which could be explored in future research.

The tissue analysis results were surprising in that, of the nutrients where there was a difference in the treatments, the 'Food' medium had higher values for all except Zn. This contradicted our hypothesis that the media producing healthier looking, greener plants would have higher leaf tissue nutrient concentration. Mg, Fe and Mn are particularly important to chlorophyll production and photosynthesis, but were not reduced in leaves with chlorosis. Nutrient concentrations in the media were sufficient to supply the plant, but the EC levels may have resulted in a high salt stress that could explain the differences observed between the treatments.

The level of K in the leaf tissue from the 'Food' treatment at 10.2% would be considered at luxury K consumption levels, and was nearly double that of the basil from the 'Control' at 5.5. The high K in leaf tissue may cause an antagonism of Mg or Mn availability or an "induced deficiency," despite Mg and Mn levels in the leaf tissue being acceptable. Dzida et al. (2018)

found that as K increased in basil tissue, both Mg and Ca declined, which is inconsistent with our results in which there were no differences in Mg and Ca between the treatments despite the large difference in K. This relationship between K and Ca was also observed by Yermiyahu et al. (2015), again in basil. At higher concentrations, K has an antagonistic effect on uptake of other cations, especially Mg (Kabu & Toop, 1970; Nurzynska-Wierdak et al., 2012), but if the Mg is still at a normal level in the tissue regardless of K concentration, it is possible that there is an antagonism or physiologically induced deficiency happening within the leaves. The relationships between the media and nutrient concentration in leaf tissue warrants more investigation.

Of note is the small amount of power in the experiment due to the number of replications and the complicating factor of environmental conditions year to year with replication having been done over years. While there are variations in the means between treatments that may at first appear to be substantial, when evaluated using either Tukey's HSD or Welch's t-test, there is no significant difference between some treatments that may have been initially hypothesized. If, however, there was greater power through additional replication, significant differences may have been resolved.

FUTURE RESEARCH AND CONCLUSIONS

Future research to build upon the short-term impact on transplants could be a study of transplants planted out into the field to assess long-term ramifications of transplant media on harvestable yield and disease presence/severity. In addition, the compost root media could be used for growing plants to full maturity in containers and the impact on yield/health in this environment. Container plant culture in protected environments is a subsector of the specialty crop industry that is rapidly expanding (Rogers, 2017). Looking beyond yield to changes in the microbiome of the plant and soil as well, and how that may cascade to disease resistance.

Additional physical characteristics of the compost media may further demonstrate differences created by specific feedstocks. Water holding capacity, easily available water, water buffering capacity, total and volatile solids, particle size distribution, and porosity would all warrant investigation and relating these characteristics to other existing composting literature.

The biological differences resulting from adjustment of starting carbon to nitrogen ratio were an initial motivating factor for this research when there was an original intention of using the composts for compost tea research. Analysis of biology, especially of fungal versus bacterial populations, would add to the body of knowledge about how bacterial/fungal ratios in compost may vary. More recent advances in testing for biology are opening up this area of compost research (St. Martin et al., 2020). If analyzed to the species level, a similar statistical analysis using PCA that was used in this experiment for physio-chemical characteristics could be employed to assess differences in the biology of different composts. Fungal to bacterial ratios could also be assessed, as it has been found in multiple studies that higher C:N starting feedstocks result in an increase in fungal:bacterial ratios (Eiland et al., 2001) but other aspects of feedstocks could also be related to this ratio and assessed by the feedstocks chosen herein.

Adjustment of specific characteristics of media for future research would help to elucidate which aspects of the media are playing a larger role in transplant performance, and the target levels of these nutrients or other parameters for a 100% compost-based media. The high pH of these composts may have played a larger role affecting transplant health, superseding the effect of EC and specific nutrients. Isolating just the effect pH by its adjustment with elemental S at either the start of composting or at the end of thermophilic composting would help to test pH specifically. Similarly, Ca adjustments with gypsum or another form of soluble Ca would affect the relative ratios of nutrients that also may be playing a large role in transplant health using the media. Adjustment of specific chemical characteristics such as these in conjunction with biological data on the composts would further our understanding of these interactions.

With the differences observed in transplant growth as affected by compost age, there is the indication that physiochemical characteristics linked to the degree of maturity are worth exploring further. More research on trends of maturation beyond one year of storage and the effects of specific storage conditions could help the composting industry to expand without fear of product losing value. Inoculation of a stable but not-yet-mature compost with mature compost could be assessed for changes in microbial diversity and rate of maturation.

There were many differences observed between treatments both for the physiochemical characteristics and performance of transplants in the compost media. Based on this research, compost can be used as a growing media without additional components (at 100%) successfully. Differences in the physiochemical properties could be further adjusted or composts of different qualities blended to achieve the most desirable transplant characteristics. For example, a more mature compost with high EC and lower pH could be blended with a less mature compost with lower EC and higher pH. Effects of such compost blends warrants further exploration.

APPENDIX

Table 1.1. Compost feedstock recipes.

Compost name	Base Mix (Grass and Leaves 50:50)	Additional Feedstock (33% by volume)	Purpose
Base Mix	100%		Base for comparison
More Leaves	67%	Dry leaves	Higher C:N
More Grass	67%	Fresh grass	Lower C:N
Wrapped	100%		Nutrient/Moisture retention
Manure	67%	Manure	Lower C:N, more nutrients
SCG	67%	SCG	Lower C:N, more nutrients
Paper	67%	Paper	Higher C:N
Shavings	67%	Shavings	Higher C:N
Peat	67%	Peat	Higher C:N, lower pH
Control Recipe		Peat, shavings, 2x hay, straw	Tested mix for transplant production, combining many of same feedstocks from other recipes

Table 1.2. Compost properties of organic matter and carbon content, carbon to nitrogen ratio (C:N) and dry bulk density (mean +/- SE, n=3) by compost treatment. Samples collected for analysis approximately six months after the start of the thermophilic composting phase.

Compost	Organic Matter %		Carbon %		C:N		Dry Bulk Density g cm-3	
Manure	46 (0.8)	a	27 (0.5)	a	11.5 (1.2)	a	0.36 (0.015)	d
SCG	76 (6.5)	bc	44 (3.8)	bc	11.9 (0.8)	a	0.34 (0.015)	cd
More Grass	61 (4.2)	abc	36 (2.5)	abc	14.2 (1.6)	a	0.28 (0.003)	c
Wrapped	60 (0.9)	abc	35 (0.5)	abc	15.9 (0.7)	a	0.26 (0.007)	bc
Base Mix	62 (0.6)	abc	36 (0.3)	abc	19.1 (1.5)	ab	0.27 (0.012)	bc
More Leaves	56 (2.9)	ab	33 (1.7)	ab	16.1 (0.6)	a	0.28 (0.012)	c
Paper	53 (1.8)	ab	31 (1.1)	ab	18.3 (0.2)	ab	0.31 (0.021)	cd
Shavings	75 (1.3)	bc	43 (0.7)	bc	31.2 (4.4)	c	0.15 (0.003)	a
Control Compost	67 (12.2)	abc	39 (7.1)	abc	28.8 (4.9)	bc	0.14 (0.028)	a
Peat	80 (0.7)	c	47 (0.4)	c	34.7 (0.7)	c	0.20 (0.007)	ab
P	0.001		0.001		<0.001		<0.001	

* means followed by the same letter in the same column are not statistically different (p<0.05) according to Tukey's HSD test

Table 1.3. The pH, EC and water soluble nutrient values (mean +/- SE, n=3) from the saturated media extract for compost media. Samples collected for analysis approximately six months after the start of the thermophilic composting phase.

Compost	pH	EC	NO3-N	NH4-N	P	K	Ca	Mg	Na	Cl
	5.2-6.3	dS m-1	ppm	ppm	ppm	ppm	ppm	ppm	ppm	ppm
Acceptable range*	5.2-6.3	0.75-3.5	100-199	0-10	6-10	150-249	>200	>70	<115	<180
Manure	7.9 (0.1) c**	10.6 (1.1) d	734 (132) c	1.8 (0.3) a	40 (8) a	2241 (326) c	880 (174) b	199 (3) b	377 (6) b	1210 (487) a
SCG	7.8 (0.6) bc	4.5 (0.2) abc	4.5 (1.5) a	47 (15) b	118 (52) b	891 (406) ab	308 (8) ab	130 (34) ab	52 (8) a	569 (281) a
More Grass	7.7 (0.2) c	6.7 (1.3) c	446 (68) bc	1.3 (0.7) a	45 (5) a	1505 (348) bc	510 (202) ab	138 (45) ab	92 (60) a	647 (215) a
Wrapped	8.3 (0.2) c	6.2 (0.1) c	323 (23) ab	0.8 (0.2) a	17 (3) a	1381 (139) bc	480 (120) ab	122 (19) ab	89 (61) a	709 (175) a
Base Mix	8.1 (0.2) c	4.2 (0.2) abc	250 (9) ab	0.8 (0.3) a	22 (4) a	965 (68) ab	380 (82) ab	96 (12) ab	80 (50) a	495 (166) a
More Leaves	8.2 (0.2) c	5.0 (0.7) bc	250 (44) ab	1.0 (0.3) a	14 (2) a	1097 (191) ab	400 (48) ab	89 (3) a	31 (1) a	636 (231) a
Paper	8.8 (0.3) c	4.2 (0.3) abc	88 (64) a	1.9 (1.0) a	6 (1) a	829 (152) ab	520 (95) ab	102 (5) ab	126 (30) a	696 (295) a
Shavings	8.1 (0.4) c	1.8 (0.2) ab	1 (0.9) a	5.6 (4.8) a	30 (11) a	482 (70) ab	200 (43) a	49 (8) a	56 (32) a	242 (35) a
Control	6.3 (0.2) ab	3.2 (1.2) abc	208 (110) ab	3.2 (0.6) a	64 (13) ab	801 (231) ab	245 (151) a	78 (36) a	31 (7) a	231 (54) a
Peat	5.7 (0.3) a	1.3 (0.3) a	32 (18) a	0.6 (0.1) a	33 (7) a	218 (30) a	115 (63) a	42 (12) a	25 (10) a	103 (23) a
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.013	0.003	<0.001	0.124

* Acceptable range numbers are for peat-based root media for container grown plants as a reference for use of compost as root media. Adopted from Noguera et al., 2003 and Warncke and Krauskopf, 1983

** means followed by the same letter in the same column are not statistically different ($p<0.05$) according to Tukey's HSD test

Table 1.4. Nutrient values (mean +/- SE, n=3) from total nutrient analysis of compost media. at 6 months maturity for nutrients with significant differences between treatments. Samples collected for analysis approximately six months after the start of the thermophilic composting phase.

Compost	N		P		K		Ca		Mg		S		Zn		Mn		Cu		B		Na	
	%		%		%		%		%		%		%		%		%		%		%	
Manure	2.40 (0.26)	bc*	0.52 (0.04)	d	1.55 (0.21)	b	4.05 (0.52)	c	0.95 (0.11)	b	0.32 (0.01)	bc	154 (27)	c	297 (18)	b	51 (6)	b	50 (7)	ab	0.197 (0.02)	c
SCG	3.69 (0.07)	d	0.32 (0.01)	bc	1.61 (0.03)	ab	2.33 (0.23)	abc	0.59 (0.01)	ab	0.33 (0.03)	bc	59 (3)	ab	187 (14)	ab	40 (6)	ab	47 (6)	ab	0.05 (0.01)	ab
More Grass	2.55 (0.24)	cd	0.33 (0.06)	c	1.63 (0.44)	b	3.38 (0.32)	abc	0.58 (0.06)	ab	0.32 (0.02)	c	82 (4)	abc	260 (3)	ab	20 (2)	a	62 (11)	b	0.018 (0.015)	a
Wrapped	2.20 (0.13)	abc	0.26 (0.04)	bc	1.51 (0.23)	b	3.66 (0.32)	bc	0.61 (0.06)	ab	0.27 (0.02)	abc	75 (0.3)	abc	233 (22)	ab	18 (1)	a	68 (11)	b	0.021 (0.008)	a
Base Mix	1.89 (0.13)	abc	0.24 (0.03)	abc	1.01 (0.10)	ab	3.74 (0.28)	bc	0.65 (0.08)	ab	0.25 (0.02)	abc	80 (7)	abc	273 (31)	ab	19 (2)	a	60 (6)	b	0.018 (0.007)	a
More Leaves	2.03 (0.13)	abc	0.22 (0.03)	abc	0.98 (0.21)	ab	3.49 (0.42)	abc	0.58 (0.07)	ab	0.22 (0.01)	abc	76 (7)	abc	273 (19)	ab	18 (2)	a	62 (10)	b	0.021 (0.001)	a
Paper	1.68 (0.06)	abc	0.17 (0.02)	abc	0.69 (0.10)	ab	8.82 (0.92)	d	0.59 (0.09)	ab	0.21 (0.00)	abc	140 (40)	bc	202 (12)	ab	17 (2)	a	44 (9)	ab	0.077 (0.001)	b
Shavings	1.45 (0.22)	ab	0.15 (0.03)	ab	0.73 (0.11)	ab	2.28 (0.45)	abc	0.42 (0.06)	a	0.18 (0.02)	a	65 (6)	ab	301 (20)	b	14 (2)	a	39 (12)	ab	0.021 (0.001)	a
Control Compost	1.50 (0.42)	ab	0.13 (0.02)	ab	1.12 (0.25)	ab	1.25 (0.29)	a	0.53 (0.19)	ab	0.19 (0.07)	ab	42 (5)	a	215 (69)	ab	26 (17)	ab	14 (6)	a	0.027 (0.004)	a
Peat	1.34 (0.02)	a	0.09 (0.01)	a	0.44 (0.05)	a	1.68 (0.14)	ab	0.37 (0.03)	a	0.17 (0.00)	a	41 (1)	a	154 (12)	a	12 (2)	a	24 (3)	ab	0.032 (0.004)	ab
P	<0.001		<0.001		0.006		<0.001		0.028		0.001		0.001		0.025		0.007		0.005		<0.001	

* means followed by the same letter in the same column are not statistically different ($p<0.05$) according to Tukey's HSD test

Table 1.5. Emergence rates for cucumber, kale and tomato transplants in the ten compost media and control. Mean % (+/- SE), n=3.

Compost	Cucumber	Kale	Tomato
Manure	84% (6)	85% (4)	71% (7)
SCG	86% (5)	81% (7)	79% (6)
More Grass	95% (2)	87% (2)	84% (3)
Wrapped	98% (1)	81% (6)	82% (4)
Base Mix	93% (3)	82% (6)	82% (4)
More Leaves	83% (5)	88% (4)	93% (2)
Paper	97% (1)	87% (4)	93% (2)
Shavings	95% (2)	82% (7)	87% (3)
Control Compost	96% (2)	91% (2)	89% (4)
Peat	91% (4)	86% (7)	82% (5)
Control Media	97% (2)	89% (3)	92% (2)

Table 1.6. Effect of compost feedstock recipes on plant growth responses of dry weight, wet weight, root rating, plant height and number of leaves for cucumber, kale and tomato. Mean values (+/- SE), n=3.

	Shoot Dry Weight (g)			Shoot Wet Weight (g)			Root Rating (1-5)		
	Cucumber	Kale	Tomato	Cucumber	Kale	Tomato	Cucumber	Kale	Tomato
Manure	0.34 (0.03) c*	0.23 (0.02) d	0.27 (0.04) bc	3.88 (0.25) b	2.81 (0.26) d	2.39 (0.24) c	3.43 (0.30) abc	2.90 (0.33) cd	3.21 (0.38) bc
SCG	0.37 (0.03) c	0.10 (0.02) bc	0.26 (0.02) bc	3.78 (0.61) b	1.41 (0.20) bc	2.31 (0.39) bc	3.22 (0.45) ab	1.56 (0.26) ab	3.47 (0.24) bc
More Grass	0.34 (0.02) bc	0.24 (0.03) d	0.30 (0.03) bc	3.54 (0.26) b	2.31 (0.21) cd	2.65 (0.30) c	4.23 (0.19) cde	3.60 (0.28) cde	4.19 (0.15) c
Wrapped	0.35 (0.01) c	0.26 (0.03) de	0.22 (0.02) bc	4.13 (0.25) b	2.90 (0.28) d	2.15 (0.25) bc	3.67 (0.30) abcd	3.46 (0.21) cde	3.14 (0.28) bc
Base Mix	0.31 (0.03) bc	0.24 (0.02) de	0.31 (0.03) bc	3.46 (0.28) b	2.65 (0.23) cd	2.68 (0.36) c	4.56 (0.13) e	3.83 (0.24) de	4.09 (0.25) c
More Leaves	0.42 (0.02) c	0.22 (0.02) d	0.29 (0.03) bc	3.24 (0.31) b	2.08 (0.25) bcd	2.31 (0.24) bc	4.30 (0.33) cde	4.03 (0.24) e	3.56 (0.16) bc
Paper	0.35 (0.03) c	0.22 (0.02) cd	0.23 (0.02) bc	3.17 (0.17) b	2.44 (0.23) cd	1.98 (0.23) bc	4.08 (0.24) bcde	4.00 (0.26) e	3.46 (0.30) bc
Shavings	0.12 (0.01) a	0.20 (0.01) a	0.01 (0.00) a	0.94 (0.12) a	0.27 (0.05) a	0.17 (0.02) a	2.97 (0.15) a	1.08 (0.26) a	0.42 (0.14) a
Peat	0.40 (0.02) c	0.33 (0.03) e	0.34 (0.09) bc	3.65 (0.36) b	2.95 (0.29) d	2.68 (0.64) c	4.39 (0.13) de	4.02 (0.28) e	2.72 (0.37) b
Control Compost	0.36 (0.03) c	0.22 (0.03) bcd	0.41 (0.11) c	3.54 (0.46) b	2.10 (0.34) bcd	2.94 (0.76) c	4.40 (0.11) de	3.35 (0.22) cde	3.46 (0.40) bc
Control Media	0.22 (0.01) ab	0.10 (0.02) b	0.10 (0.01) ab	1.41 (0.09) a	0.90 (0.22) ab	0.56 (0.08) ab	3.69 (0.17) abcde	2.51 (0.16) bc	2.44 (0.25) b
P	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

* means followed by the same letter in the same column are not statistically different (p<0.05) according to Tukey's HSD test

Table 1.6 (cont'd)

	Plant Height (cm)						Number of Leaves					
	Cucumber		Kale		Tomato		Cucumber		Kale		Tomato	
Manure	13.6 (0.97)	b*	12.6 (0.67)	d	17.9 (1.41)	cd	2.77 (0.13)	bc	5.44 (0.13)	c	5.38 (0.21)	cde
SCG	14.2 (1.38)	b	8.3 (0.60)	bc	19.8 (0.48)	d	3.15 (0.16)	c	4.91 (0.21)	bc	5.50 (0.21)	cde
More Grass	13.5 (0.70)	b	11.3 (0.35)	cd	19.0 (0.54)	d	2.63 (0.14)	bc	5.04 (0.19)	c	5.94 (0.10)	e
Wrapped	14.8 (0.65)	b	12.0 (0.52)	d	16.7 (0.91)	bcd	2.98 (0.07)	c	5.60 (0.18)	c	5.18 (0.44)	bcde
Base Mix	13.9 (0.68)	b	12.8 (0.61)	d	18.8 (0.44)	d	2.56 (0.15)	bc	5.49 (0.25)	c	5.80 (0.12)	de
More Leaves	12.0 (0.94)	b	11.7 (0.56)	d	17.0 (0.42)	cd	2.41 (0.15)	b	5.28 (0.46)	c	5.55 (0.14)	cde
Paper	13.7 (0.78)	b	13.0 (0.48)	d	16.8 (0.59)	bcd	2.60 (0.16)	bc	5.25 (0.23)	c	5.63 (0.15)	de
Shavings	6.1 (0.32)	a	4.3 (0.27)	a	4.3 (0.28)	a	1.68 (0.16)	a	2.47 (0.23)	a	1.63 (0.22)	a
Peat	13.1 (0.35)	b	12.1 (0.6)	d	14.0 (1.43)	bc	2.88 (0.07)	c	5.61 (0.29)	c	4.31 (0.49)	bc
Control Compost	12.9 (0.64)	b	11.2 (0.97)	cd	15.6 (1.91)	bcd	2.83 (0.10)	bc	4.77 (0.30)	bc	4.67 (0.36)	bcd
Control Media	9.0 (0.34)	a	8.1 (0.50)	b	11.6 (0.70)	b	2.13 (0.05)	a	3.81 (0.39)	b	3.72 (0.24)	b
P	0.009		<0.0001		<0.0001		<0.0001		<0.0001		<0.0001	

* means followed by the same letter in the same column are not statistically different (p<0.05) according to Tukey's HSD test

Table 1.7. Mean values (+/- SE) for five plant metrics of dry weight, wet weight, root rating, plant height and number of leaves per plant for cucumber grown in compost treatments at different ages.

Treatment	Age	Shoot Dry Weight (g)		Shoot Wet Weight (g)		Root Rating (1-5)		Plant Height (cm)	
Manure	2	0.31 (0.01)	a	2.79 (0.15)		4.54 (0.08)		10.7 (0.65)	
	3	0.38 (0.01)	b	3.13 (0.19)		4.83 (0.11)		11.6 (0.44)	
	4	0.35 (0.02)	ab	2.96 (0.23)		4.79 (0.08)		11 (0.38)	
P		0.024		0.517		0.134		0.485	
SCG	2	0.2 (0.01)	a	1.63 (0.07)		3.92 (0.04)	a	8.5 (0.54)	
	3	0.31 (0.03)	b	2.58 (0.36)		4.79 (0.11)	b	9.8 (1.26)	
P		0.0147		0.058		0.002		0.396	
More Grass	2	0.27 (0.01)	a	2.25 (0.19)		4.83 (0.04)		9.5 (0.91)	
	3	0.32 (0.02)	b	2.21 (0.08)		4.96 (0.04)		9.6 (0.86)	
	4	0.33 (0.00)	b	2.58 (0.11)		4.88 (0.07)		10.1 (0.21)	
P		0.010		0.183		0.317		0.819	
Wrapped	2	0.33 (0.02)	b	2.88 (0.22)	b	4.42 (0.04)	a	11.5 (1.09)	b
	3	0.36 (0.01)	b	2.67 (0.08)	b	4.83 (0.04)	a	10.4 (0.15)	b
	4	0.15 (0.01)	a	1 (0.19)	a	4.83 (0.17)	a	6.5 (0.60)	a
P		<0.001		<0.001		0.043		0.006	
Base Mix	2	0.35 (0.01)		3.17 (0.15)		4.79 (0.04)		11.3 (0.57)	
	3	0.39 (0.04)		3.17 (0.48)		4.67 (0.04)		11.5 (0.83)	
	4	0.37 (0.05)		2.79 (0.37)		4.79 (0.11)		10.9 (0.62)	
P		0.78		0.658		0.437		0.845	
More Leaves	2	0.33 (0.02)		2.75 (0.14)		4.63 (0.00)	a	10.8 (0.43)	
	3	0.33 (0.02)		2.21 (0.30)		4.96 (0.04)	b	9.4 (0.87)	
	4	0.30 (0.02)		2.08 (0.22)		4.67 (0.04)	a	9.5 (1.07)	
P		0.536		0.176		0.003		0.447	

Table 1.7 (cont'd)

Treatment	Age	Shoot Dry Weight (g)		Shoot Wet Weight (g)		Root Rating (1-5)		Plant Height (cm)	
Paper	2	0.32 (0.01)	ab	2.71 (0.15)		4.54 (0.11)		10.5 (0.10)	
	3	0.37 (0.01)	b	2.58 (0.08)		4.88 (0.13)		10.3 (0.35)	
	4	0.31 (0.00)	a	2.33 (0.08)		4.75 (0.07)		11.1 (0.17)	
P		0.023		0.149		0.084		0.139	
Shavings	2	0.10 (0.00)	a	0.58 (0.04)	a	3.88 (0.07)	a	4.6 (0.27)	a
	3	0.39 (0.03)	b	2.75 (0.38)	b	4.92 (0.08)	b	10.7 (0.65)	b
	4	0.43 (0.05)	b	3.17 (0.29)	b	4.96 (0.04)	b	10.9 (0.37)	b
P		<0.001		0.001		<0.001		<0.001	
Peat	2	0.38 (0.02)		2.67 (0.41)		4.83 (0.11)		10.9 (1.09)	b
	3	0.28 (0.04)		1.79 (0.36)		4.67 (0.15)		7.9 (0.58)	ab
	4	0.28 (0.01)		1.75 (0)		4.75 (0.13)		7.4 (0.15)	a
P		0.056		0.148		0.679		0.028	
Control Compost	2	0.25 (0.00)	a	1.92 (0.04)	ab	4.54 (0.08)	a	8.3 (0.38)	a
	3	0.25 (0.01)	a	1.63 (0)	a	4.83 (0.11)	ab	8.4 (0.39)	a
	4	0.36 (0.03)	b	2.71 (0.41)	b	4.92 (0.04)	b	9.5 (0.43)	b
P		0.005		0.043		0.043		0.004	
Control Media	-	0.26 (0.05)		1.54 (0.44)		5 (0.00)		7.3 (1.05)	

* means followed by the same letter in the same column are not statistically different ($p<0.05$) according to Tukey's HSD

Table 1.8. Selected chemical characteristics of compost at different ages.

