EXAMINING BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) COMPOSTING FOR URBAN AG SPECIALTY CROP PRODUCTION

by

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Dedicated to the two heftiest men I know: ***Beef*** *Truck Sheppard & WWF World Heavy Weight Champion Macho Man* ***Randy*** *Savage, my cats.*

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ABSTRACT

Urban farmers face many unique challenges associated with the urban environment in which they produce. One of the most expensive and limited resources is access to healthy soils. There is often low organic matter and industrial contaminants present in urban soils, resulting in the need for remediation, such as capping and importing topsoil and compost. Recently, black soldier fly larvae (*Hermetia illucens; BSF)* have been recognized as an efficient organism used to break-down organic matter and produce a soil amendment comparable to traditional fertilizers. These fly larvae can feed on a wide range of organic waste (plant material, biosolids, food waste, etc.), can break down contaminants such as pharmaceuticals or pesticides, and impact the bioavailability of heavy metals. The resulting material is a digestate that can be applied as a soil amendment, much like the vermicomposting processes of worms. Fly pupae can be harvested and used as a nutrient dense feedstock for livestock or reared to adults to continue the cycle of composting. Knowledge gaps remain regarding the impact of feedstock on the nutritional quality of the digestate for crop production and the application and implementation of BSF composting on-farm. We found that larval weight is unaffected by diet streams, however, larval length is improved on food waste streams. Additionally, crop growth varies when grown with BSFL digestate.

CHAPTER 1. LITERATURE REVIEW

1.1 Introduction to Black soldier fly

Black soldier fly (*Hermetia illucens* L.; Diptera: Stratiomyidae; BSF) is an insect native to the Neotropics and is currently distributed globally (Marshall et al. 2015). This fly is a detritivore/decomposer and has received much attention in recent years for its various applications to waste reduction and food production. The life cycle from egg to reproductive adult is 44 days, however, larvae stop feeding around 21 days and enter the pupal stage. BSF larvae (BSFL) progress through six instars (Klammsteiner et al., 2020) and shed a layer of chitin ($C_8H_{13}O_5N$) with each molt. They can tolerate a wide range of temperatures (24-40°C), and humidity levels (30-90%) and retain reproductive fitness (Sheppard et al., 2002).

BSF pre-pupae exhibit an interesting behavior known as tonic immobility where larvae will cease to move if exposed to external stimuli; this is typically coupled with increased chitin production and darker pigmentation suggesting developmental progression to the pupal stage (Giannetti et al., 2022). During this transition BSFL exhibit a "self-harvesting" behavior where prepupa BSFL will exit their food habitat in search of a dry environment where the pupae rest until emergence as adults. This self-harvesting behavior, coupled with BSFL's diet breadth make them an ideal candidate for compost production and the flies themselves provide a source of protein, fat, and essential amino acids for animal feed.

1.2 BSFL Survival and Development

Black soldier fly larvae feed on a variety of organic waste products. They have been applied in the management of biosolids and food/plant waste efficiently, with great potential for large scale management (Sheppard et al., 1994). In harvesting the larvae or pupae from this process, industries have developed a process to capture the protein, lipid and amino acids within the insect. BSFL have become one of the few insects being explored as a resource for food and feed. Industry, guided by research, has been refining the processes around feedstocks and rearing densities to optimize the nutritional values that can be derived from harvesting the insects themselves. Based on research conducted by Addeo et al. (2021), BSFL fed on the single sourced diet of vegetables were smaller in weight and length in comparison to BSFL reared on butchery wastes (fats and meat trimmings), however, overall development of larvae (total larval biomass, length:height ratio, growth rate, larval yield, and protein conversion ratio) were similar.

In another study, Fischer & Romano (2021) compared fruit waste (apples, oranges, bananas), vegetable waste (carrots, broccoli, green beans), and starches (potato, spaghetti, basmati rice). They found that while larvae reared on starch diets were heavier and contained the most fat, larvae reared on vegetable diets had higher protein values with less fat, demonstrating the impact of larval diet on the nutritional composition of the pupae (Fischer & Romano, 2021).

Like many organisms, there is evidence for increased larval growth on mixed diets, such as kitchen scraps (Nguyen et al., 2015) compared to single-item feed, such as manure or fruit and vegetable wastes. The kitchen scraps are likely to have more nutritional variety available to the consumer, both human and detritivore. However, BSFL provided single-source manure (swine, dairy, and poultry) were still able to develop into adulthood and reduce manure dry matter by 50%-75% (Bortolini et al., 2020; Miranda et al., 2019). This supports evidence that BSFL may have diet-dependent adaptations in the midgut to ensure exploitation of poor feeding substrates (Bonelli et al., 2020)..

Larvae may also participate in cannibalism to reduce competition in nutrient poor situations. Nguyen (2010) reported a rate of 46.6% cannibalism among BSFL groups when experiencing limited access to food. This cannibalism may explain the low survival rates of BSFL documented by Logan et al. (2021) compared to previous studies (Craig Sheppard et al., 1994; Xiao et al., 2018). It is important to keep in mind that although BSFL may have midgut adaptations for protein optimization, diets high in fat content may not produce the most fit larvae (Lalander et al., 2019). However, a documented benefit of elevated saturated fatty acids within larvae have been linked with cold adaptation (Meneguz et al., 2018) further supporting that diet greatly impacts BSFL health.

1.3 BSFL-derived Compost

In contrast to the attention given to BSF as a protein and fat source for food and feed, less has been devoted to the value of the digestate (frass) resulting from the rearing process. An exception is Anyega et al. (2021), who tested BSF compost sourced from spent worts in comparison with Evergrow (commercial organic fertilizer), a mineral fertilizer (NPK 23:23:0), and composted spent worts, in addition to combinations of BSF compost + NPK and spent worts + NPK. The objective was to compare the effects of commercial fertilizers with BSF compost along with mineral fertilizers mixed with composted, organic fertilizers. They reported that BSFL compost improved growth, yield, and the nutritional quality of vegetable crops, especially when combined with a mineral fertilizer (Anyega et al., 2021). This is likely due to either slow release of nutrients or mineralization.

The addition of gypsum and biochar into the diet of BSFL can increase the carbon: nitrogen ratios (C:N) of BSFL frass, which can be collected and applied as a soil amendment (Beesigamukama, et al., 2020a). BSFL compost from different diets alter resulting chemical composition and mineralization dynamics (Rummel et al., 2021). Explored by Green and Popa (2012), they showed that BSFL presence led to increased concentrations of ammonium (NH_4^+).

However, this could be due to low C:N ratios which are known to rapidly mineralize (Chiam et al., 2021). Ammonium is known to volatize into ammonia or nitrate gas if exposed to alkaline or acidic soils, respectively, which can injure sensitive plants such as tomatoes (Kawasaki et al., 2020), raising concerns over the application to specialty crops. In contrast to the tomato sensitivity, C:N ratios greater than 20 are prone to N immobilization, which may favor plants that have a rhizobiome. The zone around roots within the soil that are influenced by microbes, that can more efficiently exploit immobilized N (Klammsteiner et al., 2020).

Additional research has shown that maize, a nitrogen-hungry crop, accumulated higher levels of nitrogen on spent wort-derived BSFL compost compared to a locally available organic fertilizer (SAFI; Beesigamukama et al., 2020c) . This information, in conjunction with the BSFL compost in that study having lower NPK values (BSFL compost = 2.1:1.16:0.17; local organic fertilizer = 3.0:1.23:1.49) provides evidence that nitrogen being released from BSFL compost is happening over a greater period of time than SAFI fertilizer. This could lead to nitrogen immobilization however, as soil microorganisms prefer the high levels of ammonium nitrogen that were present in BSFL compost (Beesigamukama, et al., 2021a). Excess ammonium could raise cause for concern related to its ability to damage plants (mentioned above).

Black soldier fly larvae have also been investigated as a sustainable pathway to recycle chicken manure (Bortolini et al., 2020). However, BSFL health may be compromised on this study's diet due to high levels of lignin from the combination of chabazite-rich zeolitic tuff, water, and chicken feed, making it less optimal for co-product development. In a comparison study, Quilliam et al. (2020), examined feedstocks of chicken manure and spent worts to generate BSF compost in relation to using the raw ingredients themselves as the amendment. The process of BSF digestion resulted in a nutritionally superior soil amendment in comparison to the raw ingredient on its own (Quilliam et al., 2020). The additional process of BSF digestion has the advantage of increasing the value of these soil amendments, highlighted above, as poultry manure and spent worts are commonly used on their own, but with the added benefit of the protein and fat that can be harvested from the flies themselves.

An additional material generated from BSF rearing is the chitin-rich exoskeleton from larval molting, adult emergence, and the adult flies themselves. Nutritional composition of BSFL frass, larval skins, and adult flies vary greatly in regard to nitrogen content (3.3, 4.8, and 11.3, respectively) but are similar in content of other important compounds for plant growth: phosphorus (3.4, 2.8, 2.4, respectively) and potassium (2.4, 2.5, 1.1, respectively) (Gärttling et al., 2020). HexaFrass™, a BSFL soil amendment (used in Setti et al., 2019), had a similar NPK of 3-2-1, but pointed out that 2.3% of their total nitrogen in BSFL compost is described as slow-release, while the other 0.7% is considered fast-release. This observation supports the previously described improved maize development on BSFL compost (Beesigamukama, et al., 2020c).

1.4 Urban Agriculture

Growers within urban settings face certain restrictions related to the land they have access to, through purchase or lease agreements, ranging from access to water, food and worker safety in relation to exposure to soil contaminates with access to healthy, productive soils at the top of the list, which affects plant growth (Oberholtzer et al., 2016). In traditional growing environments, practices such as cover cropping and reduced tillage are tools that can be applied to increase soil health. However, another viable approach for improving soil health is the addition of soil amendments. This leads to the need to outsource materials to create suitable conditions for plant growth. UA farmers often purchase topsoil or compost to build raised beds, thus increasing the cost of production. Black soldier fly larvae rearing could resolve the obstacles of outsourcing for soil amendments while providing additional income. The introduction of BSFL rearing specifically onto peri-urban and urban production systems could lower the input costs, thus increasing the sustainability of urban farming.

1.5 Application of BSFL Compost

1.5.1 Insect frass effects on soil

Other insect frass studies have shown spongy moth (*Lymantria dispar*) insect frass had the most rapid carbon mineralization within the first 10 days compared to the total 120 days of incubation time (Lovett & Ruesink, 1995). Other research supporting these rapid mineralization trends report that compost decomposition increases with N concentration but decreases when C:N ratios are higher (Kagata & Ohgushi, 2012). These nutrient relationships are potentially described by microbial immobilization, denitrification, and/or ammonia volatilization (Kagata & Ohgushi, 2012). Further evidence of nutrient binding is highlighted by Klammsteiner et al. (2020), suggesting that C:N ratios >20 bears risk of N immobilization. These conditions, as previously mentioned, may favor plants with rhizobiomes that are able to exploit N more efficiently.

In another study, elemental analyses of control soil along with compost additions of an organic fertilizer and BSFL compost show that the control soil was lacking in many of the macro- and micronutrients essential for plant growth (Chiam et al., 2021). When comparing NPK values, plants grown in the control (N *above detection levels*-0.6-26.26; mg kg $^{-1}$) required additions of phosphorus (P) and potassium (K), which both organic compost (0.83-4.58-100.9; mg kg $^{-1}$) and BSFL compost (5.15-293.67-1929.67 mg kg $^{-1}$) were able to provide (Chiam et al., 2021). These observations show that BSFL compost works well as a soil conditioner.

1.5.2 Subsequent effects on plant growth

Increases in plant performance after applying BSFL compost as a soil conditioner can be seen with maize production in sub-Saharan Africa when compared to a standard organic fertilizer known as SAFI (Beesigamukama, et al., 2020c). This was likely due to higher levels of nitrogen that maize plants were able to accumulate from BSFL frass. Other studies have reported complex results when growing lettuce plants (Chiam et al., 2021). Lettuce grown in soil with 30% compost inoculation resulted in greater dry weights and saw greater dry weight with the addition of supplemental fertilizers. However, when given increasing rates of compost, lettuce plant growth was inhibited, providing evidence that there is an ideal rate of application, prompting further research.

BSFL compost (unknown diet source) was found to have positive effects on plant growth of the following potted plants: basil, borage, parsley, and phacelia (Borkent & Hodge, 2021). Further evidence from this study suggests that smaller, more frequent applications of BSFL compost may be better for plant and soil health. BSFL compost generated from okra (Beesigamukama, et al., 2020c) was able to support maize growth, and improved grain yields further supporting that BSFL compost is a justified alternative to commercially available organic composts.