Compost	Age at time of sampling	pH	EC dS / m-1	NO3-N ppm	NH4-N ppm	P ppm	K ppm	Ca ppm	Mg ppm	Na ppm	Cl ppm
Manure	<1yo	7.9(0.1)	10.6(1.14)	734(132)	1.8(0.2)	39.7(8)	2241(326)	880(174)	199(3)	5.16(0.54)	16.0(5.8)
	2yo	8	20	1410	0.8	34.8	4689	1043	450	580	1773
	3yo	6.8	17.84	1776	1.3	43.3	3024	1113	531	456	723
	4yo	5.9	23.5	2430	2.2	240	2835	1391	900	380	879
SCG	<1yo	7.6(0.6)	4.5(0.22)	4.5(1.5)	47(15.3)	118(52)	890(405)	307(8)	130(34)	1.65(0.35)	17.8(8.1)
	2yo	7.1	7.51	192	0.9	173.3	2211	261	189	80	987
	3yo	6.1	12	1080	0.7	120	2403	470	300	96	381
More Grass	<1yo	7.7(0.2)	6.7(1.27)	446(68)	1.3(0.7)	44.7(5.0)	1505(347)	510(202)	138(45)	1.80(1.10)	12.9(2.9)
	2yo	8	11.27	498	0.7	10.5	4011	417	194	42	1118
	3yo	7.2	11.27	1116	1.5	22.4	1647	835	336	42	369
	4yo	6.1	13.64	1472	0.5	74.3	1932	1137	394	76	493
Wrapped	<1yo	8.3(0.2)	6.2(0.07)	323(22.6)	0.8(0.2)	16.6(2.6)	1380(138)	480(120)	122(19)	2.00(1.35)	16.3(3.8)
	2yo	7.7	13.38	882	1.0	22.4	3069	678	276	48	1400
	3yo	6.9	17.6	1710	5.1	20	2529	1304	560	30	827
	4yo	7.9	7.03	172	1.4	10.6	1875	348	164	26	818
Base Mix	<1yo	8.1(0.2)	4.2(0.23)	250(8.6)	0.8(0.3)	22(4.0)	965(68)	380(82)	78(36)	2.67(1.67)	16.5(5.5)
	2yo	7.8	11.27	828	0.7	17.9	2472	678	276	48	1036
	3yo	6.8	13.38	1320	5.9	22.4	1836	939	365	42	404
	4yo	7.1	11.76	1264	1.3	16.7	1458	1053	384	112	413

Table 1.8 (cont'd)

Compost	Age at time of sampling	pH	EC dS / m-1	NO3-N ppm	NH4-N ppm	P ppm	K ppm	Ca ppm	Mg ppm	Na ppm	Cl ppm
More Leaves	<1yo	8.2(0.2)	5.0(0.66)	250(44)	1.0(0.3)	13.7(2.2)	1097(191)	400(48)	89(3)	0.93(0.19)	17.8(4.8)
	2yo	7.8	12	984	0.6	14.5	2133	783	324	54	780
	3yo	6.9	6.11	576	1.0	28.8	909	452	212	64	254
	4yo	6.5	11.75	1160	1.4	27	1221	1000	360	95	413
Paper	<1yo	8.8(0.3)	4.2(0.27)	88(64)	1.9(1.0)	5.9(1.3)	829(152)	520(95)	102(5)	4.33(1.18)	23.3(9.2)
	2yo	7.9	10.61	1145	1.5	7.4	2025	991	342	270	2591
	3yo	7.9	10	1030	1.1	5.8	1307	870	290	145	308
	4yo	7.9	9.39	915	0.7	6.2	1157	913	328	195	385
Shavings	<1yo	8.1(0.4)	1.8(0.18)	1.1(0.9)	5.6(4.8)	29.6(10.7)	482(70)	200(43)	49(8)	4.83(2.86)	20.6(4.5)
	2yo	8.4	2.47	6	0.1	24	732	183	54	34	440
	3yo	6.7	10	975	1.7	30	1508	826	320	95	433
	4yo	6.7	4.46	368	1.6	37.2	369	400	142	98	309
Control Compost	<1yo	6.3(0.2)	3.2(1.21)	208(110)	3.2(0.6)	64.3(13.2)	801(231)	245(151)	78(36)	1.50(0.20)	11.5(2.5)
	2yo	6.2	4.69	332	0.1	200	1518	157	91	58	207
	3yo	4.9	6.55	612	33.7	99.7	789	383	232	56	277
	4yo	6.7	5.63	489	0.8	41.7	837	339	174	72	190
Peat	<1yo	5.7(0.3)	1.3(0.27)	32(18)	0.6(0.1)	32.9(7.2)	217(30)	115(63)	42(12)	2.77(0.78)	11.4(0.9)
	2yo	5.2	4.69	386	0.5	129.3	729	174	141	36	273
	3yo	5.5	4.0	328	0.4	72.1	446	243	142	68	185
	4yo	7.9	11.22	1260	1.9	55.7	446	365	147	116	287

* numbers in parenthesis are standard errors for mean values from all 3 years compost was made. All other values based upon analysis of a single sample.

Table 1.9. Mean harvest weights (+/- SE, n=6) of basil, and pH of media from 1-year old compost media, grown in 2015.

Compost Treatment	Harvest Weight (g)		pH	
Manure	175 (15)	bc*	8.4 (0.09)	bcd
Food	197 (24)	c	8.2 (0.09)	bc
More Grass	164 (9)	bc	8.6 (0.04)	cde
Wrapped	134 (18)	b	8.7 (0.05)	e
Base Mix	169 (11)	bc	8.6 (0.05)	de
More Leaves	178 (18)	bc	8.7 (0.03)	e
Paper	137 (13)	bc	8.7 (0.01)	e
Shavings	38 (7)	a	8.2 (0.10)	b
Peat	351 (10)	d	7.4 (0.09)	a
Control Compost	418 (21)	e	7.5 (0.10)	a
P	<0.001		<0.001	

* means followed by the same letter in the same column are not statistically different (p<0.05) according to Tukey's HSD test

Table 1.10. Basil harvest weight, root media pH, and recently mature leaf tissue analysis from 'Control' (with normal leaf coloration) and 'Food' (chlorotic leaf coloration) compost media treatments.

	Root Media		
	Control Compost	Food Compost	P
Harvest weight	424 (27)	220 (31)	0.003
pH	7.4 (0.15)	8.3 (0.09)	0.003
N	3.3 (0.19)	5.2 (0.09)	<0.001
P	0.57 (0.01)	0.88 (0.04)	<0.001
K	5.5 (0.31)	10.2 (0.24)	<0.001
S	0.24 (0.01)	0.33 (0.01)	<0.001
Ca	1.00 (0.08)	1.06 (0.07)	0.592
Mg	0.45 (0.03)	0.49 (0.03)	0.367
B	16 (0.7)	23 (1.5)	0.006
Zn	83 (7.2)	59 (2.6)	0.022
Cu	3.0 (0.41)	4.5 (0.29)	0.024
Mn	54 (7)	70 (6)	0.133
Fe	66 (2)	53 (13)	0.389
Al	7.5 (4.5)	18.5 (12.3)	0.434

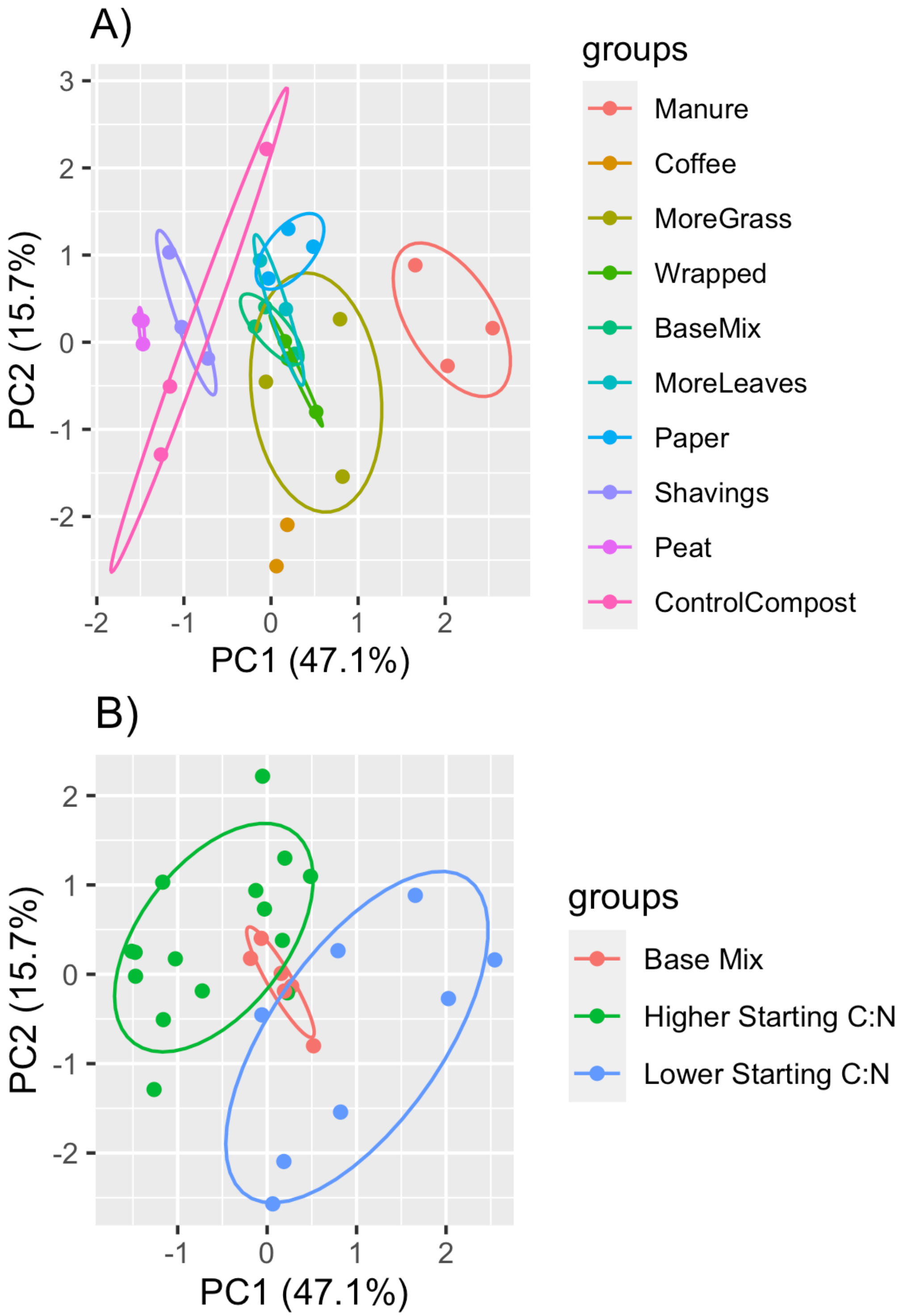

Figure 1.1. Principal component analysis biplot of the first and second principal components for compost physiochemical properties of A) all thermophilic composts and B) treatments grouped by starting C:N ratio. The percentages of the total variance accounted for by each principal component are indicated in parentheses in axis titles.

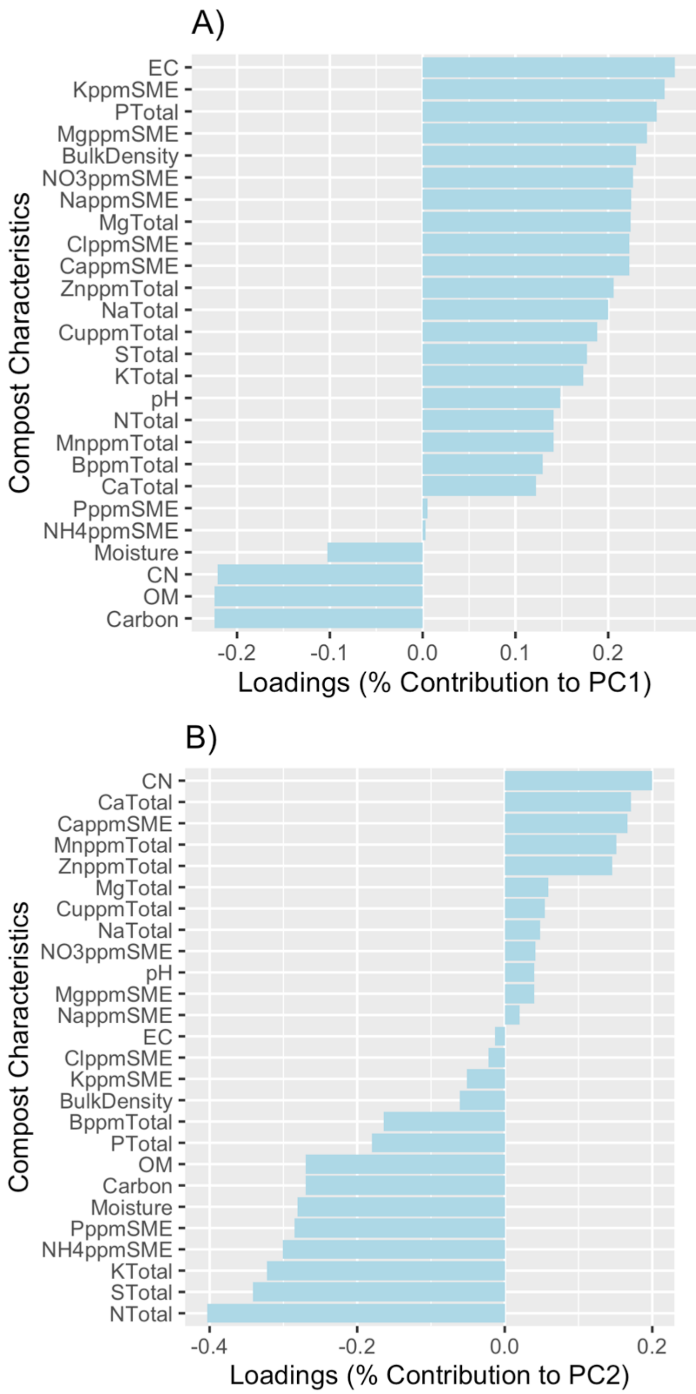

Figure 1.2. Loading contributions of compost physiochemical characteristics for interpretation of PCA values for A) PC1 and B) PC2.

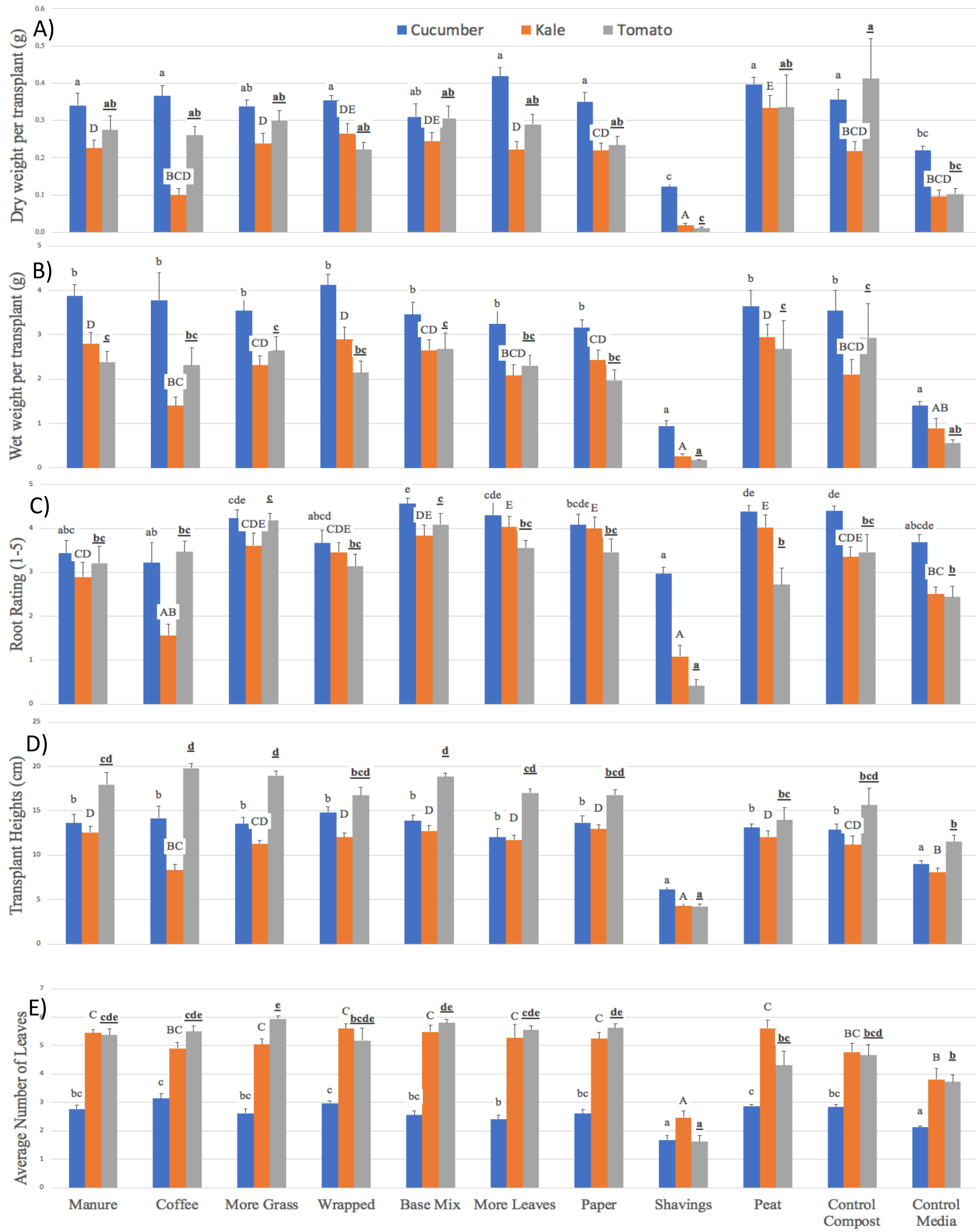

Figure 1.3. Plant growth data for all plant species for A) dry weights, B) wet weights, C) root rating, D) plant heights and E) number of leaves. Columns denoted by the same letter within the same species are not different according to Tukey's HSD test (p<0.05).

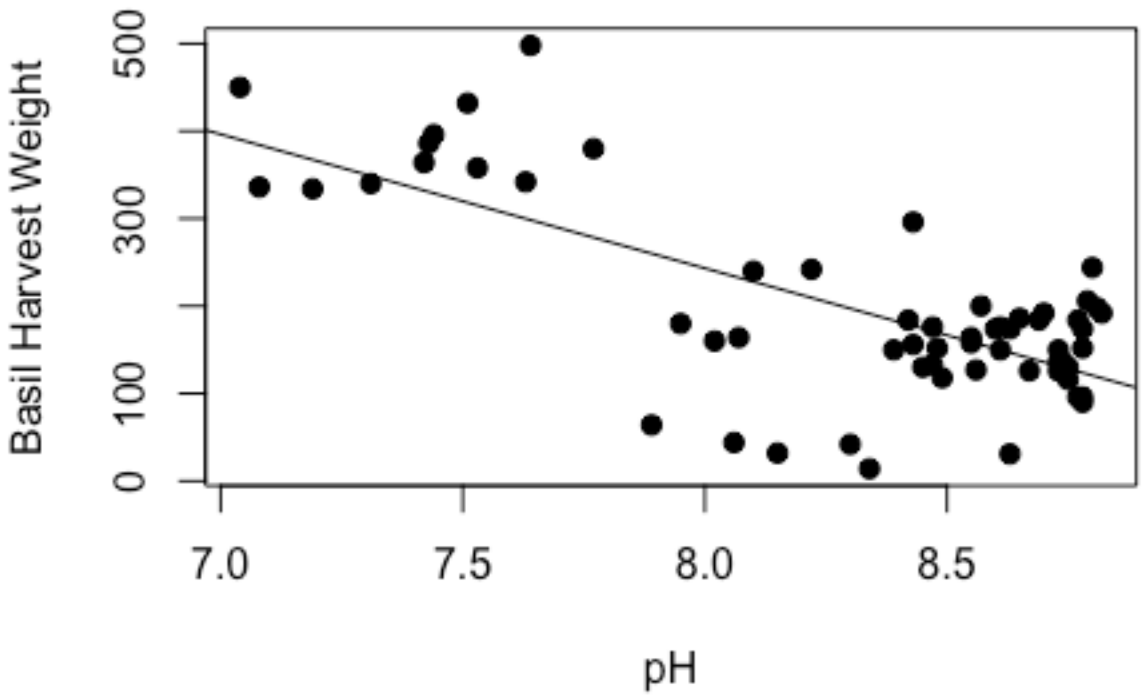

Figure 1.4. Linear regression of relationship between pH and basil harvest weight. $P<0.001$, $R^2=0.50$.

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CHAPTER II

BIOCHAR AND ANAEROBIC DIGESTER EFFLUENT IN COMPOSTING: EFFECTS ON FINISHED COMPOST FOR GROWING ORGANIC CUCUMBER TRANSPLANTS

ABSTRACT

Properly designed and aged compost is a promising alternative to peat based substrates for cultivation of transplants and plants in containers. The objective of these studies was to evaluate biochar and liquid anaerobic digester effluent (ADE) as amendments at the start of composting, for both the effects on finished compost characteristics and transplant growth using compost-based root media. Thermophilic compost was made in static piles of 1yd^3 starting volume with a base recipe of equal volumes dried municipal leaves and fresh cut grass for the purpose of producing transplant growing root media. Amendments to the base recipe included 1) 10% biochar by volume; 2) 20 gallons/cubic yard added liquid anaerobic digester effluent (ADE); 3) a combination of 10% BC and 20 gallons ADE; and 4) the base recipe without additions as a control. In a separate experiment, vermicompost was also produced over a period of nine months, with and without biochar addition at 10% by volume. Compost samples were analyzed for both total and water soluble nutrients as well as physical properties at five months maturity for thermophilic composts and fully mature vermicomposts aged over one year. The two different composting methods were employed to assess if the effects of biochar on the physical and chemical characteristics were consistent in both systems. Differences in finished compost characteristics included higher soluble nutrient values driven by the addition of ADE but not biochar, while biochar inclusion increased C:N and decreased bulk density for the thermophilic composts. A greenhouse cucumber bioassay was conducted with the finished thermophilic composts and a control treatment consisting of a standard peat based medium. Cucumber transplants were evaluated one week after seeding for emergence, and at three weeks after seeding for above ground biomass, height, number of leaves and root rating, with data analyzed as a two-factor ANOVA of biochar and ADE, and with Dunnett's test for comparisons

to the peat medium. Transplants in all compost media outperformed the peat-based control for all metrics except root rating. Within compost mixes, addition of biochar improved all metrics of transplant growth. In contrast, addition of ADE reduced several metrics of transplant health including shoot dry weight and root ratings. The 100% compost media supplied adequate fertility and physical characteristics to grow cucumber seedlings, though the underdeveloped root systems would likely cause problems when planting into a field. The nature of the effect of biochar on compost should be reviewed further as results were dependent on what type of composting employed (thermophilic or vermicomposting). In thermophilic compost used as a root media, co-composted biochar may be beneficial, though more research is needed to optimize the use of this amendment.

INTRODUCTION

Transplant use provides a number of benefits for vegetable farmers and the quality of transplants impact management practices and potentially yield. By avoiding germination problems encountered with direct seeding, transplants ensure uniform plant establishment and more dense plant populations (Burnett et al., 2016; Sterrett, 2001). Keeping young plants in one easy to monitor and control location helps with protection from diseases and pests until they are ready to be planted out, at which time they should be better able to withstand such pressures. This in turn helps with improved ground cover (weed) management. Use of transplants can better ensure predictable harvest times as well. They shorten the production cycle, providing more time for cover crops to improve soil quality. The shortening of the production cycle is particularly important for certain crops based on growing season length in areas such as the Midwest. Nearly every organic vegetable farm, regardless of size, uses transplants, but because organic transplants can be difficult to source, farmers often produce their own, with inherent difficulties.

The qualities of the root media can have long-lasting ramifications on yield (Dufault, 1998; Jack et al., 2011; Raviv et al., 1998). Transplant/container culture involves a restricted root zone, in which sufficient water, nutrients and aeration for the growing transplant must be provided (Raviv, 2011). Desired root media characteristics include good water holding capacity, high pore space and low bulk density (Sterrett, 2001).

Organic agriculture is expanding and with it the demand for root media that are approved for use in an organic system. Fertilization regimen in organic agriculture is limited in options from natural sources such as fish emulsion, feather, bone or blood meal, and producers are often weighing the benefits against the high costs and environmental footprint of these choices (Clark & Cavigelli, 2005; Kuepper & Everett, 2004). For organic vegetable farmers the general recommendation has been to invest in a uniform, high quality transplant growing medium, which are most often peat-based, and may or may not include compost, as well as using these limited fertilizer options.

Peat-based root media may be the most commonly used in horticulture, however, there is growing demand for an alternative. The positive physical characteristics of peat that make it an ideal media component have been difficult to match. These attributes include its low bulk density, high pore space and water holding capacity (Raviv, 2011). However, peat harvest may result in environmental degradation, the regeneration time is long, and increasing production and transportation costs make it increasingly expensive (Clark & Cavigelli, 2005; Lazcano et al., 2009; Pelaez-Samaniego et al., 2017; Ridout & Tripepi, 2011; Treadwell et al., 2007). Still, most container substrates on the market have peat in the range of 80% and upwards (Ceglie et al., 2015).

Composting has been employed by farmers for centuries, with compost being used to increase soil organic matter and nutrients, soil water holding capacity and nutrient retention, and to provide other benefits. Using local resources to turn what would be a waste stream into a valuable resource can help to create a circular economy in which nutrients flow back to the land (Peng & Pivato, 2019). Compost use in root media cultivation, either at 100% or as a component, provides a much more regenerative/sustainable system. Development of affordable compost-based transplant media using feedstocks that are readily available and generally consistent throughout the United States could increase the adoption of compost based media and reduce use of fertilizers.

Two methods of aerobic composting are thermophilic or “hot” composting which employs microorganisms at elevated temperatures (130°F minimum), and vermicomposting which employs microorganisms and composting worms at ambient temperatures, to aid in the breakdown and humification of feedstock materials (Dominguez & Edwards, 2011; Edwards et al., 2010). Both methods are an effective means of cycling nutrients and produce nutrient-rich compost, with vermicompost often having higher nutrient levels but this is based largely upon starting feedstocks. A primary difference often cited between compost derived from these methods are the microorganisms present which can have variable effects on plants.

Compost, whether thermophilic or vermicompost, provides soluble and long-term nutrients, water holding capacity, etc. Composts with high total porosity and air-filled porosity are more likely to be used as a transplant media (Raviv, 2005). However, compost variability can be a concern (Beozzi et al., 2017; Michael Raviv, 2011), making more careful management of transplants necessary (Burnett et al., 2016). Concerns with compost have been high salinity levels, poor physical characteristics such as high bulk density, phytotoxicity, high pH and heavy metals (Beozzi et al., 2017; Ceglie et al., 2015; Rogers, 2017). For widespread adoption on a larger scale, growers would need assurances that a compost can reliably provide a uniform, stable, and readily available product (Sterrett, 2001). There have been numerous studies with compost used in root media. Many have used compost as a component and not at 100%, often using different percentages to arrive at the optimal or cut-off at which the negatives outweigh the positives of its inclusion in media. The goal herein is to study compost effects on transplants in isolation by using 100% compost as a growing media. Understanding these shifts by individual growth media components, in this case different types of compost, will provide information helpful for making combinations that optimize root and overall transplant health.

To develop compost specifically for use as a growing media, biochar is an attractive component as it has many of the same characteristics that make peat desirable for growing plants in containers such as biological stability, good water holding capacity, and micropores (Lehmann & Joseph, 2009). It has also been compared with perlite/vermiculite for these same reasons. It does, however, convey additional benefits to a root media. Biochar is pyrolyzed biomass consisting primarily of polycyclic aromatic hydrocarbons and is relatively recalcitrant in nature (Cheng et al., 2008; D. J. Lehmann & Joseph, 2009). Some of the physical and chemical properties affected by biochar are increases in water holding capacity and soil aggregation, modifications of pH, changes in nutrient dynamics, and reduction of bulk density, amongst others (Guo et al., 2020; McCormack et al., 2013). It has many of the same benefits as compost, particularly in storing carbon in the soil, raising soil organic matter, increasing water holding capacity and nutrient holding capacity. Biochar can be incorporated into compost as an amendment prior to the composting process as an effective means of retaining nutrients, though results vary (Steiner et al., 2015). Despite these positive attributes, experimental results are inconsistent because biochar varies by feedstock type, pyrolysis temperature, and retention time, and interacts differently across different soil types, crops, etc. (Laghari et al., 2016; Sun et al., 2014). Depending on the source, biochar can be a relatively expensive input on a farm (Mihreteab et al., 2016), and the fine dust-like nature of the material can make it potentially dangerous to the applicator depending on the means of land application (Lehmann & Joseph, 2009). One means of overcoming these obstacles could be pinpointed application by having the biochar in larger percentages at the root zone of plants that are to be transplanted out into the field. As biochar does not easily degrade, there will be eventual accumulation of biochar in the soil as new transplants are put into the field each year in the case of an annual cropping system. Some past research indicated that biochar can bind up nutrients in the first few years, but co-composting biochar can alleviate this issue by charging it with nutrients (Jeffery et al., 2011; Kammann et al., 2014; Wang et al., 2019). Similarly, for use in root media, biochar that has not been co-composted may bind nutrients and have negative effects on transplant growth as seen by Nair and Carpenter (2016). There is a labile fraction of carbon in biochar that can result in immobilization of nutrients, but this fraction is likely to have been consumed during the composting process so that the remaining carbon is in the more recalcitrant form (Chan & Xu, 2009). While biochar has been researched in compost production, the focus has been biochar effects on N retention and organic matter processing with little to no attention paid to other macro- and micronutrients (Steiner et al., 2015). A primary question being addressed in this study is the effect of softwood biochar on compost, both thermophilic and vermicompost, and biochar effects on plant growth when included as a component of compost-based growing media.

For a plug tray of transplants, the root zone volume is limited and available nutrients are easily lost by leaching during irrigation (Bilderback, 2002), but compost and biochar may help to alleviate these losses.

Anaerobic digester effluent (ADE) is a resource that could be better used through composting in creation of a circular economy in which nutrients/resources are conserved in the agricultural system. Anaerobic digesters have been growing in popularity as a means of generating electricity and decreasing volume of organic matter, especially for dairy facilities in the US (Pelaez-Samaniego et al., 2017; US EPA, n.d.). While much of the carbon is converted to methane, the remaining effluent or digestate (solid and liquid fractions, which are generally separated) retains nutrients in generally high concentrations, especially for ADE from dairy manure (Fuchs & Drosg, 2013; Pan et al., 2018; Tambone et al., 2010). The liquid ADE is particularly high in N and K, while more of the P is retained in the solid fraction (Logan & Visvanathan, 2019; Milles, 2014; Möller & Müller, 2012; Peng & Pivato, 2019). While the solids from ADE have been investigated as a composting feedstock, little attention has been paid to the liquid ADE fraction. The liquid is often seen as a negative for ADE facilities primarily due to its high water content that makes it expensive for transport (Akhiar et al., 2017; Lin et al., 2014; Peng & Pivato, 2019). Other concerns include high ammonia content, potential plant or human pathogens, and smell (Alburquerque et al., 2012; Bustamante et al., 2013; Pelaez-Samaniego et al., 2017; Zeng et al., 2016) which may be alleviated by composting ADE with biochar. Lin et al. (2014) noted that the predominant forms of nitrogen in ADE are NH_3 and NH_4^+, which would be more likely to be lost to volatilization or leaching, whereas composted ADE in their study was 74% organic nitrogen which leads to more long-term N turnover. The biochar in a composting system would be more likely to retain that ammoniacal-N, preventing its volatilization and leaching.

The first objective was to characterize the effects of biochar and anaerobic digester liquid effluent on finished compost physical and chemical characteristics. We hypothesized that biochar in both the thermophilic compost and vermicompost experiments would result in greater nutrient retention (of both soluble and total nutrients, thereby also increasing EC), and that this nutrient retention effect would be more pronounced in the presence of elevated nutrient levels. We hypothesized that biochar added to compost would result in an increase in the water holding capacity, organic matter/carbon, C:N and pH, while lowering bulk density of the finished compost. We further hypothesized that ADE would elevate nutrient concentration and EC. The second objective was to contribute towards the development of affordable compost-based transplant media for production of vegetable transplants by assessment of the composts made in Experiment 1 by growing cucumber seedlings. We hypothesized that the compost media could grow acceptable cucumber transplants without modification, and that the amendments added at the time of compost production would result in differences in the transplant morphological features assessed, with biochar in particular increasing plant growth. While much of the research surrounding compost for root media assesses specific composts and their use for growing plants, this research aim is to address how individual components/feedstocks impact the finished compost and its use for transplant production in order to guide compost producers or farmers to be able to achieve the desired compost characteristics.

MATERIALS AND METHODS

Experiment 1: Thermophilic Compost Treatments, Experimental Design and Management

Composts were made at the Michigan State University (MSU) Horticulture Teaching and Research Center (HTRC) in Holt, MI (42°40" N, 84°28" W). Experimental treatments consisted of four compost recipe combinations. The base feedstock mix ("Mix") was grass freshly cut from organically managed/certified property at the HTRC and senesced municipal leaves from the city of East Lansing, MI, at equal parts by volume which were thoroughly mixed by layering in a PTO-driven manure spreader and ejecting into a pile. Experimental treatments consisted of 1) the base recipe without additions; 2) base mix plus biochar at 10% by volume ("Mix+BC"); 3) base mix plus liquid ADE ("Mix+ADE"); and 4) base mix plus biochar at 10% and ADE ("Mix+BC+ADE").

The biochar used in the experiment was from Biogenic Reagents (Circle Pines, MN), made from softwood biomass. Biochar characteristics were supplied by the company and guaranteed a minimum surface area of 400 m^2/g, minimum carbon at 90%, maximum ash and volatile matter both at 5%, and a pH in the range of 7-9. For each treatment with biochar, 22lbs or 22.5 gallons were added and mixed as the base mix was put into the compost bay to make a homogeneous pile. The calculated rate of biochar was approximately 10% by volume at the start of composting. With a volume reduction by the end of the composting process of nearly half, the % biochar in the compost would be considerably higher due to its recalcitrance relative to the base mix. Final proportion of biochar may near 20% by volume.

Anaerobic digester liquid effluent (ADE) was obtained from the MSU Anaerobic Digester Research and Education Center (ADREC) located at the MSU Dairy Facility on College Road, East Lansing, MI. The ADREC digester is an electricity co-generation facility with the capacity to store two million gallons of ADE. Twenty gallons of ADE were added to all piles for which AD was a part of the treatment; treatments that did not receive ADE were moistened with 20 gallons of water.

Compost bays were made using standard (40"x48") wooden pallets (lined with cardboard) to allow for approximate starting size of a cubic yard for each static pile. The 12 compost piles were maintained under a moveable (sled) open-ended high tunnel for rain exclusion. Treatments were organized in a randomized complete block design (RCBD). Materials were moistened by a hose with a breaker as piles were constructed. Temperature data were manually recorded daily taking the average of two 18in compost thermometers to ensure proper turning of all piles based upon thermophilic heating curves. Piles were turned manually with a pitchfork when internal temperatures fell below 130°F (four times). Compost was started in July 2015, turned four times and left to mature/cure at the end of August 2015.

Experiment 2: Vermicompost Treatments, Experimental Design and Management

Vermicompost beds were constructed at the same location inside a stationary 30'x72' unheated high tunnel. A base mix of pre-consumer kitchen preparation food residue, municipal leaves, shredded office paper, sphagnum peat, used coffee grounds and spelt dust was pre-composted for approximately 2 weeks (approximate ratio of 10:10:2:2:1:1 by volume). The uniform mixture was placed in six 21ft^2 (3'x7') vermicomposting beds (static piles) to a depth of approximately 1.5 feet, being added in shallow layers (~4" at a time) over a period of a month to drop the temperature of the pre-composted mixture and ensure it did not heat again to allow for the addition of worms. Experimental treatments were the base mix on its own, or with biochar thoroughly mixed in at approximately 8% by volume. Volume reduction at the termination of vermicomposting averaged 40%; assuming the recalcitrance of biochar left the majority of BC intact, the final %BC by volume would be approximately 13%. The biochar in Experiment 2 was the same biochar from Biogenic Reagents as used in Experiment 1. There were three replications of each treatment totaling six experimental units, arranged as a randomized complete block design (RCBD).

After the pre-composted material with or without biochar was added to beds, approximately twenty pounds of composting worms (*Eisenia fetida*) were added per bed on top to move into the bedding /food mixture. This is referred to as a "batch" system of vermicomposting compared to a "bin" system of vermicomposting where food is added at regular intervals to existing bedding (Edwards et al., 2010). Beds were started in the fall and left for nine months (September-May) to be converted by worms into finished vermicompost. This included the winter months, where worms moved to the lower parts of the beds that were warmer, and spring months were worms returned to the surface layers. Worms were extracted using crates of fresh pre-composted material on the surface. The finished vermicompost was screened through half inch hardware cloth to provide a uniform product. Vermicompost samples were submitted for laboratory analysis for chemical and physical properties.

Experiments 1 and 2 Data Collection

Composite samples from ten areas in each compost pile (thermophilic composts) or bed (vermicomposts) immediately after they had been mixed were taken for laboratory analysis of chemical and physical properties. This was approximately five months after composting had begun for thermophilic compost, and after nine months being vermicomposted. Key responses measured from mature compost samples were for nutrients including both total nutrients by ashing (% N, P, K, Ca, Mg, S, Na; ppm Zn, Mn, Fe, Cu, B, Al) and water soluble nutrients using saturated media extract (ppm nitrate-N, ammonium-N, P, K, Ca, Mg, Na, Cl), chemical characteristics of electrical conductivity (EC), pH, % organic matter and physical characteristics of bulk density and percent moisture. Calculations for the percent carbon and the carbon to nitrogen ratio were obtained from the data and the percent of each nutrient contribution to the electrical conductivity was also calculated using the equation

[1] % Element = ((ppm element) / (EC mg/L * 700)) * 100 (Warncke & Krauskopf, 1983).

For Experiment 1 only, additional data of EC and pH data were collected for all the thermophilic composts again using a 1:2 dilution of compost to deionized water (v/v). This was at the time of growing cucumber transplants in the fall of 2016, when compost had additional time to mature to approximately 15 months old

Experiment 3: Transplant Experimental Design, Management and Data Collection

Finished composts from Experiment 1 (Mix, Mix+ADE, Mix+BC, Mix+BC+ADE) were used as planting media (100% compost) for cucumber cv. Marketmore 76 (*Cucumis sativus*) transplants. An additional treatment of Sunshine Potting Mix (Sun Gro Horticulture) which is a peat-based media, commercially available and approved for use in organic production systems, was also included. For the four compost media treatments, there were 12 replications each, (four replications from each of the original three compost replications), and four replications from the peat-based media, totaling 52 experimental units, each of which consisted of four 4-cell packs, totaling 16 plants. All units were arranged in a randomized complete block design (RCBD), each block being arranged on a single greenhouse bench. Flats were watered daily and no supplemental fertilizer was added.

The experiment was carried out in the research glass greenhouses of MSU in November 2016 when the compost had been curing for approximately one year, 15 months from starting the compost piles. Supplemental lighting was supplied with three overhead 400W HPS lamps suspended 4’ above a 3’x18’ bench for a 16 hour photoperiod. Air temperature was maintained at a minimum heating setpoint of 65F with ventilation at 75F. Transplants were watered daily by overhead watering.

Compost media were filled into cells with settling and seeded, planting two seeds per cell. Seven days after planting, emergence data were collected with the number not emerged seeds being recorded to obtain a percentage for emergence (number of seeds emerged out of 32 possible per experimental unit). Delayed emergence led to an additional collection of data for emergence at 10 days as well. Cells were then thinned to one plant per cell by shoot removal.

Transplant data was taken at three weeks, with a minimum of two fully expanded leaves. The data collected to assess quality of transplants were above ground biomass (both wet and dry weights), root rating (categorical variable 0-5 of qualitative rating per transplant plug, Figure 2.1), average plant height (cm) and number of true leaves. All 16 plants were used for obtaining wet and dry weights, and total number of leaves. Samples were dried in a forced draft oven at 60°C for 72 hours. For other metrics the observational unit was a subsample of eight plants selected at random from each experimental unit. Plant height was a visual average of these eight plants, while root rating was a cumulative total for the eight plants in the observational unit. The root rating was a proxy for root biomass due to the difficulty of separating roots from the media; this also correlates to the ease with which transplants are able to be pulled from a plug tray and how well they fill out the volume of the plug. Data was converted to per plant by dividing by the applicable number of plants used to obtain the data. The ratio of roots to shoots was calculated using the root ratings and dry weights, and treatment differences analyzed for this variable as well, though this ratio is not reflective of what would typically be reported for root:shoot where both variables would have been by mass and not a categorical variable for root rating.

Statistical Analysis

For all three experiments, data analysis was carried out using R statistical software package, and additional packages for R including lme4 for linear regression, lsmeans for pairwise comparisons and ggbiplot for PCA (R Core Team, 2019). Unless otherwise noted, $\alpha<0.05$ was designated as level of significance. Data were assessed to ensure that ANOVA assumptions were not violated and were log transformed when needed prior to analysis. Sattherthwaite's degrees of freedom was employed to account for potential nonequal variance in the data. All data presented in figures and tables are non-transformed.

Chemical and physical properties of the thermophilic compost (Experiment 1) and transplant data (Experiment 3) were analyzed for treatment significance by two-way analysis of variance (ANOVA) with main factors being presence/absence of biochar, and presence/absence of ADE. To account for the blocking effect, a random effect term was included: block (rep) for the compost data, and transplant block (rep) nested within compost block for transplant data. Significant differences between treatment means were determined by pairwise comparison and separated according to Tukey's highly significant difference (HSD) test ($P\leq0.05$). The transplants from the peat-based media used as a commercially available control were also analyzed, comparing this treatment to the compost treatments using Dunnett's Test.

The data from Experiment 2 (chemical and physical compost properties) were analyzed for treatment significance by one-way analysis of variance (ANOVA).

Principal component analysis (PCA) was conducted on the standardized compost physical and chemical characteristics data (for Experiment 1 and 2, separately). PCA is a tool for data compression, to capture the main features in the data set and to extract information from multivariate, high-dimensional data sets. With the large number of dependent variables, PCA is a descriptive technique used to explore and visualize the data for relationships among variables (physiochemical characteristics) and potential differences among composts. The PCA was run for 28 different variables- the soluble and total nutrients, EC, pH, percent organic matter, carbon to nitrogen ratio (C:N), bulk density and moisture (with two sets of data for EC and pH in Experiment 1, taken when compost was 5 and 15 months old). While % soluble salts were calculated from the ppm of each nutrient and the EC (Equation 1), these numbers were not included in the PCA analysis because this data was already captured by the ppm of each nutrient. Visual representation of the PCA was made by creating bi-plots of the first and second PCs. Loading contribution of compost characteristics to PC1 and PC2 were used for interpretation of the PCA.

RESULTS

Experiments 1 and 2: Compost Physiochemical Characteristics

There were a number of differences observed between treatments for the various physiochemical properties of the finished compost (Tables 2.1, 2.2, 2.3). Dry bulk density was negatively correlated with biochar amendment, decreasing from 0.37 to 0.32 g/cc with addition of biochar (a 14% decrease) but was not affected by ADE in Experiment 1 (Table 2.1). However, the vermicompost did not differ in bulk density values, which were higher at 0.47-0.50 g/cc compared to the thermophilic composts. While neither main factor of biochar or ADE were significant for moisture level (44% to 54%), the interaction was ($p=0.041$) with an increase in moisture with inclusion of biochar if ADE was not present, but a decrease in moisture with inclusion of biochar when ADE was present. No difference in moisture was detected between vermicompost treatments.

Inclusion of biochar increased the C:N of the finished thermophilic compost (Table 2.1). From Experiment 2, vermicompost C:N was also increased with inclusion of biochar. In both experiments the decrease of C:N by biochar was 8-9%. For both experiments, the magnitude of the differences observed was small with values ranging from just 21 to 23 for Experiment 1, and 11 to 12 for Experiment 2. Organic matter and carbon content was not changed by biochar treatment in either thermophilic or vermi- compost.

The pH values of the more mature (15mo) composts were influenced by ADE, decreasing from 8.3 to 8.1 with the inclusion of ADE in the compost (Table 2.2). Although there were differences between treatments, the magnitude of the differences was quite small. There were no differences for pH values in the composts sampled at 5mo which were higher than the values at 15mo, indicating the continued maturation of the compost over this time. Biochar was not shown to affect the pH in either the thermophilic or vermi- composts.

For the EC of the younger compost samples (5mo) ADE increased these values, from 4.3 to 5.7 dS/m ($p=0.035$, Table 2.2). The EC taken at the time of growing the transplants was influenced by both biochar and ADE amendments ($p=0.016$ and $p<0.001$, respectively). Here again the addition of ADE was the primary driver, with compost mixes without ADE at 7.6, and inclusion of ADE increasing EC to 9.9 dS/m. In Experiment 2, biochar addition to the vermicompost decreased EC from 12.0 to 10.8 dS/m ($p=0.007$). EC values for both vermicompost treatments were very high, in the upper ranges of what we have observed in years of composting research and composting literature.

Regarding soluble nutrients, addition of ADE increased concentration of all nutrients except Mg and NH_4-N (Table 2.3). From addition of biochar to the compost, in contrast, no differences in any of the nutrients was observed for Experiment 1. There was an effect of biochar in the vermicompost, however, with decreases in soluble NO_3-N, K, Ca and Mg. This mirrors the lower EC of the Vermi+BC treatment. For Experiment 1, differences between treatments in nutrient values were detected for only soluble P and Na when looking at pairwise comparisons of all four treatments. Mix+BC+ADE had a mean value of 11.4 ppm P, higher than any of the other treatments. Additionally, soluble P was the only nutrient for which an interaction was observed between biochar and ADE, where biochar had no effect in the absence of ADE but increased P in the compost with ADE. Isolating the main effect of ADE then for the soluble nutrients, the following percent increases in nutrients were observed with the inclusion of ADE in the compost: NO_3-N +144%, P +43%, K +21%, Ca +38%, Na +233%, and Cl +30%.

The only difference observed between treatments for total nutrient values in Experiment 1 was an increase in zinc with ADE, though a number of other nutrients were marginally significant ($p<0.1$, data not shown). No differences between total nutrients were observed in Experiment 2.

Principal Component Analysis: PCA explained 59% of the variation in the data when taking the first and second principal components together (Figure 2.2a). Plotted against each other, both of the treatments that received ADE are grouped, and those without are grouped together. Presence or absence of biochar appears to be inconsequential or dwarfed by the impact of presence/absence of ADE when looking at the physical and chemical characteristics of the compost. The majority of the variables were for nutrient levels. ADE having the greater impact is not surprising as significant elevation of nutrients was expected.

With further analysis/segregation of the data, however, to isolate the effect of biochar only by comparing Mix v Mix+BC and Mix+ADE v Mix+BC+ADE, the trend emerges that the treatments are distinctly grouped by presence/absence of biochar in the compost recipe, which is also true when evaluating the biochar treatment effect in the vermicompost (Figure 2.2 b, c, d). Principal component analysis of the compost characteristics explained approximately 69% of the variation in the data when taking the first two principal components together for all three groups comparing biochar amended compost to the control.

The bi-plots of the first and second principal components demonstrate that the treatments are different from each other in regards to their physiochemical characteristics as the contrasts in the original variables pull the treatments apart in the visualization. The loadings of each variable as to how much each is related to the principal components is inconsistent for the different compost pairings displayed (Figure 2.3).

Experiment 3: Transplant Performance

Transplants grew in all media (Figure 2.4), with differences between treatments observed for early emergence and all plant metrics (Table 2.4). The differences in growth were driven by both inclusion of biochar and ADE as both of these main factors were found to be significant, with no interaction between biochar and ADE.

Seedling emergence data taken at seven days after sowing ranged from 91% in the Mix+ADE treatment to 96% for Mix+BC+ADE, with these two treatments differing from one another but not from the other treatments which were of intermediate percent emergence (Table 2.5). Difference between treatments was driven by inclusion of biochar as a compost amendment, increasing emergence by just over 3% (Table 2.4). There was also a marginally significant interaction between biochar and ADE ($p=0.072$) with biochar increasing emergence when ADE was also present. Differences, however, did not last as final emergence data taken at ten days after sowing displayed no differences for all compost media or control (range from 97-98.5%, data not shown).