Additional research has proven that BSFL compost can perform as well as inorganic fertilizers by providing similar yields for Swiss chard (Chirere et al., 2021) and perennial ryegrass (Klammsteiner et al., 2020), however, more research is needed to account for BSFL compost variability. These researchers hypothesized that additional chitin from larval instar casings could influence C:N ratios and the subsequent by-product, chitosan, could provide underrated plant benefits and pathogen resistance (Klammsteiner et al., 2020; Quilliam et al., 2020). However, additional research (Tan et al., 2021) has shown opposite results where lettuce grown on mineral fertilizer had greater yield (AG biomass; harvestable crop part for lettuce) compared to BSFL compost (upcycled food waste and wood waste BSFL diet).

Regardless of the source or production method, compost maturity must be attained to avoid phytotoxicity (Kawasaki et al., 2020). Organic wastes can have high moisture and organic matter which can inhibit germination and damage growth, along with high levels of salts or harmful pathogens. Further evidence in the same study (Kawasaki et al., 2020) showed the importance of analyzing and measuring the rates of compost addition to maximize the benefits. When 1/20 soil volume BSFL compost was applied to komatsuna seedlings there was no yellowing, suggesting adequate N availability. However, if applied at 1/10 soil volume, germination was inhibited.

Conversely, Menino et al. (2021) showed that ryegrass grown on vegetable waste sourced-BSFL compost from Entogreen® (onions and potatoes), applied at rates based on dehydrogenase activity (HDA), improved overall growth compared to no amendment added (fresh weight cuts 1-5, dry weight cuts 2-5). HDA was used to indicate oxidative microbial metabolism productivity within the soil, which is just one factor of soil health that can be measured. Treatments that had higher HDA resulted in greater dry weights for ryegrasses, specifically during later harvests. Because of these knowledge gaps and conflicting results, it is clear that further investigation is required on how larval diet and density affect BSFL compost, which in turn will affect plant growth and yield along with soil health.

1.6 Creating a circular economy

A circular economy, as defined by the EPA, refers to a systems-focused economy that involves restorative and regenerative industrial and economic activities aimed at waste reduction through the use of innovative materials, products, and systems. Because BSFL is a detritivore and can easily bridge agro-industrial loops by converting waste materials into two usable co-products, they present a unique opportunity to close agricultural loops and create an economic value for organic waste streams.

BSFL’s ability to reduce overall waste material for manure (44%), kitchen waste (67.9%), fish (74.2%), and vegetable/fruit waste (98.9%) (Nguyen et al., 2015) make them an ideal candidate for agro-industrial waste reduction, including processed and unprocessed livestock carcasses (Logan et al., 2021). Several authors have made observations that this waste reduction is overall more sustainable and could support circular economies and the revaluing of agro-industrial wastes (Beesigamukama, et al., 2020a; Bortolini et al., 2020; Setti et al., 2019; Tan et al., 2021). The most opportune environment for BSFL composting are peri-urban and urban environments as they provide a variety of easily accessible agro-industrial inputs while having proximity to diverse agro-industrial and organic waste streams (Quilliam et al., 2020). An added advantage of BSFL digestion is the reduction in biogas and methane production compared to the alternative anaerobic composting (Czekała et al., 2020).

Lastly, previous research has pointed out that given the right conditions, there is ample opportunity to create circular economies (closed agricultural feedback loops) through the incorporation of BSF production (Bava et al., 2019). “In the context of a circular economy, it is paramount to study the residual effect of [BSFL compost], particularly in soils with low mineral and water retention, so as the most responsive cultures to be considered in crop rotations, so as in monoculture[s].” Menino et al. (2021) is a call for continued research for multiple BSFL compost application rates, diet sources, and target crops in addition to pest and pathogen studies to measure chitin and chitosan influence on plant and soil health.

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CHAPTER 2. NUTRITIONAL VIABILITY OF SINGLE SOURCE WASTE STREAM DIETS FOR

2.1 Introduction

Urban farmers face challenges uniquely associated with producing in urban environments. Access to healthy, productive soils is at the top of the list, which affects plant growth. Due to zoning and land-use history, urban farmers often face restrictions around land and resource access, through purchase or lease agreements, ranging from access to water to worker safety in relation to exposure to soil contaminates (Oberholtzer et al., 2016). This leads to the need to outsource materials to create suitable conditions for plant growth, usually by purchasing topsoil/compost to build raised beds, thus increasing the cost of production. A potential solution to both resource limitations could be the introduction of black soldier fly rearing into urban production systems.

Black soldier fly larvae (*Hermetia illucens*; BSFL) are a common detritivore that has been gaining popularity in sustainable agricultural. Two factors driving the interest and application of BSF in agriculture is their potential to generate compost and the feed value of the flies themselves for animal production. At faster production rates than traditional composts (Beesigamukama, et al., 2021b), reducing the compost maturity period from 3 (rapid) – 6 months (typical) to 5 weeks, BSFL compost can be generated more quickly. BSFL behave similarly to earthworms that are added to composts, a practice known as vermicomposting. These detritivores, earthworms and BSFL, burrow through soil, consuming dead or decaying organic matter. Tunnels that are generated through their movements produce a similar effect to manually shifting composts around to introduce fresh air and release trapped gasses, known as aerobic composting. However, because larvae and earthworms are constantly moving in the substrates they inhabit, aeration occurs without the need for turning. The last most common form of composting is anaerobic, which requires the least manipulation, but takes the longest to mature as gases are unable to escape and microbial communities are unable to access fresh oxygen. However, microbes that do not require oxygen are still active and allow these composts reach maturity. Anaerobic and BSFL composts have previously been compared which revealed that BSFL compost released less biogass making this method more environmentally friendly.

About one third of all food waste that is prepared for human consumption in the world was either lost or wasted in the years leading up to 2013(Food and Agriculture Organization of the United Nations, 2013), with trends likely rising along with the global population. Additionally, in urban areas, disposing of livestock manures, specifically chicken manure due to poultry being more widely accepted in urban spaces than other livestock (Bouvier, 2012), can pose a particularly unique problem for urban producers. Excess chicken waste has been provided to BSFL as a feed stock and have been successfully reared to egg-laying adulthood (Bortolini et al., 2020). In addition to this local waste stream, industrial and commercial wastes, such as paper mill and sewage sludges along with brewer's grains, may be collected and used as BSFL diet for nutritional BSFL composts (Alvarenga et al., 2015; Anyega et al., 2021; Lalander et al., 2013; Quilliam et al., 2020). Sewage sludge, municipal slaughterhouse sludge, and paper mill wastes, after being fed to BSFL, resulted in high levels of inorganic nitrogen compounds, which are easier for plant uptake (Alvarenga et al., 2015).

Each of these waste streams give ample inputs for introducing agricultural feedback loops into urban ag systems, allowing for nutrient retention within the system and adding value to a discarded resource. Previous authors have highlighted the benefits of generating agricultural feedback loops in the context of circular economies (Bava et al., 2019); hereafter used interchangeably in this paper. While previous work supports the integration of BSF to create an agricultural feedback loop

(Bava et al., 2019; Beesigamukama, et al., 2020a; Menino et al., 2021; Quilliam et al., 2020; Setti et al., 2019), our study focused on larval development on organic waste streams generated in urban environments. We are interested in investigating the larval development, feed quality of the harvested flies, and suitability of the digestate as a soil amendment for urban food production. This information can lead to the development of BSF compost integration to urban agriculture. Our overall objective was to investigate urban-generated waste streams as BSFL diets to answer the following two questions: 1) How does diet impact BSFL development? and 2) How does the feed stock impact the nutritional quality of the resulting digestate as a soil amendment?

2.2 Materials and Methods

2.2.1 Laboratory Colony Establishment

Black soldier fly larvae were initially purchased from the following vendors to establish a colony at Purdue University: Fluker's farms (West Baton Rouge Parish, LA), Symton (College Station, TX), the Critter Depot (Lancaster, PA), and Josh's Frogs (Owosso, MI). The entire contents of the shipments were transferred into 4 x 9.75 x 12.25-in, 2-gallon containers and stored in a room at 22.3 ± 0.04 ˚C and 37.5 ± 0.31% relative humidity, used to maintain the colony. Each bin was placed inside of a mesh bug dorm to contain any of the BSFL that crawled out of the bins.

Larvae were fed a mixed diet consisting of Gainesville diet (50% wheat bran, 30% alfalfa meal, and 20% corn flour), food wastes generated from lab members, and culled produce collected from local farms. Colony diet was delivered at a rate of 163 mg/larva/day, but as populations grew and we were unable to accurately estimate the number of larvae in colony bins, we switched to an alternative method of adding a similar mixed diet when the food items in the colony had been depleted (i.e. no visible undigested food).

Some of the BSFL migrated out of the rearing bins and were collected from the bottom of the mesh bug dorms. The remaining pupae and prepupae were hand sifted from the two-gallon colony storage bins. We selectively removed darkly pigmented larvae, prepupae, or pupae that were present. Pupating individuals were placed in a shallow bin and transferred to the Entomology Environmental Laboratories (EEL) greenhouses, where large cages are maintained for the adult colony.

The adults were maintained in the EEL greenhouse where they were exposed to natural light and day length, over the course of one year, which are qualities required for mating and oviposition. In these cages the adults were provided with high density foam in trays filled with water for hydration. BSF adults prefer to oviposit their eggs in a sheltered habitat near a viable food source. To facilitate oviposition and egg collection we implemented food traps consisting of bowls containing fresh food and young larvae covered with a mesh lid. Fluted cardboard stacks were bound with a rubber band or binder clip and placed directly on the mesh lid.

Egg cards were collected from the cages daily and placed on a small amount of feed in an environmental growth chamber (24.7 ± 0.02 ˚C, 65.7 ± 0.04 % RH) in Smith Hall at Purdue University. Egg hatch and larval growth were closely monitored. When the larvae reached approximately six days in age, they were transferred either to the experimental bins examining larval diet or into the larger pool to maintain the colony.

2.2.2 Sole-source Diet Rearing Trials

Four food waste streams were selected to evaluate BSFL development and digestate quality. The materials were chosen based on their availability to urban farmers. Waste streams included were processed food, organic food, spent worts and biosolids. Processed food waste consisted of table scraps collected from lab members and included fast foods (cheeseburgers, French fries), baked goods (carrot cake, muffins), pasta, grains and associated fats and oils (condiments and dressings). Organic food waste refers to plant material collected from the Meigs Horticultural Research Farm (40.29, -86.88; part of the Throckmorton Purdue Agricultural Center in Lafayette, IN; weeds, culled plants and produce), expired produce sourced from local grocery stores, and rotten produce sourced from lab members. This waste stream was not necessarily produced from Certified Organic Farms and should not be confused as such. Biosolids were collected from the sheep herd at the Purdue Animal Sciences Research and Education Center (ASREC; West Lafayette, IN). Spent worts, or brewer's grains, were obtained from two local breweries near West Lafayette, IN.

Three replicates examining the impact of sole-source diets were carried out. The first trial started with 25,000 larvae that were purchased from Symton (College Station, TX) and divided by weight into four rearing containers, resulting in roughly 6,250 larvae per treatment. Larvae for trials two and three were collected from the BSF colony established at Purdue. In all trials, approximately 6-day old larvae were placed in 4 x 9.75 x 12.25-in, 2-gallon containers and stored in a room at 20.8 ± 0.05 ˚C and 32.8 ± 0.11% relative humidity. Each trial consisted of one container for each diet source.

Larvae in feeding trial one were fed at a rate of 23-28 mg food per larva per day. This feeding rate resulted in slowed growth rates and lengthened development times. Larvae in feeding trials two and three were fed at 163 mg food per larva per day, identified as optimal feeding rates to produce the maximum amount of remaining digestate (Parra Paz et al., 2015). The discrepancy in feeding rates between trials was the result of miscalculations which were recognized by the slow development time for all diet groups in trial one.

On a weekly basis, a subset of larvae were collected from each bin to monitor development, with length (mm) and weight (g) measurements taken. Due to their small size, groups of 10 larvae were pooled and weighed. Larval length was measured by placing the 10 individuals that were weighed into the freezer and preserved to prevent larval movement impacting the measurements. Larvae were later removed from the freezer and each individual length was measured in mm with a ruler (trial one) or digital caliper (trials two, three).

2.2.3 Digestate Analysis

Following BSF pupation, vacant digestate was harvested and sent to A&L Laboratories (Fort Wayne, IN) for compost analyses package C6. This test package quantified solids, nitrogen, phosphorus, potassium, sulfur, calcium, magnesium, sodium, iron, aluminum, manganese, copper, zinc, total organic matter, total carbon, C:N ratio, pH, and soluble salts. Total organic matter was measured using the Walkley-Black method. pH was measured using a calibrated electrode into a water slurry. Mineral analyses were conducted using the Mehlich III Extraction methods. We analyzed all four BSF treatments (processed food waste, organic food waste, spent worts, and biosolids) in addition to a commercially available source of compost to serve as a reference when interpreting the analyses (Black kow composted manure; Oxford, FL).

2.2.4 Statistical Analysis

Larval development over time was examined by generating a scatterplot placing length or weight per 10 larvae plotted across time. A linear regression line was fit for each feeding trial (n=3) by treatment (n=4). The slope of the lines were then compared across treatments using an ANOVA. In addition, the final length and weight per 10 larvae were compared to examine differences between the overall size of the final larval stage, prior to pupation, across the different treatments using ANOVA. All statistical tests were performed using JMP Pro 16.0 (SAS Institute).

2.3 Results

2.3.1 Sole-source Diet larval development

The scatter plots examining development over time of each treatment by trial can be found in the figures 2.4-2.7. The slope of the lines generated from these plots are reported in Table 2.1. Growth rate, as measured by weight, was not influenced by treatment ($F_{3,11} = 2.34$, $p = 0.17$, however length was influenced between treatment and replicate (ANOVA, $F_{11,119} = 19.07$, $p = 0.04$.) Significant differences were only detected among the larval length data. Individuals from the OFW treatment grew to be longest in comparison to the BS treatment; PFW and SW were not different from any of the treatments, differences between treatments are denoted by A, AB, and B (Table 2.1).

Similar to the growth rates themselves, diet source did not impact final average larval weight (Table 2.3), but we saw a trend for higher final weights being achieved in the PFW and SW treatments (Fig. 2.1). In contrast, the final average larval length was dependent on an interaction between treatment and trial (Table 2.3). The longest larval length was observed in the first trial in the PFW treatment (Fig. 2.2). The lowest larval length were recorded in BS trial 1, PFW trial 2, and SW trials 2-3 (Fig. 2.2). All other treatments and trials had intermediate lengths recorded.

2.3.2 Digestate Quality

BSFL digestates from single-source feed stocks were compared to the commercially available Black Kow (BK) composted cow manure (Oxford, FL) to evaluate potential nutritional contributions as a soil amendment. BK is advertised as 0.5-0.5-0.5 NPK per bag, however our quality check resulted in 1.63-0.02-0.1 NPK (Fig. 2.3). Comparatively, the most similar in terms of phosphate (P2O5) and potash (K2O) to BK is the spent worts (SW) treatments with an NPK of

4.18-0.11-0.01, which is the second highest nitrogen content produced in this study. Processed food waste digestate had the highest NPK values overall with 4.94-2.59-4.21. NPK values for organic food waste (OFW) and biosolids (BS) are 0.89-0.5-3.07 and 3.16-2.5-2.95, respectively (Fig. 2.3). The amont of organic matter, pH, and soluble salt values from the test are other characteristics that impact soil health and plant performant. BSF compost had higher values for both pH, 7.7, and soluble salts, 7.22 ppm, compared to the lower BK compost with a pH of 6.7, and soluble salts at 0.81 ppm.

2.4 Discussion

BSFL development due to diet has had mixed results within previous studies (Addeo et al., 2021), however, in the current study larval weight was not different, but larval length was overall shorter on spent worts diets. This could be attributed to nutrient shortages forcing larvae to focus on fat and protein storage to promote reproductive fitness rather than chitin synthesis to increase length. Evidence shows that midgut diet-dependent adaptations ensure the exploitation of nutritionally poor substrates (Bonelli et al., 2020). This was further supported in our study as larvae in trial one were starved at a rate of 23-28 mg/larva/day as offspring but still produced adults within each feeding trial, albeit they were noticeably smaller.

BSFL fed manure have been shown to have varying performance (Bortolini et al., 2020; Hepperly et al., 2009; Logan et al., 2021; Quilliam et al., 2020; Xiao et al., 2018). In the current study, rehydrated sheep manure at feeding rates of 23-28 mg/larva/day and 163 mg/larva/day proved that BSFL are able to survive and produce offspring on diets lacking in all essential amino acids. However, BSFL reared on sheep manure were observably more lethargic than BSFL reared on other diets.

Larval diet has been significantly linked to resulting BSFL compost composition, however, larvae are able to survive on poor substrates or, at starved levels, and produce BSFL composts that are similar, or greater in quality, to composted manures (Kawasaki et al., 2020). These qualities, in tandem with positive effects on plant growth (Anyega et al., 2021; Beesigamukama, et al., 2020; Borkent & Hodge, 2021; Chavez & Uchanski, 2021; Chirere et al., 2021; Tan et al., 2021), highlight a niche for BSFL composting in Urban Agriculture Systems. The integration of BSFL composting creates a value for the organic waste stream that can be captured to feed the larvae and the resulting digestate has the potential to serve as a soil amendment as it is similar, or superior, in compost properties to commercially available products. Adding value to waste streams and generating soil amendments that would otherwise be purchased are some of the aspects of urban agriculture circular economies created through the integration of BSF (Bortolini et al., 2020; Klammsteiner et al., 2020; Setti et al., 2019).

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2.5.1 Tables and Figures

Table 2.1 Larval growth rates. The growth rate per treatment per trial (N =3) generated from linear regressions using a scatterplot matrix graphing weight and length across time. (OFW = organic food waste; PFW = processed food waste; SW = spent worts; BS = biosolids). Letters in parentheses next to treatment designations indicate significant differences between growth rates.

Trial	**Larval weight (g/10 larvae) growth rates**			
	OFW	PFW	SW	BS
1	0.0016	0.0043	0.0014	0.0009
2	0.0042	0.0113	-0.0043	0.0025
3	0.0047	0.0533	-0.0049	0.0025
	Larval length (mm) growth rates			
	OFW (A)	PFW (AB)	SW (AB)	BS (B)
1	0.1112	0.2098	0.1207	0.2237
2	0.2926	0.1576	-0.0487	0.158
3	0.3407	0.2871	-0.0444	0.2237

Table 2.2 Results of ANOVA test comparing the growth rate of larval weight (per 10 larvae; g) and length (mm) across four treatments per trial (N =3; values found in table 2.1). (OFW = organic food waste; PFW = processed food waste; SW = spent worts; BS = biosolids).

	F-statistic	p-value
Weight growth rate	$F_{3,11} = 2.16$	0.171
Length growth rate	$F_{3,11} = 4.45$	0.041

Table 2.3 Results of ANOVA tests comparing the average larval weight (g per 10 larvae) and length (mm) among treatment, trial, and the interaction of treatment x trial. Three replicated were completed for each diet (N = 3). (OFW = organic food waste; PFW = processed food waste; SW = spent worts; BS = biosolids).

	Final larval weight (g/ 10 larvae)	
	F-statistic	**p-value**
Treatment	$F_{3,3} = 2.34$	0.150
	Final larval length (mm)	
Trial	$F_{2,108} = 5.46$	0.006
Treatment	$F_{3,108} = 14.01$	<0.0001
Trial x Treatment	$F_{6,108} = 26.14$	<0.0001

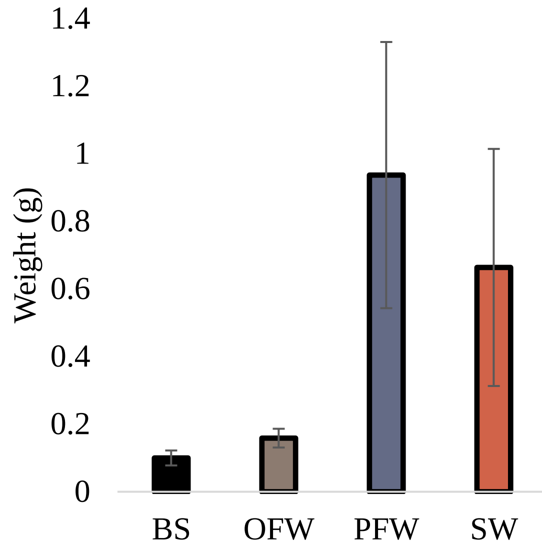


Figure 2.1 Average larval weight of 10 larvae from four diet streams, replicated three times. (OFW = organic food waste, PFW = processed food waste, SW = spent worts, BS = biosolids). No effect was detected from diet on final average larval weight (F3,11 = 2.34, p = 0.15).

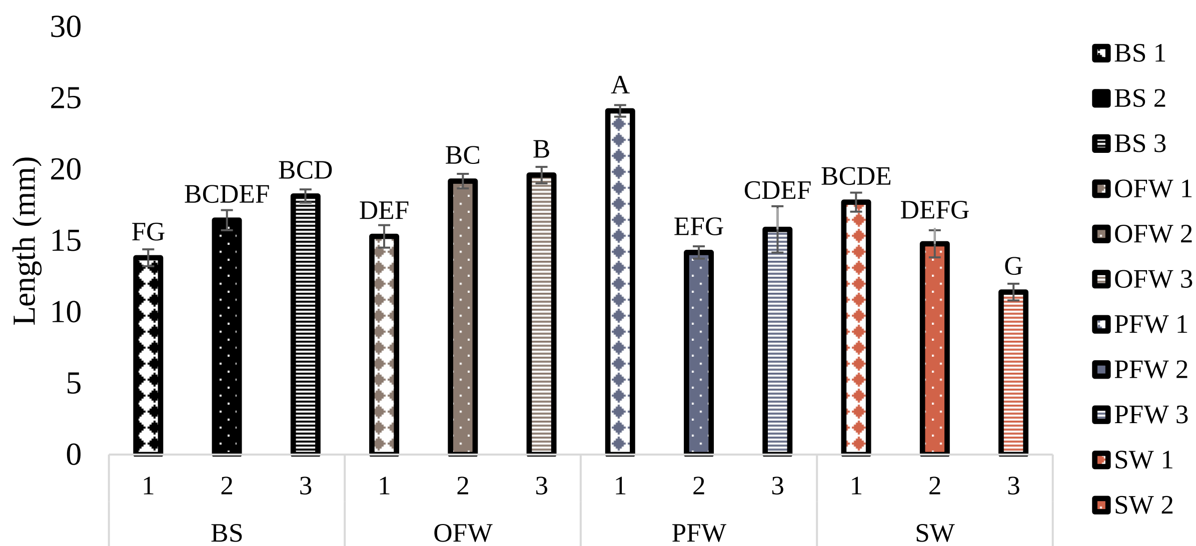
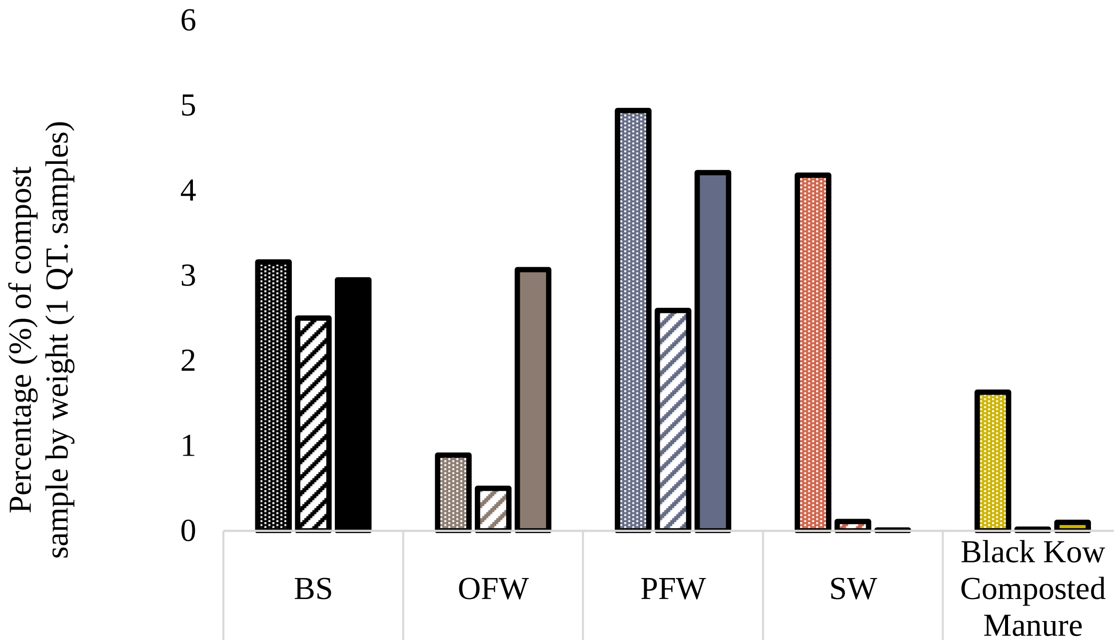


Figure 2.2 Average final larval length from four diet streams, replicated three times. (OFW = organic food waste, PFW = processed food waste, SW = spent worts, BS = biosolids). (A-G values denote statistically measurable differences between treatments). An effect was detected between replicate and treatment. ANOVA, $F_{11,119}$ = 19.07, p = 0.0001; Effects test; Treatment by replicate, $F_{6,6}$ = 26.14, p = 0.0001.