Compost treatment differences observed were similar for both the shoot dry and wet weights (Table 2.4); mixes containing biochar had greater shoot weights than those without, however neither ADE nor biochar additions increased shoot weight relative to the control Mix. The differences observed between the peat-based medium and the compost media was more pronounced in the wet weights where all treatment means were different than this control, whereas for dry weight only Mix+BC was different from the peat control at $p<0.05$ (Table 2.5).

Within mixes, plant height had a similar trend as the aboveground biomass, with biochar increasing plant height by approximately 14%, and ADE decreasing plant height by 10% (Table 2.4). As compared with the peat-based medium, all compost media resulted in taller transplants ($p<0.05$ for all, Table 2.5).

The number of leaves per transplant ranged between 2.15 in the Mix to 2.29 in the Mix+BC+ADE (Table 2.4). Within compost mixes, biochar addition resulted in a small increase (approximately 5%) in leaf number, but ADE had no detectable effect. As compared against the peat-based medium, however, all compost root media had more leaves ($p<0.001$ for all, Table 2.5).

For root rating between the compost media treatments there were large differences in root growth in response to amendment with biochar and ADE (Table 2.4). Within mixes, biochar increased root ratings by 41%, while ADE reduced root ratings by 24%. Root rating was the one transplant metric for which the peat-medium control had a greater value than the compost media treatments ($p<0.001$ when compared against all other treatments), with the highest rating (5) of roots for all transplants assessed (Table 2.5). This contributed further to the higher root rating : dry weight ratio of the peat-control which was higher again than all compost-based media (p<0.001). Within the compost media only, biochar increased and ADE decreased this indicator by of partitioning to roots relative to shoots (16% and -18%, respectively).

DISCUSSION

With any study involving biochar or ADE it is important to note the specifications for the production of each material, such as feedstocks, production temperatures, etc. because these can all change the properties of the resultant biochar or ADE and how they will interact with compost (Laghari et al., 2016; Sun et al., 2014). For our biochar, a feedstock of pine biomass residue from forestry operations produced using pyrolytic fractionation has resulted in a biochar material that contained little to no nutrients, and had a pH range of 7-9. While softwood biochars have some nutrient content, it is much lower when compared to biochars made from manure or sewage sludge (Ippolito et al., 2020). The same is true for ADE as the ingestate material will greatly affect the quality of the digestate effluent. The primary ingestate for the digester from which we sourced the ADE in this study is dairy manure/material with additional inputs of food residue, fats, oils and grease. Solid and liquid fractions of digestate effluent were separated by a screw press. Analysis of fresh liquid ADE provided by ADREC had an average of 303ppm N, 67ppm P, and 264ppm K.

The hypothesis that C:N would increase with the addition of biochar to the compost was supported by both Experiment 1 and 2. The pairwise comparison only revealed differences between treatments in the vermicomposts, however, and not the thermophilic composts. This may have been influenced by the small degree of magnitude in the differences observed, low power and having multiple comparisons. C:N values observed (21-23) were slightly higher than the typical desired/expected range of 15-20 for finished compost. The small range in the C:N data between thermophilic compost treatments may have been in part due to the compost not being fully mature at the time of sampling as C:N ratios decrease with increased compost maturity. C:N is likely to have decreased more over the ensuing 10 months between the compost samples being analyzed and the transplants being grown. Due to the highly recalcitrant nature of BC, it is likely that the C:N of those treatments containing biochar would have declined less than the other treatments and differences would become more apparent. The more mature vermicompost did display a difference of a higher C:N with addition of biochar, though as previously noted the magnitude of the difference was small.

The hypothesis that dry bulk density would decrease with the inclusion of biochar in compost was supported in Experiment 1 but not in Experiment 2. This is supported by other studies as well (Guo et al., 2019; Husni & Samsuri, 2012). The bulk density of a softwood biochar is approximately 0.14-17g/cc (Guo et al., 2020; Harada et al., 2020), so it could be further hypothesized that higher percentages of biochar as a compost feedstock would further decrease bulk density based on the bulk density of our control compost at 0.39g/cc.

Contrary to our hypothesis, biochar addition to compost did not affect pH values of the finished compost; however, ADE was marginally significant ($p=0.057$), decreasing pH with its addition. Despite the statistical differences in pH, the spread of the data was very small, with all media having a much higher pH than would be typically recommended for a growing media (5.2-6.3; Warncke & Krauskopf, 1983). Compost used for transplant media may need to age or mature for longer periods of time to reduce pH, or be amended with peat or sulfur, or finished with vermicomposting.

While there was no difference between treatments for EC of the less mature (5mo) compost, mean EC of Mix+ADE was higher than the Mix ten months later. The net increase of

EC units of the Mix+ADE over the control is 47.5%; a study by Zhang et al. (2016) using straw-feedstock biochar and poultry manure compost resulted in an increase in EC of 7.0-37.5%. Herrera et al. (2008) found a similar result of high EC compost having a negative effect on tomato transplants. Theirs was a substitution experiment with different percentages of a high EC municipal solid waste compost with either old peat or white peat. The higher EC coincides with an increase in a number of the elemental nutrient concentrations in the treatment Mix+ADE (Table 1.1). Raw ADE is known to be high in nutrients as previously discussed in the introduction, and its addition to the compost was primarily to elevate nutrient levels so these results are to be expected.

For the purpose of this experiment the environmental parameters were managed as much as possible which included the exclusion of rain. However, in many composting settings this would not be true and the effects of biochar in compost may be greater as it may reduce nutrient leaching from the compost during rain events. Biochar effects on nitrogen loss in particular from composting systems have been documented in a number of different experiments (Guo et al., 2020; Major et al., 2009; Steiner et al., 2010). Using composting systems that do not exclude rain, the use of biochar may have more of an impact on nutrient retention than seen in the current study.

The nutrient balance of K, Ca and Mg must also be regarded in an evaluation of the compost media. Besides quantity of soluble K, Ca, and Mg in ppm, there are also recommendations for percent of the total soluble nutrients to take into account. Percent K recommended range is between 11 and 13% (Warncke & Krauskopf, 1983), whereas these values ranged between 25.1% to 27.4% in the thermophilic compost treatments. Percent Ca was nearly in the recommended range of 14-16% with values ranging from 12.5 to 14.1%. For Mg the values ranged between 2.8 to 3.5%, which is lower than the recommended 4 to 6%. While NO_3-N is not generally included in nutrient balance recommendations, the values herein were lower than recommended, ranging from 3.6 to 6.9%. Other nutritional analyses of commercially available compost-based planting media have been made that generally have a nutrient balance higher of Ca to K.

The treatments with ADE contained higher ppm levels of Zn as well, in particular Mix+ADE (Table 2.3), which is the treatment which consistently performed the poorest in all metrics of plant health (Table 2.4). Manure based digestates analyzed by Alburque et al. (2012) found high Zn concentrations, which can pose a problem, as well as Cu. Cu levels were not elevated in our study but were highest for the Mix+ADE treatment (21 and 16ppm Cu for Mix+ADE and all other treatments, respectively). There were a number of other factors that made the Mix+ADE treatment different than the others that could have contributed towards the decreased transplant growth, however.

Effects of biochar and ADE compost amendments on plant growth

The hypothesis that transplants could be produced in the 100% compost media and display acceptable characteristics was generally supported for cucumbers in this study, though individual growers may differ in what they would consider acceptable transplants. The roots of all compost treatments were under-developed relative to the peat-based media. Increases in root volume and branching have a long-term positive impact on post-transplant success in the field as plants would have higher capacity to exploit soil resources (López-Bucio et al., 2003). The peat-based control in this experiment had the highest ratings for roots and the highest root to shoot ratio. With the lower nutrient concentrations of this peat media this is in line with a number of experiments in which nutrient stress from deficiencies and particularly N deficiency stimulated more exploratory forms of roots and a higher adsorbing surface area ratio of roots to leaf area (Chung, 1983; Winsor & Massey, 1978). This is contradicted, however, by the findings of Lazcano et al. (Lazcano et al., 2009) wherein increasing proportions of compost to peat had a positive impact on root growth for treatments using vermicompost.

The hypothesis that inclusion of biochar in compost would be a primary factor in positive transplant growth responses was also supported. This may be attributed to a number of differences observed to be affected by biochar. The lower bulk density of the treatments with biochar and coupled with the positive growth attributes of transplants grown in the compost media with biochar provides further support to the need for lower bulk density media for transplant growth. Bulk density is often cited as a negative aspect of inclusion of compost in root media due to its high bulk density (Raviv, 2011), however it appears that inclusion of biochar then could help to ameliorate this problem for compost adoption in root media. Although total pore space was not measured in this study, it is inversely correlated with bulk density and this may favor root growth (Ceglie et al., 2015). In comparison, the Sunshine potting medium had a bulk density of 0.20 g/cc.

Delayed emergence or non-uniformity of emergence can negatively impact a crop production plan and transplant growth. The difference between the day seven seedling emergence and day ten emergence in the compost media indicates a delay particularly for the Mix+ADE treatment, and this may have had a lingering effect which may account for lower final biomass. The compost media with higher early emergence performed better for nearly all other plant metrics measured at day 21. The higher EC and concentration of certain elements such as Na and Cl in treatments with ADE are likely to have contributed to this delayed emergence, similar to the findings of Zhu et al. (2008) and Ebrahimi (2014).

The high EC of all the compost media may be seen as preventing the compost from successfully growing transplants without dilution. While EC above a certain number is considered prohibitively high, the composition of the ions contributing to EC should be taken into account. EC that is high due to the presence of Na and Cl may be detrimental to plant growth, high EC due to plant nutrients such as N, K and Ca may not be as detrimental. An SME EC value above 5 for a root medium is considered very high, and anything above 6 would be thought to produce severe salt injury symptoms (Warncke & Krauskopf, 1983), however, in the present work high EC value composts still produced acceptable transplants. However, that the highest EC compost, Mix+ADE, had the lowest root rating which supports the negative effects that EC can have. Reducing EC by blending high EC composts with lower EC composts, or materials such as peat, and having a better idea of a target range for EC when the nutrients are coming from compost as opposed to more soluble synthetic forms, could help in the development of compost root media.

One somewhat surprising result of our study was the negative effect of ADE on several plant growth indicators. ADE, particularly effluent with manure or post-consumer food residue as a feedstock which is what was used in this study, is known to have high salt concentrations of a number of different ions, but Na and Cl in particular can become a problem. Looking at just the Mix+ADE and Mix+BC+ADE treatments, there are elevated levels of Na and Cl in each compared to the control Mix. Sodium as a non-nutritive salt is contributing towards the higher EC also found for these treatments as compared to the treatments without ADE. The Mix+ADE consistently had values for all plant metrics lower or equal to the other treatments. Compared specifically to the Mix+BC+ADE the parameters of early emergence, dry and wet weights and root rating were all lower (Table 2.4), while the values for nearly all nutrients were not different between them (Tables 2.3) with the exception of soluble P. Thomas et al. (2013) found that biochar was able to ameliorate the negative effects of sodium chloride sorption of salts. Manure based composts are high in many important nutrients, but also Na which can be a negative as possibly seen in this experiment. Biochar may be able to counteract the addition of Na while still maintaining high nutrient levels in the compost for superior plant growth.

Interveinal chlorosis was observed in five of the 12 replications of transplants from the treatment Mix+ADE. Chlorosis of new leaves in particular may be due to high pH, root issues, or imbalances of nutrients (Ding et al., 2006). Of particular interest are Mg, Mn, and Fe all of which are needed for production of chlorophyll. Manganese and iron were analyzed as parts per million of the total nutrients (ash) and no significant differences were observed when comparing other treatments to Mix+ADE for these elements, though this does not necessarily rule out the possibility of an imbalance of these nutrients. It is important to note the changes that would have happened since the analysis of the compost samples especially in regards to the soluble nutrients and possible changes to their relative proportions. The increase of Na in composts with ADE may have thrown off the balance of the other nutrients and made it more difficult for these other nutrients to be taken up by the plants; leaf tissue analysis would help assess this but was not performed in this study.

Of note is the small amount of statistical power in the experiment due to the low number of replications. While there are variations in the means between treatments that may at first appear to be substantial, when evaluated using Tukey's HSD there is no significant difference between some treatments that were hypothesized. Greater power through additional replication may have identified more differences as significant.

FUTURE RESEARCH AND CONCLUSIONS

Based on knowledge gained from this experiment, future experiments could include biochar added at different percentages to assess the relationship of percent change in biochar to changes in specific physiochemical characteristics and the different percentages of co-composted biochar effects on transplants. Increasing percentages could determine a target rate beyond which the impacts of increased biochar have a negative effect on transplants. Comparisons could also be made of co-composted biochar in media versus fresh biochar as media component for growing transplants. Similarly, testing different rates of ADE added into a composting system could help assess whether the negative effects that were observed in the transplants could be ameliorated at a lesser rate while still achieving some benefits of nutrient additions from ADE. Use of the composts in transplant media could also be adjusted using different percentages mixed with another component such as peat, trying to determine a target percentage of root medium.

Further experimentation could compare performance of different plant species to assess the consistency of biochar impacts in root media. The differential responses of plant variables between treatments may be more pronounced in other plant species if tested, such as in results by Kaya et al. (2016) in which high salt content resulted in dry matter and chlorophyll reductions that were more pronounced in pepper transplants as compared with cucumber. Cucumbers have a much shorter production phase (two to three weeks) as compared with other vegetables such as brassicas or solanaceous transplants at four to six weeks, or alliums at 10-12 weeks. The nutrient load of root media and having more slow-release nutrients may have a greater impact when production cycle is longer and no supplemental fertilizer is used.

Future research to build upon the short-term impact on transplants could be a study of transplants planted out into the field to assess long-term ramifications of transplant media on harvestable yield and disease presence/severity. Elad et al. (2010) have shown that biochar can induce systemic resistance in plants, one means by which biochar in media may have long term consequences in the field. In addition, the compost root media could be used for growing plants to full maturity in containers and the impact on yield/health in this environment. Container plant culture in protected environments is a subsector of the specialty crop industry that is rapidly expanding (Rogers, 2017). If sowing and growing plants in the compost media to full maturity in a controlled environment (no transplanting), the decreased root growth observed initially for some treatments may not be an issue as there would not be concern over damage to sensitive roots during transplanting.

Such long-term field-study impacts of use of biochar in transplant media could incorporate an economic analysis as to a percentage increase/decrease in yield and profits versus the cost of incorporating biochar into media. A multi-year field study could also account for the gradual accumulation of biochar in the soil over time and the effect this has on soil properties.

Additional parameters that could be measured in future work include physical characteristics such as water holding capacity, total solids, volatile solids, aeration porosity, easily available water, water buffering capacity, and total porosity/pore space, CEC, air-filled pore space / air-capacity. Study of the biological changes in compost made with and without biochar, as well as adjustment of nutrient levels with ADE, and the relation of these variables with transplant performance would further the understanding of the effects of biochar. If analyzed to the species level, a similar statistical analysis using PCA that was used here for physiochemical characteristics could be employed to assess differences in the biology of different compost as a result of adding BC.

For all plant metric data, the main factors of biochar and ADE were significant. Based upon the data for these transplant metrics, it can be concluded within the confines of this study that the inclusion of ADE into the compost media resulted in a decrease in transplant quality, while the inclusion of biochar had a positive effect on transplants in these compost-based media. Although the values for many of the transplant metrics were higher in the compost based media compared to the peat-based media, the opposite was true for root rating, which is of particular importance for transplants. The poor performance of the compost media for this metric indicates that all of these composts may need to be blended with other media components to achieve more desirable root growth while still maximizing the positive effects on the above ground metrics. That the effect of biochar on compost was inconsistent between the thermophilic composts and vermicomposts, however, gives pause to any broad statements about biochar at large. More research into biochar / compost systems is needed.

APPENDIX

Table 2.1. Experiment 1 effects of biochar and liquid anaerobic digester effluent (ADE) addition to compost on physical characteristics of dry bulk density and moisture as well as organic matter, carbon and C:N. Experiment 2 effects of biochar addition to vermicompost on bulk density, moisture, organic matter, carbon and C:N. Mean (+SE, n=3) for all.

Experiment 1 Compost	Rate of Biochar	Dry Bulk Density g/cc	Moisture %	Organic Matter %	Carbon %	C:N
Biochar x ADE Interaction						
Mix	0%	0.39 (0.01)	44.1 (2.7)	70.3 (1.1)	40.8 (0.6)	22 (0.8)
	10%	0.31 (0.02)	53.7 (3.2)	69.2 (2.0)	40.1 (1.2)	23 (0.6)
Mix + ADE	0%	0.36 (0.01)	51.2 (3.6)	64.9 (2.7)	37.6 (1.6)	21 (1.0)
	10%	0.32 (0.01)	46.3 (1.0)	67.8 (3.2)	39.4 (1.9)	23 (0.4)
Main Effect of Biochar						
No Biochar		0.37 (0.01) a				21.4 (0.6) a
Biochar		0.32 (0.01) b				23.2 (0.3) b
Significance (p-values)	Biochar	0.007	0.438	0.721	0.709	0.017
	ADE	0.422	0.959	0.210	0.214	0.238
	Interaction	0.161	0.041	0.429	0.431	0.219
Experiment 2 Compost	**Rate of Biochar**	**Bulk Density**	**Moisture (%)**	**Organic Matter (%)**	**Carbon (%)**	**C:N**
Vermicompost	0%	0.47 (0.03)	63.2 (0.4)	64.4 (0.6)	37.4 (0.3)	11 (0.3) a
Vermi + BC	10%	0.50 (0.00)	64.1 (0.3)	65.6 (0.5)	38.0 (0.3)	12 (0.1) b
p-value		0.374	0.162	0.174	0.200	0.031

Means followed by the same letter in the same column are not statistically different (p<0.05) according to Tukey's HSD test

Table 2.2. Experiment 1 effects of biochar and liquid anaerobic digester effluent (ADE) addition to compost on compost pH and EC at 5 and 15 months old (mo). Experiment 2 effects of biochar addition to vermicompost on pH and EC. Mean (+/- SE, n=3) for all.

Experiment 1 Compost	Rate of Biochar	pH 5 mo	pH 15 mo		EC (SME, 5mo) dS m-1		*EC (15mo) dS m-1	
Biochar x ADE Interaction								
Mix	0%	8.6 (0.12)	8.3 (0.09)		4.0 (0.43)		7.0 (0.33)	
	10%	8.7 (0.00)	8.3 (0.03)		4.7 (0.36)		8.3 (0.44)	
Mix + ADE	0%	8.6 (0.03)	8.1 (0.03)		5.9 (0.23)		9.5 (0.61)	
	10%	8.3 (0.12)	8.2 (0.03)		5.6 (0.90)		10.2 (0.69)	
Main Effect of ADE								
No ADE		8.7 (0.06)	8.3 (0.04)	a	4.3 (0.3)	a	7.6 (0.4)	a
ADE		8.5 (0.08)	8.1 (0.03)	b	5.7 (0.4)	b	9.9 (0.4)	b
Significance (p-values)	Biochar	0.463	0.143		0.627		0.016	
	ADE	0.057	0.010		0.035		<0.001	
	Interaction	0.098	0.742		0.282		0.218	

Experiment 2 Compost	Rate of Biochar	pH	EC	
Vermicompost	0%	6.7 (0.06)	12.0 (0.00)	a
Vermi + BC	10%	6.6 (0.09)	10.8 (0.24)	b
P	P	0.561	0.007	

*EC values for 15mo compost were converted from EC values obtained from a 1:2 compost:water dilution using a conversion factor of 2.8 * EC (1:2). Conversion factor was calculated from ratio of SME EC to 1:2 EC readings from Warncke and Krauskopf, 1983

Means followed by the same letter in the same column are not statistically different (p<0.05) according to Tukey's HSD test

Table 2.3. Experiment 1 effects of biochar addition and anaerobic digester effluent (ADE) on compost soluble nutrients. Experiment 2 effects of biochar addition to vermicompost on soluble nutrients. Mean (+/- SE, n=3) for all.

Experiment 1 Compost	Rate of Biochar	NO3-N ppm	P ppm	K ppm	Ca ppm	Mg ppm	Na ppm	Cl ppm
Biochar x ADE Interaction								
Mix	0%	145 (17)	7.3 (1.1) b	997 (78)	362 (21)	95 (7)	36 (0.3)	616 (44)
	10%	121 (22)	6.0 (0.2) b	1170 (107)	413 (24)	109 (9)	36 (3.5)	758 (68)
Mix + ADE	0%	268 (29)	7.8 (0.4) b	1349 (37)	520 (30)	116 (8)	123 (5.5)	854 (23)
	10%	284 (99)	11.4 (1.1) a	1266 (126)	545 (78)	123 (25)	117 (9.3)	932 (123)
Main Effect of ADE								
No ADE		113 (13) a	6.7 (0.6) a	1084 (71) a	387 (18) a		36 (1.6) a	687 (48) a
ADE		276 (46) b	9.6 (0.9) b	1308 (62) b	533 (38) b		120 (4.8) b	893 (58) b
Significance (p-values)	Biochar	0.561	0.132	0.603	0.379	0.517	0.573	0.185
	ADE	0.018	0.004	0.049	0.040	0.256	<0.001	0.028
	Interaction	0.768	0.008	0.190	0.572	0.874	0.852	0.487

Experiment 2 Compost	Rate of Biochar	NO3-N	P	K	Ca	Mg	Na (ppm)	Cl (ppm)
Vermicompost	0%	1009 (4) a	163.8 (5.1)	2388 (28) a	567 (16) a	227 (3) a	247 (4.8)	562 (15)
Vermi + BC	10%	895 (7) b	145.5 (18.4)	2184 (48) b	514 (7) b	210 (6) b	223 (7.0)	521 (19)
p-value		<0.001	0.394	0.021	0.037	0.038	0.052	0.166

Means followed by the same letter in the same column are not statistically different (p<0.05) according to Tukey's HSD test

Table 2.4. Effects of biochar addition and anaerobic digester effluent (ADE) in compost media on emergence and plant growth responses. Mean (+/- SE), n=4.

Root Media	Early Emergence (%)		Shoot Dry Weight (g)		Shoot Wet Weight (g)		Plant Height (cm)		Leaves #		Root Rating (1-5)		Root Rating : Dry Weight	
Main Effect of Biochar														
No Biochar	92 (1.2)	a	0.22 (0.01)	a	2.87 (0.13)	a	12.0 (0.5)	a	2.17 (0.04)	a	2.18 (0.14)	a	9.95 (0.45)	a
Biochar	95 (1.1)	b	0.26 (0.01)	b	3.34 (0.13)	b	13.8 (0.5)	b	2.28 (0.05)	b	3.07 (0.17)	b	11.53 (0.40)	b
Main Effect of ADE														
No ADE	93 (1.1)		0.25 (0.01)	a	3.22 (0.13)		13.4 (0.5)	a	2.21 (0.04)		2.99 (0.15)	a	11.80 (0.39)	a
ADE	93 (1.2)		0.23 (0.01)	b	2.99 (0.14)		12.1 (0.5)	b	2.24 (0.05)		2.27 (0.17)	b	9.68 (0.41)	b
p-values														
Biochar	0.043		<0.001		0.001		0.007		0.041		<0.001		0.005	
ADE	0.888		0.019		0.099		0.021		0.537		<0.001		<0.001	
Interaction	0.072		0.328		0.602		0.326		0.837		0.567		0.612	

Means followed by the same letter in the same column are not statistically different (p<0.05) according to Tukey's HSD test

Table 2.5. Comparison of compost-media treatments with peat-medium control for mean (+/- SE, n=12) emergence and plant growth responses. Asterisks indicate significant differences between treatments and the peat-based control using Dunnett's test.