	BS	OFW	PFW	SW	Black Kow Composted Manure
Nitrogen (N)	3.16	0.89	4.94	4.18	1.63
Phosphate (P2O5)	2.5	0.5	2.59	0.11	0.02
Potash (K2O)	2.95	3.07	4.21	0.01	0.1

Figure 2.3 Clustered bar graph comparing NPK (nitrogen – phosphate – potash) from BSFL composts from four diets (PFW = processed food waste, OFW = organic food waste, SW = spent worts, BS = biosolids) in addition to commercially available Black Kow composted cow manure (BK) (N = 5).

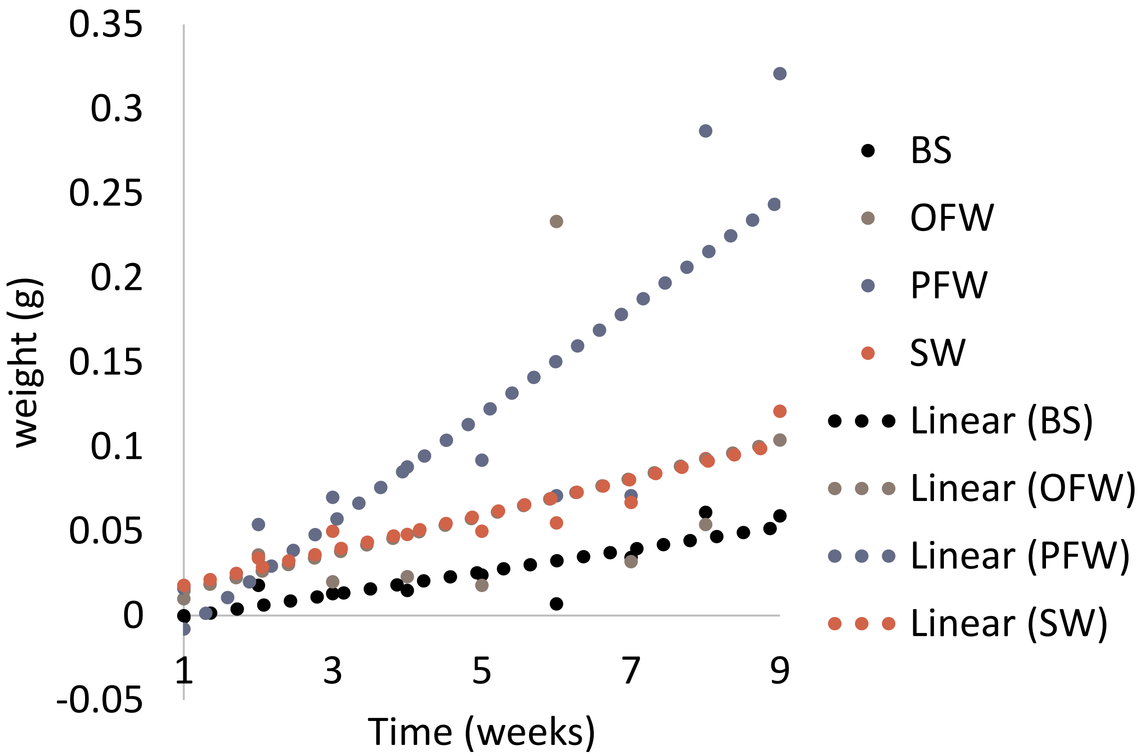


Figure 2.4 Larval growth weight (g) for 10 larvae across time (weeks), feeding rate 23-28 mg/larva/day, replication one (N = 1; OFW = organic food waste, y = 0.0111x + 0.0036, R2 = 0.1812; PFW = processed food waste, y = 0.0317x – 0.0398, R2 = 0.6528; SW = spent worts, y = 0.0105x + 0.0071, R^2 = 0.8629; BS = biosolids, y = 0.0067x – 0.0076, R^2 = 0.7049).

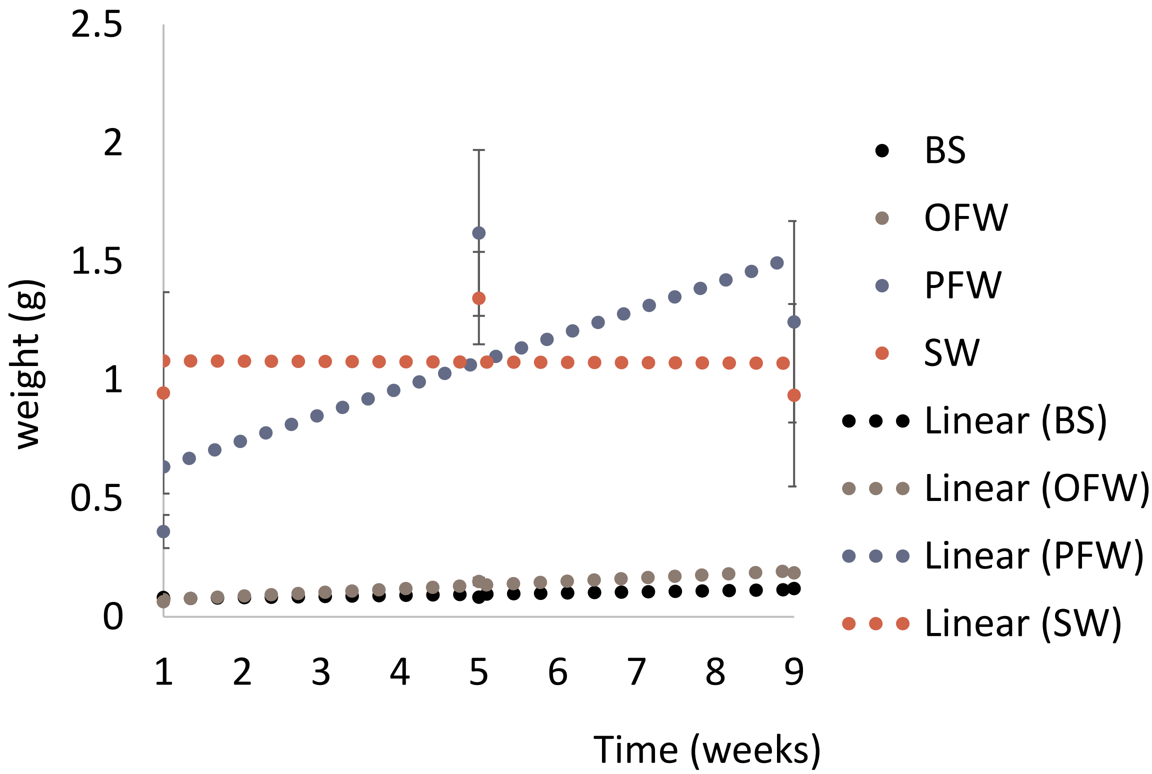


Figure 2.5 Larval growth weight (g) for 10 larvae across time (weeks), feeding rate 163 mg/larva/day, replication two and three (N = 2; OFW = organic food waste, y = 0.0152x + 0.0574, R2 = 0.9496; PFW = processed food waste, y = 0.1106x + 0.5219, R2 = 0.4678; SW = spent worts, y = -0.0012x +1.0813, R^2 = 0.0005; BS = biosolids, y = 0.0049x + 0.071, R^2 = 0.7979).

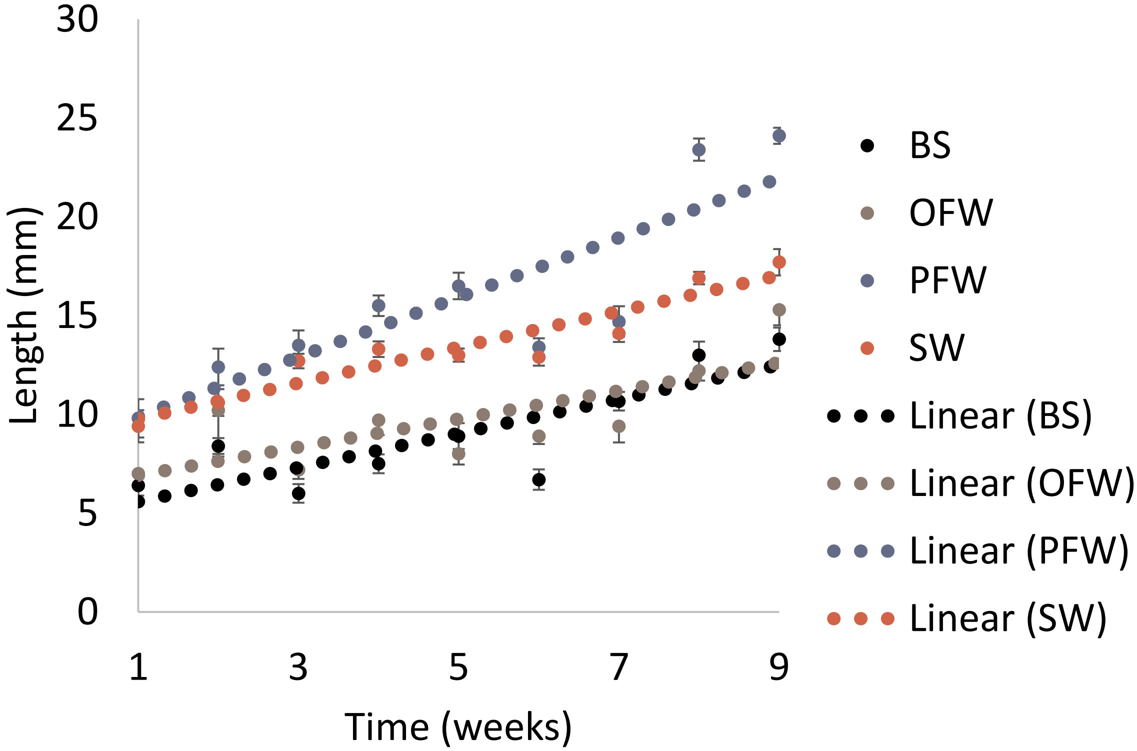


Figure 2.6 Larval growth length (mm) across time (weeks), feeding rate 23-28 mg/larva/day, replication one (N = 1; OFW = organic food waste, y = 0.7131x + 6.1997, R2 = 0.5548; PFW = processed food waste, y = 1.5083x + 8.3806, R2 = 0.7303; SW = spent worts, y = 0.9083x + 8.8583, R^2 = 0.8843; BS = biosolids, y = 0.8656x + 4.713, R^2 = 0.6866).

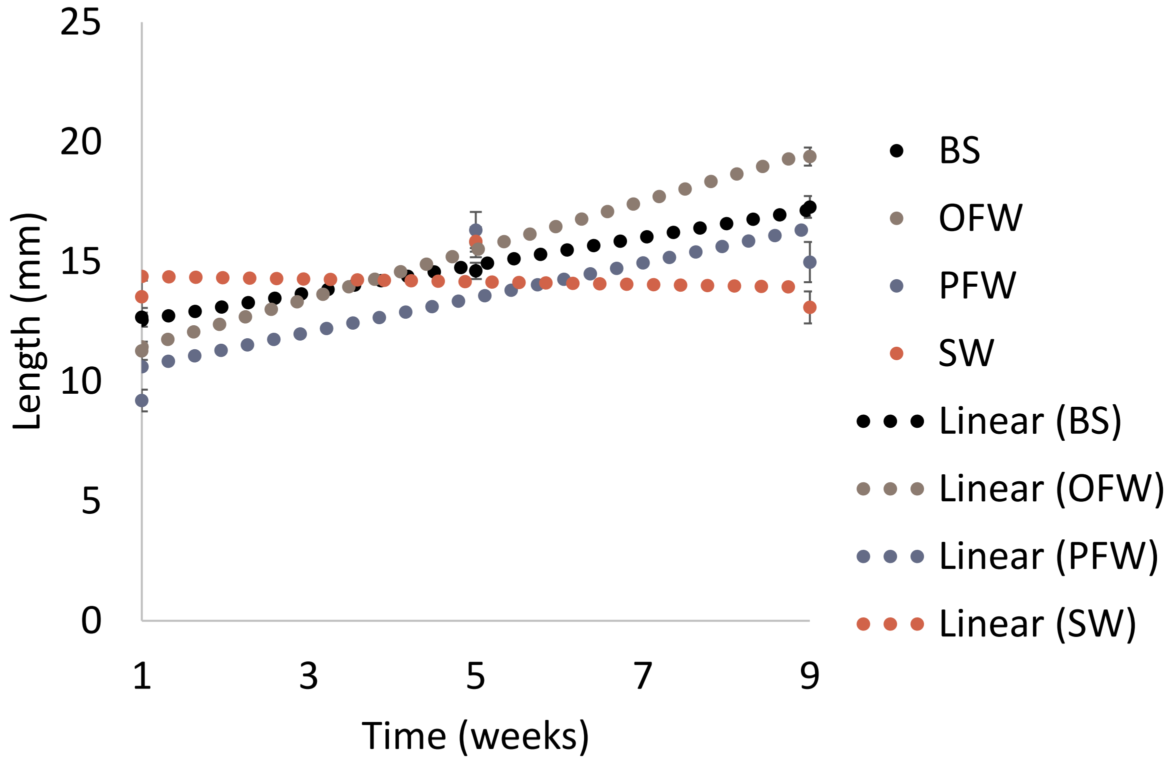


Figure 2.7 Larval growth length (mm) across time, feeding rate 163 mg/larva/day, replication two and three (N = 2; OFW = organic food waste, y = 1.0137x + 10.423, R2 = 0.9954; PFW = processed food waste, y =, R2 =; SW = spent worts, y = -0.0564x + 14.439, R2 = 0.0232; BS = biosolids, y = 0.5762x + 11.975, R^2 = 0.9917).

CHAPTER 3. EVALUATION OF BLACK SOLDIER FLY (*HERMETIA ILLUCENS*) DIGESTATE AS A SOIL AMENDMENT FOR VEGETABLE PRODUCTION

3.1 Introduction

Adding soil amendments, any material that can be added to the soil to increase soil properties, is a strategy to increase soil health. With the addition of soil amendments, soil health can be more easily maintained leading to healthier plants and higher crop yields (Altieri & Nicholls, 2003). Depending on the amendment, improvements provided by the practice include stabilized soil pH, increased carbon and nitrogen and increased crop yield (Hepperly et al., 2009). Organic matter and beneficial microorganisms that aid in the maintenance of healthy soil can be introduced in the form of soil amendments (Magdoff, 1993). Compost is one of the most applied soil amendments to introduce additional organic matter into the soil due to the high amount of organic matter in these amendments. The other leading use of compost is adding it to raised beds to add supplementary nutrients for crop growth and productivity, much like the application of fertilizers.

Traditional composting is where organic material, excluding meat or oily material due to potential attraction of unwanted pests such as bears or blow flies, can be placed into a pile where one of two processes will happen: aerobic or anaerobic digestion. Aerobic composting relies on microorganisms that require oxygen to breakdown organic material meaning the material should be thoroughly mixed to introduce new air. Anerobic composting relies on microorganisms that do not require oxygen meaning new air does not need to be introduced. Aerobic composting is generally faster than the anaerobic method, however, this method can still take three to six months to develop a mature compost that has reached 160° F at its peak activity to kill off pathogens.

An alternative approach to composting is through the application of farming invertebrates that feed on decomposing or detritus material. Vermicomposting is the addition of earth worms, most commonly red wigglers (*Eisenia fetida,* Savigny, 1826) and red earth worms (*Lumbricus rubellus*, Hoffmeister, 1843) which provides the benefits of aerobic composting without having to turn the compost as frequently (R & M, 2019). Vermicomposts can potentially provide more benefits than a traditional compost such as increased crop yield and pathogen suppression (Arancon et al., 2003) due to shed worm castings. One of the main drawbacks to this method of composting is the fastidiousness of earth worms. Earth worms are unable to break down organic matter that is rough or dry because they are not equipped with strong mouthparts. Additionally, it is recommended to avoid the addition of animal products or other oily products to vermicomposting as they may attract unwanted pests and cause odor as the worms take longer to break down those materials resulting in greater decomposition.

Black soldier fly larvae (*Hermetia illucens*; BSFL; Linnaeus, 1758) are an alternative to both traditional and vermicomposting. When BSFL were placed in similar materials used in a traditional compost, compost maturity was reached in five weeks, compared to the standard three months, when fed brewer's grains (Beesigamukama, et al., 2021b). BSFL have been applied to manage biosolids, food and plant waste, along with viscous mixtures known as sludges typically resulting from industrial waste. This insect is able to digest these materials at efficient rates and in large quantities (Sheppard et al., 2002). This includes manures that may contain harmful pathogens such as *E. coli* and *salmonella*, where BSFL have been shown to reduce pathogen load post-digestion (Erickson et al., 2004). BSFL have also been shown to reduce pesticide and pharmaceutical residues, specifically fungicides, antiepileptics, and antibiotics that were present in lab diets (Lalander et al., 2016). Lastly, BSFL has been shown to break down 100% of a cooked chicken diet and reach pupation at the same rate as control groups (Logan et al., 2021) showing great potential for food waste diversion from landfills.

The integration of BSFL composting offers a unique opportunity to capture organic waste streams and recycle them back into a usable agricultural resource, creating a more circular economy. Providing waste for BSFL to feed on generates two desirable products; BSFL that can be fed to other livestock and the resulting digestate that can be applied as a soil amendment. This digestate can be applied in the same way that traditional or vermicompost is used to improve soil health. At the end of the growing season, any organic material waste such as culled crops can be harvested to feed BSFL, which can be harvested as a protein-rich source for potential small-scale livestock, such as poultry. This closed loop in agricultural production practices could place a value on waste streams and reduce input costs related to purchasing commercial compost or other soil amendments. Previous research has argued in support of BSFL composting, describing this loop as a circular economy (Bava et al., 2019).

Our research investigated the application of black soldier fly digestate to vegetable production. This study examined the fertility value of digestate produced by BSFL along with the effects it had on the performance of tomato, cucumber, and carrot production. Evaluating the nutritional quality of BSFL digestate in comparison to a commercial compost and across a variety of vegetable crops contributes to our knowledge and application of BSFL composting to increase soil health and food production.

3.2 Materials and Methods

3.2.1 Crop Selection

Three focal crops were selected based on their popularity on small farms, representation of crop families (Solanaceae, Cucurbitaceae, Apiaceae), and growth habits (fruit and root crops). Seeds were obtained from commercial seed suppliers and included: Tyria cv. cucumbers, Celebrity cv. tomato and Naval cv. carrot from Johnny's Selected Seeds (Winslow, ME) and Short N' Sweet cv. carrot from Burpee (Warminster Township, PA).

3.2.2 Field Location

The field experiment was conducted at the Meigs Horticultural Research Farm (40.29, -86.88) in Lafayette, IN. All three focal crops were grown in a field that was planted with soybean in the previous year. Lime was applied at one ton per acre in the spring of 2020 to raise the pH to neutral values. Raised earthen beds were built on three-foot centers with 6.5-foot spacing between the rows. Plastic drip irrigation was installed, and plastic mulch covered the beds for the tomato and cucumber crops; carrot beds remained open. Beds were 250 feet long, oriented in a north-south direction. Each crop was planted into two rows and blocked by crop type.

3.2.3 Source of Amendments

BSF larvae were purchased from Popworms! (Brownwood, TX) and Symtom, Inc. (College Station, TX) to establish a research colony maintained in Smith Hall, Purdue campus, West Lafayette, IN. Larvae were split into groups of 6,000 individuals and reared on single source food streams (food waste, plant waste, spent worts from a local brewery and sheep manure). BSF digestates generated from these individual feedstocks were pooled for field application and laboratory analysis. The commercial composted manure product Black Kow (Oxford, FL) was included in the study as a conventional compost control, in addition to a third treatment, the control that received no amendment. Both composts were sent for compost analyses at A&L Great Lakes Laboratories (Fort Wayne, IN). Compost package C6 was performed and measures solids, nitrogen, phosphorus, potassium, sulfur, calcium, magnesium, sodium, iron, aluminum, manganese, copper, zinc, organic matter, carbon, C:N ratio, pH, and soluble salts using standard methods.

3.2.4 Planting and Treatment Applications

Carrots were directly seeded into beds using a Jang Clean Seeder in single rows on May-14, 2021. Seed spacing was set to 2.5 inches, however, due to the small size of the seeds they were closer. This resulted in a row length of 16.66 ft (~5.07 meters) for the cv. Short N' Sweet and 30.66 ft (~9.34 meters) for the cv. Naval. The composts were added to subplots on Jun-22. Each row was evenly divided into three blocks and one treatment applied to each block (BSFL digestate, Black Kow, no amendment). To apply the composts, trenches were dug 6 inches away from the carrot seedlings at a depth of 6 inches and evenly filled with each compost treatment. Naval cv. carrots received composts at a rate of 99.64 grams per row foot (g/Rft) and Short N' Sweet cv. carrots received composts at a rate of 151.02 g/Rft. Carrots cultivars did not receive the same application rate due to a lack of available compost.

Tomatoes and cucumbers were seeded on Apr-29 into 72-cell trays filled with Sungro germination mix (peat, vermiculite, and starter nutrients; Agawam, MA) and germinated under mist irrigation in a greenhouse at Throckmorton Purdue Agriculture Center. Seedlings were transplanted into the field on May-24 using a rainflo model 1670 water wheel transplanter. Both crops were transplanted into two parallel, adjacent beds with two alternating rows for crops in each bed starting on the Northwest side of the plot (Fig. 1). Seedlings were transplanted into single rows; 24-inch spacing for cucumber plants and 48-inch spacing for tomato plants. One block was made up of 5 plants; the outer plants served as a buffer and data was collected from the 3 plants in the center of each block. T-posts were installed for each cucumber treatment along with mesh netting to create a trellis to support plant growth. Cages were installed around individual tomato plants for the same purpose. Cucumber plants had 7 blocks of three treatments, tomatoes had 6 blocks of three treatments, and carrots only had one block with three treatments due to the closer seed spacing and limited seed supply.

Tomato and cucumber plants received one application of compost from May-25 to Jun-4 where the compost was applied at the base of the plant under bed cover as a side dressing (Jr. & Barnett, 2016). Cucumber plants received a rate of 89.85 grams per row foot (g/Rft) of compost and tomato plants received composts at a rate of 44.93 g/Rft. Differences in rates can be attributed to the different plant densities per row foot (i.e. tomato planted at 48-inch spacing and cucumbers planted 24-inch spacing). Each plant received the same volume of compost application.

3.2.5 Data Collection

Pest and pathogen surveys were completed weekly during the summer of 2021. Subplots comprising the three center plants in each 5-plant block were surveyed by examining the whole plant for pests and counting the total number of aphids on each of three leaves per plant, all arthropod observations were recorded. Beginning three weeks after transplanting/germination plant growth measurements were taken biweekly. Pest and pathogen surveys were conducted on a biweekly basis from Jun-10 and ended Jul-23 for cucumber plants, ended early due to bacterial wilt introduced by cucumber beetles. Similar surveys were taken for tomatoes that started on Jun-10 and ended on Aug-13, the full planned duration. Carrot plants pest and pathogen surveys were taken on Jul-14 and Jul-23. Stem diameter was measured using a digital caliper (Carrera precision, 6” digital caliper alloy). Plant height was measured from the stem-soil junction to the apical meristem using a meter stick. Marketable cucumber fruits were harvested and weighed Jul-14 and Jul-23. Marketable tomato fruits were harvested and weighed Aug-13, Aug-27, and Sept-11. On the final harvest day, immature fruits were harvested and measured in addition to marketable fruits to account for all fresh weight generated by plants.

At the conclusion of the fruit harvest, the entire plant biomass was harvested and measured. Cucumber plants were harvested on Jul-30. The above-ground portion of the plant was clipped at the soil surface. The roots were excavated using a hand trowel and loose soil dislodged in the field. Fresh weight was not taken for these plants due to bacterial wilt infection (*Erwinia tracheiphila:* Smith 1895) drying some plant parts. Roots and shoots were stored in separate paper bags, dried, and weighed. Carrots were harvested on Aug-4 using a broad fork. The combined fresh and dried weights of roots and shoots were recorded along with dried above and below ground biomass. Tomato plants were harvested on Aug-17. Roots and shoots were stored in a greenhouse located at the Throckmorton Purdue Agricultural Farm (Lafayette, IN) for 3 weeks to dry. The plant material was further dried using a drying oven for 7-14 days at 75°C to ensure plant material was completely void of moisture. Five bags of plant material were randomly selected and weighed, dried for an additional 24 hours, and weighed again. We were looking for changes in weight greater than 5% suggesting more moisture could be evaporated from plant material. After material was dried, dry weight was taken of roots (below ground biomass, BGB) and shoots (above ground biomass excluding fruits, AGB). BGB and AGB were recorded from 20 randomly selected carrots from each treatment and variety, resulting in 120 samples.

Soil samples were collected at the end of the growing season on Oct-13 using a 1-inch (2.54 centimeter) diameter soil corer at a depth of 25.4 centimeters and sent to A&L Great Lakes Laboratories (Fort Wayne, IN) for soil analysis. Soil samples were composite samples from one to two replicates within soil treatments Samples for carrots were taken within treatments separated by cultivar, resulting in six samples (one of each treatment by cultivar) . A total of 27 soil samples taken from all crop plots were sent for analyses packages S1 and S3 which includes total organic matter, available phosphorus, exchangeable potassium, magnesium, calcium, soil pH, buffer pH, cation exchange capacity, percent base saturation of cation elements along with sulfur, zinc, manganese, iron, copper, and boron. Soil organic matter was measured using the Walkley-Black method, pH was measured using a calibrated electrode into a water slurry, and mineral analyses were conducted using the Mehlich III extraction methods at A&L Great Lakes Laboratories (Ellis et al., 1998).