Root Media	Rate of Biochar	Early Emergence (%)	Shoot Dry Weight (g)	Shoot Wet Weight (g)	Root Rating (1-5)	Plant Height (cm)	Leaves #	Root Rating: Dry Weight
Peat-Based Media		98 (1.5)	0.20 (.01)	1.75 (0.06)	5.00 (0.00)	7.7 (0.7)	1.63 (0.03)	24.98 (1.46)
Mix	0%	93 (1.5)	0.24 (.02)	3.00 ** (0.19)	2.59 *** (0.14)	12.9 ** (0.7)	2.15 *** (0.05)	11.14 *** (0.50)
	10%	94 (1.8)	0.27 * (0.01)	3.44 *** (0.19)	3.39 *** (0.22)	14.0 *** (0.8)	2.27 *** (0.06)	12.45 *** (0.54)
Mix + ADE	0%	91 . (1.9)	0.20 (0.02)	2.75 * (0.19)	1.77 *** (0.17)	11.0 * (0.5)	2.19 *** (0.06)	8.76 *** (0.57)
	10%	96 (1.2)	0.26 (0.01)	3.25 *** (0.19)	2.76 *** (0.22)	13.2 *** (0.7)	2.29 *** (0.07)	10.61 *** (0.48)

Significance levels for Dunnett's Test: '***' 0.001, '**' 0.01, '*' 0.05, '.' 0.1

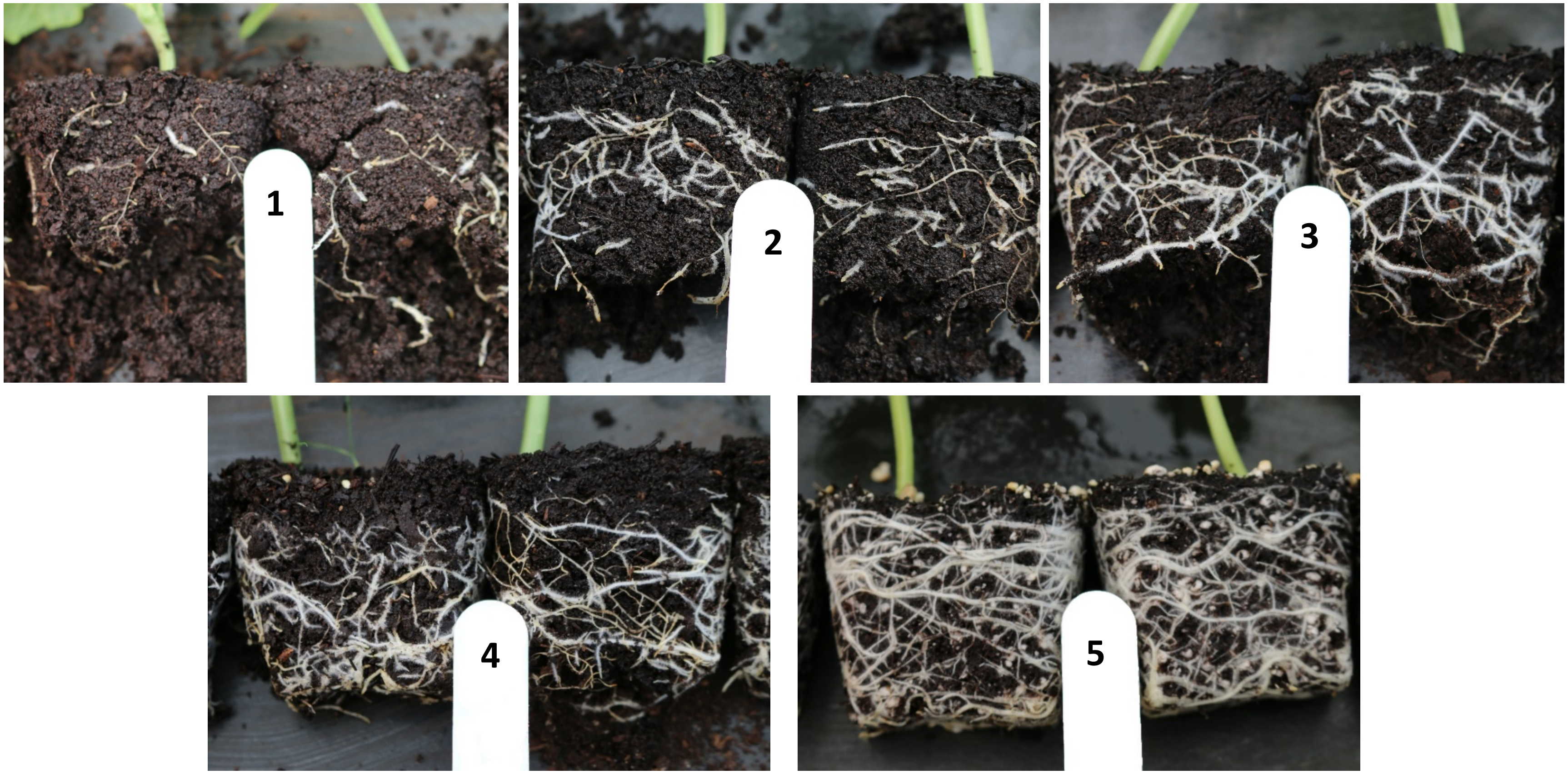

Figure 2.1. Root ratings scale for transplant root assessment ranging from 1 to 5 which were used in the visual assessment of transplants.

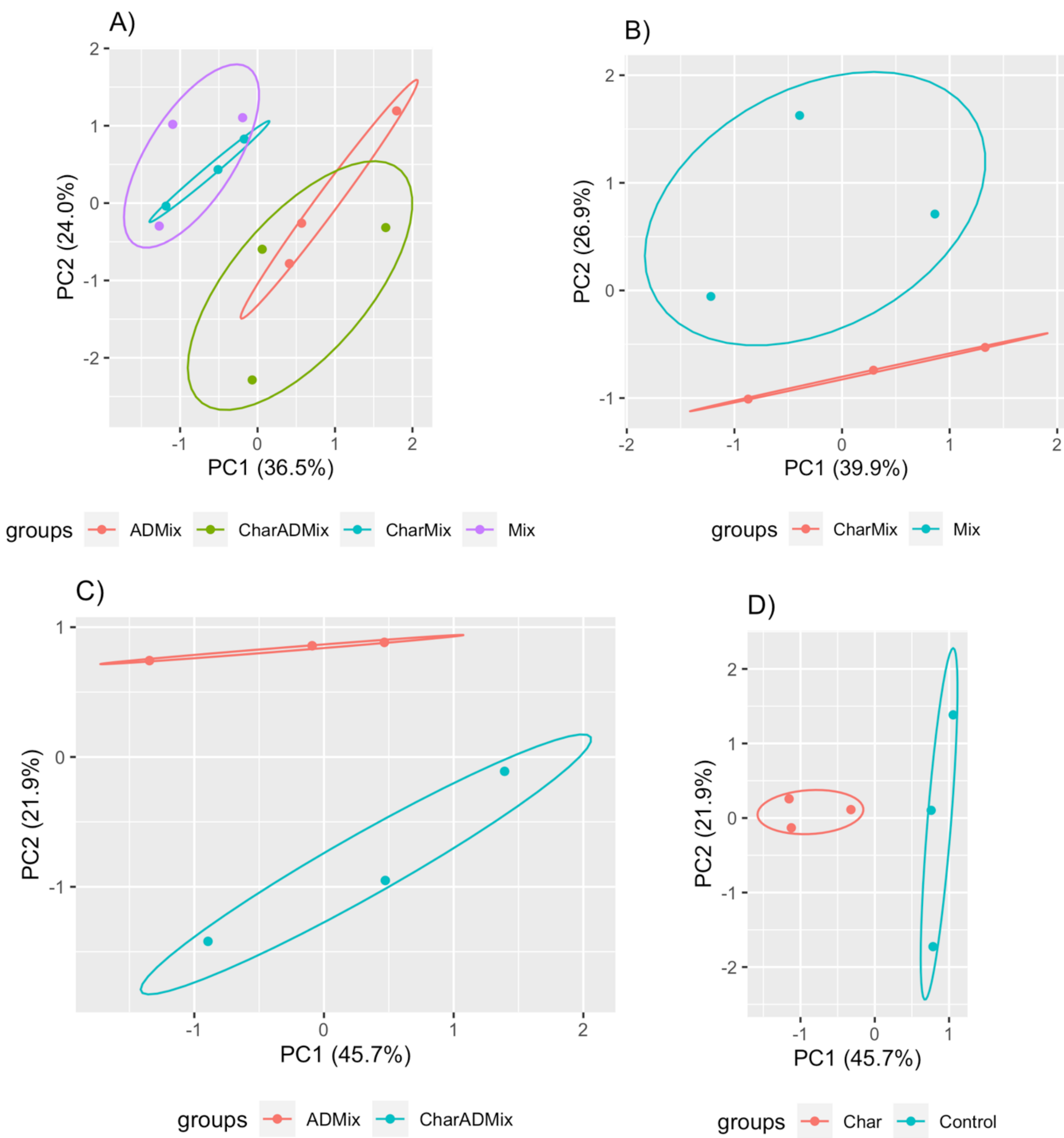

Figure 2.2. Principal component analysis biplots of the first and second principal components for compost physiochemical properties of A) all four thermophilic composts, B) Mix and Mix+BC only, C) Mix+AD and Mix+BC+AD only, and D) Vermicomposts. The percentages of the total variance accounted for by each principal component are indicated in parentheses in axis titles.

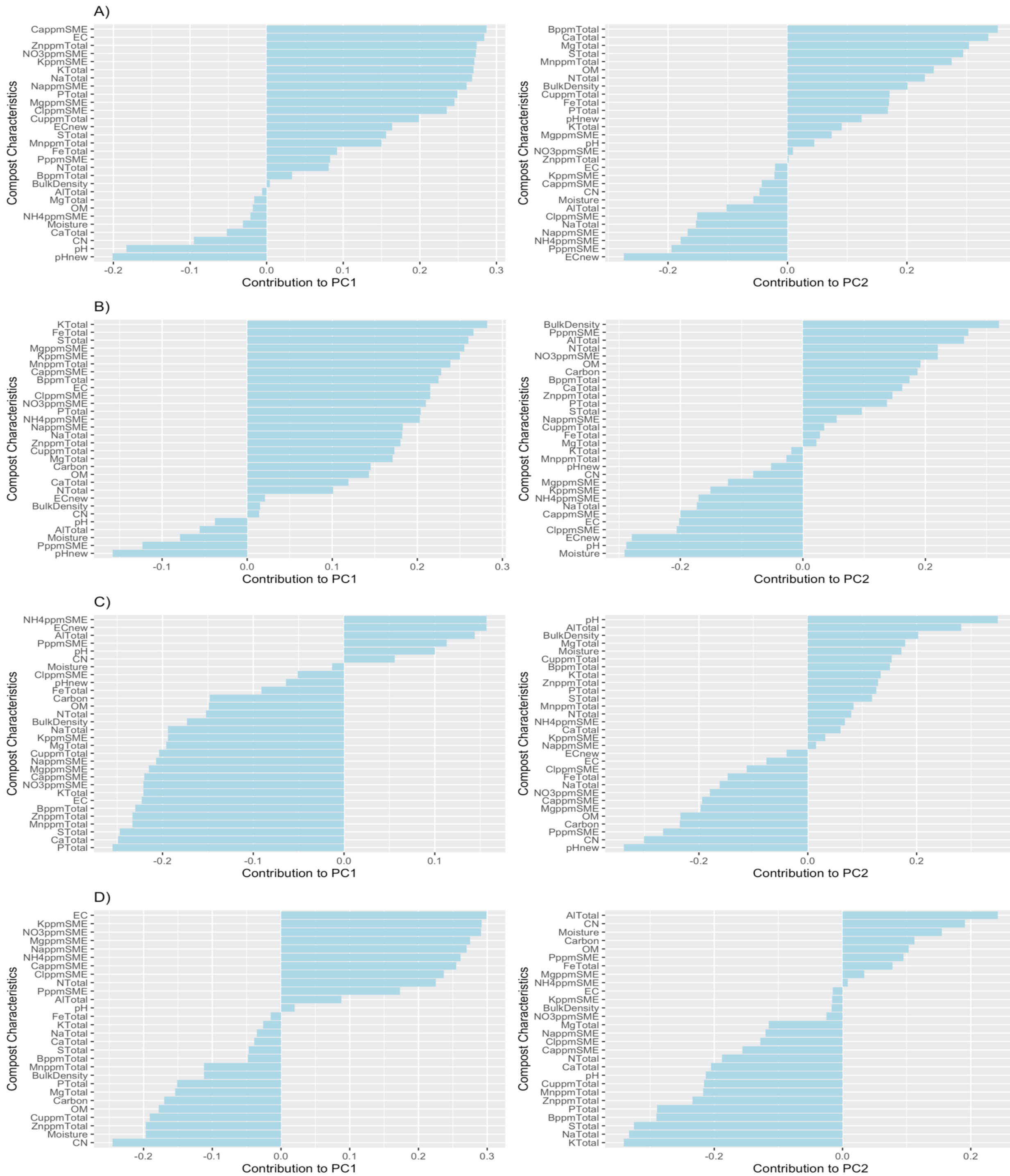

Figure 2.3. Loading contribution of compost physiochemical characteristics for interpretation of PCA values of PC1 and PC2 for A) all four thermophilic composts, B) Mix and Mix+BC only, C) Mix+AD and Mix+BC+AD only, and D) Vermicomposts.

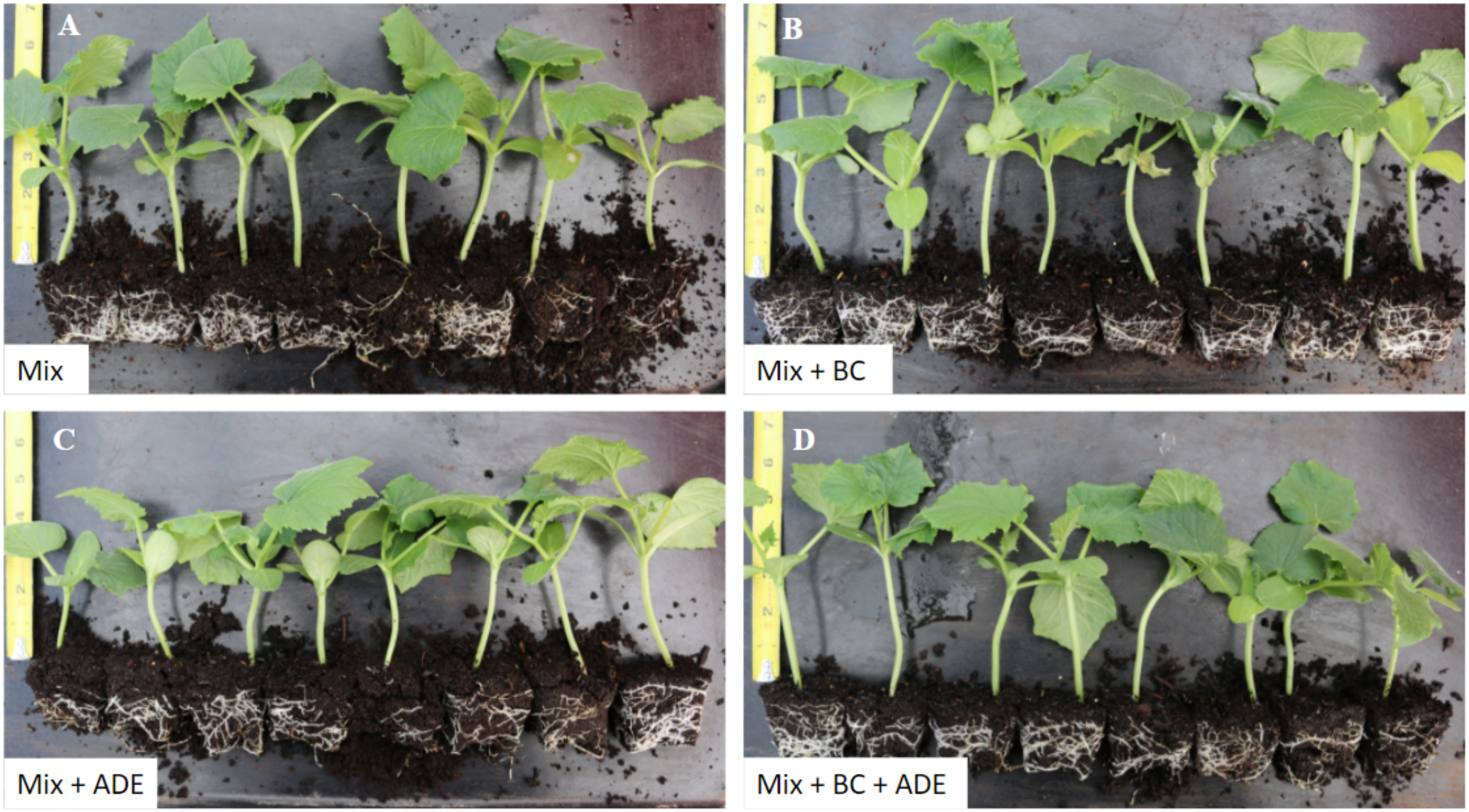

Figure 2.4. A subset of eight plants from one of the replications from each compost treatment A) Mix; B) Mix+BC; C) Mix+ADE; and D) Mix+BC+ADE.

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CHAPTER III

FEEDSTOCK EFFECT ON COMPOSTING WORMS AND VERMICOMPOST NUTRIENTS AND MICROBIAL COMMUNITY FUNCTION

ABSTRACT

A laboratory bioassay was used to determine the effects of five common kitchen preparation food residue feedstocks on earthworms and the microbial community, and the nutrient quality of the finished vermicompost. Carrot, melon, pineapple, onion, spent coffee grounds (SCG), and a mixture of all five at equal proportions by volume were added to earthworm cultures established in compost twice weekly for five weeks at rates estimated to be one-half worm body mass per day and compared with a no feed control treatment. Worm populations as indicated by mass did not change over the course of the bioassay and were the same in all treatments, indicating no negative effect of any food on the worms. The microbial community was assessed by using Biolog-Ecoplates™ for community-level physiological profiles (CLPP). The SCG vermicompost had equivalent or higher estimated microbial biomass, substrate richness and functional diversity relative to other treatments. The food mixture (which included a lower percentage of SCG) had intermediate values between that of SCG and the other feedstocks. Nutrient values for both soluble and total nutrients differed by treatment as well. For example, the SCG treatment had lower NO_3-N but higher total N (2.4%) compared to the food mix (2.1%) as well as all single-feedstock treatments (1.5-1.7%).

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INTRODUCTION

Vermicompost is organic material that has been processed under protected/managed conditions by a dense population (target of 1 lb/ft^2) of composting worms, in a time frame resulting in a mixture of worm castings and other organic matter (C. A Edwards et al., 2010). There are only about six species of worms out of thousands of species that are used commercially as composting worms. The most prevalent worldwide across tropical, subtropical and temperature climates is *Eisenia fetida*, more commonly called the red wriggler or tiger worm (James & Guimaraēs, 2010). Beyond the presence of worms having processed the material, the primary distinction between vermicompost and thermophilic or hot compost is the temperature at which material is converted to stable humic-rich organic matter. While the primary microbial activity of thermophilic compost is above 130°F, vermicompost microbial activity occurs at mesophilic temperatures, generally between 50-80°F (Dominguez & Edwards, 2011). Vermicompost frequently sells for a higher price than hot compost if being sold, driven in part by limited production and thereby supply as well as conceptions of its superior attributes as compared to thermophilic compost, whether accurate or not. The particle size is often smaller, as a result of passing through the worm. There is an important distinction, however, between vermicompost and worm castings which is the material that did specifically pass through the gut of a worm, whereas vermicompost is a mixture of castings and other organic matter. The microbial community in vermicomposts has been demonstrated to be markedly different than that of thermophilic composts, even if made from the same starting feedstocks (Neher et al., 2013).

The amount of food wasted in the US and around the world has been gaining heightened attention with recent estimates that as much as 40% of food produced in the US is not consumed by people. The most recent data compiled by the USEPA (from 2017) was that of the 270 million tons of municipal solid waste generated in the US, 27 million pounds (10%) was composted, despite the fact that 60% of the waste was compostable, whereas 139 million tons (52%) was disposed of in landfills (US EPA, 2017). Of what was landfilled, 21.9% is food waste, whereas only 2.7% of composted material is food waste. Long-term nutrient extraction from the soil and deposition to landfills instead of resupplying the soil is not sustainable. Food waste is an ideal food for worms to produce vermicompost due to its high moisture content and nutrients. However, there is concern over some of the more common kitchen preparation food waste materials regarding possible negative effects on composting worms (Angima et al., 2011; Dickerson, n.d.; Sherman, 2017). Some concerns stem from the pH or the perceived pH of material in that it may be too acidic for maintaining a healthy worm population (Harris, n.d.; Shaw, 2011). For the MSU project of vermicomposting dining hall pre-consumer kitchen preparation residue there is often a predominance of pineapple skins, melon rinds/insides, onions layers and skins, and carrot peels. Onion is a food source that has been singled out to not feed to worms, as well as high acid food which would include pineapple (Angima et al., 2011; Dickerson, n.d.; Sherman, 2017). These are prevalent food residues from MSU making these of particular interest in this study. Other common food residues are melon rinds/insides, and carrots/peels.

Spent coffee grounds (SCG) are also prevalent coming from dining halls and coffee shops. Coffee is an abundant food residue with annual production at approximately six million tones worldwide, or 650 kg SCG per 1 ton of green coffee, which has been generally landfilled (Mussatto et al., 2011). This abundant material could be labeled a resource instead of environmental pollutant through its valorization but more research has been called for (Stylianou et al., 2018). It has been identified as a preferred feedstock for worms and often as a sole food source by some vermicomposters (Shaw, 2011), though the specifics of how this feedstock affects worms and finished vermicompost has not been sufficiently evaluated. This is another prevalent food residue coming from MSU campus to the worm composting facility.

Feedstocks are anticipated to affect the biology of the compost, especially as the nutrients and food type (proteins, starches, sugars, etc.) contained therein can inhibit or promote growth of certain genera/species (bacterial, fungal, higher organisms) over others. Abundances and diversity of microorganisms at different stages of vermicomposting have been reported as well (Chen et al., 2015; Gómez-Brandón et al., 2011, 2012; Gopal et al., 2017; Vivas et al., 2009). Still other studies have looked at finished vermicompost for presence of plant-beneficial microbes (Gopal et al., 2009; Pathma & Sakthivel, 2013; Raphael & Velmourougane, 2011). Knowledge of the microbiology resulting from specific feedstocks could be related to these and other studies if attempting to achieve a desired microbial population.

A vermicomposting laboratory bioassay was developed to determine the effects of the five feedstocks on the worms, the microbial community, and the nutrient quality of the compost. The feedstocks were single ingredients of melon (rind and seed cavity), carrot (primarily peel), pineapple rinds, onion (skin and layers) and SCG, as well as a mix of all together. Food waste feedstocks for vermicomposting are more likely to be mixed instead of single-source; however, knowledge of how each might affect the finished vermicompost and earthworms advances vermicompost technology. At MSU specifically, this information could help to guide what is vermicomposted on campus if a specific residue was found to be particularly deleterious or beneficial to the worms or vermicompost. We hypothesized that the worms would survive in all treatments, but there would be differences between treatments in worm biomass at the termination of the vermicomposting process. Specifically, we hypothesized that the mixture would be the food that enhanced growth of the biomass of the worm the most. Regarding microbial community, we hypothesized that there would be differences in the community-level physiological profiles (CLPP) between treatments, and the mixture would have the highest level of microbial diversity and abundance as determined by Biolog EcoPlates™. Lastly, we hypothesized that the worm food sources would affect pH, EC and specific nutrient concentrations of the vermicompost produced.

MATERIALS AND METHODS

Feeding Treatments and Experimental Design

Eisenia fetida were isolated from earthworm bins at the vermicompost research facility and established in recycled cafeteria food service plastic bins of 4” x 20” x 4” depth with two liters of a moistened immature compost as bedding (compost feedstocks of softwood shavings, hay, straw, and peat). The compost bedding had been maintained in an indoor laboratory space at MSU (70°F), and was not fully decomposed, still having some structure of original materials such as stems of grass/straw. Each bin (0.55ft^2 surface area) was established with 150g (0.6 lb/ft^2) composting worms at the start of the experiment. The feedstock treatments were 1) Control (no food added), 2) carrot, peels and whole, 3) melon, rinds and seed cavity, 4) pineapple, skins, 5) onion, skins and whole, 6) spent coffee grounds (SCG), and 7) a food mixture of 2-6 at equal parts by volume. Samples of all feedstocks were oven dried to obtain moisture content which was approximately 90% for each.

Buckets were provided to the food preparation kitchen at MSU for the purpose of separating the common food preparation residues. The food was prepared for worm trial by grinding with a food processor to a puree, with the exception of the moist SCG that were used directly. The food was primarily the discarded portion (melon rinds, pineapple skins, onion skin, carrot peel) though for the carrot and onion there were some whole vegetables that were no longer edible.

Each experimental unit was one worm bin/tray with two liters volume of starting bedding material. There were four replications of each treatment for a total of 28 vermicomposting bins, arranged on a vertical/stacked shelving unit (Figure 3.1A). Replications were assigned positions at random, each shelf having one replication, comprising a randomized complete block design (RCBD).

Vermicompost Management and Data Collection

Worms were fed the equivalent of half of the earthworm body weight per bin, per day, distributed by 262g feedings twice weekly. The food was put on the compost/bedding surface and covered with a thin layer of shredded leaves, then moistened with water from a 0.5 L hand pump sprayer (Figure 3.1B). Fresh food was placed on only one half of the bin each week, alternating which half received food. This was in accordance to feeding protocol in use at a larger scale at the MSU HTRC mid-scale vermicomposting facility to ensure worms could move away from the added food if necessary. Bins were weighed weekly to guide water applications and maintain moisture uniformity as supplemental water was added. Multiple layers of newspaper were also layered on top of the bins (above the surface of the compost) to maintain low light and humid conditions. Feeding lasted a total of five weeks, with four additional weeks for the worms to finish composting the freshest material, during which time the vermicompost was mixed and watered weekly.

The earthworms were re-isolated at the termination of the experiment and weighed.

At the end of the trial a sample of each vermicompost, collected from the thoroughly mixed content of each container, was analyzed for chemical and physical properties. Key responses measured from mature compost samples were for nutrients including water soluble nutrients using saturated media extract (SME) (pH, EC, ppm nitrate-N, ammonium-N, P, K, Ca, Mg, Na, Cl), and total nutrients (% N, P, K, Ca, Mg, S, Na; ppm Zn, Mn, Fe, Cu, B, Al), organic matter (%) and moisture (%). Percent carbon and the carbon to nitrogen ratio were calculated from the data and nutrient balance (percent of each nutrient contribution to the electrical conductivity) was also calculated using the equation

[1] % Element = ((ppm element) / (EC mg/L * 700)) * 100 (Warncke & Krauskopf, 1983).

CLPP of the microbial communities was determined using Biolog EcoPlates™ (Biolog Inc., CA, USA) to provide an assessment of the functional diversity and estimation of microbial biomass. The culturable soil microbial population was exposed to 31 distinct carbon sources and a water control well within a 96-well plate, each replicated three times. A procedure based on field soils by Garland and Mills (1991) was adapted for use with compost. Ten grams moist compost from each treatment replicate sample (n=3) were shaken in 90 ml of a sterilized saline solution (0.85% NaCl, w/v) for 60 min, pre-incubated for 18 h at 23 °C, and then brought to a final dilution of 10^3 before 150 µl aliquots were added to each of the 96 wells (1 plate per treatment replicate). Reduction of tetrazolium dye in the wells by the respiration of microbes results in color change (clear to purple) indicating use of the carbon source. Plates were incubated at 23°C for 7 days, during which time color development in each well was measured at 24 hour intervals as absorbance at 590 nm using a microplate reader (Model 680, Bio-Rad Laboratories, Hercules, CA). The overall degree of substrate use was expressed as average well color development (AWCD), calculated as the mean difference between carbon source absorbances (Ri) and the absorbance reading for the control well (C) within plate replicates (Garland and Mills, 1991):

[2] $$AWCD = \frac{\sum Ri - C}{31}$$

Substrate richness (S) was calculated by the number of carbon substrates that were oxidized (from 0 to 31 possible substrates utilized). The Shannon-Wiener diversity index (H) and evenness (E) were calculated using the following equations (Spellerberg & Fedor, 2003; Zak et al., 1994):

[3] $H = \sum Ri(\ln Ri)$

[4] $E = H/\log S$

where Ri is the ratio of the corrected absorbance values of each well to the sum of absorbance value of all wells. The Shannon-Wiener diversity index provides information on the functional diversity of the compost microbial community. Evenness is a measure of the equitability of activity across all of the utilized substrates (Zak et al., 1994). Patterns of substrate use were investigated after first dividing the control-corrected absorbances (Ri - C) by AWCD within plate replicates to reduce potential bias due to differences in inoculum density among samples (Garland, 1997).

Statistical Analysis

Chemical, physical, and biological properties of the composts and final weight of the worm population were analyzed for treatment significance by analysis of variance (ANOVA). Significant differences between means of treatments were determined by pairwise comparison using Tukey's Honestly Significant Difference (HSD) test ($P \leq 0.05$). For the worm population data only, a decrease in the no-food Control was anticipated and therefore not included in the pairwise comparison that was to assess the foods' effect on the worms. Data analyses were carried out using R statistical software package, and multcomp package (glht function) for pairwise comparisons (R Core Team, 2019). For all data, normality was determined by univariate procedures and Sattherthwaite's degrees of freedom was employed to account for potential nonequal variance in the data. Where normality assumptions were violated, the data were log transformed prior to analysis.

RESULTS

Worm Growth and Health. Based on visual assessments at each feeding, there were no obvious negative impacts on the worm populations for the duration of the experiment. Additionally, worms appeared to readily move into the fresh material shortly after it was applied. Final mass of worms for some food treatments differed. The Carrot, Pineapple and Mix treatments mass of worms (range of 139-148g) were different only from the Melon treatment (120g) and not from the Onion and SCG (Table 3.1). The mass decrease observed from the original 150 g ranged from 1-21% for food treatments while the worm mass of the Control decreased by 64%. The water equivalent of what the other treatments received in the food (feedstocks at 89% moisture) was added to the no-food bins to ensure that it was not moisture that was a limiting factor on growth.

Microbial Community Analysis. Compared to the Control, differences in substrate richness and functional diversity (Shannon-Wiener Diversity Index) were only detected for Carrot, SCG and the Mix treatments (Table 3.2). The SCG had greater functional diversity than the Control, Melon, Pineapple and Onion treatments, but was equivalent to the Carrot and Mix. Substrate richness ranged from 21.2 (Control) to 27.7 (SCG), and the functional diversity index ranged from 2.74 (Control) to 3.08 (SCG). Substrate evenness, however, did not differ between treatments (2.07-2.13). The estimation of microbial biomass (using AWCD) had a smaller range of values from 0.46 (Onion) to 0.70 (SCG); SCG had greater microbial biomass than all treatments other than Carrot and the Mix for which it was equivalent.

Chemical Analysis. All treatments other than SCG and Mix produced similar results in regards to final percent organic matter or percent carbon with values ranging from 48-53.7% (Table 3.3). The SCG treatment, however, had a higher percent organic matter at 73.7% than all other treatments, while the Mix (58.3%), with SCG incorporated into the feed at 20% by volume, was also higher than all but the Control and Pineapple. The percentage C, having been calculated from the organic matter followed the same trend, with SCG with the highest values at 42.3%. The C:N ratio of all treatments were not different from one another, spanning from 16.3 (Mix) to 20.7 (Control). Moisture content was marginally significant ($p=0.055$) where it would appear that SCG may have had greater moisture than the other treatments (at $p<0.1$).

There were differences in the EC and pH values observed (Table 3.4). EC values were highest for the Melon and Carrot treatments at 13.7 dS/m (though equivalent with Onion). EC of SCG was the lowest by a factor of almost four in comparison with all other treatments at 2.5dS/m (Table 3.4). The pH differed by treatment with the Control at the lowest pH of 5.5 and Carrot the highest at 9.1 followed by Pineapple (8.2). The other food treatments had equivalent pH, near neutral.

There were differences observed between treatments for all soluble and total nutrients with the exception of NH_4-N (Tables 3.4, 3.5). Of the soluble nutrients, the nutrient values from SCG were all lower than or equivalent to the other treatments, of particular note are NO_3-N (5ppm), and Ca (405ppm). The Control, in contrast had higher values for a number of soluble nutrients, including Mg (279ppm) and NO_3-N (988ppm). Some total nutrient concentration values of note are the higher N in SCG (2.4%), higher K in compost from Carrots (2.9%), higher S in compost from Onions (0.38%), higher Zn in compost from Melons (104.7ppm), and higher Mn in compost from Pineapple (318ppm, although not different from Onions, and then Onions from others). The treatments Carrot and Melon had similar profiles of nutrients, differing from one another only for total K (Carrot 2.9% > Melon 2.2%) and Zn (Melon 104.7ppm > Carrot 75.7ppm) and in soluble Mg (Melon 172ppm > Carrot 80ppm) and Na (Melon 445ppm > Carrot 235ppm).

DISCUSSION

The hypothesis that composting earthworms would survive in all treatments is supported by the data of the worm biomass at the termination of the experiment, as was the hypothesis that there would be differences in earthworm biomass between treatments at the termination of the experiment. The hypothesis that the Mix would enhance the growth of the biomass the most was not supported as no treatments increased the biomass of the worms, and the final earthworm biomass in the Mix was not different than all food treatments except Melon. The primary difference observed was between the food treatments and the no-food control where it was expected that worm biomass would decline, which it did. Inconsistent with our findings, Liu and Price (2011) observed a reduced worm growth/survival in SCG treatments, though this effect was decreased with cardboard additions which may have had a similar effect as the compost bedding used in our study.

The data in this experiment are from one point in time after a total period of 56 days, five weeks of feeding, and four additional weeks wherein no new food was added but the worms had additional time to process material. At this high density of worms present, along with the stable temperature and moisture conditions, it is a reasonable expectation that the majority of the feedstock would be processed by the worms (Clive A. Edwards, 2011). The time frame, however, would not have allowed for reproduction of worms and their growth/decline may have become more apparent with a longer experimental duration. It is important to note that individual worms were not counted. While the worm population can be inferred by the data collected, the reality could be that some worms died while others grew larger. Also common in vermicomposting can be worms leaving a bed system if conditions become unfavorable, and this was not observed in any of the treatments. The relative consistency of final worm mass regardless of treatment indicates a stable density of approximately 300g-worms/ft^2 in this container system, but a longer term study would help to evaluate what the optimal socking density would be under these conditions as the worm population would have the chance to stabilize over time by reproduction.

There were differences observed between treatments in their CLPP as hypothesized, with the impact of SCG on the microbial community being of particular interest. In SCG vermicompost all parameters measured/calculated were higher or equal to the other treatments. Some possible relationships may be with the higher organic matter and nitrogen present. The coffee seeds may contain more complete, complex, or diverse compounds to be digested, which enhanced the microbial community resulting in the CLPP seen. The hypothesis that the Mix would have the highest level of microbial functional diversity and abundance was not supported, though it was equivalent to the SCG in all of the parameters. The Mix might be assumed to have the most complex assemblage of different compounds as it contains all of the individual foods, but if this is true, it did not result in a more diverse microbial community relative to the other treatments.

Changes in the microbial community over time were not measured here, but rather the microbial community function assessed at one point in time. Findings by Gopal et al. (2017) in which samples were taken at four different intervals (when worms were introduced to material at day 15, 45, 75 and 105 days) displayed significant differences in microbial community structure at the different points in time, with the highest abundance of microbes at day 75 of their study, which based on when they inoculated their substrate with composting earthworm was approximately equal in timing to our analysis of microbial community function. Whether the biological differences seen between vermicomposts with the different substrates would be consistent over time would need to be evaluated with a longer duration study in which material is assessed multiple times.

Feedstock treatment did affect the concentrations of all soluble and total nutrients except NH_4-N. Differences observed between the SCG feedstocks and the others are the most distinct in all the treatments, particularly with regard to N. SCG had the highest total N at 2.4%, while there was nearly an undetectable quantity of soluble NO_3-N (Tables 3.3 and 3.4). The differences between soluble N and total N may in part be due to the N being assimilated in the bodies of the microbes, which may create a kind of slow-release fertilizer over time as microbes cycle and decay, and mineralize more soil organic matter. The recalcitrant nature of SCG (Shofie et al., 2015) may also be contributing to this lack of soluble N for such a short duration vermicomposting experiment. There could also be inhibition of mineralization and nitrification. The SCG treatment final volume was approximately 50% larger than the other treatments as the material did not appear to be as digested or reduced in volume at the termination of the study. Regarding other soluble and total nutrients, SCG was lower or equal to the other treatments for all (with the exception of total N). Our results contradict that of Adi and Noor (2009) that addition of SCG enhances the quality of vermicompost in regards to nutrients as opposed to kitchen scrap vermicomposting alone. The only nutrient that was enhanced in their trial as well as ours was the total N.

The observation that the Control treatment had the highest values for soluble NO_3-N and Mg is of interest, as well as having high concentration of P, though this was not higher than the Mix, Melon or Onion. One possible explanation is that the microbial biomass was lower than the other treatments, possibly due to no fresh / degradable food added, and not as many nutrients were sequestered in the microbial biomass itself. Soluble P (measured by SME) for peat-based media is often related to the pH (lower pH, higher P) and the pH is lowest in the Control, but there is not a consistent trend in these results.

There has been concern circulated over pH of bedding/feedstock material becoming too acidic for composting earthworms, such as if using a high acid food like pineapple (pH 3.2-3.7, Bridges & Mattice, 1939). Out of all of the feedstocks tested, however, the pineapple feedstock treatment had the second highest pH of the finished vermicompost. That the final pH values were so high contradicts the findings of Garg et al., (2012) in which the acidity of their finished vermicompost was more acidic than the starting feedstocks. The decomposition of the material is attributed to the decrease in pH as humic acids and other ions are formed.

Future research: While the results of this experiment indicated that there was no negative effect on the worm population, the short duration of the experiment did not allow sufficient time to assess any potential effects on reproduction. A longer study in which reproduction is assessed by looking at cocoons and actual worm numbers for population instead of mass as an indication of density would further elucidate the effects of specific feedstocks.

The experimental design of the feedstock vermicomposting bioassay can be used for testing other parameters of a vermicomposting system such as temperature, the limits of EC where worm health drops off (as measured either by mass as it was herein, or by number of worms), pH, addition of various minerals, or use of different feedstocks. Additional feedstocks have a conception of being negative or positive, as taught by extension publications, in workshops and online with frequency such as citrus (negative) or banana peels (positive), but they have not been evaluated specifically (Dickerson, n.d.; Sherman, 2017). The compost bedding was not sufficient to sustain the worm population, and so establishing a different baseline against which to test other feedstocks would be warranted. A limitation with this bioassay, however, was the amount of time required to isolate worms for the bins and improvements in the method could be made.

Further analysis of microbial communities may be benefited from more refined techniques to determine genus/species composition. Beyond production and testing of vermicompost, future work may involve experiments to evaluate how these microbial communities affect plant growth and the long-term impact on the rhizosphere when vermicompost is incorporated into soils or used for transplant production.

APPENDIX

Table 3.1. Microbiology of composts determined by Biolog Ecoplates for species richness, Shannon-Wiener Diversity Index, substrate evenness, and AWCD by vermicompost feedstock treatment.

Feedstock	Species Richness		Shannon-Wiener Diversity Index		Substrate Evenness	AWCD	
NoFood	21.2 (.9)	a	2.74 (.04)	a	2.07 (.01)	0.47 (.04)	a
Carrot	25.8 (.6)	bc	2.98 (.03)	bcd	2.11 (.01)	0.56 (.03)	ab
Melon	24.6 (1.4)	abc	2.89 (.07)	abc	2.09 (.02)	0.49 (.05)	a
Pineapple	22.4 (.6)	ab	2.82 (.04)	ab	2.09 (.02)	0.53 (.03)	a
Onion	23.4 (.5)	ab	2.86 (.03)	ab	2.09 (.02)	0.46 (.02)	a
Coffee	27.7 (.5)	c	3.08 (.03)	d	2.13 (.01)	0.70 (.05)	b
Mix	27.4 (.6)	c	3.03 (.03)	cd	2.11 (.01)	0.60 (.03)	ab
P	<0.001		<0.001		0.132	<0.001	

* means followed by the same letter in the same column are not statistically different (p<0.05) according to Tukey's HSD test

Table 3.2. Compost properties of organic matter (%), carbon (%), carbon to nitrogen ratio, and moisture by compost treatment after 5 weeks of feeding and 4 weeks of incubation.

Compost	OM		C		C:N	Moisture
	%		%			%
NoFood	53.7 (0.3)	ab*	31 (0)	ab	20.7 (1.2)	61.3 (3.3)
Carrot	48 (2.5)	a	28 (1.5)	a	16.7 (0.9)	63.7 (1.3)
Melon	51 (0.6)	a	29.7 (0.3)	a	18 (1.2)	64.3 (0.9)
Pineapple	53.7 (0.7)	ab	31.3 (0.3)	ab	19.3 (0.9)	66.3 (2.7)
Onion	52.7 (0.9)	a	30.3 (0.7)	a	18 (1.2)	63.3 (1.8)
Coffee	73.7 (0.7)	c	42.3 (0.3)	c	17.7 (0.7)	71 (0.6)
Mix	58.3 (1.2)	b	34 (0.6)	b	16.3 (1.2)	62.7 (1.2)
P	<0.001		<0.001		0.121	0.055

* means followed by the same letter in the same column are not statistically different (p<0.05) according to Tukey’s HSD test

Table 3.3. The pH, EC and water soluble nutrient values from the saturated media extract for vermicompost treatments after 5 weeks of feeding and 4 weeks of incubation.

Compost	pH		EC		NO3-N		NH4-N		P		K		Ca		Mg		Na		Cl	
			dS m-1		ppm		ppm		ppm		ppm		ppm		ppm		ppm		ppm	
Control	5.5 (0.1)	a*	10.3 (0.5)	bc	988 (51)	e	4.7 (0.9)	a	146 (6)	d	1305 (78)	a	1080 (52)	bc	279 (14)	d	100 (2)	a	403 (18)	a
Carrot	9.1 (0.3)	d	13.7 (1.2)	d	694 (104)	bc	3.7 (0.7)	a	58 (3)	ab	3569 (355)	d	1490 (98)	d	80 (9)	a	235 (24)	b	997 (116)	c
Melon	7.3 (0.1)	b	13.7 (0.7)	d	769 (25)	bcd	3.7 (0.7)	a	92 (5)	bcd	2856 (163)	cd	1620 (104)	d	172 (30)	bc	445 (27)	c	1146 (33)	c
Pineapple	8.2 (0.1)	c	7.9 (0.2)	b	302 (49)	b	2.3 (0.3)	a	35 (0)	a	2060 (73)	b	820 (10)	b	102 (18)	ab	56 (2)	a	788 (44)	bc
Onion	6.8 (0.3)	b	11.5 (0.6)	cd	827 (128)	d	3.7 (0.9)	a	132 (22)	cd	2304 (36)	bc	1110 (30)	c	195 (20)	c	90 (6)	a	481 (23)	ab
Coffee	7.1 (0)	b	2.5 (0.2)	a	5 (2)	a	7 (2)	a	59 (9)	ab	727 (56)	a	405 (9)	a	84 (10)	a	65 (3)	a	232 (13)	ab
Mix	7.3 (0.1)	b	9.4 (0.2)	bc	572 (53)	cd	6 (1.2)	a	89 (16)	bcd	2286 (36)	bc	1050 (30)	bc	141 (11)	abc	175 (8)	b	843 (161)	bc
P	<0.001		<0.001		<0.001		0.098		<0.001		<0.001		<0.001		<0.001				<0.001	

* means followed by the same lower-case letter in the same column are not statistically different (p<0.05) according to Tukey's HSD test

Table 3.4. Total nutrient analysis of vermicomposts after 5 weeks of feeding and 4 weeks of incubation.

Compost	N	P	K	Ca	Mg	S	Zn	Mn	Cu	B	Fe	Al
	%	%	%	%	%	%	ppm	ppm	ppm	ppm	ppm	ppm
NoFood	1.5 (0.1) a*	0.15 (0.019) a	0.8 (0.13) a	1.8 (0.17) b	0.48 (0.03) ab	0.15 (0.023) a	64 (8) b	181.3 (12.2) ab	20.7 (0.9) a	21.7 (3.7) a	5122 (570.3) ab	2500 (120.4) ab
Carrot	1.7 (0.02) ab	0.36 (0.013) c	2.9 (0.13) d	2.1 (0.14) bc	0.53 (0.04) abc	0.22 (0.006) abc	75.7 (1.8) bc	210.3 (10.2) abc	27.7 (2.8) ab	40.3 (2.4) b	5476 (792.8) b	2845 (344) b
Melon	1.7 (0.12) ab	0.36 (0.01) c	2.2 (0.06) c	2.4 (0.06) c	0.63 (0.02) c	0.28 (0.006) c	104.7 (0.9) d	219 (4) abc	30 (1.2) b	37.3 (1.9) b	5162 (372.3) ab	2632 (66.8) b
Pineapple	1.6 (0.07) ab	0.21 (0.015) a	1.8 (0.14) bc	2.3 (0.12) bc	0.61 (0.01) bc	0.21 (0.017) abc	76 (4.9) bc	318 (23.4) d	25.7 (2.7) ab	36.3 (4.7) b	5002 (331.1) ab	2493 (165.4) ab
Onion	1.7 (0.09) ab	0.36 (0.009) bc	1.6 (0.05) b	2.4 (0.05) c	0.55 (0.02) abc	0.38 (0.012) d	93.7 (2.7) cd	257.7 (27.4) cd	21.7 (0.3) a	36 (1.2) b	4961 (95.1) ab	2423 (74.1) ab
Coffee	2.4 (0.09) c	0.15 (0.003) a	0.9 (0.01) a	1.3 (0.06) a	0.45 (0.04) a	0.19 (0.003) ab	41.7 (1.2) a	150 (5) a	27.7 (0.9) ab	17.3 (0.3) a	2766 (62.6) a	1549 (82.7) a
Mix	2.1 (0.19) bc	0.29 (0.024) b	1.8 (0.15) bc	2.1 (0.11) bc	0.54 (0.03) abc	0.26 (0.024) bc	72.3 (4.3) b	236.3 (6.4) bc	32.3 (0.9) b	30.3 (2.7) ab	5607 (831.4) b	2626 (319.2) b
P	<0.001	<0.001	<0.001	<0.001	0.006	<0.001	<0.001	<0.001	0.002	<0.001	0.029	0.011

* means followed by the same letter in the same column are not statistically different ($p<0.05$) according to Tukey's HSD

Figure 3.1. A) Laboratory set-up for vermicomposting feeding trial with the seven treatments, one replication per shelf and a total of four replications and B) Close-up of feeding of pineapple on one half of worm bin (back) and covered with ground leaves before being wet by misting (front).

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CHAPTER IV

AERATED COMPOST TEA FOR DISEASE SUPPRESSION IN HIGH TUNNEL TOMATO AND FIELD GROWN WINTER SQUASH

ABSTRACT

Compost tea (CT) is a watery extract of compost; CTs have been tested for a variety of host plant/pathogen systems for disease suppression. Disease pressure can decrease yields and pesticide options are limited for organic growers. The effectiveness of aerated (48hr) CT (1:5 v:v compost:water) from three composts [leaf/grass/coffee (LGC) and dairy manure (DMC) thermophilic composts and vermicompost (VC)] were evaluated in five separate experiments: two with tomatoes and tomato leaf mold (*Fulvia fulva*), varieties 'Celebrity' and 'Big Beef'; and three with winter squash and powdery mildew (*Podosphaera xanthii*), varieties 'Waltham Butternut,' 'Delicata,' and 'Blue Ballet Hubbard' in field trials in 2016. Treatments included the three undiluted CTs, a spreader-sticker (NuFilm P), VC with the spreader-sticker, and a no-spray control. Experimental treatments provided some degree of disease suppression relative to the control early in the crop production cycle for both varieties of tomato, and butternut squash, but not for hubbard or delicata. CTs made from the three composts differed in their efficacy, with treatment VC+NuFilm P providing the most consistent early reductions in the incidence and overall severity of disease in tomatoes, for example. For the host/pathogen combinations in which there had been early disease suppression, this effect disappeared over the course of the season, and no differences in final disease suppression or crop yield between treatments were detected. Foliar spray of CT as part of an integrated pest management system may contribute to early season disease suppression of *F. fulva* on tomatoes and *P. xanthii* on butternut squash, but efficacy declines later in the season as the disease progresses.

INTRODUCTION

The general definition of compost tea (CT) is a watery fermented extract of compost, aimed at obtaining soluble nutrients and multiplying beneficial microbes from the compost in an aqueous solution that can be applied to plants or soil (Martin, 2014; Scheuerell & Mahaffee, 2002). There are many factors that could influence the effectiveness of compost teas which include the quality of the compost, water source and ratio with compost, added nutrients, aeration, fermentation time, temperature, pH, dilution, application equipment, application timing, adjuvants, and application rates (Edwards et al., 2011; Ingham, 2000; Litterick et al., 2004; Neher et al., 2013; Scheuerell, 2002). The brewing process of compost teas influences the resultant characteristics of the extract, the primary distinction being whether a CT is aerated or non-aerated (W. Brinton et al., 2004) with certain advantages attributed to both methods. There are guidelines from the National Organics Standards Board (NOP, 2011) to address concerns for safety (regarding possible transmission of human pathogens primarily), but more research and guidelines have been called for by the scientific community and farmers (De Corato, 2020; Kelley et al., 2004; Scheuerell, 2002; personal communication).