3.2.6 Data Analysis

Crop measurements, including above and below ground weight, stem diameter, and height growth, were compared across treatments using an ANOVA with a Tukey HSD to confirm differences between treatments. Soil analyses were compared using ANOVA as well, however no differences were found when examining soil properties. Compost samples could not be statistically analyzed due to having one compost analysis per diet stream, however, nitrogen, phosphate, and potash (NPK) levels were visually compared using a clustered bar graph. The statistical software JMP Pro 16.0 (SAS, Inst.) was used to perform statistical analyses.

3.3 Results

3.3.1 Digestate

The full list of compost nutrients and physical characteristics are reported in Table 3.1. Comparing the composite BSF digestate to a sample of the commercial Black Kow composted cow manure, we see stark differences in the NPK (nitrogen – phosphorus – potassium) values, which are common metrics to describe macronutrient contents in a soil amendment. BSF digestate had an NPK value of 4.5-2-3.7, while Black Kow's NPK value was 1.6-0.02-0.1, slightly different than what is advertised on the bag (0.5-0.5-0.5). Black kow has a pH of 6.7 and BSF has a pH above of 7.7. BSF soluble salts, at 7.22 dS/m, are considerably high compared to Black Kow, which is at 0.81. Lastly, C:N (carbon:nitrogen) ratios for both Black Kow and BSF digestate are considered low at 15.1:1 and 10.2:1, respectively.

3.3.2 Crop Performance

All crop plant final heights and weights were recorded prior to harvest. Final cucumber height ($F_{2,18} = 4.6768$, $p = 0.0252$; Table 3.2) and stem diameter ($F_{2,18} = 4.1080$, $p = 0.0363$; Table 3.2) showed that cucumber plant growth is improved on BSFL compost in comparison to the control group, however, there were no differences between BSFL compost and BK compost. There were no differences between carrot height and the number of leaves between compost treatments ($F_{5,89} = 1.54$, $p = 0.19$; $F_{5,89} = 1.46$, $p = 0.21$; Table 3.2). Tomato final height and stem diameter did not differ between soil amendment treatments ($F_{2,17} = 1.12$, $p = 0.35$; $F_{2,17} = 2.96$, $p = 0.08$; Table 3.2).

Dried carrot AGB ($F_{2,2} = 2.47$, $p = 0.0891$; Table 3.3a) had no differences between treatments but did see differences between cultivars ($F_{1,1} = 5.80$, $p = 0.0176$; Table 3.3a), which was to be expected when comparing a standard and a dwarf cultivar of carrots. However, Short N' Sweet cultivar had more dried AGB within the BSF compost compared to Naval cultivar carrots grown on Black Kow. Dried carrot BGB ($F_{2,2} = 4.70$, $p = 0.0109$; Table 3.3a) showed differences between growth with cultivar Naval having overall more biomass, but within treatments, BSFL compost was proven to improve carrot growth when compared to Black Kow.

These data also showed evidence in support of differences between treatments applied to cucumber crops for BGB ($F_{2,32}$ = 3.59, p = 0.0399; Table 3.3b) between BSFL compost and control treatments, however, there were no differences between BSFL compost and Black Kow. Dried and fresh AGB for tomato plants were not different between treatments ($F_{2,53}$ = 0.57, p = 0.5711; $F_{2,53}$ = 0.13, p = 0.8803, respectively), however, there was evidence that cucumber BGB is affected by treatment for fresh ($F_{2,53}$ = 3.29, p = 0.0449; Table 3.3c) and dried material ($F_{2,53}$ = 7.32, p = 0.0016; Table 3.3c) with the control group (no amendment applied) having more BGB. This suggests that the soil system these crops were grown in is nutrient limited, prompting for greater soil amendment application rates for future studies.

3.3.3 Soil

There was no evidence for differences between soil samples post growing season, within soil amendment or crop treatments.

3.3.4 Pest Surveys

Pests were not encountered on a consistent basis to warrant overarching effects on plant growth, however, cucumber beetle counts reflect the number of cucumber plants lost due to bacterial wilt. Additionally, tomato block four treatment BSF was the only block/treatment combination to have tomato hornworm (*Manduca quinquemaculata*; Haworth, 1803) herbivory damage. Caterpillars were removed when discovered to prevent potential spread in the area as they are a pest. Tomato plants were able to recover in this treatment/block combination.

3.4 Discussion

We have found that BSFL compost is a suitable soil conditioner for cucumber and carrot specialty crop production. This is in line with BSFL compost being reported as a suitable crop fertilizer (Kawasaki et al., 2020) due to its final nutrient composition being similar to manure composts. BSFL compost has been reported to contain less nitrite, which could help reduce concerns related to health risks for people (Kawasaki et al., 2020). However, BSFL have also been reported to have higher levels of ammonium relative to other composts as a result of nitrification of nitrite by microbes. These compounds could volatize as ammonia or nitrate gas if exposed to alkaline or acidic soils, respectively, posing risks to sensitive plants, such as tomatoes, if applied at very high concentrations. Due to this, it is recommended that BSFL compost receive additional aging after BSFL digestion but prior to application to allow for stabilization. This is corroborated with other studies that found additional composting of BSFL compost resulted in higher C:N ratios (Song et al., 2021). However, extending the compost maturity period could alter the nutritional value of the amendment (Kagata & Ohgushi, 2012), since increasing the C:N ratio would likely cause the amendment to decompose more slowly.

Research is accumulating that examines the impact of insect-derived soil amendments on plant performance, specifically looking at the frass collected from insect protein production. For example, plant performances on food waste derived frass in comparison to NPK fertilizers demonstrated that lettuce plants are utilizing BSF compost more efficiently (Tan et al., 2021), however above ground biomass was reduced for BSFL compost treatments. This is further evidenced by nitrogen accumulation within maize plants during silking seasons when compared to an organic fertilizer (SAFI) (Beesigamukama, et al., 2020c). A proposed use of BSFL compost would be in smaller, more frequent doses. Overtime, smaller more frequent applications could increase organic matter within the soil (Borkent & Hodge, 2021), and potentially synergize with nitrogen hungry plants by providing more readily accessible nutrients during the entirety of the shoot and root growth period. This study also found that BSFL compost provided a general increase in crop performance at increasing compost applications regardless of base soil nutrients.

BSFL compost has the potential to replace unsustainable agricultural inputs for crop production if ideal waste-to-input rates and sources are identified that can provide the most fertility benefit as a soil amendment. Other research has shown that frass quality critically influences its effects on plant growth and causes significant differences in the amount of mineral N that is released (Beesigamukama, et al., 2020c; Gärttling et al., 2020), further supporting that there is an ideal waste source and diet rate to be found. Our study was only able to test one rate of one diet source (pooled from urban sourced waste streams described in chapter 2). Other authors have also reported there is room to find an ideal application rate as overapplication can influence plant germination or lead to growth inhibition (Kawasaki et al., 2020). For example, Song et al., 2021, reported increased germination rates when additional BSFL compost was added, while (Chiam et al., 2021) observed lettuce development on BSFL compost and found that if provided too much soil amendment, growth was stunted if plants were provided too much amendment. However, in nitrogen-demanding crops such as maize, growth when supplied with spent wort sourced BSFL compost at rates of 30-60 kg N Ha-1 yields only increased at higher rates (Beesigamukama, et al., 2020c). This suggests that BSFL compost sourced from spent worts may have increased quality to some commercially available organic fertilizers that are currently applying fresh or composted spent worts to their crops.

The combination of BSFL compost with mineral or organic NPK fertilizers is an additional route of research that requires more input. Currently, there is evidence that tomato plant height in greenhouse and field settings followed an increasing growth trend through experiments when supplied with a mixed soil amendment (Anyega et al., 2021). Additional metrics that should be considered for application of these materials that could directly impact plant health include pH, soluble salts, and carbon:nitrogen ratios. pH determines the availability of nutrients that a plant can uptake; typically plants perform best in soils with a pH level of 6.0 to 7.0, which is slightly acidic to neutral. High levels of soluble salts can harm plants since this can cause water to be drawn away from plant roots. Carbon:nitrogen ratios influence the rate at which soil microbes can break down organic materials and make nutrients available for plants; higher ratios such as 41:1 can lead to immobilization and therefore lower concentrations of available N, but lower ratios such as 8:1 promote rapid mineralization and available N. These conditions may favor plants that have a rhizobiome, the zone around roots within the soil that are influenced by microbes, that can more efficiently exploit immobilized N (Klammsteiner et al., 2020). Due to high pH and EC being observed in BSFL compost, it is recommended to dilute it, however, this could be done with supplemental fertilizers to reach ideal nutrient input. In addition to non-insect wastes being included in soil amendment practices, adult BSF remains have been shown to provide extremely high levels of nitrogen when compared to frass and larval casings (Gärttling et al., 2020).

All of these attributes make BSFL an ideal candidate for completing an agricultural loop within peri-urban environments (Quilliam et al., 2020) due to their proven success with specialty crops within this study. BSFL addition would encourage waste streams to be valued as inputs into a circular economy that could provide soils lacking in organic matter, struggling with water retention, or low in minerals an alternative method for restoration (Menino et al., 2021), while generating soil amendments and larvae to use as small-scale livestock protein supplements.

3.5 References

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3.6 Tables and Figures

Table 3.1 Compost analysis results from A&L Great Lakes Laboratories (moisture at 70C, solids, nitrogen, phosphorus, phosphate, potassium, potash, sulfur, magnesium, calcium, sodium, iron, aluminum, copper, manganese, zinc, pH, soluble salts, ash at 550C, organ matter, total organic carbon, and carbon: nitrogen ratios). Unit values represent percentage of dry analyses excluding moisture, milligrams per kilograms (mg/kg), and deci-siemens per meter (dS/m). Compost treatments BK (Black Kow) and BSF (Black soldier fly) were compared.

Treatment	Unit	Black Kow composted manure	BSF digestate
Moisture @ 70C	%	59.83	8.71
Solids	%	40.17	91.29
Total Nitrogen (N)	%	1.63	4.54
Phosphorus (P)	%	0.01	0.88
Phosphate(P2O5)	%	0.02	2.02
Potassium (K)	%	0.08	3.13
Potash (K2O)	%	0.1	3.76
Sulfur (S)	%	0.1	0.46
Magnesium (Mg)	%	0.12	0.39
Calcium (Ca)	%	0.33	1.35
Sodium (Na)	%	0.01	1.27
Iron (Fe)	%	0.06	0.09
Aluminum (Al)	%	0.04	0.08
Copper (Cu)	mg/kg	5	19
Manganese (Mn)	mg/kg	33	63
Zinc (Z)	mg/kg	4	80
pH	-	6.7	7.7
Soluble Salts	dS/m	0.81	7.22
Ash @ 550 C	%	50.88	7.06
Organic Matter (Loi @ 550 C)	%	49.12	92.94
Total Organic Carbon (C)	%	24.56	46.47
Carbon:Nitrogen Ratio (C:N)	-	15.1:1	10.2:1

Table 3. 2 ANOVA results comparing final crop growth measurements for cucumber height (cm) and stem diameter (mm), tomato height (cm) and stem diameter (mm), and carrot height (cm) and number of leaves grown on BSF (black soldier fly) and BK (Black Kow) composts.

	F-statistics	p-values
Final cucumber height	$F_{2,18} = 4.68$	0.0252
Final cucumber stem diameter	$F_{2,18} = 4.11$	0.0363
Final carrot height	$F_{5,89} = 1.54$	0.1875
Final carrot # of leaves	$F_{5,89} = 1.46$	0.2121
Final tomato height	$F_{2,18} = 1.12$	0.3529
Final tomato stem diameter	$F_{2,17} = 2.96$	0.0823

Table 3.3 Effect tests and ANOVA results for crop biomass for carrot, cucumber, and tomato plants based on fresh and dry weights. Table 3.3A: fresh and dried weight of carrot plants, comparing variety, treatment, and a cross between them. Table 3.3B: Dried cucumber plant weight comparing treatment. Table 3.3C: Fresh and dried tomato plant weight, comparing treatment.