Soluble nutrients from compost are transferred into the CT as well as biological organisms, making quality and maturity of compost used for CT one of the most important factors for efficacy and mode of action. Microbial life in CT include bacteria, fungi, actinomycetes, protozoa such as ciliates, flagellates and amoebas, and nematodes (Ingham, 2000). The ratio of compost to water and dilution prior to application is also a major determinant of quantity of nutrients and biology being applied with CT application (Islam et al., 2016). CT may be applied as a soil drench a foliar spray, the latter is a means of inoculating the phyllosphere with beneficial microorganisms (Ingham, 2000). The suppressive abilities of composts or CTs have been diminished by heat treatment, indicating the importance of the biology for disease management (Elad & Shtienberg, 1994; Koné et al., 2010; Malandraki et al., 2008; Siddiqui et al., 2009). Metabolites and secondary compounds such as plant growth hormones and regulators, and free enzymes produced by microorganisms may be driving efficacy instead of the biology itself (Edwards et al., 2011; Larbi et al., 2006).

Efficacy of a CT against a disease system starts with the compost used to make the CT (Elad & Shtienberg, 1994; Koné et al., 2010). The effect of feedstocks and production method influence the finished compost product in terms of biological, physical and chemical properties (Neher et al., 2013). The two primary means of composting are thermophilic (hot) composting and mesophilic vermicomposting with worms, most often *Eisenia fetida*. The biological differences between these methods has been quantified in various studies, with vermicompost generally having higher levels of both bacteria and fungi (Dominguez & Edwards, 2011). This is in part due to the interaction of the gut fauna within the worm itself interacting with the compost product. Whether CT is made from composts that have manure feedstocks or vegetative matter only have been shown to affect efficacy as well for certain host/patho systems (Elad & Shtienberg, 1994). Feedstock effects on biological, chemical, and physical characteristics of compost and thereby CT may impact efficacy.

A primary desired benefit of CT is the ability of teas to suppress plant diseases. The biology present in composts/CT lends the suppressive ability (Edwards et al., 2011; Marín et al., 2013). Various studies have shown suppression of a range of fungal and bacterial plant pathogens in different agricultural systems, though with inconsistent efficacy (Hadar, 2011; Litterick et al., 2004; Martin et al., 2012; Scheuerell, 2002; Scheuerell & Mahaffee, 2006). Mechanisms of disease suppression include competition for nutrients, antibiosis, secreting secondary metabolites that are toxic to pathogens, parasitizing pathogens, or activating plant defense responses (Agrios, 2005; W. F. Brinton, 1995; On et al., 2015; Scheuerell, 2002; Segarra et al., 2009). Mode of action may be different for different host/disease systems. A study by On et al. (2015) demonstrated a synergistic effect of specific bacteria found in composts for disease reduction, adding further support to idea that a consortium of biology may be more effective than specific biocontrol agents on their own. However, while a CT may be effective against one disease, it may not be for another, and therefore broad generalities are difficult to make. Many research studies present extreme disease pressure and artificial conditions that would not mirror the natural field environment so the complicated interactions in the field may also produce different results. Compost tea as a preventative or protective measure is more likely to provide protection as opposed to reactive efforts to reduce populations already above economic impact threshold (Scheuerell & Mahaffee, 2002).

When CT is applied as a foliar spray, a spreader-sticker (surfactant) may be used (Mahaffee & Scheuerell, 2006). Spreader-stickers help a spray solution cover evenly and effectively over the surface of leaves, thus helping the CT to adhere to the surface and making it more likely to be effective (Rajkovic & Markovic, 2012). Such adjuvants have been shown to have some degree of disease suppression applied alone (Bruggen et al., 2016). There have been studies that employed a spreader-sticker (McGrath, 2009), and others that have not (Segarra et al., 2009). There is a paucity of research as to the degree to which a spreader-sticker may aid in the suppressive ability of a CT and more work is needed in this area (Martin, 2014). NuFilm P (Miller Chemical and Fertilizer, LLC) is a spreader-sticker approved for use in organic systems that could provide increased efficacy of CT treatments but needs to be evaluated.

Tomatoes are one of the most important agricultural fruits and vegetables grown and consumed around the world (Blancard, 2012; Wang et al., 2009). While there are many diseases that affect tomatoes, one of the most prominent in greenhouse/high tunnel grown tomatoes is *Fulvia fulva* (syn. *Mycovellosiella fulva*) is commonly referred to as tomato leaf mold (Blancard, 2012). *F. fulva* is a parasitic fungus that attacks the leaves, appearing early as light green to pale yellow spots and progressing to brown necrotic lesions (Figure 4.1). Its damage can be considerable and cause significant yield losses. Primary means of management are cultural measures such as cultivar selection for resistant varieties, proper ventilation, removal of old and/or infected leaves, and fungicide application though no fungicides that are approved for organic systems are labeled for use against *F. fulva* (Bardin & Gullino, 2020; Blancard, 2012; Caldwell et al., 2013).

Powdery mildew (PM) is an obligate biotroph, a common fungal pathogen of many plants and particularly the genus *Cucurbitaceae* including winter squash (Hacquard, 2014). PM can give a white fuzzy appearance to leaves in early stages and results in complete leaf necrosis as the disease progresses (Grubinger, 2005; Pérez-García et al., 2009, Figure 4.1). PM contributes to decreases in harvestable yield, immature fruits, and premature death of plants (Caldwell et al., 2013; Rur et al., 2018). *Podosphaera xanthii* is one species of fungus that is commonly referred to as powdery mildew and is a significant disease affecting winter squash varieties (Barickman et al., 2017; Pérez-García et al., 2009). Varietal selection of those breed for resistance to PM has been a primary means of control, as well as removal of infected tissue, and commercially available fungicides that have varying degrees of efficacy (Caldwell et al., 2013). Development of fungicide resistance has been demonstrated to be a particular problem with *P. xanthii* around the world (Pérez-García et al., 2009; Rur et al., 2018).

CT may be more important for biological/ecological agricultural systems, especially those that are organically certified, which have limited options for managing diseases (Bruggen et al., 2016; Zaker, 2016). Additionally, crops such as tomatoes that have a scalar harvest over time often would have time-to-harvest intervals making options even more limited (Bellini et al., 2020). A result of some studies has been that disease management provided by CT or other biofungicides would not be commercially acceptable if there were other means of controlling disease, but also that use of CT may be best used in an integrated pest management system instead of being relied upon as a sole method of disease control (Barickman et al., 2017; Evans & Percy, 2014; Marín et al., 2013; Martin, 2014). Additionally, resistance to many fungicides traditionally used for these diseases has become an issue, as well as concerns for both environmental and human health, making development and better understanding of biofungicides even more pressing (De Corato, 2020; Mahaffee & Scheuerell, 2006; Pérez-García et al., 2009; Rur et al., 2018; Zaker, 2016). Determination of CT treatments as biofungicides that would maximize efficacy could increase adoption of its use and the options available to organic growers.

The objective of this study was the evaluation of aerated compost teas, made with three composts that varied by starting feedstocks and composting method, for their effects on two host-disease systems: tomato leaf mold on tomatoes, and powdery mildew on winter squash. An additional objective was the assessment of the use of a sticker-spreader and if such use enhances the suppressive abilities of compost tea by looking at its effects both on its own and in conjunction with the vermicompost CT treatment. We hypothesized that all compost tea treatments would have significant reduction in disease as compared with the no-spray control but the extent of disease reduction would vary. We also hypothesized that the spreader-sticker would have some degree of efficacy on its own, but the synergistic effect of the vermicompost tea and the spreader-sticker would be greater than either treatment on their own. Yield data from compost tea treatments was hypothesized to be greater than in the control and correlate with degree of reduction of disease. We further hypothesized that these effects would be consistent across different genera, and species/varieties within genera. While results are limited to these specific host/disease systems, assessing composts that have such contrasting characteristics on multiple host/disease systems may help us understand whether one type of compost may be more effective than another for disease suppression more broadly when used to make CT.

MATERIALS AND METHODS

Five separate experiments were conducted at Michigan State University (MSU) Student Organic Farm (SOF) at the Horticulture Teaching and Research Center (HTRC) in Holt, MI (42°40” N, 84°28” W) in the summer of 2016. Each experiment evaluated the effects of compost tea treatments on a different crop or cultivar. These included two varieties of tomatoes, and three varieties of winter squash. Working within the existing planting schedule and plan for a working farm, the number of rows and plants were limited so both size and number of replications were made within these constraints.

Compost Tea Treatments and Experimental Design

Compost: For all 5 experiments, three types of compost tea were used with two factors being considered: method of composting and compost feedstocks. The two composting methods employed were themophilic (hot) composting, and worm or vermicomposting. A thermophilic compost was made with the feedstocks municipal fall-collected leaves, fresh cut grass from organic certified land, and spent coffee grounds at approximate volume ratio of 2:2:1. An approximately ten cubic yard turned windrow was managed over the course of two months, then stored in wooden crates (approximately 1yd^3) in an unheated high tunnel for approximately 1 year prior to use in the trial. The second thermophilic compost had a primary feedstock of dairy manure, and is a commercially available compost called Dairy Doo® produced in managed windrows by Morgan's Composting, Inc. (Sears, MI). The final compost was vermicompost made with the feedstocks of municipal fall-collected leaves and pre-consumer kitchen preparation food scraps. These feedstocks were first pre- hot composted for two weeks and then surface applied to a wedge windrow vermicomposting system in a high tunnel that allowed approximately six months for the earthworms (sp. *Eisenia fetida*) to process the material.

Total nutrient analysis by ashing of each compost included N, P, K, Ca, Mg, S, and Na (%) and ppm Zn, Mn, Fe, Cu, B, and Al. Saturated media extract (SME) analysis included pH, electrical conductivity (EC), nitrate-N, ammonium-N, P, K, Ca, Mg, Na, and Cl (ppm).

Compost tea preparation: Compost teas were brewed with forced aeration for 48 hours prior to application, using a built brewing system of 15-gallon plastic totes with PVC piping with holes drilled into it arrange along the bottom attached to a 1750gal/hr air pump providing forced air. The ratio of compost to water was 1:5 v/v for all CT applied; ten gallons were brewed at a time with 2 gallons of compost contained in a 5-gallon capacity paint strainer bag. Three CTs were made with identical methods, the variable being the type of compost used. At the end of 48 hours the sleeve of compost was removed and spraying of CT began within 1 hour after aerated brewing finished.

Quality control parameters of oxygen concentration and temperature were monitored for each batch of CT made from each compost. Oxygen content remained consistent in each batch, averaging just over 100ppm O_2 throughout the duration of brewing (measured using ExStik®II dissolved oxygen meter by Extech). Brewing was in a laboratory setting with ambient temperatures of approximately 21°C, and temperature of CT averaged just below 20°C.

Treatments and application: Each of the 5 separate field experiments were arranged in a randomized complete block design with 3 replications of six treatments: CT made from the leaf/grass/coffee compost (LGC), CT made from dairy manure based Dairy Doo® compost (DMC), CT made from the vermicompost (VC), VC with NuFilm P spreader-sticker added just prior to application (VC+Nu), NuFilm P alone added to water (NuFilm), and a no-spray control (Control). The unsprayed control treatment was included to compare actual practices used by farmers in the field, and in particular the farm management plan at the SOF for these crops. While brewing method (aerated versus non-aerated) effect on efficacy of teas is another important distinction, aerated only was selected, as number of treatments was limited by the size of the plot available. Additionally, aerated methods have been gaining in popularity, and allow for a faster turn-around time when CT is needed, allowing farmers to take weather forecast into account when deciding when to brew CT.

Undiluted CTs were filtered through three layers of cheese cloth to remove any remaining debris when put into Chapin pump backpack sprayer. CT was sprayed onto foliage using backpack sprayer with handheld boom, allowing for full coverage by wetting leaves on all surfaces to the point of runoff (approximately 50gal/acre when plants were full grown). Sprays were applied on a weekly basis for a total duration of 13 weeks from June (6/8) to September in 2016. Weather was monitored to ensure that leaves were not wet prior to application and would have sufficient time to dry before any rain event.

Tomato Leaf Mold (*Fulvia fulva*) Trials

In the two tomato experiments, we evaluated the effects of CT on high tunnel grown tomato (*Solanum lycopersicum*) cvs. 'Big Beef' and 'Celebrity.' For each experiment, treatments were applied to experimental units of two plants, with three replications of the six spray treatments with one plant buffer between each. Plant spacing was 24" within row, one row per 30" bed. Plants were top trellised and suckers removed as the plants grew to allow for good air flow, and plants watered with drip irrigation.

Winter Squash Powdery Mildew (*Podosphaera xanthii*) Trials

In three separate experiments, we evaluated the effects of CT on three different varieties of winter squash. *Cucurbitaceae moschata* 'Waltham Butternut,' and *C. pepo* 'Delicata' experiments were planted in 150' beds and four plants (2 plants per bed spanning 2 beds, approximately $144ft^2$) comprising an experimental unit with a block of four plants between for a buffer. *C. maxima* 'Blue Ballet Hubbard" squash plants were arranged in one bed of 150' with three plants (approximately $108ft^2$) per experimental unit with one additional plant between for a buffer. Buffers were in place to eliminate the factor of spraying overlap, and account for the vining nature of winter squash growing into one another. There were three replications of each of the six spray treatments for a total of 18 experimental units per species. For all winter squash, spacing was 24" within row, and 6' between rows, with irrigation provided by drip tape. The field was cultivated prior to placing 4-week-old squash transplants, and weeded again at three weeks after transplanting. Late season weed pressure was very high.

Data collection

For both tomato and winter squash, plants were monitored weekly for disease. Disease incidence and severity of tomato leaf mold was assessed twice for tomatoes, once shortly after disease had appeared (7 August, 2 months after spray trial began), and once when disease had spread significantly (12 September) based on visual observation and before any final harvest of fruits. Winter squash varieties were visually rated for powdery mildew, for both incidence and severity, twice for 'Delicata' (8/5 and 9/9) but only once for 'Butternut' and 'Hubbard' (on 8/6) due to high weed pressure eventually ending their trial early and final attempt at rating having insufficient leaves to assess.

Disease incidence is the percentage leaves with disease, calculated from the number of leaves with disease out of a total of 30 leaves per experimental unit. Disease severity is the percentage of leaf area that is symptomatic on diseased leaves only. Percentages of disease severity on individual leaves were averaged for all leaves assessed that had disease incidence per experimental unit and this average was used for the severity measure. Overall severity is a measure of the total leaf area infected (%) for the whole plant and is calculated using the equation

[1] (Incidence * Severity)/100.

For tomatoes harvest data were also collected, for both total number of fruit and total weight throughout the multiple harvests during the growing season, data collected from mid June to late September approximately every 4 days. Winter squash harvest data was not possible at this farm.

Statistical Analysis

Data analyses were carried out using R statistical software package, and additional packages for R including lme4 for the linear mixed model and multcomp for Tukey's HSD pairwise comparisons (R Core Team, 2019). Unless otherwise noted, statistical significance is determined at a<0.05. Data were analyzed for normality and heterogeneity of variance prior to analysis of variance. Where normality assumptions were violated, the data were log transformed prior to analysis and Sattherthwaite's degrees of freedom was employed to account for potential nonequal variance in the data.

One-way ANOVA with random effect to account for the blocking effect of the RCBD design was performed for each species/variety, as well as mean separation ($p \leq 0.05$ unless otherwise noted). Mean separation by Tukey's Highly Significant Difference (HSD) test was performed for pairwise comparisons ($p \leq 0.05$ unless otherwise noted). This means of statistical analysis was done for the individual varieties and their respective diseases, for disease incidence, severity, and overall severity, as well as for the harvest data collected for the tomato varieties. To address the hypothesis that the efficacy of VC was affected by the use of the spreader-sticker NuFilm P, we conducted a separate two-way ANOVA on the subset of four treatments representing all four combinations of VC and NuFilm P.

RESULTS

Tomato Disease and Yield

For 'Big Beef' disease ratings from the early assessment there was a treatment effect on overall severity ($F=10.052$, $p=0.001$), incidence ($F=9.192$, $p=0.002$) and severity of infection on infected leaves only ($F=4.5$, $p=0.021$; Table 4.1, Figure 4.2). The overall severity of *F. fulva* as a measure of the percent leaf area infected on the entire plant ranged between 8.0% (Control) and 1.2% (VC+Nu). All treatments were lower than the Control, and additional VC+Nu was also lower than LGC and DMC. The percent of leaves infected (disease incidence) ranged between 90.0% (Control) and 40.0% (VC+Nu); VC, NuFilm and VC+Nu were all lower than the Control, as well as some additional treatment differences. Severity of infection on infected leaves ranged between 8.8% (Control) and 3.0% (VC+Nu) with no differences between experimental treatments, but all were lower than the Control.

For 'Celebrity' there was a treatment effect on overall severity (F=8.546, p=0.002), incidence (F=8.351, p=0.002) and severity of infection on infected leaves only (F=5.288, p=0.012; Table 4.1). The overall severity ranged between 10.9% (LGC) and 2.6% (VC+Nu). VC+Nu treatment was different from all but DMC, and DMC was different from the Control and LGC treatments. The percent of leaves infected (disease incidence) ranged between 94.4% (VC) and 52.2% (VC+Nu), with the only differences observed being between VC+Nu and all other treatments. Severity of infection on infected leaves ranged between 12.2% (LGC) and 4.6 (VC+Nu) with VC+Nu being different from the Control, LGC and VC, and DMC different from LGC.

Suppressiveness of CT sprays diminished over the season in both varieties (data not shown). Data taken later in the season (on 12 September) when disease pressure was significantly higher yielded no differences between any treatments for overall severity (F=6.637, p=0.677 and F=0.585, p=0.712 for 'Big Beef' and 'Celebrity', respectively). The range of the percent leaf area infected at this time was between 26-45% for 'Celebrity' and 34-46% for 'Big Beef' (data not shown). Similarly, neither incidence or severity were different by treatment.

In the evaluation of the subset of data to specifically assess the interactive effects of VC CT and the spreader-sticker NuFilm P, the two-factor ANOVA performed on the data from the first disease rating for overall severity demonstrated significance of the main effects of both VC and NuFilm, as well as their interaction for both varieties of tomato (Table 4.4). For 'Big Beef' a decrease in overall disease severity with the application of NuFilm was only observed in the absence of the VC CT (Figure 4.3A), whereas when VC CT was already being applied, NuFilm did not appear to increase its efficacy. The interaction for ‘Celebrity’ was marginally significant (p=0.062, Figure 4.3B), and displayed a different trend with NuFilm decreasing overall severity on its own and in concert with VC CT, but even more effectively when combined with VC CT than when on its own.

Yield differences between treatments were not observed for total number of fruit harvested, total weight harvested over the season, or fruit weight (grams per tomato) for either variety (Table 4.2). ‘Big Beef’ mean harvestable weight per plant ranged from 6.9-11.8 pounds per plant, while ‘Celebrity’ harvest weights ranged from 14.4-17.7 pounds per plant. The total number of tomatoes by variety was greater for ‘Celebrity’ with 35-42 tomatoes per plant whereas ‘Big Beef’ yielded 15-24 tomatoes per plant. In this trial, yield was not affected by spray treatment and not affected by disease severity.

Winter Squash Disease

Winter squash varieties were visually rated for powdery mildew, for both incidence and severity on 5 August (Table 4.3, Figure 4.4). Level of infection was higher for ‘Delicata’ (4.2-12.5% overall leaf area infected) followed by ‘Hubbard’ (0.8-2.5%) and then ‘Butternut’ (0.8-1.8%). Delicata and Hubbard squash displayed no differences in overall disease severity, incidence, or severity of infected leaves by treatment (Table 4.3, Figure 4.4 for overall severity).

For the ‘Waltham Butternut’ squash trial there was a treatment effect on overall severity (F=4.297, p=0.024), marginal effect on severity of infection on infected leaves only (F=2.609, p=0.092) while no differences were observed between treatments for incidence (F=2.363, p=0.116; Table 4.3). The overall severity ranged between 1.8% (Control) and 0.8% (both VC+Nu and LGC), with all treatments besides NuFilm being different from the Control, and no differences between spray treatments. Disease incidence ranged between 69.3% (Control) and

50.7% (VC+Nu), and despite a lack of significance observed in the linear model, Tukey's HSD comparison resulted in separation between VC+Nu and the Control, with all others being not different statistically. Severity of infection on infected leaves ranged between 2.6% (Control) and 1.5 (VC+Nu and LGC) with VC+Nu and LGC being marginally different from the Control.

Excessively weedy conditions complicated the experiment, ending the spray trial of 'Hubbard' and 'Butternut' earlier than anticipated, and while a final disease rating was attempted the plants were so compromised by the weeds any data were discarded. Downy mildew (*Pseudoperonospora cubensis*) was also present on the 'Butternut' making it difficult to assess powdery mildew only. Delicata squash, however, were rated a second time on 9 September, when disease pressure had increased by between 284% (Control) and 785% (VC+Nu), which had originally displayed the least signs of disease (data not shown). The spread of the overall severity data for the final rating of 'Delicata' was from 36% for the LGC and VC CT treatments to 48% leaf area infected for the Control with no differences between treatments as was the case for the rating of disease earlier in the season for Delicata ($F=1.164$, $p=0.390$; data not shown). There were very few fruits for harvest of 'Blue Ballet Hubbard' and due to unexpected circumstances, no harvest data were collected for 'Butternut' or 'Delicata.'

The two-factor ANOVA used to evaluate the interaction of VC CT and NuFilm P for the Butternut squash overall severity of infection early in the season indicated the significance of VC CT ($p=0.012$), which decreased overall severity independent of NuFilm (Table 4.4). NuFilm was marginally significant ($p=0.087$), also decreasing overall severity with its use independent of VC CT. The combination of VC CT and NuFilm did not impact efficacy ($p=0.669$, Figure 4.3C).

DISCUSSION

The primary hypothesis that all compost tea treatments would have significant reduction in disease as compared with the no-spray control was supported in some of the experiments but not others. The Control in all experiments was the same or higher than all of the experimental treatments for the metrics of overall severity which is of the most interest, incidence and severity of disease on infected leaves. Of the experiments where differences were observed, for 'Big Beef' tomatoes and 'Butternut' squash all CT sprays were different from the Control, while for 'Celebrity' tomatoes the LGC CT was not different but the other CTs were. There appeared to be a lack of any efficacy in reduction for 'Delicata' and 'Hubbard.' Within compost tea literature, there are many examples of disease reduction but also many where there was no effect which is consistent with our results (Martin, 2014).

That the extent of the disease reduction would vary was again supported by some of the experiments but not all. For the tomatoes, there was variation between spray treatments. Of particular interest is the difference in efficacy of the LGC and the VC CT sprays where in the 'Celebrity' tomatoes these were not different than the Control, while they did reduce disease severity for 'Big Beef' tomatoes. For all the winter squash trials, there were no differences between the experimental sprays, and only for 'Butternut' was there even a difference between spray treatments and the Control.

The hypothesis that the spreader-sticker would have some degree of efficacy on its own when compared against the Control was supported in both tomato experiments. This was seen from the full set of data and pairwise comparisons, and was even more apparent in the subset of data examining the main effects of VC and NuFilm P. This is in contrast to the three winter squash experiments in which NuFilm P had no effect on any of the varieties; however, for

‘Butternut’ squash when analyzed in the two-way ANOVA the results were marginally significant (p=0.087, Table 4.4).

The results of increased efficacy in VC+Nu (for ‘Celebrity’ tomatoes) and of efficacy of NuFilm P on its own further substantiates findings by Rajkovic and Markovic (2012) in which they used NuFilm P in conjunction with biofungicide AQ10 for powdery mildew on oak trees, as well as NuFilm P on its own demonstrating efficacy for disease reduction. Rur et al. (2018) evaluated spreader-sticker Yuccah for effects with and without a biocontrol agent also demonstrating greatest efficacy in PM control by use of a spreader-sticker. This is, however, in contrast to findings by Yohalem et al. (1996) in which the spreader-sticker Latron had no effect on CT efficacy for apple scab. In our trial whether there was any additive effect of VC CT and NuFilm was dependent on species/variety. For ‘Celebrity’ tomatoes VC+Nu overall severity was lower than for either treatment on its own. This did not hold true for ‘Big Beef’ tomatoes or butternut squash, however, where the treatments were not different from one another. This should be reviewed further, and testing spreader-stickers with other CTs to determine if there is in fact likely to be an additive effect or not, and if this would be dependent on type of CT, or plant/pathogen system.

What differences there were between treatments did not appear to have a lasting effect. There was also no impact on yield of tomato in either variety by treatment. This refuted the hypothesis that compost tea treatments would result in increases in yield. This is contrary to other findings in which irrespective of disease presence it was found that yield could be increased by foliar feeding of nutrients found in CT sprays (Haggag & Saber, 2007; Martin, 2014). In a study of effect of foliar application of Ca and Mg on tomatoes, Ilyas et al. (2016) suggests their addition can increase growth and yield of tomatoes. Increases of disease severity are generally inversely proportional with yield in many plant/disease systems (Gaunt, 1995), but at least the early reduction in disease did not correlate with increases in yield in these experiments, and for the yield data there was nearly significant differences of some treatments, but for decreases in yield which is the opposite of what would have been expected.