A. ANOVA table	Variety		Treatment		Variety x Treatment	
Crop Metric	F-Statistic	P-value	F-Statistic	P-value	F-Statistic	P-value
Carrot Fresh Weight	$F_{1,1}$ = 3.23	0.0748	$F_{2,2}$ = 1.55	0.2169	$F_{2,2}$ = 0.81	0.4459
Carrot Dried Above Ground Weight	$F_{1,1}$ = 5.80	0.0176	$F_{2,2}$ = 2.47	0.0891	$F_{2,2}$ = 0.09	0.9156
Carrot Dried Below Ground Weight	$F_{1,1}$ = 25.12	<0.0001	$F_{2,2}$ = 4.70	0.0109	$F_{2,2}$ = 0.01	0.9944

B. ANOVA table	Treatment	
Crop Metric	F-Statistic	P-value
Cucumber Dried Above Ground Weight	$F_{2,2}$ = 1.11	0.3406
Cucumber Dried Below Ground Weight	$F_{2,2}$ = 3.59	0.0399

C. ANOVA table	Treatment	
Crop Metric	F-Statistic	P-value
Tomato Fresh Above Ground Weight	$F_{2,2}$ = 0.13	0.8803
Tomato Dried Above Ground Weight	$F_{2,2}$ = 0.57	0.5711
Tomato Fresh Below Ground Weight	$F_{2,2}$ = 3.29	0.0449
Tomato Dried Below Ground Weight	$F_{2,2}$ = 7.32	0.0016

Table 3.4 Soil analyses generated by A&L Great Lakes Laboratories, grouped, and averaged by plant and treatment type. Organic matter, phosphorus, potassium, magnesium, calcium, pH, CEC meq/100g, sulfur, zinz, manganese, iron, copper, and boron. (BK = Black Kow commercial compost, BSF = Black soldier fly digestate, Control = field soil with no amendment applied). No observable statistical differences.

Plant Type	Carrot			Cucumber			Tomato		
Treatment	BK	BSF	Control	BK	BSF	Control	BK	BSF	Control
Organic matter	3.20	3.05	3.00	2.93	3.10	3.05	3.13	2.90	2.97
Phosphorus bray-1 equiv ppm-P	26.50	21.50	31.00	17.75	26.50	21.00	22.67	28.67	27.00
Potassium	130.50	122.00	129.50	132.75	141.25	133.50	119.33	122.00	128.00
Magnesium	377.50	405.00	410.00	416.25	416.25	430.00	380.00	406.67	406.67
Calcium	2325.00	2350.00	2425.00	2425.00	2462.50	2450.00	2350.00	2350.00	2383.33
pH	6.70	6.85	6.90	6.73	6.68	6.75	6.33	6.57	6.63
CEC meq/100g	15.90	16.60	16.15	17.00	17.23	17.00	18.03	17.43	17.30
Sulfur	8.00	8.00	8.50	8.00	8.25	8.00	8.00	8.67	8.67
Zinc	1.80	1.70	2.40	1.70	1.83	1.75	2.00	2.13	2.17
Manganese	28.50	27.00	30.00	29.00	26.00	28.50	21.33	26.67	27.67
Iron	25.00	19.00	22.50	20.00	21.75	21.75	28.00	27.67	27.33
Copper	2.20	2.05	2.25	2.05	2.18	2.10	2.27	2.37	2.43
Boron	0.50	0.55	0.60	0.50	0.55	0.55	0.53	0.53	0.53

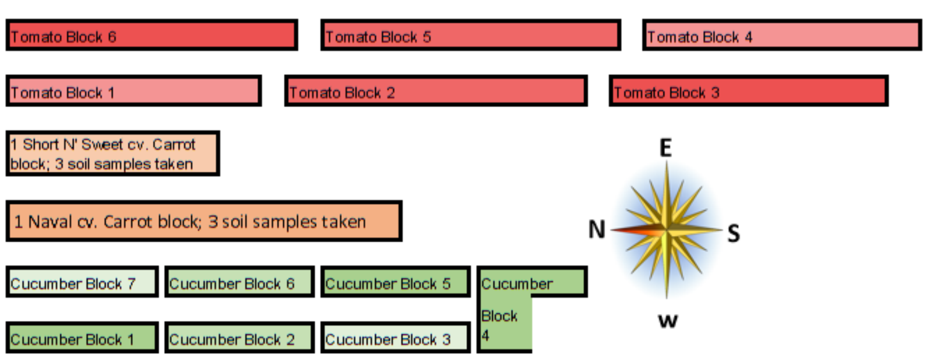


Figure 3.1 Map of field at Meig's horticultural farm where carrot, cucumber, and tomato plants were grown and where soil samples were taken from. Blocks are marked, each block had one control, black kow, and bsf treatment within.

Figure 3.2 Cucumber data

Figure 3.2A. Cucumber height (cm) growth comparing soil amendment treatments BK (Black Kow; 62.33 cm), BSF (Black soldier fly; 73.17 cm), and control (no amendment; 60.71 cm). BSF compost improved cucumber height compared to the control group; however, BK was not different from either BSF or control treatments ($F_{2,18} = 4.68$, $p = 0.02$).

Figure 3.2B. Cucumber stem diameter (mm) comparing soil amendment treatments BK (Black Kow; 11.84 mm), BSF (Black soldier fly; 13.06 mm), and control (no amendment; 11.59 mm). BSF compost improved stem diameter in comparison to the control group; however, BK was not different from either BSF or control treatments ($F_{2,18} = 4.11$, $p = 0.04$).

Figure 3.2C. Cucumber yield data by count, comparing soil amendment treatments BK (Black Kow; n = 79), BSF (Black soldier fly; n = 134), and control (no amendment; n = 79). There were no differences observed between cucumber yields ($F_{2,19} = 20.41$, $p = 0.67$).

Figure 3.2D. Average dried weight of below ground cucumber biomass (grams; g) grown on different soil amendments BK (Black Kow; 6.80 g), BSF (Black soldier fly; 6.17 g), and control (no amendment; 4.71 g). BK had more BGB than the control treatment, however BSF had no differences between the compost treatments ($F_{2,2} = 3.59$, $p = 0.04$).

Figure 3.2E. Average dried weight of above ground cucumber biomass (grams; g) grown on different soil amendments BK (Black Kow; 190.32 g), BSF (Black soldier fly; 209.77 g), and control (no amendment; 184.33 g). There were no observable differences between compost treatments ($F_{2,2} = 1.11$, $p = 0.34$).

Figure 3.2 continued

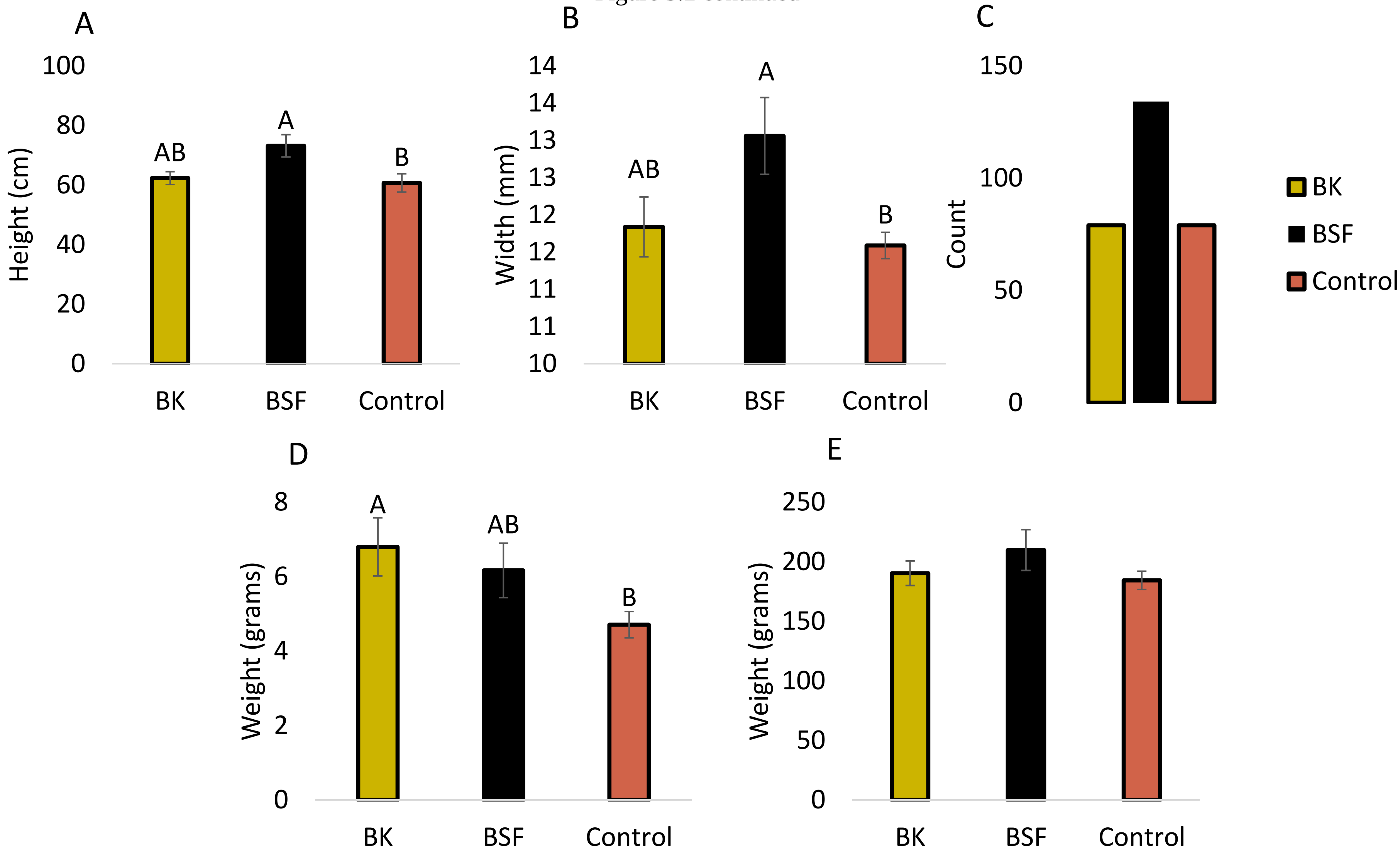


Figure 3.3: Tomato data;

Figure 3.3A. Tomato height (cm) growth comparing soil amendment treatments BK (Black Kow; 85.47 cm), BSF (Black soldier fly; 85.00 cm), and control (no amendment; 81.17 cm). No differences were observed in tomato height on varying composts ($F_{2,18} = 1.12$, $p = 0.35$).

Figure 3.3B. Tomato stem diameter (mm) comparing soil amendment treatments BK (Black Kow; mm), BSF (Black soldier fly; mm), and control (no amendment; mm). There were no observed differences between composts and the control for tomato stem diameter ($F_{2,17} = 2.96$, $p = 0.08$).

Figure 3.3C. Tomato yield by weight (kilograms; kg), comparing soil amendment treatments BK (Black Kow; 30.74 kg), BSF (Black soldier fly; 27.06 kg), and control (no amendment; 30.99 kg). There were no differences observed between tomato yields ($F_{2,17} = 0.09$, $p = 0.91$).

Figure 3.3D. Average dried weight of below ground tomato biomass (kilograms; kg) grown on different soil amendments BK (Black Kow; 30.74 kg), BSF (Black soldier fly; 27.07 kg), and control (30.99 kg). BK had more BGB than the control treatment, however BSF had no differences between the compost treatments ($F_{2,2} = 7.32$, $p = 0.001$).

Figure 3.3E. Average dried weight of above ground tomato biomass (grams; g) grown on different soil amendments BK (Black Kow; 71.34 g), BSF (Black soldier fly; 76.22 g), and control (no amendment; 112.66 g). There were no observable differences between compost treatments ($F_{2,2} = 0.57$, $p = 0.57$).

Figure 3.3 continued

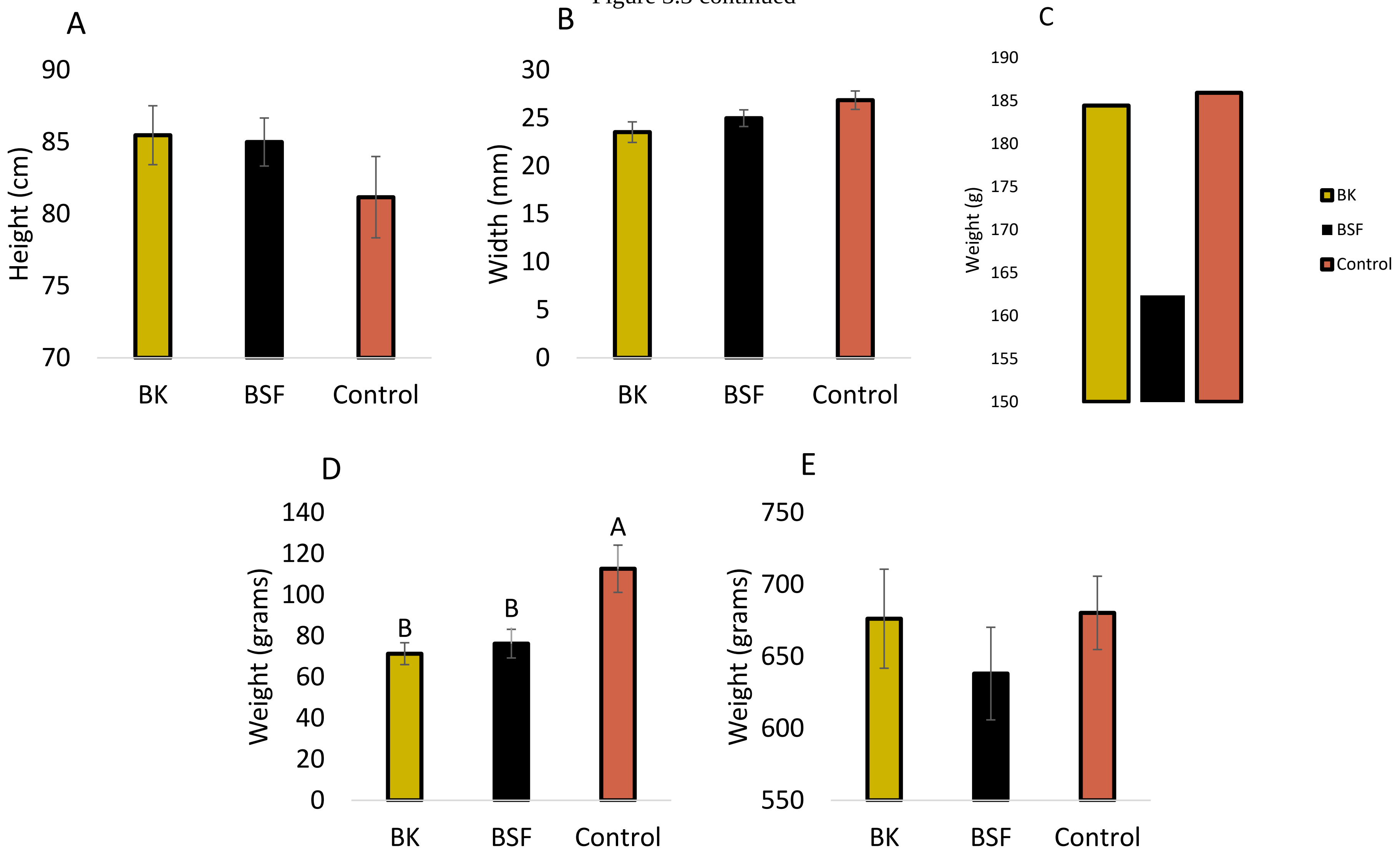


Figure 3.4: Carrot data;

Figure 3.4A. Carrot height (cm) growth comparing cultivars Naval and Short N' Sweet on soil amendment treatments BK (Black Kow), 28.75 cm and 24.08 cm, respectively; BSF (Black soldier fly), 27.76 cm and 27.31 cm, respectively; and control (no amendment) 25.93 cm and 28.54 cm, respectively. There were no effects between treatments or cultivars ($F_{5,89} = 1.54$, $p = 0.19$).

Figure 3.4B. Carrot number of leaves (n) comparing cultivars Naval and Short N' Sweet on soil amendment treatments BK (Black Kow), n = 6.85 and 7.25, respectively; BSF (Black soldier fly), n = 7.82 and 6.62, respectively; and control (no amendment) n = 7.40 and 8.23, respectively. There were no effects between treatments or cultivars ($F_{5,89} = 1.46$, $p = 0.21$).

Figure 3.4C. Average dried weight of above ground carrot biomass (grams; g) grown on different soil amendments treatments BK (Black Kow), 8.97 g and 4.70 g, respectively; BSF (Black soldier fly), 12.25 g and 7.75 g, respectively; and control, 19.84 g and 5.45 g, respectively. There were no observable differences between compost treatments ($F_{2,2} = 0.09$, $p = 0.91$) and cultivars.

Figure 3.4D. Average dried weight of below ground carrot biomass (grams; g) grown on different soil amendments treatments BK (Black Kow), 7.50 g and 11.94 g, respectively; BSF (Black soldier fly), 11.97 g and 15.63 g, respectively; and control, 10.87 g and 13.74 g, respectively There were no observable differences between compost treatments ($F_{2,2} = 0.01$, $p = 0.99$).

Figure 3.4 continued

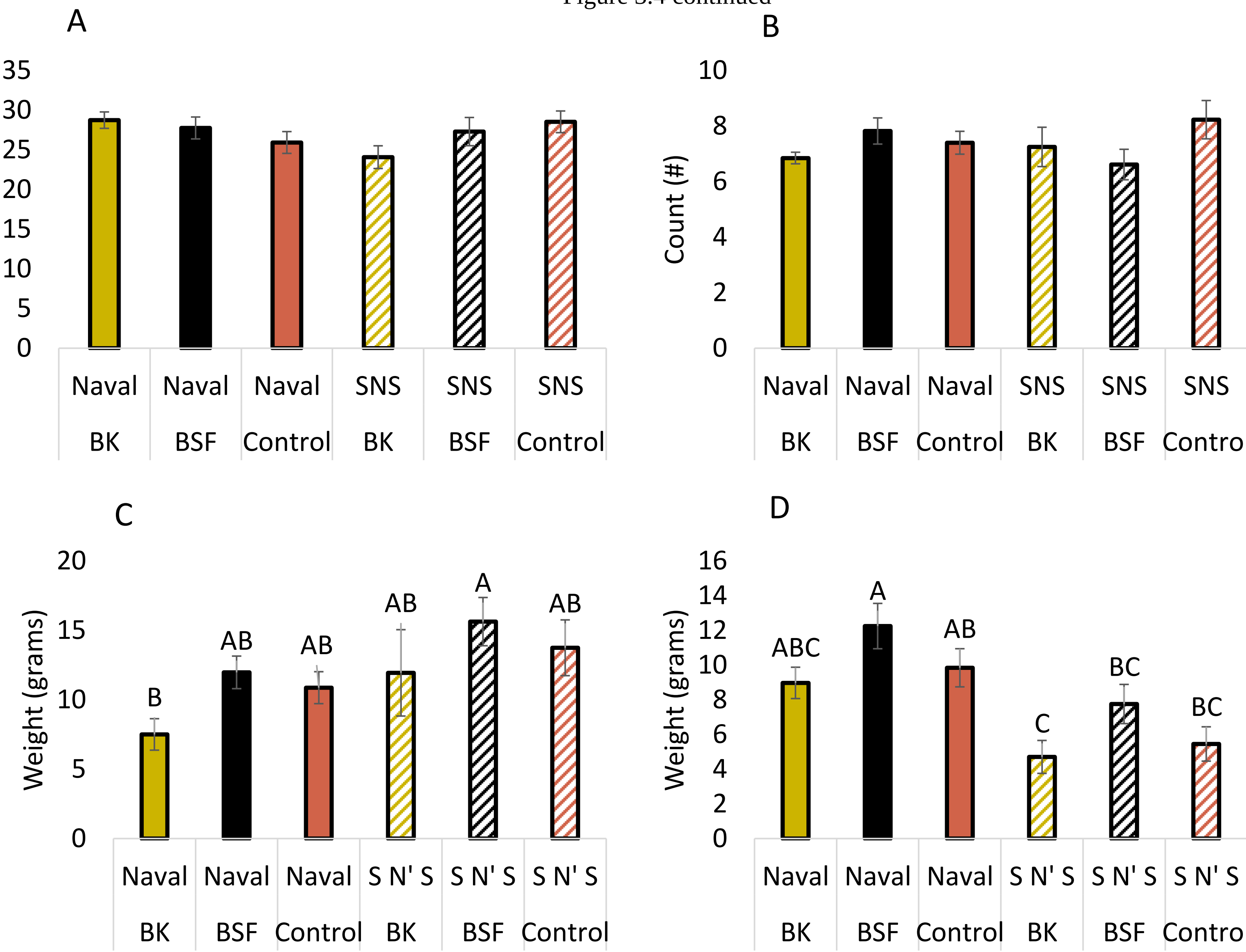


Figure 3.5 Soil data;

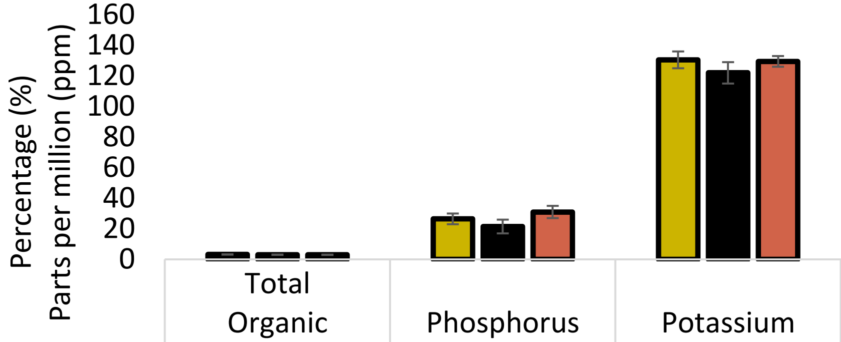
Figure 3.5A. Soil properties for cucumber plant total organic matter (percentage; %) and phosphorus and potassium (part per million; ppm) were compared for differences in soil post plant growth. The were no observable differences between soil amendment treatments for total organic material ($F_{2,11} = 0.87$, $p = 0.45$), phosphorus ($F_{2,11} = 2.87$, $p = 0.11$), and potassium ($F_{2,11} = 1.03$, $p = 0.39$).

Figure 3.5B. Soil properties for tomato plant total organic matter (percentage; %) and phosphorus and potassium (part per million; ppm) were compared for differences in soil post plant growth. The were no observable differences between soil amendment treatments for total organic material ($F_{2,8} = 0.32$, $p = 0.74$), phosphorus ($F_{2,8} = 1.45$, $p = 0.31$), and potassium ($F_{2,8} = 1.35$, $p = 0.33$).

Figure 3.5C. Soil properties for carrot plant total organic matter (percentage; %) and phosphorus and potassium (part per million; ppm) were compared for differences in soil post plant growth. The were no observable differences between soil amendment treatments for total organic material ($F_{2,5} = 1.44$, $p = 0.36$), phosphorus ($F_{2,5} = 1.39$, $p = 0.37$), and potassium ($F_{2,5} = 0.71$, $p = 056$).

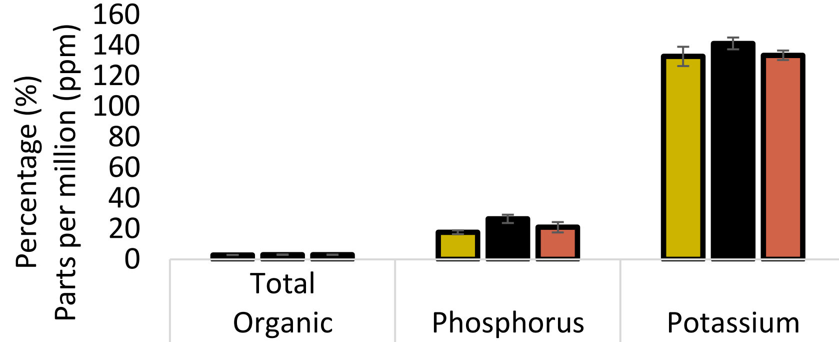
Figure 3.5 continued

A



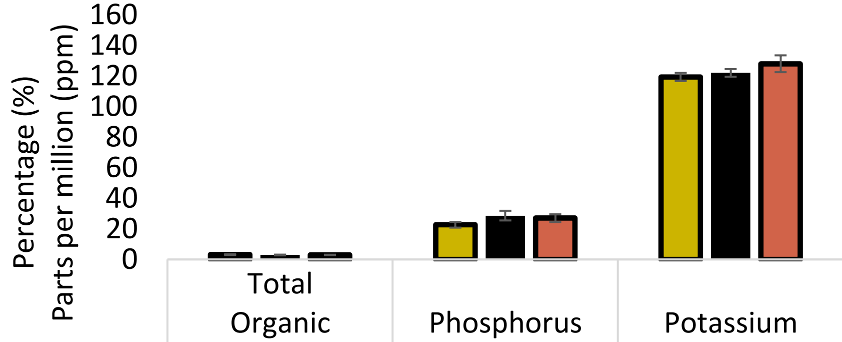
	Total Organic Matter	Phosphorus	Potassium
BK	3.20	26.50	130.50
BSF	3.05	21.50	122.00
Control	3.00	31.00	129.50

B



	Total Organic Matter	Phosphorus	Potassium
BK	2.93	17.75	132.75
BSF	3.10	26.50	141.25
Control	3.05	21.00	133.50

C



	Total Organic Matter	Phosphorus	Potassium
BK	3.13	22.67	119.33
BSF	2.90	28.67	122.00
Control	2.97	27.00	128.00