In looking at the results of the five different experiments together and comparing significance by treatments, the hypothesis that effects of CTs would be consistent across different genera, and species/varieties within genera is not supported. While some of the CT sprays were effective for tomatoes, this was largely not the case for winter squash. Within tomatoes, the effect of the LGC and VC CTs in particular were inconsistent, while the DMC CT and VC with NuFilm were consistent in their reduction of overall severity. Within winter squash, efficacy was only observed for 'Butternut,' thereby undercutting the assertion that these CT sprays are effective against *P. xanthii*.

For the two different tomato experiments the varieties responded differently to some treatments. The plant morphology of the two different tomato varieties may have had an impact on the efficacy of the CT and spreader-sticker sprays. For the 'Celebrity' tomatoes (a semi-determinant variety), the plants were dense/compact with foliage. This was in contrast to the 'Big Beef' (an indeterminant variety) for which the top trellising kept plants very vertical, growing tall with less foliage lower in the plant. This likely allowed increased airflow which is helpful for suppression of *F. fulva* and other fungal diseases (Blancard, 2012). Free standing water can increase the presence of the disease, and with more dense foliage in the 'Celebrity' tomatoes foliage is likely to have stayed wet longer and this could be part of the reason for the greater overall disease levels in this variety for all treatments. This would give greater weight to the decrease in disease observed for DMC and VC+Nu treatments despite this complicating factor. To assess this more directly, data on the average length of time the foliage remained wet in both varieties could be taken in future experiments.

The common name powdery mildew is used for a variety of different fungal species that produce similar morphological features and responses to host plants. The lack of efficacy of CT against powdery mildew has been demonstrated in other host/patho systems such as Koné et al. (2010) or Kelley et al. (2004), whereas other studies have found significant control of the disease (Marín et al., 2013; Naidu et al., 2012; Segarra et al., 2009) though some of this work has been done on other fungal species responsible for PM. It can be difficult to compare across studies, even if the specific host/pathogen system is the same due to the differences in CT used for treatments.

Documentation of efficacy of CTs to reduce disease presence/severity caused by foliar pathogens has been less successful in field trials as compared to trials in more controlled environments. Martin (2014) reviewed 42 field trials for different host/pathogen systems finding that 57% of CT treatments had less disease compared with the control, whereas 89% of the 47 *in vitro*, greenhouse and/or container experiments reviewed resulted in CT treatments with decrease in disease. This was attributed at least in part to the diverse interactions of biotic and abiotic factors that are generally not present in a more controlled setting. In these experiments the high degree of variability may contribute towards the lack of statistical significance between treatments. The low number of replications (n=3) per treatment contributed to this, and future experimentation should aim to increase replication where possible. Replication of the experiments in subsequent seasons as well would add to the knowledge of how reliable these results might be.

Regarding differences between the plant/pathogen systems in this study it is important to note the role of leaf wetness. While the presence of water on the leaf surface will aid in the transmission of and the infection by *F. fulva*, the opposite is true of *P. xanthii*. Based on the biology of the pathogens, suppression results observed in tomato are very likely real treatment effects, while for squash, it is possible that effects are just due to application of water.

The primary decision for the control treatment to be "no spray" as opposed to a water spray was what the default method that would be employed on a farm, in which they would be unlikely to ever spray just water on foliage. This choice was especially important in the high tunnel environment in which rainfall is excluded and the practice on this farm was for drip irrigation in which the leaves were never wet unless by the intentional spraying for this study. Also only having a negative control with the no spray treatment, inferences as to how CT would have compared to a positive control of a conventional fungicide known to treat these diseases cannot be made under these conditions.

FUTURE RESEARCH AND CONCLUSIONS

Biological data for the constituent bacteria and fungi in each of the respective compost teas if collected could be related to the relative efficacy. Collection of such data by MiSeq analysis was originally intended for this study but was not possible. If in the future specific organisms could be isolated against the diseases of interest it could even be possible to establish a new specific biological control organism that could be targeted in compost and CT to increase efficacy. There are multiple biocontrol organisms that have already been identified for efficacy against *F. fulva* and *P. xanthii* (Gao et al., 2016; Lee et al., 2011; Zhang et al., 2018) that may be present in some composts, and species specific data from multiple types of composts would elucidate whether there is a factor of feedstock or production measures on presence of these specific organisms in compost or CT.

Based on this study, CT sprays from the vermicompost and dairy-manure based compost used in this study can provide some disease management benefits of *F. fulva* tomatoes and butternut squash. Similar to prior published research, certain compost teas were disease suppressive while others were not. Later in the season differences between treatment efficacy declined in all experiments, indicating that while CT may be effective at delaying the onset and early spread of disease the efficacy diminished over time in the present study. CT could be incorporated into a spray regimen for disease management but appears unable to suppress disease effectively on its own over the course of the growing season.

Farmers have expressed their desire for more information on the topic of compost teas, looking for more concrete recommendations consistent with chemical pest management strategies. More research into CTs with methods that are reproducible by farmers may increase the adoption of the use of CTs if found to be effective against specific host/pathogen systems such as *F. fulvia* demonstrated here. More research of compost teas is needed to better understand the mechanisms of disease reduction and how to reliably produce CT that will decrease disease severity.

APPENDIX

Table 4.1. Overall severity, incidence, and severity for ‘Big Beef’ and 'Celebrity’ tomatoes infestation by *F. fulvia*. Data taken on 7 August 2016, two months after weekly spraying commenced when plants showed moderate signs of disease.

Tomato Variety	CT Treatment	Overall Severity (Total % Leaf Area Infected)		Incidence (% Leaves Infected)		Severity (% Infection on Infected Leaves)	
'Big Beef'	Control	8.0 (1.4)	c	90.0 (6.9)	d	8.8 (1.0)	b
	LGC	3.9 (1.5)	b	75.6 (6.2)	cd	5.0 (1.8)	a
	DMC	3.1 (0.2)	b	66.7 (5.1)	bcd	4.6 (0.4)	a
	VC	2.3 (0.8)	ab	51.1 (8.7)	ab	4.2 (0.9)	a
	VC+Nu	1.2 (0.2)	a	40.0 (6.7)	a	3.0 (0.5)	a
	NuFilm	2.1 (0.3)	ab	52.2 (7.3)	abc	4.0 (0.3)	a
P		0.001		0.002		0.021	
'Celebrity'	Control	9.6 (3.6)	c	92.2 (4.8)	b	10.2 (3.4)	bc
	LGC	10.9 (3.1)	c	88.9 (1.1)	b	12.2 (3.4)	c
	DMC	3.9 (1.0)	ab	74.4 (8.0)	b	5.2 (1.1)	ab
	VC	9.4 (3.3)	bc	94.4 (2.9)	b	9.8 (3.2)	bc
	VC+Nu	2.6 (1.3)	a	52.2 (7.3)	a	4.6 (1.6)	a
	NuFilm	5.4 (1.4)	bc	76.7 (10.2)	b	6.8 (1.0)	abc
P		0.002		0.002		0.012	

* means followed by the same letter in the same column are not statistically different according to Tukey’s HSD test at p<0.05.

Table 4.2. Harvest data for 'Big Beef' and 'Celebrity' tomatoes by number of tomatoes harvested, total weight harvested, and grams (g) per tomato over the course of the season per plant.

Tomato Variety	CT Treatment	Number tomatoes per plant	Pounds tomatoes per plant	Grams per tomato
'Big Beef'	Control	20.3 (4.0)	11.8 (3.7)	0.55 (0.08)
	LGC	23.7 (2.8)	11.5 (1.3)	0.49 (0.02)
	DMC	23.5 (2.0)	11.7 (1.7)	0.49 (0.03)
	VC	18.3 (0.6)	9.4 (1.1)	0.51 (0.05)
	VC+Nu	22.0 (1.9)	11.6 (0.9)	0.53 (0.01)
	NuFilm	15.5 (0.3)	6.9 (0.9)	0.44 (0.05)
P		0.177	0.411	0.651
'Celebrity'	Control	42.3 (3.6)	17.7 (0.7)	0.42 (0.02)
	LGC	37.0 (3.3)	16.0 (0.7)	0.44 (0.04)
	DMC	37.7 (1.4)	15.3 (0.7)	0.41 (0.01)
	VC	32.3 (2.2)	13.8 (0.7)	0.43 (0.01)
	VC+Nu	41.7 (2.2)	17.4 (0.6)	0.42 (0.03)
	NuFilm	34.8 (5.8)	14.4 (2.2)	0.41 (0.01)
P		0.079	0.060	0.925

Table 4.3. Overall severity, incidence, and severity for 'Waltham Butternut,' 'Delicata,' and 'Blue Ballet Hubbard' squash infestation by *P. xanthii.* Data taken on 5 August 2016, two months after weekly spraying commenced when plants showed moderate signs of disease.

Squash Variety	CT Treatment	Overall Severity (Total % Leaf Area Infected)		Incidence (% Leaves Infected)		Severity (% Infection on Infected Leaves)
'Waltham Butternut'	Control	1.8 (0.5)	b	69.3 (9.3)	b	2.6 (0.4)
	LGC	0.8 (0.2)	a	53.3 (10.4)	ab	1.5 (0.1)
	DMC	0.9 (0.1)	a	53.3 (6.7)	ab	1.8 (0.2)
	VC	0.9 (0.2)	a	56.0 (4.6)	ab	1.7 (0.4)
	VC+Nu	0.8 (0.2)	a	50.7 (10.4)	a	1.5 (0.1)
	NuFilm	1.2 (0.2)	ab	54.7 (9.3)	ab	2.2 (0.2)
P		0.024		0.116		0.092
'Delicata'	Control	12.5 (2.9)		67.8 (7.8)		18.3 (3.7)
	LGC	7.3 (2.3)		56.7 (3.8)		12.5 (3.1)
	DMC	6.2 (2.5)		57.8 (13.7)		10.0 (3.4)
	VC	8.7 (1.9)		62.2 (4.0)		14.1 (3.3)
	VC+Nu	4.2 (0.6)		51.1 (2.9)		8.2 (0.8)
	NuFilm	8.6 (0.3)		63.3 (3.8)		13.7 (0.9)
P		0.161		0.647		0.211
'Blue Ballet Hubbard'	Control	2.5 (0.7)		49.3 (9.3)		5.6 (2.4)
	LGC	2.7 (1.4)		37.3 (8.7)		6.1 (3.1)
	DMC	0.8 (0.2)		50.7 (4.8)		1.5 (0.3)
	VC	1.8 (0.6)		42.7 (6.7)		4.2 (1.6)
	VC+Nu	1.8 (1.1)		44.0 (6.1)		3.6 (1.9)
	NuFilm	0.3 (0.1)		36.0 (4.6)		0.9 (0.3)
P		0.265		0.494		0.353

* means followed by the same letter in the same column are not statistically different ($p<0.05$) according to Tukey's HSD test

Table 4.4. Significance (p-value) of the main effects of vermicompost compost tea (VC CT), the spreader-sticker NuFilm P, and their interaction on overall severity of *Fulvia fulva* on tomatoes and *Podosphaera xanthii* on butternut squash.

	Tomatoes		Winter Squash
	Big Beef	Celebrity	Butternut
Disease	*F. fulva*		*P. xanthii*
VC CT	<.001	0.046	0.012
NuFilm	<.001	0.002	0.087
VC+NuFilm	0.034	0.062	0.669

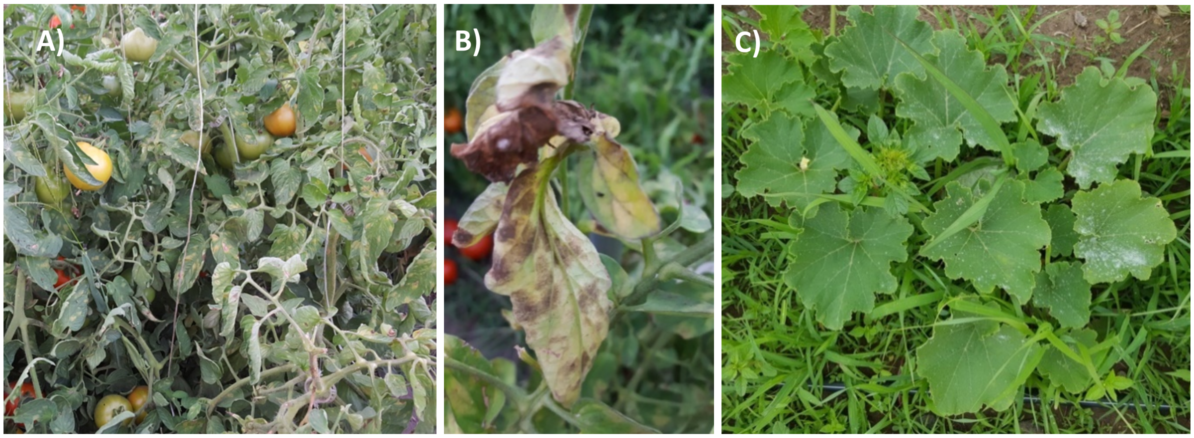

Figure 4.1. A) Tomato leaf mold on ‘Celebrity’ tomatoes, B) Close up of tomato leaf infected, and C) Powdery mildew symptoms on ‘Blue Ballet Hubbard’ squash.

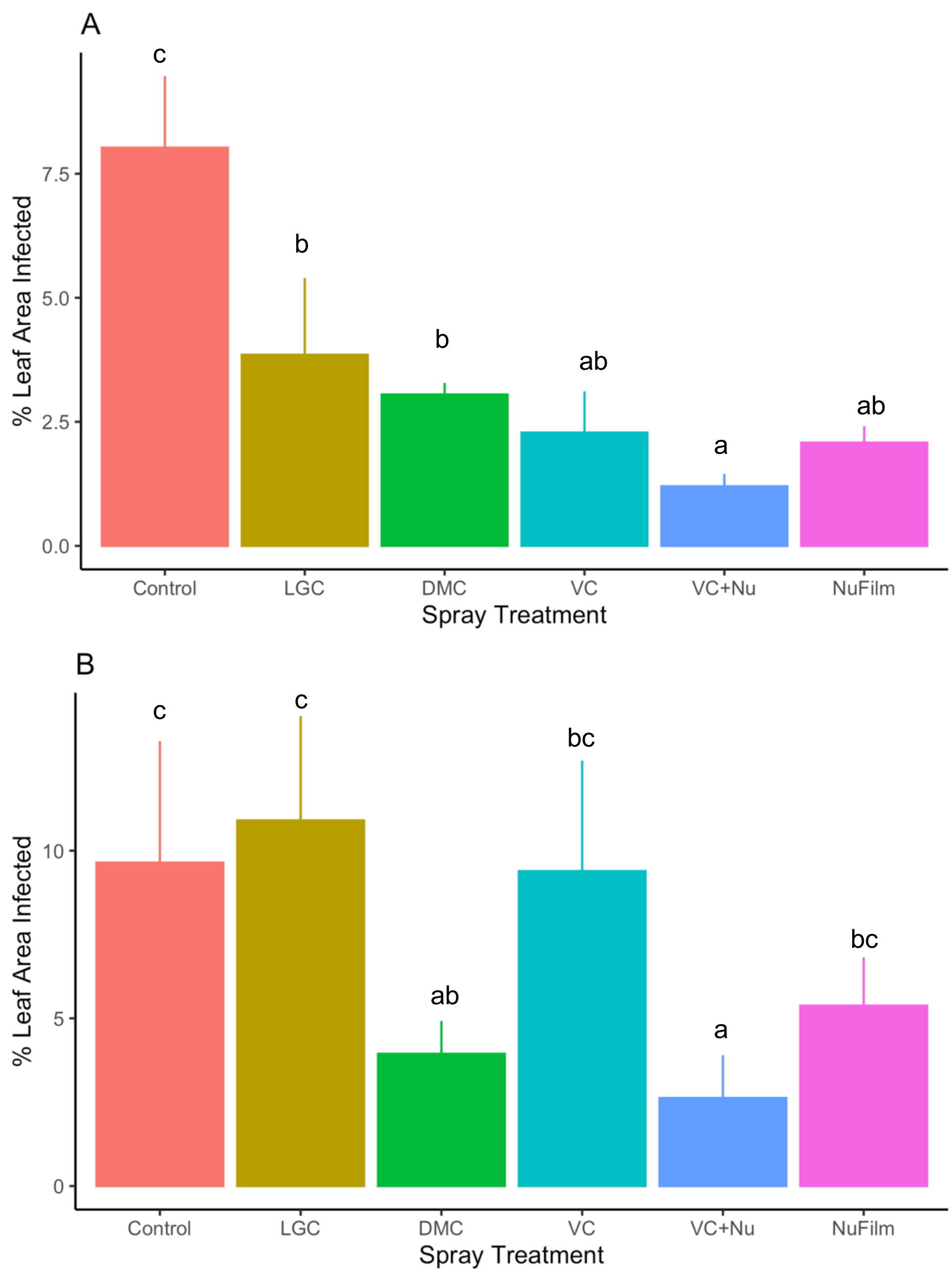

Figure 4.2. Overall severity (incidence*severity) as % leaf area infected by *F. fulvia* for tomato varieties A) 'Big Beef' and B) 'Celebrity' by compost tea treatment. Columns labeled with the same letter are not significantly different than one another ($p \leq 0.05$). LGC = CT from leaf/grass/coffee thermophilic compost; DMC = CT from dairy manure based themophilic compost; VC = CT from vermicompost (no manure); VC+Nu = VC with NuFilm P added; NuFilm = NuFilm P only.

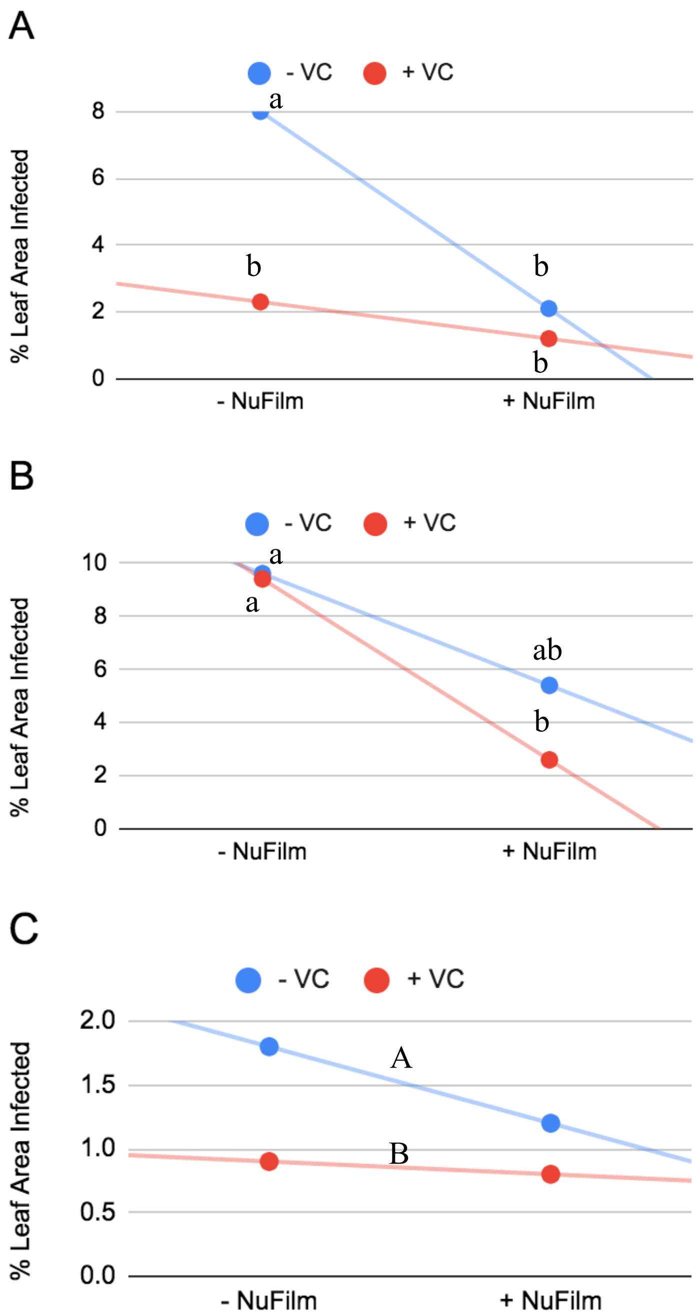

Figure 4.3. Overall severity (% leaf area infected) for the treatments Control (- VC, - NuFilm), VC, NuFilm and VC+Nu of *F. fulvia* on tomato varieties A) 'Big Beef' B) 'Celebrity' and of *P. xanthii* on C) 'Waltham Butternut' squash from disease ratings taken on 5 and 7 August in the early stages of disease. Letters indicate differences by treatment according to Tukey's HSD test ($p<0.05$), capital letters in C) are for main effects of VC only, as there was no interaction.

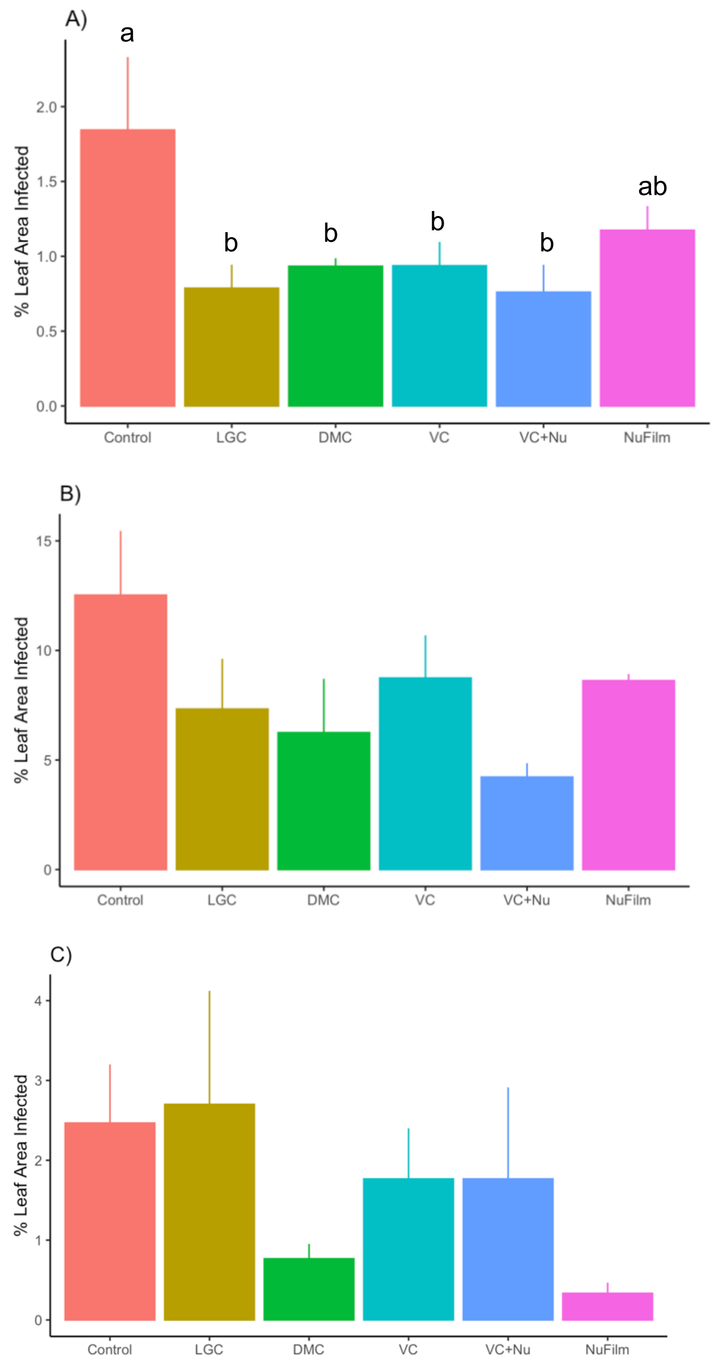

Figure 4.4. Overall severity (incidence*severity) as % leaf area infected by *P. xanthii* for three winter squash species/varieties A) (*Cucurbitaceae moschata*) 'Waltham Butternut', B) *C. pepo* 'Delicata', and C) *C. maxima* 'Blue Ballet Hubbard' by compost tea treatment. Columns labeled with the same letter are not significantly different than one another ($p \leq 0.05$). LGC = CT from leaf/grass/coffee thermophilic compost; DMC = CT from dairy manure based themophilic compost; VC = CT from vermicompost (no manure); VC+Nu = VC with NuFilm P added; NuFilm = NuFilm P only.

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